School of Medicine
Marmara Med J

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Instructions to Authors

About Journal

The Marmara Medical Journal, Marmara Med J, is a multidisciplinary, academic publication of Marmara University, School of Medicine. It is an open access, double blind peer-reviewed journal. It publishes manuscripts that focus on clinical and laboratory medicine, health care policy and medical education, ethics, and related topics. It includes original research papers, case reports, reviews, articles about clinical and practical applications and editorials, short reports, letters to the editor and occcasionally a photo-quiz.

The Marmara Medical Journal is continuously published since 1988 and its archive with full-text manuscripts can be reached under www.dergipark.org.tr/marumj/archive.

Frequency: Three times a year (January, May, October)

Year of first print issue: 1988

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The manuscripts published in the Marmara Medical Journal are indexed and abstracted in: Thomson Reuters/ Emerging Sources Citation Index (ESCI), EBSCO, SCOPUS, EMBASE/Excerpta Medica, DOAJ (Directory of Open Access Journals), CrossRef, ULRICH'S Database, Google Scholar, The British Library, Turkish Academic Network and Information Center (ULAKBİM)-Turkish Medical Database, TURK MEDLINE–Türk Sağlık Bilimleri (Index of Turkish Health Sciences), Türkiye Makaleler Bibliyografyası (Bibliography of Articles in Turkish Periodicals), Türkiye Klinikleri Tip Dizini (Turkish Citation Index).

Aims and Scope

The Marmara Medical Journal, Marmara Med J, is a peer-reviewed, multidisciplinary academic publication of Marmara University, School of Medicine, which is authored by physicians both nationally and internationally.

The journal aims to publish papers of general interest relating to advances in medical practice and novel treatments that will be of interest to general practitioners, medical students, and senior practitioners and specialists. Marmara Medical Journal also aims to publish all types of research conducted by medical students.

The Marmara Medical Journal is among the most widely read and cited scientific publications for physicians among journals of its kind nationally and increasingly gaining new readers and authors internationally with its English only format since 2016.

The journal consists of manuscripts on recent developments in general and internal medicine and new methods of treatment based on original research. We greatly welcome research papers, case reports, reviews and occasionally a photo-quiz of an interesting medical encounter in English, only.

Each manuscript is strictly assessed by a select Editorial Board. and refereed critically by two or more reviewers, at least one from another institution. The editor reserves the right to reject or to return the manuscript to the author(s) for additional changes.

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The Marmara Medical Journal has an open access policy. All articles in the journal are permanently available online for all to read.

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The Marmara Medical Journal publishes original scientific research papers, case reports, manuscripts about clinical and practical applications and editorials, short reports, letters and occasionally a photo-quiz.

Manuscripts submitted under multiple authorship are reviewed on the assumption that all listed authors concur with the submission and that a copy of the final manuscript has been approved by all authors and tacitly or explicitly by the responsible authorities in the laboratories where the work was carried out.

Manuscripts are accepted for review with the understanding that no substantial portion of the study has been published or is under consideration for publication elsewhere.

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Pages should be numbered consecutively and organized as follows:

1. Title Page
2. Abstract
3. Keywords
4. Introduction
5. Materials and Methods
6. Results
7. Conclusion
8. References

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State why the investigation was carried out, note any relevant published work, and delineate the objective of the investigation.

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New methods or significant improvements of methods or changes in old methods must be described. Methods for which an adequate reference can be cited are not to be described, except for providing information about the aims of the method. Details regarding animal housing conditions should be given. All clinical studies must contain :

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ii. Walker M, Hull A. Preterm labor and birth. In: Taeusch HW, Ballard RA, eds. Avery's Diseases of the Newborn. Philadelphia: WB Saunders, 1998: 144,153.

iii. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 2017; 67: 1265-73. doi: 10.1016/j.jhep.2017.07.027.

iv. WONCA Ad Hoc Task Force on Tobacco Cessation.

http://globalfamilydoctor.com/publications/new/november/09.htm
(Accessed on …….)

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1-3] or [1,3].

Figures, Tables, Units

Diagrams and illustrations should be given Arabic numerals. All figure legends should be grouped and written on a separate page. Each Figure should be in one of the following preferred formats: Tiff, JPEG, PDF, and EPS.Tables should be numbered consecutively with Roman numerals in order of appearance in the text. Type each table double-spaced on a separate page with a short descriptive title directly above and with essential footnotes below.

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Statement of Human Rights

Title:

This is to certify that the procedures and the experiments followed for the manuscript were in accordance with the ethical standards of the …… Ethics Committee on human experimentation and with the ethical standards in the Declaration of Helsinki 2013, as well as the national law.

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Title:

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Case Report

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Assessment of the knowledge and awareness of a sample of young researcher physicians on reporting guidelines and the EQUATOR network: A single center cross-sectional study

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ABSTRACT

Objective: This study aimed to investigate knowledge and awareness of a sample of young researcher physicians about reporting guidelines and the EQUATOR network.

Materials and Methods: One hundred young researcher physicians were enrolled for the study and evaluated using a questionnaire which assessed the level of usage and knowledge of reporting guidelines and the EQUATOR network.

Results: Thirty-eight percent of the participants were aware of the EQUATOR network. The most recognized reporting guidelines were CONSORT (32%), PRISMA (35%), and the least recognized ones were ARRIVE (12%) GRRAS (12%). The percentage of participants who were aware of the EQUATOR network and reporting guidelines were deficient. Seventy-three percent of the participants declared that they requested more information about reporting guidelines when they were asked whether they wished to be informed about reporting guidelines. There were statistically significant differences between specialists and residents regarding the level of knowledge on reporting guidelines and reporting guideline use except for PRISMA and SAMPL guidelines and their level of the desire to be informed about guidelines were similar. When participants whose publication number was above and below the mean of publications of all participants were compared, the level of reporting guidelines usage and knowledge of PRISMA, GRRAS were similar and both groups showed equal levels of desire to be informed about guidelines.

Conclusion: The use and awareness of the EQUATOR network and reporting guidelines are low among young researcher physicians. To improve the quality of manuscript writing and the acceptance rates, knowledge and awareness of the EQUATOR network and reporting guidelines should be increased among young physicians doing research.

Keywords: EQUATOR network, Reporting guidelines, Research

INTRODUCTION

Inaccurate and variable reporting of medical research can contribute to wasted research and sources [1]. As a result of poor methodology or improper reporting, published studies cannot often be replicated by researchers, compared with the existing literature, included in evidence synthesis or translated into clinical practice [1,2]. A reporting guideline is a simple, structured tool for health researchers to use while writing manuscripts. Reporting guidelines are statements that provide advice on how to report methods and results of a research.

Reporting guidelines are more than just some thoughts about what needs to be in an academic paper. Reporting guidelines are defined as: "A checklist, flow diagram, or structured text to guide authors in reporting a specific type of research, developed using explicit methodology". They provide a minimum set of items that are necessary for a transparent and clear account of what was done and what was found in a research study [2]. A reporting guideline provides a minimum list of the information needed to ensure a manuscript can be: understood by a reader, replicated by a researcher, used by a doctor to make a clinical decision, and included in a systematic review [2,3].

The Enhancing the QUAlity and Transparency Of Health Research (EQUATOR) network is an "umbrella" organization that brings together researchers, medical journal editors, peer reviewers, developers of reporting guidelines, research funding bodies and other collaborators with a mutual interest in improving the quality of research publications and of research itself. The EQUATOR network aims to achieve accurate, complete, and transparent reporting of all health research studies to support research reproducibility and usefulness, increase the value of health research and to minimize avoidable waste of financial and human investments in health research [2]. The EQUATOR network's online Library for Health Research Reporting currently lists almost 409 reporting guidelines [3]. Some of them are core guidelines for the most commonly conducted research types, and some of them are specialized guidelines which are

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specific to several research areas [4]. Popham et al. previously defined Consolidated Standards of Reporting Trials Statement for Reporting Randomized Controlled Trials (CONSORT); Transparent Reporting of Evaluations with Nonrandomised Designs (TREND) for nonrandomized trials; Strengthening the Reporting of Observational Studies in Epidemiology (STROBE), Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Standards for Reporting of Diagnostic Accuracy Studies (STARD) as core guidelines because they are frequently recommended guidelines by journals, have permanent websites, have explanations and examples that may be found in separate publications at the time of Popham et al.'s preparation of their manuscript [4]. CONSORT for randomized controlled studies, PRISMA for systematic reviews and meta-analysis, Consensus-based Clinical Case Reporting (CARE) for case report, STROBE for observational studies, STARD for diagnostic accuracy studies, Animal Research: Reporting: In Vivo Experiments Guidelines (ARRIVE) for experimental animal studies and Statistical Analyses and Methods in the Published Literature (SAMPL) for statistical analysis are the basic guidelines that represent a range of study design.

The use of reporting guidelines and submission of checklists of the guidelines are promoted by the majority of high impact journals and journals which are indexed in the Science Citation Index or Science Citation Index Expanded [5,6]. Following them helps in preparing high-quality research, facilitates peer review, and increases the chances of manuscript acceptance [6]. Improved awareness and knowledge about reporting guidelines and the EQUATOR network may increase accurate reporting, thus providing adequate evidence to synthase and translate into clinical practice and increase acceptance rates. Several editorials are published to encourage researchers to use reporting guidelines. The level of knowledge and awareness of these reporting guidelines among authors, reviewers, and editors have been investigated in various scientific fields [2,3,5-9]. But, previously, only one study investigated the level of knowledge and awareness of these reporting guidelines among young physicians. However, the physicians' samples were limited to pediatricians [10].

The objective of this study was to investigate knowledge and awareness of a sample of young physicians engaged in research regarding reporting guidelines and the EQUATOR network. A further aim of this study was to increase young physicians' and readers' awareness about reporting guidelines and the EQUATOR network as well as increasing awareness of guidelines other than core guidelines.

MATERIALS and METHODS

One hundred young researcher physicians from Marmara University Training and Research Hospital were enrolled in this cross-sectional study undertaken between January 2019 and March 2019. Oral and written informed consents were obtained from all participants. Ethics committee approval was obtained from the Ethics Committee of Marmara University Medical School (approval number: 206). This study was reported in accordance with the checklist of items in the STROBE statement. Inclusion criteria were: (1) Being between the ages of 25-45; (2) Being a resident, specialist, fellowship program resident, fellowship specialist, assistant professor or associate professor; (3) Working less than 15 years in their respective field. Participants who were previously involved in a study investigating knowledge and awareness of reporting guidelines were excluded. One hundred young researcher physicians were interviewed. Knowledge and awareness on reporting guidelines were investigated via a questionnaire which was adopted from the survey invented by Oncel et al [10]. Data on age, sex, years in medical practice, professional degree, and specialty were recorded. Participants were requested to fill out a non-standardized 28-item questionnaire. Participants were asked whether they had participated in any scientific study before the completion of the questionnaire, how many publications they have made; whether they had acted as a reviewer, and if yes, their numbers of reviews; whether they had used reporting guidelines in their publications; whether they had known about the EQUATOR network; which reporting guidelines they had known and whether they had used these as authors or reviewers; and whether they wanted to be informed about these guidelines.

Statistical analysis

IBM SPSS Statistics for Windows, Version 20,0 (Armonk, NY) was used to perform the analysis. The histogram and normality plots and Kolmogorov-Smirnov normality test were used to evaluate the distribution of variables before test selection. For the categorical data, descriptive analysis was performed and presented as frequency and percent values. For comparisons chi-square, Fisher's exact test and independent-samples t-test were used, and $p < 0.05$ was accepted as significant.

RESULTS

The recruitment flowchart is presented in Figure 1**.** A total of 110 participants were asked to complete the questionnaire, 10 of them were excluded, and 100 participants were enrolled and interviewed. Characteristics of the participants were presented in Table I. Thirty-eight percent of the participants were aware of the the EQUATOR network. The most recognized reporting guidelines were CONSORT (32%), PRISMA (35%), and the least recognized ones were ARRIVE (12%) and GRRAS (12%). The percentage of participants who were aware of the EQUATOR network and reporting guidelines were very low (Figure 2). Also, the rate of residents who knew about the EQUATOR Network and reporting guidelines were very low (Figure 3). Thirty-seven percent of the participants answered ''Yes" to the question ''Do you use reporting guidelines during publication of research?" When the participants were asked at which stages of production or publication of research they use reporting guidelines, 24% of the participants reported they used them while submitting their research to a journal, 14% of them used them during writing the manuscript, 12% of them reported that they used them while preparing research proposals for ethics review, 5% of the participants used reporting guidelines when determining study protocol. Only 19% of the participants answered "Yes" to the question ''Do you use reporting

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guidelines when serving as a reviewer for a scientific journal?" 73% of the participants declared that they requested more information about reporting guidelines when they were asked whether they wished to be informed about reporting guidelines. Participants were categorized into two groups as residents and medical specialists (specialists, fellowship residents, assistant professors and associate professors). When the specialist and residents were compared, there were statistically significant differences between them with regards to the level of knowledge of reporting guidelines and reporting guideline use except for PRISMA and SAMPL guidelines but their level of desire to be informed about guidelines were similar (Table II). When the participants with publications below and participants with publications above the mean number of publications were compared concerning their level of reporting guidelines usage and knowledge of PRISMA and GRRAS, the results of the groups were similar and both groups stated equal levels of desire to be informed about guidelines (Table III). When participants from Surgery and Internal Medicine were compared, participants from Surgery used reporting guidelines less than participants from Internal Medicine during publication of a research study (Table IV).

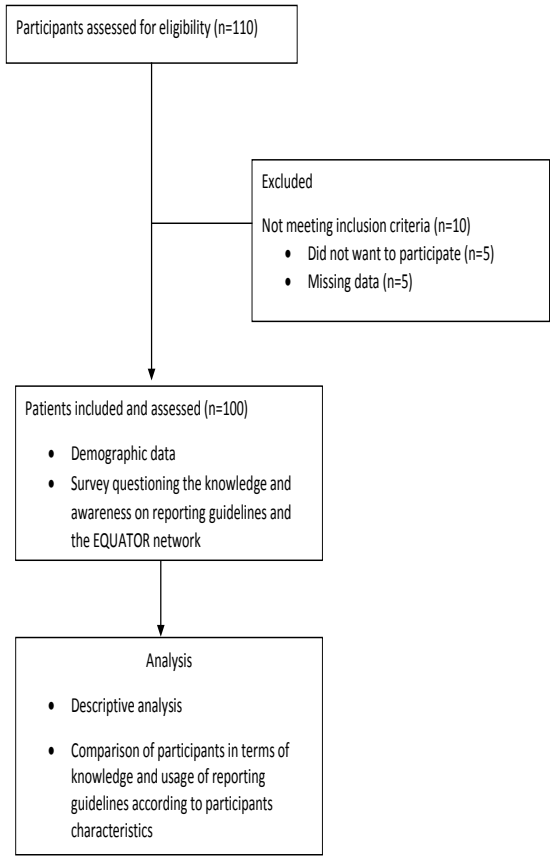

Figure 1. Flow chart of the study.

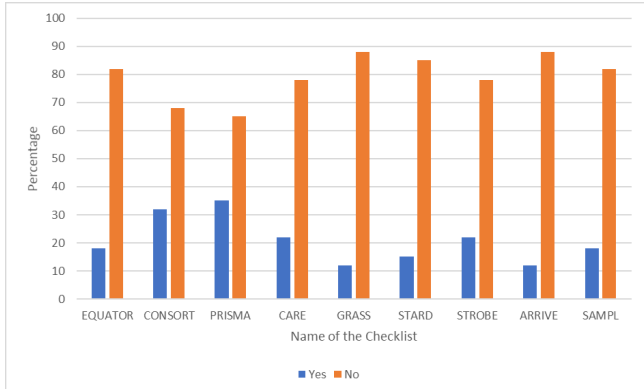

Figure 2. The percentage of participants who are aware of reporting guidelines and the EQUATOR network

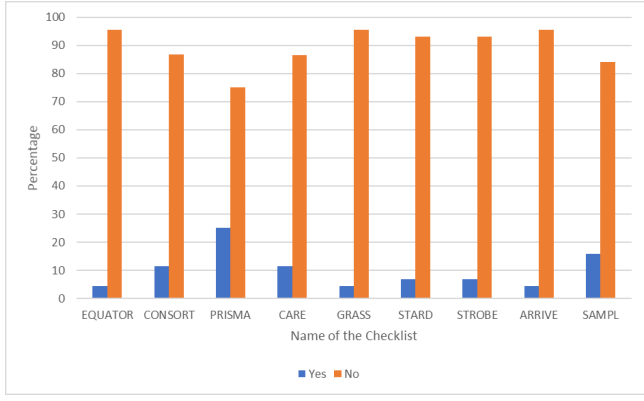

Figure 3. The percentage of residents who know about reporting guidelines and the EQUATOR network

Table I. Characteristics of the participants.

Age	**32.69±5.91**
Gender (female/male)	**39/61**
Years in medical practice	**8.3±6.27**
Professional degree	
Resident	44%
Specialist	23%
Fellowship Resident	10%
Assistant Professor	11%
Associate Professor	12%
Medical specialty	
Internal medicine	64%
Surgery	36%
Having participated in a research previously	87%
Number of publications	11.77±17.55
Publications in Turkish	3.5±5.63
Publications in English	8.05±12.92
Publications	
Turkish	64%
English	65%
Previously acted as a reviewer	**35%**

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Table II. Comparisons between specialists and residents

	Specialist (n=56)	Resident (n=44)	p
Having participated in a research previously	54 (96%)	33 (75%)	**0.002**
Number of publications	19.36±20.33	2.11±3.24	**0.005**
Proportion of participant who have publication in Turkish	47 (83%)	17 (38%)	**0.005**
Number of publications in Turkish			**0.005**
Proportion of participant who have publication in English	52 (92%)	13(29%)	**0.005**
Number of publications in English	13.95±14.81	0.55±0.98	**0.005**
Number of participants who use reporting guidelines	31 (55%)	6 (13%)	**0.005**
Number of participants who use reporting guidelines during publication process	38 (67%)	14 (31%)	**0.005**
Number of participants who act as reviewers	34 (60%)	1 (0.2%)	**0.005**
Number of participants who use reporting guidelines as a reviewer	17 (30%)	1 (0.2%)	**0.005**
Number of participants who know the EQUATOR Network	16 (28%)	2 (0.4%)	**0.002**
Number of participants who know CONSORT	27 (48%)	5 (11%)	**0.005**
Number of participants who know PRISMA	24 (42%)	11 (25%)	**0.06**
Number of participants who know CARE	17 (30%)	5 (11%)	**0.02**
Number of participants who know GRRAS	10 (17%)	2 (0.4%)	**0.04**
Number of participants who know STARD	12 (21%)	3 (0.6%)	**0.04**
Number of participants who know STROBE	19 (33%)	3(0.6%)	**0.001**
Number of participants who know ARRIVE	10 (17%)	2(0.4%)	**0.04**
Number of participants who know SAMPL	11 (19%)	7 (15%)	**0.63**
Number of participants who want further information about reporting guidelines	45 (80%)	28 (63%)	**0.06**

Table III. Comparisons between participants whose number of publications are under the mean of all participants and whose number of publications are over the mean of all participants

	Above mean (n=33)	Below mean (n=67)	p
Having participated in a research previously	33 (100%)	54 (80%)	**0.007**
Number of publications	30.73±19.5	2.43±2.84	**0.005**
Proportion of participant who have publication in Turkish	32 (96%)	32 (47%)	**0.005**
Number of publications in Turkish	8.39±7.51	1.09±1.5	**0.005**
Proportion of participant who have publication in English	33 (100%)	32(47%)	**0.005**
Number of publications in English	21.91±14.64	1.22±1.78	**0.005**
Number of participants who use reporting guidelines	16 (48%)	21(31%)	0.09
Number of participants who use reporting guidelines during publication process	22 (66%)	30 (44%)	**0.04**
Number of participants who act as reviewers	27 (81%)	8 (24%)	**0.005**
Number of participants who use reporting guidelines as a reviewer	11 (33%)	7 (10%)	**0.01**
Number of participants who know EQUATOR network	11 (33%)	7(10%)	**0.005**
Number of participants who know CONSORT	18 (54%)	14 (20%)	**0.001**
Number of participants who know PRISMA	16 (48%)	19 (28%)	**0.05**
Number of participants who know CARE	12 (36%)	10(14%)	**0.02**
Number of participants who know GRRAS	7 (21%)	5 (%7)	0.05
Number of participants who know STARD	8 (24%)	7 (10%)	0.06
Number of participants who know STROBE	13 (39%)	9	**0.003**
Number of participants who know ARRIVE	9 (27%)	3	**0.001**
Number of participants who know SAMPL	10 (30%)	8	**0.03**
Number of participants who want further information about reporting guidelines	25 (75%)	48	0.66

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Table IV. Comparisons between participants from Surgery and Internal Medicine

	Internal Medicine (n= 64)	Surgery (n=36)	p
Having participated in a research previously	57 (89%)	30 (83%)	0.41
Number of publications	10.13±15.43	14.69±20.71	0.21
Proportion of participant who have publication in Turkish	41 (64%)	23 (63%)	0.72
Number of publications in Turkish	2.63±4	5.06±7.55	**0.03**
Proportion of participant who have publication in English	44 (68%)	21 (58%)	0.54
Number of publications in English	7.17±12.02	9.61±14.43	0.36
Number of participants who use reporting guidelines	26 (40%)	11 (30%)	0.32
Number of participants who use reporting guidelines during publication process	39 (60%)	13 (36%)	**0.02**
Number of participants who act as reviewers	22 (34%)	13(36%)	0.41
Number of participants who use reporting guidelines as a reviewer	12 (18%)	6 (16%)	0.39
Number of participants who know EQUATOR network	14 (21%)	4 (11%)	0.18
Number of participants who know CONSORT	21 (32%)	11 (30%)	0.81
Number of participants who know PRISMA	22 (34%)	13 (36%)	0.86
Number of participants who know CARE	16 (25%)	6 (16%)	0.33
Number of participants who know GRRAS	9 (14%)	3 (8%)	0.39
Number of participants who know STARD	10 (15%)	5 (13%)	**0.81**
Number of participants who know STROBE	14 (21%)	8 (22%)	**0.97**
Number of participants who know ARRIVE	7 (10%)	5(13%)	**0.66**
Number of participants who know SAMPL	12 (18%)	6(16%)	**0.79**
Number of participants who want further information about reporting guidelines	49 (76%)	24 (66%)	**0.28**

DISCUSSION

According to the results of the present study, the level of knowledge and awareness about reporting guidelines and the EQUATOR network among young physician researchers is low. Independent of being expert or resident or of the number of publications and of the medicine field, they all wanted to be informed about reporting guidelines and the EQUATOR network.

Recently, editors of journals from variable medical fields have published editorials to increase the awareness of researchers among reporting guidelines and aimed to promote transparent and accurate reporting of research. Also, adherence of journals to reporting guidelines have been investigated by checking author instructions of the journals [2,5,7-9,11]. In line with the results of the current study, the endorsement of reporting guidelines was found to be low in journals. Similar to the results of the current study, CONSORT and PRISMA were the most recommended guidelines that were found in author instructions [8,9].

In a study conducted by Oncel et al [10], the rate of participants who were previously involved in a research was 63.8%, which was found as 87% in our study and the rate of participants who have used reporting guidelines before was 26.6%, which was found to be 37% in our study. In the present study, CONSORT (32%) and PRISMA (35%) were the most recognized guidelines. Similarly, Oncel et al., detected that the most known guidelines were PRISMA (10.3%) and CONSORT (9.3%) among a sample of pediatricians [10]. Marusic et al., assessed the endorsement of reporting guidelines in Rheumatology journals [8]. They detected that only ten Rheumatology journals among twenty-eight journals recommended the use of reporting guidelines in instructions to authors in 2012, and the most recommended guideline was CONSORT. In another study, Kunath et al., investigated which reporting guidelines were recommended in journals publishing in the field of Urology [9]. Then it was detected that fourteen journals (25.5%) among the fifty-five journals mentioned at least one reporting guidelines in their author instructions. CONSORT was the most frequently recommended guideline in author instructions of Urology journals while reporting guidelines other than CONSORT was mentioned by the 6% of the journals publishing in the field of Urology. These results were compatible with the results of the present study that showed CONSORT was the most recognized reporting guideline, and the other was less known among young researchers. In 2014, Grindlay et al., assessed the knowledge and views of the Editors-in-Chief of veterinary journals [7]. Thirty-six of 68 editors (52%) mentioned that they were aware of a reporting guidelines and 20 of these editors (35.1%) stated that they refer reporting guidelines in their journals' instructions for authors. CONSORT and ARRIVE were the most recommended guidelines in instructions for authors. This result was opposite to the finding of the present study which found ARRIVE as one of the least known guidelines. This can be explained with the fact that ARRIVE is the reporting guideline for animal research, which is the leading research area of veterinary journals. When the editors were asked whether more information about guidelines would be useful, 88% of them answered "Yes" [7]. Similar to these results, all of the participants in this study wanted to be informed more about guidelines.

Reporting guidelines can be accounted as guides that may be used by reviewers and to evaluate the details of scientific studies objectively [10]. According to the results of the present study, the use of reporting guidelines by young physician researchers while serving as a reviewer for a scientific journal was found to be very infrequent. In line with the results of the present study, Oncel et al., reported that only 4 (4.5%) of 224 pediatrician researchers stated that they use guidelines while reviewing an article [10].

Eighty-seven percent of the participants have previously been involved in research, but only 38% of participants of the study were aware of the EQUATOR network. In the study by Oncel

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et al.,63.8% of the participants had participated in research and similarly, their level of knowledge and awareness about the EQUATOR network was low (5.8% of the participants). Fuller et al., also demonstrated that familiarization with the EQUATOR network was low, 91% of the participants were unfamiliar with the EQUATOR network [1].

Oncel et al., compared pediatricians working at a university hospital to pediatricians working in a setting other than a university hospital in terms of their knowledge and awareness status. They did not determine any difference between them. Knowledge and awareness status were detected to be low in both. This study aimed to compare residents with specialists in terms of their knowledge and awareness about reporting guidelines. It also compared participants from Surgery and participants from Internal Medicine. A further analysis was made to compare participants with publications above or below the mean number of publications in terms of their knowledge and awareness about reporting guidelines. Although, knowledge of participants with higher number of publications and specialists' on well-recognized guidelines such as CONSORT and PRISMA was higher, they had lower level of knowledge about other specific guidelines such as GRRAS, STARD, SAMPL and the EQUATOR network. Their level of desire to gain knowledge about the EQUATOR network and reporting guidelines were similar regardless of being specialist or having higher number of publications. This study revealed the level of awareness and knowledge of young physician researchers. They all wanted to learn more about the EQUATOR network and reporting guidelines regardless of being a specialist or having a higher number of publications. The strength of this study is in exhibiting this reality. These findings may be used to inform further studies and increase attempts to improve knowledge of the EQUATOR network and reporting guidelines. We could not be able to compare characteristics of participants who were aware and not aware of the specific guidelines as the number of participants who were aware of the specific guidelines were very small. This can be viewed as the limitation of this study.

A systematic review of results using guidelines such as CONSORT may improve the quality of reporting [8]. Using reporting guidelines should not be seen as extra paperwork and useless time-consuming materials. We believe that increased awareness and knowledge of the EQUATOR network and reporting guidelines and adherence to reporting guidelines even at the protocol stage of research or review process of a scientific paper will be a milestone for increasing the scientific quality of studies [2,6]. Scholarly publishing in many fields has undergone an evolution. Guideline use while reporting studies has become mandatory in many areas of medicine [5]. Therefore, in conclusion, to elevate the quality of medical research, the level of knowledge of the EQUATOR network and reporting guidelines should be made higher, and young researchers should be encouraged to use them.

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Dental follicle mesenchymal stem cells regulate responses in sepsis

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ABSTRACT

Objective: Sepsis-induced immune alterations are associated with secondary infections and increased risk of death. The use of mesenchymal stem cells (MSCs) has been described as a novel therapeutic strategy. We evaluated the immunomodulatory effects of human dental follicle (DF-MSCs) on lymphocytes of sepsis and septic shock patients.

Materials and Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood samples of sepsis, septic shock and healthy subjects. PBMCs were co-cultured in the presence and absence of DF-MSCs with or without interferon-gamma (IFN-γ) for 72 hours. CD4+CD25+FoxP3+regulatory T (Treg) cell frequency, lymphocyte proliferation, cytokine levels and apoptosis were evaluated via flow cytometry.

Results: DF-MSCs significantly suppressed proliferation of lymphocytes in sepsis group compared to septic shock group ($p<0.005$). DF-MSCs remarkably increased Treg ratio in sepsis compared to control group ($p<0.05$). Reduction of lymphocyte apoptosis in co-cultures of DF-MSCs and PBMC was significant in both sepsis and septic shock groups. IFN-γ stimulation of DF-MSCs ameliorated shift in the T-cell subsets from Th2 to Th1 phenotype in septic shock.

Conclusion: Our findings revealed that DF-MSCs have immunoregulatory effects both in sepsis and septic shock, by reducing interleukin-4 (IL-4) and increasing IFN-γ levels. This immunoreactivity regulation may open new therapeutic approaches for septic shock patients.

Keywords: Sepsis, Septic shock, Dental follicle mesenchymal stem cells, Immunomodulation

INTRODUCTION

Several international societies convened an international task force to review the definitions of sepsis, septic shock, and related conditions recently and sepsis was defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection by the task force [1]. It is one of the main causes of morbidity and mortality in critically ill patients despite advances in our understanding of the pathogenesis [2]. Mortality has declined modestly with improved ventilation strategies and fluid management protocols but remains high according to data from the Surviving Sepsis Campaign [3,4].

Increasing evidence suggests that both innate immune and inflammatory responses are involved in the pathophysiology of sepsis [5]. Pro-inflammatory and anti-inflammatory processes begin promptly after sepsis initiation and in a short time hyperinflammatory phase occurs, and that follows with the anti-inflammatory phase of sepsis [6]. Immunosuppression has a critical role in sepsis, and this immune response occurs by the cytokine production that leads to end-organ damage. Potential mechanisms of immunosuppression in sepsis are the deviation of response from inflammatory to anti-inflammatory phase, anergy and loss of T-helper cells (Th1) and immunity [7].

Mesenchymal stem cells (MSCs) are multipotent adult progenitor cells that have the capacity to self-renew and differentiate into various cell lineages and they can be easily isolated from various tissues, including bone marrow, umbilical cord, and adipose tissues [8,9]. They have immunomodulatory and regenerative effects that make them attractive therapeutic candidates for a potential treatment option for diseases and immune disorders [10,11]. It is considered that MSCs can give rise to multilineage progenitor cells and play an active role in immunoregulatory processes by responding to the inflammatory challenge with the production of anti-inflammatory factors [12]. In recent

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years, many studies have proven that immunomodulation with stem cells is an important mechanism underlying the benefits of strengthening repair of damaged tissue and can therefore provide a therapeutic option for sepsis [13,14]. Dental follicle mesenchymal stem cells (DF-MSCs) have more advantages than other stem cells due to their doubling time, immunomodulation effects and tissue repair capabilities. The rapid proliferative capacity together with the immunoregulatory characteristics of dental tissue-derived MSCs may prompt future studies aimed at using these cells in the treatment or prevention of inflammatory diseases [15]. Our previous studies showed that DF-MSCs regulated Th1 and Th2 responses by increasing T regulatory (Treg) cell population and kept the effectory T lymphocytes in silent stage and blocked the antigen presentation by downregulating the costimulatory molecules [16,17].

In the present study, the immunomodulatory properties of DF-MSCs were investigated by evaluating lymphocyte proliferation, apoptosis, induction of regulatory Treg cells and cytokine profile in sepsis and septic shock *in vitro*. The mechanisms have been studied by determining the changes in inflammation-associated cytokine profiles and assessing the immunomodulatory effect of interferon-gamma (IFN-γ) on DF-MSCs in sepsis.

MATERIALS and METHODS

Selection of patients and study groups

Patients between the ages of 18 and 65 years with the diagnosis of sepsis or septic shock were included in the study. According to the 2001 International Sepsis Definitions Conference Report, patients divided into two groups as sepsis (Group I: mean age 29.8±7.2, n=10) and septic shock (Group II: mean age 49.3±14.6, n = 10). Written informed consent was obtained from all patients. Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood samples of Group I, Group II and healthy subjects named as Group III (mean age 32.1±6.4, n = 10).

Exclusion criteria were those who used anti-inflammatory drugs, corticosteroids, antibiotics and who received chemotherapy, radiotherapy or immunotherapy in the last six months. Patients were included in the study according to the 2001 International Sepsis Definitions Conference Report. Septic patients were defined as a systemic response to infection with the presence of some degree of organ dysfunction. Septic shock patients were who had sepsis-induced hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include but are not limited to lactic acidosis or oliguria.

Isolation, characterization and differentiation potential of DF-MSCs

Isolation: Dental follicle tissues were obtained from 4 volunteer adult donors aged between 20 and 25 years undergoing third-molar extraction without abscess. Tissue samples were taken from individuals who were not diagnosed with a genetic disease or did not receive chemotherapy, radiotherapy or immunotherapy. DF-MSCs were isolated and cultured as described previously [18]. Briefly, dental follicles were seperately cut into 0.5-1 mm pieces and enzimatically digested with 3mg/mL collagenase type I in phosphate buffer solution (PBS – Sigma-Aldrich,USA) at 37°C. After incubation period, tissue samples were filtered through 70 micron naylon filter and obtained cell suspension was washed twice with Dulbecco's modified eagle medium (DMEM) (Gibco, Thermofisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, USA) and centrifuged at 1200 rpm for 5 minutes. Cells were then suspended in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (hereafter refered as cDMEM). Each $5x10^5$ cells were cultured in T25 flask at 37°C and $5CO_2$% humidified incubator until reaching 80-90% confluency and subjected to the next passage. Cells were used at the third passage.

Characterization: For cell surface antigen phenotyping, third passage cells were stained with fluorescein and phycoerythrin-coupled antibodies and analyzed with FACSCalibur (BD). Cells were analyzed for positive markers; CD29 (APC), CD73 (PE), CD90 (FITC), CD106 (PerCp) for MSCs and negative markers; CD14 (APC), CD34 (FITC), CD45 (FITC) and HLA-DR (PE) were used. All antibodies were provided from BD Biosciences, USA.

Differentiation potential of DFSCs: To evaluate the multipotency of DF-MSCs, we stimulated the third passage cells for osteogenic, chondrogenic and adipogenic differentiation. For osteogenic differentiation, third passage cells were treated with osteogenic medium for three weeks with medium changes twice weekly. The osteogenic medium contains basal medium supplemented with 0.1 µM dexamethasone (Sigma-Aldrich, USA), 10 mM – glycerol phosphate (Sigma-Aldrich, USA), and 0.2 mM ascorbic acid (Sigma-Aldrich, USA). The chondrogenic differentiation medium was supplemented with 0.1 µM dexamethasone, 100 µg/ml sodium pyruvate (Sigma-Aldrich, USA), 40 µg/mL proline (Sigma-Aldrich, USA), 6.25 µg/mL insulin, 6.25 µg/mL transferrin, 6.25 ng/mL selenious acid, 1.25 mg/mL bovine serum albumin (BSA – Abcam, UK), and 5.35 mg/mL linoleic acid) in the basal medium (Mesencult tm). To induce adipogenic differentiation, third passage cells were treated with the adipogenic medium for three weeks. The medium was changed twice weekly. The adipogenic medium contains basal medium (Mesencult^{tm}, Stemcell Technologies, CA) supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich, USA), 1 mM hydrocortisone (Sigma-Aldrich, USA), 0.1 mM indomethacin (Sigma-Aldrich, USA), and 10% FBS (Sigma-Aldrich, USA).

Stimulation of DF-MSCs with IFN-γ

DF-MSCs in the third passage were inoculated in 48 well plates $5x10^4$ cells in 0,5 ml of DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco, Thermo Ffisher, USA) (hereby refered as complete DMEM) in each well. DF-MSCs were incubated for 24 hours to be confluent to the plate. After the incubation period, the medium was removed from wells and changed with stimulation media (IFN-γ 0,5 µg/ml, complete DMEM) and incubated for additional 24 hours.

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Collection of venous blood samples and isolation of PBMCs

Blood samples of patients were obtained from Marmara University Training and Research Hospital, Department of Anesthesiology and Reanimation under approved guidelines of Marmara University School of Medicine Ethical Board (70737436-050.06.04). Heparinized blood was collected with central venous catheter. The blood was transported and processed in the research laboratory within 30 minutes. PBMCs were isolated by ficoll-paque density gradient solution and resuspended in cell culture media (RPMI) 1640 supplemented with 10% FBS, 1% penicillin/streptomycin.

DF-MSCs and PBMCs co-cultures

Culture conditions: PBMCs were cultured in the presence and absence of DF-MSCs (IFN-γ$^+$ or IFN-γ$^-$) with the ratio of 1:10 (MSCs: PBMC) in 0.5 ml of RPMI 1640 supplemented with 10% FBS and 1% penicillin/streptomycin in each well of 48 well plates and lymphocytes were stimulated with anti-CD3 and anti-CD28 (eBioscience, Thermo Fisher Scientific, USA) purified antibodies. PBMCs in the presence and absence of DF-MSCs (IFN-γ$^+$ or IFN-γ$^-$) were cultured for 72 hours to evaluate lymphocyte proliferation, apoptosis, and regulatory T cell ratio in cultured PBMCs.

Lymphocyte Proliferation Assay: T cell proliferation ratio was measured by the cell surface staining with carboxyfluorescein diacetate succinimidyl ester (CFSE)(e-Bioscience, Thermo Fisher, USA). Lymphocytes (5×10^5/well) cultured in 48 well plates were stained with 1.8 μM CFSE and incubated for 6 minutes. After incubation period cells were washed with 10% FBS in phosphate-buffered saline (PBS). After 72 hours of the culture period, cells were analyzed by BD FACSCalibur flow cytometer (BD FACSCalibur Biosciences, USA). In order to analyze T lymphocyte proliferation the CD3+ gated cells were subjected to CFSE signaling.

Lymphocyte Apoptosis Assay: T cell apoptosis ratio was analyzed in PBMC cultures with and without DF-MSCs (IFN-γ$^+$ or IFN-γ$^-$) after 72 hours of culturing period. T-cell apoptosis assays were performed in 48-well round-bottom plates in a total volume of 0.5 mL RPMI 1640 medium with 10% FBS and 1% penicillin/streptomycin. After 72 hours of the culture period, cells were washed twice with PBS and stained with anti-CD3 (PE), Annexin V (Fluorescein isothiocyanate; FITC) and Propidium Iodide (Peridinin-Chlorophyll-protein; PerCp) (Ebioscience) and analyzed by BD FACSCalibur flow cytometer. Analysis was performed by gating CD3+ cells for Annexin V and propium iodite (PI). All antibodies were provided from BD Biosciences, USA.

Analysis of Treg cells. After incubation period cells were washed with PBS and remaining cell pellet were evaluated for Treg cells. Treg cell ratios were analyzed by intracellular staining of FoxP3 (PerCp) and surface staining with anti-CD4 (FITC) and anti-CD25 (Phycoerythrin; PE). All staining protocols were performed according to the manufacturer's instructions. Treg cells were identified as CD4$^+$ and CD25$^+$ that labeled with FoxP3. In the analysis, CD25 gated cells were analyzed for CD4+ and FoxP3+ cells. All samples were analyzed by BD FACSCalibur flow cytometer. All antibodies were provided from BD Biosciences, USA.

Analysis of Cytokine Levels: After 3 days of the culture period, the supernatants were collected and quantified for the cytokines IL-4, IL-6, IL-10, TNF-a and IFN-γ (pg/mL). The cytokines were analyzed via flow cytometry using a BD cytometric bead array (CBA) human Th1/Th2/Th17 Kit (BD Biosciences, USA) according to the manufacturer's instructions.

Statistical Analysis

For statistical analysis, SPSS 21 software was used (SPSS, SPSS Inc., Chicago). Analysis of variance (ANOVA) for repeated measures, Student's t-test, and Fisher's exact test were used, as appropriate. Sub – group analyses were performed using ANOVA with Fisher's post hoc test. All data were checked for normal distribution (Kolmogorov – Smirnov test). Non-normally distributed data were log transformed. Results refer to the intention-to-treat population and are reported as mean ± standard deviation (S.D.), if not indicated otherwise. p value less than 0.05 was considered significant.

RESULTS

Patients with sepsis (n=10), septic shock (n=10) and healthy individuals as control (n=10) were included in this study. Inclusion and exclusion criteria for study subjects was shown in Figure 1. Peripheral blood samples were collected, and PBMCs were isolated and cultured with and without DF-MSCs (IFN-γ$^+$ or IFN-γ$^-$) with the stimulation of anti-CD3/anti-CD28 of PBMCs for 72 hours to evaluate immunomodulatory effect of DF-MSCs on lymphocytes and inflammation parameters. DF-MSCs were either stimulated with IFN-γ or non-stimulated for the observation of the immunomodulatory effect of inflammatory cytokine IFN-γ on DF-MSCs. At the end of the culture period lymphocyte proliferation, apoptosis and regulatory T cell ratios and cytokine levels were analyzed by flow cytometry.

Isolation, Characterization, Multipotency of DF-MSCs

DF-MSCs exhibited a fibroblast-like and spindle-shaped morphology in the first few days of incubation. DF-MSCs proliferated in 3 days and formed fibroblast-like colonies (Figure 2A) in the primary culture in their first passage. Third passage cells were analyzed by flow cytometry and with surface markers for positive staining for CD29, CD73, CD90, CD106 and were negative for CD14, CD34, CD45, and HLA-DR (Figure 2B). The DFSCs differentiated well into osteocytes, chondrocytes, and adipocytes. The osteogenic differentiation of DF-MSCs was evaluated with Alizarin red staining for calcium phosphate deposits. DF-MSCs showed the morphology of osteoblasts and osteocytes and calcium phosphate deposits were stained in the intracellular matrix. *The in vitro* adipogenic differentiation capability was assessed by culturing the cells in an adipogenic stimulated medium, and at the end of the culture period stained with Oil Red O. Intracellular lipid droplets were observed in these cells. The chondrogenic differentiation capability was observed

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in vitro during a twenty-one-day culture period in chondrogenic medium, and cell differentiation into chondrocytes was observed with Alcian blue staining. Proteoglycans were observed in the culture (Figure 2C).

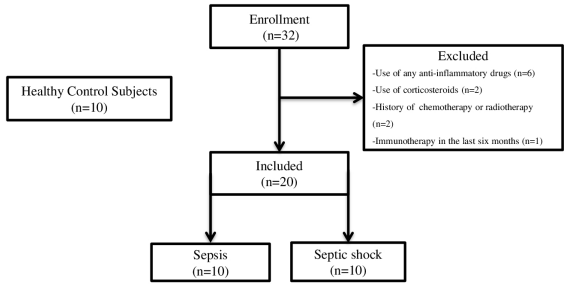

Figure 1. Flow diagram of the 20 patients with sepsis and septic shock who were identified among the 32 patients enrolled. (Healthy control subjects, n=10)

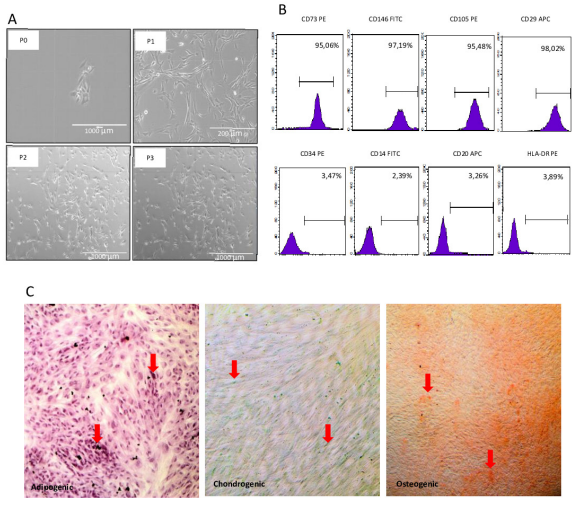

Figure 2. Isolation, characterization and differentiation of DF-MSCs. A) DF-MSCs formed fibroblast-like colonies at passage 0 (P0), P1, P2 and P3 on the fourth day of inoculation. B) DF-MSCs were analyzed in the third passage via flow cytometry and expressed positive markers of CD29, CD73, CD105 and CD146, and lack the expression of negative markers of CD14, CD34, CD45 and HLA-DR. C) DF-MSCs were differentiated into osteoblasts, chondrocytes and adipocytes after stimulation. Cells were stained with alizarin red, oil red O and alcian blue at the end of the culture period for osteogenic, adipogenic and chondrogenic differentiation of DF-MSCs respectively. Red arrows show calcium deposits in osteogenic differentiation.

DF-MSCs suppressed lymphocyte proliferation in sepsis and septic shock patients

PBMCs were isolated from peripheral blood and cultured for 72 hours. They were labeled with CFSE and incubated with and without DF-MSCs (IFN-γ$^+$ or IFN-γ$^-$), analyzed by BD FACSCalibur flow cytometry via CFSE labeling for proliferation. Based on the hypothesis that the initiation of activity of MSCs during inflammatory responses such as antigen presentation and T-cell proliferation, we treated DF-MSCs with regulatory protein IFN-γ to increase its ability in the initiation of DF-MSCs' suppressive activity. The proliferation ratio of PBMC cultures without DF-MSCs varied between the groups. Lymphocyte proliferation ratio was significantly high in anti-CD3/CD28 stimulated cultures without DF-MSCs in sepsis group when compared with septic shock and control groups ($p<0.05$). DF-MSCs, both IFN-γ prestimulated and un-stimulated significantly suppressed lymphocyte proliferation in sepsis ($p<0.001$ and $p<0.005$), septic shock ($p<0.005$ and $p<0.05$), and control groups ($p<0.01$ and $p<0.01$) compared to PBMC cultures alone (Figure 3).

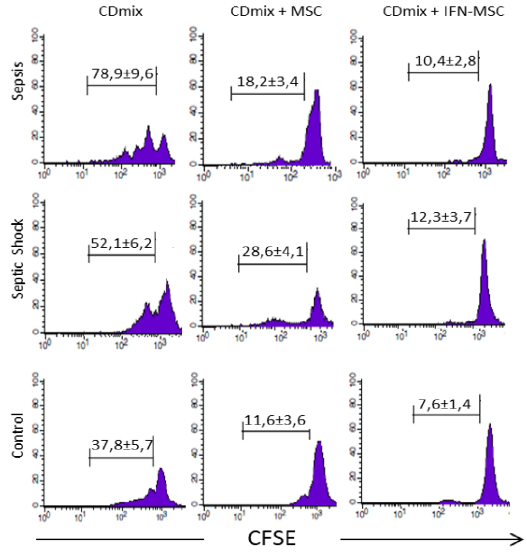

Figure 3. Lymphocyte proliferation. CFSE labeled cells were analyzed via flow cytometry and counted for proliferative responses to stimulants. Lymphocyte proliferation ratio with anti-CD3/anti-CD28 (CDmix) stimulation in PBMC of sepsis patients was significantly high compared to septic shock and healthy subjects ($p<0.05$). DF-MSCs significantly decreased proliferative response in all groups but the reduction in sepsis patients was noteworthy ($p<0.005$), and IFN-γ prestimulated DF-MSCs further decreased proliferation rates of lymphocytes in sepsis patients ($p<0.001$).

DF-MSCs increased the lymphocyte survival in sepsis and septic shock patients

PBMCs were analyzed after 72 hours of the culture period for T lymphocyte apoptosis. Lymphocytes were incubated with and without DF-MSCs (IFN-γ$^+$ or IFN-γ$^-$), and apoptosis was analyzed via BD FACSCalibur flow cytometry by labeling with anti-CD3, Annexin V and PI. The viability of lymphocyte was significantly decreased in sepsis ($p<0.005$) and septic shock groups ($p<0.001$) in the absence of DF-MSCs compared to control group. DF-MSCs with and without IFN-γ stimulation significantly increased the viability of lymphocyte by decreasing

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apoptosis compared to PBMC cultures without DF-MSCs in sepsis and septic shock groups ($p<0.01$, and $p<0.001$, respectively). IFN-γ prestimulation of DF-MSCs further decreased the lymphocyte apoptosis in all groups, but it was significant in sepsis group compared to un-stimulated DF-MSCs in the co-cultures ($p<0.01$). Apoptotic profiles differ within groups. In CDmix stimulated PBMC cultures sepsis (51.7±4.1) and septic shock patients' (59.3±5.6) lymphocytes were mostly undergone necrosis, while 4.9±2.3 of lymphocytes showed necrosis in healthy subjects. DF-MSCs with or without IFN-γ decreased necrosis both in sepsis (CDmix+MSC; 3.9± 0.7, CDmix+IFN-MSC; 4.1± 0.9) and septic shock (12.4± 3.1) patients' lymphocytes. Early apoptosis was increased in CDmix cultures of healthy individuals (11.5±1.8) without DF-MSCs, despite this, cell survival ratio of lymphocytes was high in control group (75.3±4.9) compared to sepsis and septic shock patients. IFN-γ stimulation of DF-MSCs decreased either early and late apoptosis in healthy individuals or necrosis in septic and septic shock patients. Moreover, lymphocyte survival significantly increased in sepsis, septic shock and tend to increase in healthy subjects in the presence of DF-MSCs (Figure 4).

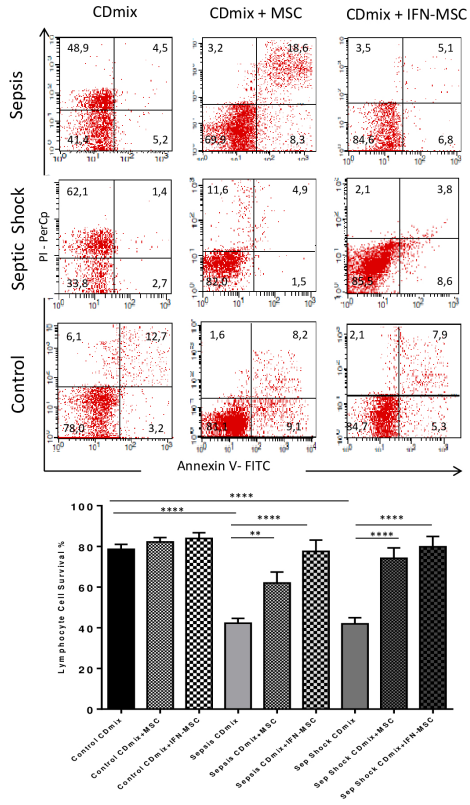

Figure 4. Lymphocyte apoptosis. Apoptotic rates of lymphocytes in PBMC cultures were quantified via flow cytometry. Cell survival ratio of lymphocytes was significantly low in sepsis ($p<0.001$) and septic shock patinets ($p<0.001$) compared to healthy subjects. Un-stimulated DF-MSCs increased cell survival and decreased apoptotic rates of lymphocytes in sepsis ($p<0.01$) and septic shock patients ($p<0.001$) significantly compared to PBMC cultures alone, and IFN-γ prestimulated DF-MSCs notably increased lymphocyte survival in sepsis group when compared with un-stimulated DF-MSCs ($p<0.01$) and PBMC cultures alone ($p<0.001$). **; $p<0.01$, ****; $p<0.001$.

DF-MSCs enhanced the number of CD4⁺CD25⁺FoxP3⁺ Treg cells

We studied the effects of DF-MSCs on the Treg cell frequency. Seventy-two hours after culture, PBMC were collected and analyzed for CD4 and CD25 with cell surface markers, and intracellular staining with the FoxP3 antibody. $CD4^+FoxP3^+$ Treg cell population differed between the groups. Treg cell ratio was lower in the sepsis group compared to septic shock and control groups in anti-CD2/anti-CD3/anti-CD28 stimulated PBMC without DF-MSCs. Un-stimulated DF-MSCs significantly increased Treg ratio in sepsis ($p<0.001$) and control group ($p<0.05$) compared to PBMC cultures alone, whereas there was no significant change observed in septic shock patients in co-cultures ($p>0.05$). IFN-γ prestimulation of DF-MSCs further increased Treg ratio in sepsis group compared to PBMC cultures alone ($p<0.001$), but there was no significant difference between co-cultures of un-stimulated DF-MSCs. In septic shock group, Treg ratio was significantly higher in PBMC cultures without stem cells compared to sepsis and control groups ($p<0.05$). DF-MSCs tend to increase the Treg ratio in septic shock group, but it was not significant (Figure 5).

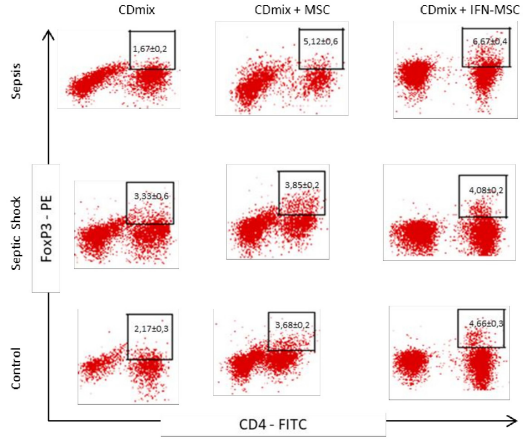

Figure 5. $CD4^+FoxP3^+$ T regulatory cell ratio. Un-stimulated DF-MSCs significantly increased Treg ratio in sepsis ($p<0.001$) and control group (0.05) compared to PBMC cultures alone, whereas there was no significant change observed in septic shock patients in cocultures ($p>0.05$). IFN-γ prestimulation of DF-MSCs further increased Treg ratio in sepsis group compared to PBMC cultures alone ($p<0.001$). Treg ratio was significantly higher in PBMC cultures without stem cells in septic shock group, compared to sepsis and control groups ($p<0.05$). DF-MSCs tend to increase the Treg ratio in septic shock group, and it was not significant.

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DF-MSCs regulated the proinflammatory cytokine levels in sepsis and septic shock patients

We analyzed the proinflammatory and anti-inflammatory cytokine levels in culture supernatants to evaluate the immunoregulatory effects of DF-MSCs in sepsis and septic shock patients. The supernatants were collected after 72 hours of culture of PBMC in the presence and absence of DF-MSCs (IFN-γ^+ or IFN-γ^-). Further, we analyzed IL-4, IL-6, IL-10, IFN-γ, and TNF-a cytokine levels after culture period.

The proinflammatory cytokine levels (IL-6 and TNF-a) were significantly higher in sepsis group PBMC when cultured without stem cells compared to septic shock and control groups (IL-6; $_{\text{sepsis-septic shock}}$<0.01, $p_{\text{sepsis-control}}$<0.001 and TNF-a; $_{\text{sepsis-septic shock}}$<0.01, $p_{\text{sepsis-control}}$<0.001). DF-MSCs significantly decreased IL-6 and TNF-a levels in sepsis and septic shock groups compared to PBMC cultures without stem cells (sepsis; p<0.001, p<0.001 and septic shock; p<0.01, p<0.01, respectively). However, IFN-γ prestimulated DF-MSCs significantly decreased IL-6 levels compared to unstimulated DF-MSCs in septic shock group (p<0.01) while there was no difference in sepsis group. IFN-γ level was significantly lower in PBMC cultures of septic shock group compared to sepsis patients (p<0.05). Unstimulated and IFN-γ prestimulated DF-MSCs significantly increased IFN-γ levels in the co-cultures of septic shock patients (p<0.01), whereas, it was significantly decreased in sepsis group and control group compared to PBMC cultures without DF-MSCs (p<0.001).

IL-4 level was higher in sepsis group when compared with control group, but it was not significant. DF-MSCs tend to decrease IL-4 levels in the co-cultures in sepsis group. Additionally, the IL-4 level was significantly higher in PBMC cultures of septic shock group compared to sepsis and control groups ($p_{\text{septic shock-control}}$<0.001, $p_{\text{septic shock-sepsis}}$<0.005). DF-MSCs decreased IL-4 levels in septic shock group significantly when compared with PBMC cultures without stem cells (p<0.005). IFN-γ prestimulation of DF-MSCs further decreased IL-4 levels compared to CDmix cultures of PBMC in septic shock group (p<0.001). We further analyzed Th_1(IFN-γ)/ Th_2 (IL-4) ratio to understand the effect of MSCs in immune deviation of sepsis and septic shock patients. Our results revealed that Th2 immunity was dominant in septic shock patients while DF-MSCs deviated it to Th1 immunity which was important for survival (Table I and Table II).

Table I. Change in the IFN-γ levels in culture groups with DF-MSCs(±IFN-γ)

Group	Culture Condition	IFN-γ Level (pg/mL) Average value	Change in the cytokine level	Change in the cytokine level in percentage (%)
Control	CDmix	647.52		
	CDmix+MSC	302.05	↓↓	53
	CDmix+IFN-γMSC	198.27	↓↓↓	69
Sepsis	CDmix	524.12		
	CDmix+MSC	156.40	↓↓	70
	CDmix+IFN-γMSC	179.23	↓↓↓	65
Septic Shock	CDmix	47.84		
	CDmix+MSC	163.35	↑↑	241
	CDmix+IFN-γMSC	217.10	↑↑↑	353

IFN-γ; Interferron gamma, MSC; Mesenchymal Stem Cell, IFN-γMSC; Interferron gamma stimulated mesenchymal stem cell

Table II. Change in the IL-4 levels in the culture groups with DF-MSCs (±IFN-γ)

Group	Culture Condition	IL-4 level (pg/mL) Average value	Change in the cytokine level	Change in the cytokine level in percentage (%)
Control	CDmix	1.86		
	CDmix+MSC	1.07	↓↓	42
	CDmix+IFN-γMSC	0.92	↓↓↓	50
Sepsis	CDmix	3.27		
	CDmix+MSC	3.19	↓↓	0,02
	CDmix+IFN-γMSC	0.89	↓↓	72
Septic Shock	CDmix	21.06		
	CDmix+MSC	4.18	↓↓	80
	CDmix+IFN-γMSC	2.86	↓↓↓	86

IL; Interleukin, MSC; Mesenchymal Stem Cell, IFN-γMSC; Interferron gamma stimulated mesenchymal stem cell

IL-10 level was significantly higher in PBMC cultures of septic shock group compared to sepsis and control groups ($p_{\text{septic shock-control}}$<0.05, $p_{\text{septic shock-sepsis}}$<0.01). DF-MSCs significantly increased IL-10 levels in sepsis group compared to PBMC cultures without stem cells (p<0.001) and further increased by pretreatment of DF-MSCs with IFN-γ. There was no significant difference between PBMC cultures and DF-MSCs cocultures in septic shock group in IL-10 levels (Figure 6).

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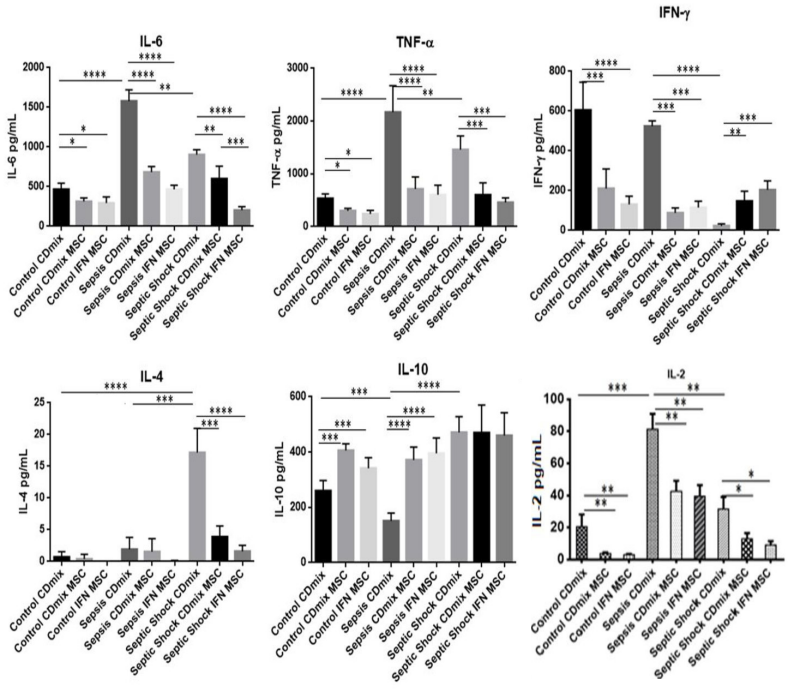

Figure 6. Proinflammatory and anti-inflammatory cytokine levels. Cytokine levels in culture supernatants were analyzed after 72 hours via flow cytometry and values were given in pg/mL. Statistical values of; IL-6, TNF-α, IFN-γ, IL-4, IL-10, IL-2 levels were shown. *: p<0.05, **: p<0.01, ***: p<0.005 and ****: p<0.001

DISCUSSION

Sepsis is characterized by overwhelming systemic activation of the immune response due to the release of various molecules from invading microbial pathogens or damaged host tissue [19]. The pathophysiology of sepsis associates interplay between pro – and anti-inflammatory responses. In the proinflammatory response phase cytokines such as tumor necrosis factor (TNF)-a, IL-1b, IL-6, and IFN-γ are produced which stimulate the effector functions of mononuclear cells [20,21]. In the current study, we evaluated the modulatory effects of DF-MSCs as an alternative source of MSCs, on lymphocytes isolated from sepsis and septic shock patients and compared those with healthy individuals. We demonstrated that DF-MSCs can modulate TNF-a and IL-6 levels in PBMC supernatants and regulate the proliferative response of T cells in sepsis patients by enhancing Treg frequency, while reducing Treg cell ratio and IL-10 levels and shifting Th2 cells in favour of Th1 cells in septic shock patients.

Sepsis leads to changes in immunity and results in immune paralysis. Anti-inflammatory stage of the host response to sepsis, characterized by apoptosis of T-cell, paralysis of macrophage and shift in the T-cell subsets from Th1 to the Th2 phenotype is mediated by Treg cells [9]. Immunodepression also occurs by decrease of IFN-γ levels and the increase of IL-10 and IL-4 levels during this T cell phenotype shift [22-24].

DF-MSCs are multipotent cells with short doubling time thus can reach the required number of cells in early passages [25]. MSCs have been recently reported to have an anti-inflammatory potential in experimental models of sepsis [26]. Recently, the immunosuppressive effect of dental mesenchymal stem cells was studied and reported that DF-MSCs have a strong immunomodulatory impact on lymphocytes [18]. However, it is still unclear whether DF-MSCs regulate sepsis-induced inflammatory responses of lymphocytes.

In this study, we demonstrated that DF-MSCs can reduce the proliferative response of T lymphocytes in PBMC of sepsis patients, by increasing $CD4^+CD25^+FoxP3^+$ Treg cell frequency and increasing IL-10 levels *in vitro*. Although, it was not noticeable in sepsis patients, the proliferation of T lymphocytes was lowered with DF-MSCs in septic shock patients. The regulatory effect of DFSCs on proliferation of lymphocytes of sepsis and septic shock patients was further augmented with the stimulation of IFN-γ. But, elevated Treg cell ratio and IL-10 levels did not change notably in septic shock patients' PBMC in the presence of DF-MSCs. We evaluated the difference in the decrease of lymphocyte proliferation in the presence of unstimulated or IFN-γ stimulated DF-MSCs in sepsis and septic shock patients together with T lymphocyte apoptosis, Treg cell frequency and cytokine level.

In this study, we investigated the apoptosis and viability ratio of lymphocytes in sepsis and septic shock patients and evaluated the effect of DF-MSCs on cell viability. Lymphocyte apoptosis was higher in sepsis and septic shock patients compared to

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healthy individuals in PBMC cultures. DF-MSCs significantly decreased apoptosis and increased viability of lymphocytes in sepsis and septic shock patients, which demonstrated the anti-apoptotic effect of DF-MSCs. These data indicated that the decrease in proliferation of T lymphocytes was not caused by apoptosis. In previous studies, it was shown that MSCs had an anti-apoptotic effect on activated lymphocytes by contact-dependent mechanisms [27,28], and modulated the function of several immune cells which released inflammatory mediators (IFNγ, IL1β and TNFα) [23,29,30]. In this regard, our results were comparable with the previous studies.

Anti-inflammatory responses with IL-10 expression by Treg cells in sepsis is a critical parameter. One of the studies showed that increase in the IL-10 level in the early phase of sepsis resulted with the improvement of the disease, but harmful, depending on the time of intervention [31]. In our study, we analyzed IL-10 levels in PBMC cultures of sepsis and septic shock patients and observed that level of this cytokine was significantly increased in septic shock compared to sepsis and control groups. Results showed that DF-MSCs did not change IL-10 levels in septic shock in comparison with PBMC cultures but significantly upregulated IL-10 secretion in sepsis group compared to PBMC cultures without stem cells. In other words, DF-MSCs modulated aggregated inflammatory response in sepsis whereas no change occurred in septic shock patients which is a critical point for the immunosuppression.

IL-2 is the key mediator of T lymphocyte proliferation and promotes the expansion of T lymphocytes [32]. In our study, IL-2 levels were high in sepsis patients which indicated increased proliferative responses and induced activated lymphocytes for clonal expansion. DF-MSCs decreased IL-2 levels in co-cultures of PBMC of sepsis patients remarkably. These data indicated that DF-MSCs regulated the lymphocyte proliferation by increasing the number of Treg cells and decreasing IL-2 levels. IFN-γ prestimulation of DF-MSCs enhanced Treg cell ratio and further decreased IL-2 levels in sepsis group, whereas there was no significant change in Treg cell ratio and IL-2 level in septic shock group and IL-2 level in healthy individuals. These data suggested that DF-MSCs may have a limited effect on effector functions of inactivated lymphocytes but have the major inhibitory effect on activated lymphocytes.

Our data indicated that immune cell profiles played a critical role in the host response to sepsis. Following the early phase, the immune cell phenotype shifted towards immune-suppression status. While, sepsis patients in early phase had Th1 (T helper 1) cell mediated immune responses with the secretion of pro-inflammatory cytokines (IFN-γ, TNF-a, IL-6, IL-12), septic shock patients had predominantly Th2 cell mediated responses with the secretion of anti-inflammatory cytokines (IL-4, IL-10) which showed the suppressive phase [33-35]. IL-4 is a cytokine with variable immunoregulatory functions, and has an important action in the differentiation of naive T lymphocyte towards Th2 type cells and anti-inflammatory mechanism suddenly occurs in this period of sepsis which could lead to aggressive immune destructions [36,37]. IL-4 causes an enhanced release of further IL-4 and other anti-inflammatory cytokines and results with immunosuppression [33]. A recent study demonstrated the increase in the mortality ratio was correlated with high expression of IL-4 [38]. In this study, we investigated IL-4 levels in PBMCs of sepsis and septic shock patients. The elevated level of IL-4 in septic shock patients was significant compared to sepsis and healthy individuals, which indicated that Th2 mediated anti-inflammatory response was dominant in septic shock patients. DF-MSCs reduced IL-4 levels in septic shock patients' co-cultures while no remarkable change was observed in sepsis and healthy subjects . This reduction in the cytokine level in septic shock patients provides hope of returning to anti-inflammatory phase of septic shock by DF-MSCs. On the contrary, some of the studies demonstrated that bone marrow or adipose tissue derived MSCs enhanced IL-4 production of T lymphocytes [39,40] in sepsis murine models. The reason for this difference in the results may be the use of different cell sources (bone marrow, adipose tissues, dental tissues) and the lymphocyte profile of sepsis or septic shock.

In a study, lymphocyte anergy or unresponsiveness occurred resulting in immune system declining, which caused the immune system susceptibility to bacterial and viral infections {41]. Clinical studies signed that the duration of sepsis-induced immune alterations was associated with increased risk of death [42]. Some biomarkers are important in disease progression for sepsis. Recent studies demonstrated that increased IFN-γ level resulted in improved patient outcomes with the restoration of immune cell functions in sepsis and septic shock [33]. In our study, we evaluated the effect of DF-MSCs on IFN-γ levels in sepsis and septic shock. IFN-γ level was significantly lower in septic shock patients compared to sepsis patients and healthy volunteers in PBMC cultures without stem cells. DF-MSCs increased IFN-γ levels in PBMC of septic shock patients, whereas downregulated in sepsis and healthy individuals. Also, IFN-γ prestimulation of DF-MSCs further increased IFN-γ levels in septic shock patients. This may be an evidence of selective immunoregulatory effect of DF-MSCs on distinctly activated T lymphocytes.

The current study demonstrates that DF-MSCs can downregulate the inflammatory responses in sepsis patients and can modulate Th2 mediated responses in favor of Th1 cells in septic shock patients. This data shows variable regulatory functions of DF-MSCs in sepsis in response to cytokine profile and inflammatory conditions, and further *in vivo* studies are required to confirm the regulatory role of DF-MSCs in sepsis.

Limitations

A power analysis was not performed to determine sample size because we did not have preliminary data of the immunomodulatory properties of DF-MSCs in sepsis and septic shock *in vitro* to perform a power analysis. Thus, this study must be considered an exploratory pilot study rather than a formal test of a hypothesis.

Conclusion

In this study, we demonstrated that IFN-γ stimulation of DF-MSCs had regulatory effects on inflammatory responses in sepsis, and reduced lymphocyte apoptosis in septic shock. DF-MSCs may be further investigated to use as a novel treatment strategy by the mean of restoring immune system homeostasis for sepsis and septic shock *in vivo* studies, and also the way of administration and dose responses can be determined.

Ethical Committee Approval

This study was approved by Marmara University, Clinical Research Ethics Committee.

Conflict of Interest

The authors declare that they have no conflict of interest

Author Contributions: Concept and Design – FG, IC; Supervision – IC; Resources – IC, TA; Materials – DG, NZ, TA; Data Collection and Processing – FG, DG, MKA; Analysis and Interpretation – FG, DG, NZ, MKA; Literature Search – FG, DG, LT; Writing Manuscript – FG, DG, IC; Critical Review – IC, FG, TA.

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Management of palatal fistulas and a simple surgical algorithm proposal

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ABSTRACT

Objectives: Despite improved techniques in repair of cleft palate, failure of healing of palatal structures resulting in a palatal fistula is one of the major challenges in the practice of reconstructive surgery. The aim of this study is to evaluate treatment success and failure in patients with palatal fistulas following cleft palate repair.

Patients and Methods: Totally 44 patients with a history of cleft palate who underwent surgery for palatal fistula were included in this study undertaken between January 1999 and August 2014. Fistulas were classified as anterior and posterior according to the repair technique and were repaired using one of the following techniques: buccal mucosal flap, tongue flap or mucoperiosteal flap.

Results: Success rate for anterior fistulas was 71.42% with tongue flap and 76.92% with mucoperiosteal flap. Success rate for posterior fistulas was 84.62% with mucoperiosteal flap and 75% with buccal mucosal flap. Difference in success rates between the anterior and posterior fistulas was not statistically significant.

Conclusion: Our study results suggest the use of mucoperiosteal flaps for both anterior and posterior fistulas smaller than 5mm as the first choice, guided by the principle of replacing absent tissue with similar tissue.

Keywords: Cleft palate, Palatal fistula, Post-palatoplasty fistula, Tongue flap, Two-flap palatoplasty.

INTRODUCTION

Despite improved techniques in repair of cleft palate, failure of healing of the palatal structures resulting in a palatal fistula is one of the major challenges in the practice of plastic and reconstructive surgery which prevents favorable outcomes in cleft palate repair. The formation of a post-palatoplasty fistula has been shown to be associated with the severity and the type of cleft, the repairing technique, timing of the repair, experience of the surgeon, tension at the suture site, hemorrhage, infection, postoperative anemia, and lack of postoperative follow-up [1-3].

The main goal of cleft palate repair is to allow for speech development and dental hygiene without disturbing midface development. Accordingly, cleft palate repair is usually performed in the first year of life before the child starts to speak. A palatal fistula, which occurs in 4 to 25% of cases, is a complication of cleft palate repair [4-10]. The indications for a fistula repair depend on the related symptoms. The most common symptoms include nasal emission causing speech distortions, leakage of fluid and food into the nasal cavity leading to poor oral hygiene, and foul smell. It is well-established that fistulas causing speech disturbances should be repaired as soon as possible, while small fistulas which do not affect speech with occasional regurgitation of fluid/food into nose can be delayed [11].

Until now, several attempts have been made for the classification of palatal fistulas according to the site [1,12,13]. Classification of fistulas based on the difficulty index has been proposed to help in preoperative management of the outcomes [13]. Initially, palatal fistulas were classified as anterior, middle (i.e., at the junction of the soft and hard palate), and posterior. [2]. In a series of 64 patients, the hard–soft palate junction was the most common site (53.1%) [14].

Although, palatal fistulas are common morbidities following cleft palate repair, there is no established treatment algorithms. In the present study, we aimed to evaluate treatment success and failure in patients with palatal fistulas following cleft palate repair.

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PATIENTS and METHODS

This retrospective study was conducted at the Department of Plastic, Reconstructive and Aesthetic Surgery, Marmara University Training and Research Hospital, between January 1999 and August 2014. A total of 51 patients with a prior history of cleft palate underwent surgery for palatal fistulas. Exclusion criteria were as follows: having a fistula following tumor surgery; having a traumatic fistula; asymptomatic fistulas and being lost-to-follow-up. Finally, a total of 44 patients who were operated for a palatal fistula following cleft palate repair were included in the study. A written informed consent was obtained from each patient. The study was reviewed and approved by the Marmara University, School of Medicine Ethics Committee (protocol number: 952). The study was conducted in accordance with the principles of the Declaration of Helsinki. Data regarding demographic and clinical characteristics of the patients including age, sex, primary cleft type, technique of primary repair, age at primary closure, symptoms, size, location, age at fistula repair, type of fistula repair, velopharyngeal function, orthodontic treatment, and outcome were retrospectively retrieved from the hospital archives. In addition, local tissue availability, scar tissue formation, inflammation, oral hygiene, and other surgical procedures were evaluated to select the most appropriate fistula repair technique. Pre-, intra-, and postoperative images were also evaluated.

In the present study, palatal fistulas were classified as anterior and posterior based on repair technique, and a fistula at the hard–soft palate junction was considered a posterior fistula.

Surgical techniques

Palatal fistulas were repaired by one of the following techniques: Buccal mucosal flap, tongue flap and mucoperiosteal flap.

Buccal mucosal flap

The buccal mucosal flap, originally described in 1969 by Mukherji as a cheek flap, is used in primary palatoplasty [15]. This technique is particularly advantageous, when the fistula is localized at the hard–soft palate junction. The buccal mucosal flap is a posteriorly based random pattern flap, and its base is located near the retromolar trigone. The distal end of the flap is located slightly below the oral commissure, and one structure which requires particular care during flap harvesting is the papilla of the parotid duct. The flap, if necessary, can be harvested from both sides and can be used both for the oral and nasal layer closure. The part of the buccinator muscle can be also incorporated in the flap to make it more robust. In that case, the flap is called a buccinator myomucosal flap [16].

Tongue flap

This is a two-stage procedure. The use of the lingual tissue in palatal fistula repair was reported more than five decades ago by Santos and Altamirano [17]. It has been shown that this technique is safe and well-tolerated by children, when applied properly. The flap is indicated for larger fistulas where there is a significant tissue deficit in the oral mucoperiosteal layer. The flap can be anteriorly or posteriorly located, depending on the site of the fistula. In our study, we used an anterior-based tongue flap. A good nasal layer repair was necessary for the treatment success. Detachment was done at the fourth postoperative week (Figure 1, 2). The donor area was almost always closed primarily.

Figure 1. Anterior palatal fistula

Figure 2. A tongue flap was used for an anterior fistula.

Mucoperiosteal flap

If the small or medium-sized fistulas were localized asymmetrically and anteriorly, a C-shaped mucoperiosteal flap was elevated around the fistula and used to achieve the nasal layer. The flap was elevated on the other side and rotated over the defect to create the oral layer (Figure 3, 4). The required rotation flap could be larger than expected. Alternatively, if the fistula was localized at the junction or at the soft palate, the incisions were made around the fistula and turned inside and sutured together to achieve the nasal closure. Then, bilateral unipedicled (two-flap palatoplasty technique) or bipedicled mucoperiosteal flaps (Von Langenbeck) were elevated, advanced toward the middle, and sutured together (Figure 5, 6).

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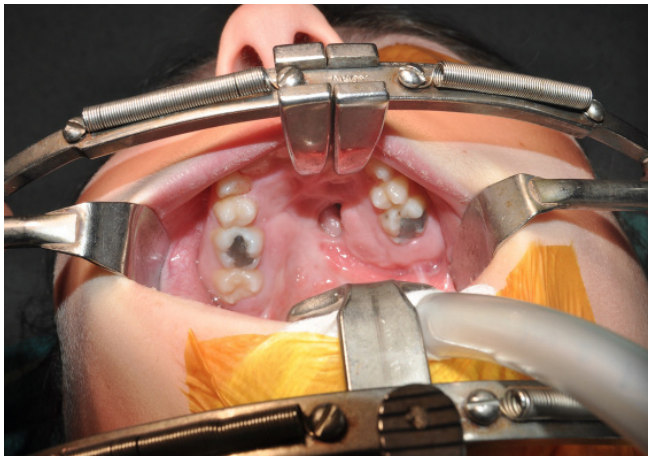

Figure 3. Palatal fistula on the hard palate

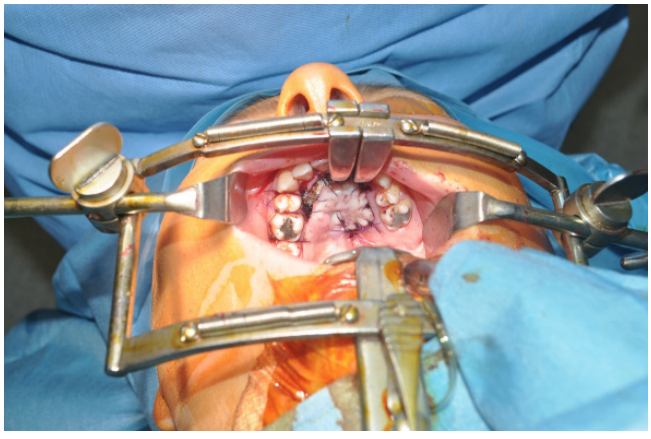

Figure 4. A rotation flap was transposed to cover the defect.

Figure 5. Fistula on the soft palate

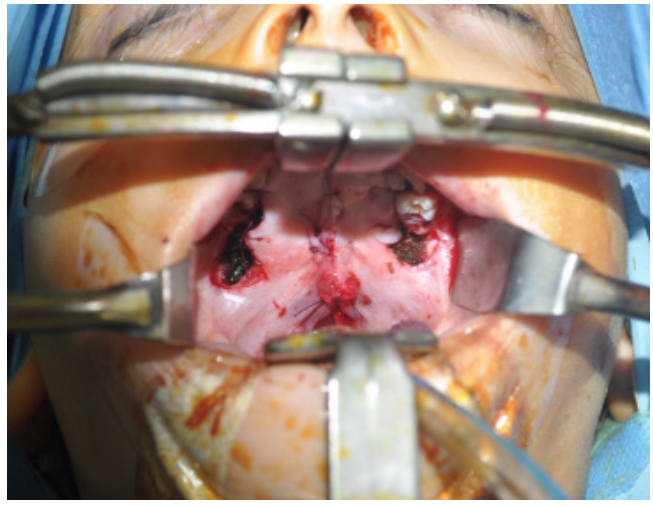

Figure 6. Bipedicled flap was advanced to cover the defect.

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software (GraphPad Software Inc., CA, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max), or number and frequency. The Fisher's exact test was used for statistical analysis. A P value of <0.05 was considered statistically significant.

RESULTS

Of all patients, 22 were males and 22 were females. The mean age was 14.3 (range, 5 to 32) years. The mean age at the time of the first repair was 19.3 (range, 9 to 120) months. The mean age at the time of fistula repair was 7.7 (range, 4 to 29) years. The mean follow-up was 5.4 (range, 2 to 7) years. Demographic and clinical characteristics of the patients are shown in Table I.

Table I. Demographic and clinical characteristics of patients

	Patients	
	n	%
Sex		
Female	22	50%
Male	22	50%
Cleft type		
Isolated cleft palate	16	36.4%
Unilateral cleft lip and palate	12	27.2%
Bilateral cleft lip and palate	16	36.4%
Fistula localization		
Anterior	27	61.4%
Posterior	17	38.6%

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The most common types of cleft were bilateral cleft lip and palates (n=16, 36.4%) and isolated cleft palates (n=16, 36.4%). Unilateral complete cleft lip and palate was seen in 12 patients (27.2%). The mean fistula size was 1.12 (range, 0.8 to 1.5) mm for anterior fistulas which were repaired with a tongue flap and 0.4 (range, 0.3 to 0.5) mm for anterior fistulas which were repaired with a mucoperiosteal flap. The mean fistula size was 0.85 (range, 0.7 to 1) mm for posterior fistulas which were repaired with a buccal mucosal flap and 0.4 (range, 0.3 to 0.5) mm for posterior fistulas which were repaired with a mucoperiosteal flap.

The number of the patients with an anterior fistula was 27 (61.4%). Among these, 14 patients (31.8%) underwent tongue flap surgery and 13 patients (29.5%) underwent mucoperiosteal flap reconstruction. Successful outcomes were obtained in 71.42% (10/14) of the patients with tongue flap and 76.92% (10/13) of the patients with mucoperiosteal flap. (Figure 3). There was no statistically significant difference in the success rate according to the operation technique (tongue flap versus mucoperiosteal flap) in the anterior fistula group (p>0.05). The number of the patients who developed a posterior fistula was 17 (38.6%). In 13 (29.5%) of them, mucoperiosteal flap was performed and four (9.1%) underwent buccal mucosal flap surgery. Successful outcomes were obtained in 84.6% (11/13) of the patients with mucoperiosteal flap and 75% (3/4) of the patients with buccal mucosal flap (Figure 4). There was no statistically significant difference in the success rate according to the operation technique in the posterior fistula group (p>0.05) (Table II).

Table II. Failure and success rates according to the operation technique

	Patients		Mean fistula size (mm)	Failure		Success		p value
	n	%		n	%	n	%	
Anterior	27	61.36%		7	25.92%	20	74.07%	p>0.05
Tongue flap	14	31.81%	11.2	4	28.57%	10	71.42%	
Mucoperiosteal flap	13	29.54%	4	3	23.08%	10	76.92%	
Posterior	17	38.64%		3	17.65%	14	82.35%	p>0.05
Mucoperiosteal flap	13	29.55%	4	2	15.38%	11	84.62%	
Buccal mucosal flap	4	9.09%	8.5	1	25.00%	3	75.00%	
TOTAL	44	100%		10	22.73%	34	77.27%	

The overall surgical success rate was 77% (34/44), with fistulas remaining in 23% (10/44) of the patients. All four of 14 patients who were treated with a tongue flap and failed were younger than six years. The failure patient group who were repaired with a mucoperiosteal flap or buccal mucosal flap had no similar history. There was no syndromic patient in the present study. The patients who were unresponsive to these treatments were reevaluated for next operations at least one year later (data not shown).

DISCUSSION

In the present study, we evaluated treatment success and failure in patients with palatal fistulas following cleft palate repair. Our study results showed that the surgical technique applied in our facility yielded comparable outcomes with the previous findings reported in the literature.

Palatal fistula is the most common complication of cleft palate surgery and fistula formation may occur even with the best hand. In the literature, recurrence rate following palatal fistula closure has been reported as 25% to 33% [1]. As the risk of fistula formation increases with every failure, all attempts should be made for successful palate repair during the first surgery.

Oronasal fistulas, which mainly demonstrate nasal regurgitation and speech problems, occur in 4 to 35% of cases [1]. Previous studies have shown that the incidence of oronasal fistulas ranges from 5 to 60% (1,2,18,19). A meta-analysis of 11 studies involving 2.505 children reported that the rate of fistula formation following primary palatal surgery was 4.9% [3]. In our study, the clinical rate of fistula formation was 6.76% during a 15-year study period.

The variety of surgical techniques indicates the lack of consensus on the optimal treatment technique in palatal fistulas. Repair of palatal fistula is a challenging procedure for cleft palate surgeons. Using intraoral flaps rather than external flaps has been reported to be associated with fewer donor site problems [16]. In a study investigating applications in intraoral reconstruction using three different techniques, Bianchi et al., reported that buccinator flaps were a good choice for the reconstruction of mid-sized oral cavity defects with a complication rate of 7%, which is not consistent with our findings [16]. In this study, although the number of repairs with the buccal mucosa flap was low, the complication rate was about 25% (1/4).

In the present study, there was a significant correlation between the anterior fistula occurrence and bilateral cleft palate. Bukowski et al., reported that patients with complete bilateral clefts (Veau IV classification) were more likely to develop a fistula [3]. Similarly, Phua et al., reported that anterior fistulas were more common in patients with a bilateral cleft lip and complete cleft palate [5].

Due to previous cleft palate repair, scar formation on the mucoperiosteal flap area makes this technique complicated for closure (2, 20, 21). Muzaffar et al., reported a success rate of 67% [2]. However, Cohen et al., reported a success rate of 33% in their study [1]. On the contrary, Emory et al., achieved successful results in 91% of cases [19]. In the present study, mucoperiosteal flaps were used for fistulas less than 5 mm, the success rate with this technique was 76.92% for anterior fistulas and 84.62% for posterior fistulas.

The tongue flap can be used to treat large anterior fistulas without causing any donor site problems. A donor site scar is acceptable, if there is no limitation on the tongue mobility or taste sensation [22]. However, the main disadvantage of this technique is that it is a two-stage procedure with possible intubation and extubation problems and impaired quality of

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life between the procedures. In addition, age is another factor which precludes to achieve successful results in young cases. Our findings demonstrated that using a tongue flap was a safe and effective method of treatment for large fistulas greater than 5 mm with a success rate of 71.42%.

A posterior fistula is more commonly related to syndromic causes. Most cases less than 5 mm can be closed with a mucoperiosteal flap, greater than 5 mm can be closed with posterior based buccal mucosal flaps. But in patients with a very large fistula, free flap transfer is recommended. However, compared to other surgical techniques, free flaps require prolonged duration of surgery and hospitalization and may lead to donor site morbidities. In the present study, none of our patients were treated with free tissue transfer. Furthermore, many surgeons use adjunctive therapies such as dermis or other barriers during fistula repair [22,23]. In the present study, however, no foreign materials or autologous dermal tissues were used.

In the literature, fistulas are often classified into three localizations: hard palate, soft palate, and junction of the hard and soft palate. Where applicable, it is recommended to repair symptomatic fistulas with mucoperiosteal flaps and large fistulas with tongue flap versus free flaps [24]. In our study, we mainly classified fistulas as anterior and posterior. Posterior fistulas include the soft palate and junction area with similar closure techniques. This algorithm simplifies the decision-making process.

Despite satisfactory cleft palate repair outcomes, the main limitations of the present study include its small sample size and retrospective design which precludes establishing a novel treatment algorithm for this patient population. In addition, in our study, we mainly classified palatal fistulas as anterior and posterior, and the fistulas located at the hard-soft palate junction were also considered posterior fistulas. Therefore, further large-scale and long-term prospective studies with a thorough classification are needed to draw a definite conclusion.

Conclusion

In conclusion, although not statistically significant, the success rate was significantly higher for tongue flap and mucoperiosteal flap in the anterior fistula group. Based on our study results, we suggest that mucoperiosteal flaps can be used for both posterior and anterior fistulas as the first choice, guided by the principle of replacing tissue with similar tissue with low failure and low morbidity rates. If the palatal tissues are not available, the tongue flap for anterior fistulas and buccal mucosal flap for posterior fistulas are preferred. Although, further studies are still needed, we believe that surgical techniques to gain a better understanding of developmental anatomy are valuable.

Compliance with Ethical Standards

Ethical approval: The study was reviewed and approved by the Marmara University, School of Medicine Ethics Committee (protocol number: 952). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Funding: The study was not supported by any funds.

Conflict of interest: The authors declare that they have no conflict of interest.

Informed consent: A written informed consent was obtained from each participant.

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Ventricular tachycardia ablation in patients with structural heart disease: single centre experience

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ABSTRACT

Objective: The aim of this study is to report our institutional experience with ventricular tachycardia (VT) ablation in patients with structural heart disease.

Patients and Methods: A total of 36 consecutive patients (31 male, age: 62.8 ± 13.2 years) who had undergone VT ablation in our institution between 01.01.2017 and 01.05.2019 were included in the analysis.

Results: A total of 27 patients with the diagnosis of ischemic cardiomyopathy and 9 patients with the diagnosis of nonischemic cardiomyopathy had undergone VT ablation. VT ablation was successful in 32 (88.9%) patients. There were no major procedural complications; however, one patient with ischemic cardiomyopathy died 48 hours after the procedure because of post-ablation cardiogenic shock. Another patient with unsuccessful VT ablation died during hospitalization due to electrical storm. Endocardial ablation was performed in 23 (63.9%) patients and epicardial or combined endocardial and epicardial ablation was performed in 13 (36.1%) patients. Substrate ablation was the dominant strategy in 29 (80.6%) patients whereas activation mapping and isthmus ablation was performed in 7 (19.4%) patients.

Conclusion: Our experience confirms the effectiveness and safety of VT ablation in patients with structural heart disease who are resistant to medical therapy and/or who receive recurrent implantable cardioverter-defibrillator shocks.

Keywords: Ventricular tachycardia, Radiofrequency ablation, Cardiomyopathies

INTRODUCTION

Ventricular tachycardia (VT) is the leading cause of mortality and morbidity in patients with structural heart disease. VT is often associated with sudden cardiac death and implantable cardioverter-defibrillator (ICD) implantation is frequently performed on these patients for prevention of arrhythmic death in accordance with current guidelines [1]. However, recurrent VT is associated with disabling recurrent shocks and may lead to hemodynamic instability.

Ventricular tachycardia ablation has emerged as an effective procedure for patients with structural heart disease and VT. The use of three-dimensional electroanatomical systems has enabled substrate mapping and ablation in sinus rhythm which has rendered VT ablation possible even in patients with hemodynamically intolerable VT. Increased knowledge about the mechanisms of scar related VT and increased experience with VT ablation have widened the applicability of VT ablation especially in patients who are at risk for VT recurrence.

In the present paper we reported our institutional experience with VT ablation in patients with structural heart disease.

MATERIALS and METHODS

Patients

A total of 36 consecutive patients (31 male, age: 62.8 ± 13.2 years) with structural heart disease who had undergone VT ablation between 01.01.2017 and 01.05.2019 at the Kartal Kosuyolu Higher Speciality Heart and Vascular Diseases Training and Research Hospital, in Istanbul were included in the analysis. Data of all patients were obtained from patient files and retrospectively evaluated. VT ablation was performed due to patients' resistance to medical therapy and/or recurrent implantable cardioverter-defibrillator shocks. Active ischemia was ruled out before the ablation procedure in all patients with ischemic cardiomyopathy. Informed consent was obtained from

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all the patients before the procedure. Kartal Kosuyolu Higher Specialty Heart and Vascular Diseases Training and Research Hospital Ethics Committee approved the study protocol (approval number 5-206).

VT Ablation

Endocardial mapping was performed to all patients and epicardial mapping was performed when endocardial mapping failed to identify desired ablation sites. Substrate mapping was performed using Ensite Precision 3D mapping system (Abbott, St Paul, Minnesota, USA). Substrate mapping and ablation was the preferred strategy for the majority of patients. Activation mapping during tachycardia was performed if the tachycardia was hemodynamically tolerated. Normal tissue was defined as tissue with bipolar voltage > 1.5 mV, dense scar was defined as bipolar voltage < 0.5 mV and scar borderzone was defined as a bipolar voltage 0.5-1.5 mV on voltage mapping. Late systolic potentials and local abnormal ventricular activities (LAVA) during sinus rhythm and mid diastolic potentials during VT were tagged as the potential ablation sites (Figures 1 and 2). Entrainment maneuvers were performed to identify isthmus if VT was hemodynamically tolerated. Pacemapping was used to identify the exit site of clinical VT. Irrigated tip catheters were used during ablation. VT stimulation was performed with one to three extrastimuli at two sites in the right ventricle at the end of the procedure to assess the effectiveness of ablation. Procedural success was assessed by non-inducibility of clinical VT during ventricular stimulation and abolishment of all late systolic potentials and LAVA's.

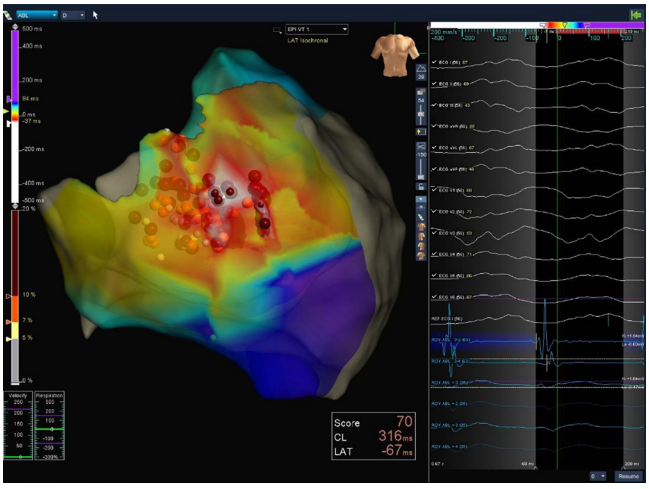

Figure 1: Mid-diastolic potentials during ventricular tachycardia (VT) recorded from endocardial surface. Sites with mid-diastolic potential during VT were assumed to be within critical isthmus and targeted for ablation

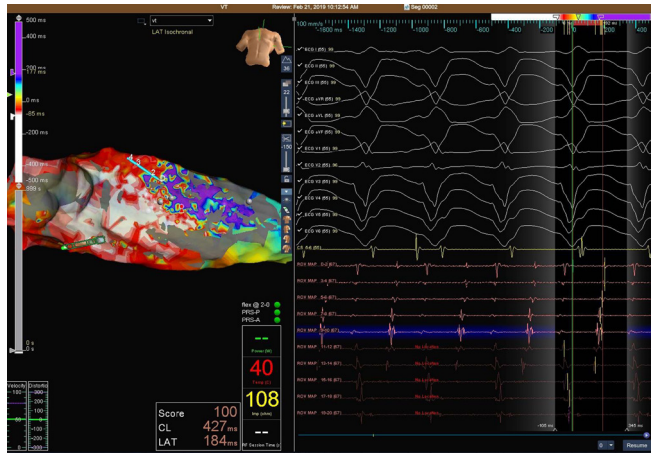

Figure 2: Mid-diastolic potentials during ventricular tachycardia (VT) recorded from epicardial space. Sites with mid-diastolic potential during VT were assumed to be within critical isthmus and targeted for ablation

Statistical Analysis

All statistical tests were performed with a commercially available statistical analysis program (SPSS 16.0 for windows, SPSS Inc., Chicago, Illinois, United States). Distribution of the data was tested by using one-sample Kolmogorov-Smirnov test. Values displaying normal distribution were expressed as the mean ± SD and values not displaying normal distribution were expressed as median with interquartile range. Categorical variables were expressed in frequencies and percentages. Significance of difference between groups regarding numeric variables with normal distribution was tested with independent samples of Student's *t*-test. Significance of difference between groups regarding categorical variables was tested with Chi-square test. A p value less than 0.05 was considered significant.

RESULTS

Demographic and procedural characteristics and outcome of patients are presented in Table-I. Most of the patients were male and majority of patients had ischemic cardiomyopathy (Table-I). There were 9 patients (75%) who were diagnosed as nonischemic cardiomyopathy and among them 1 was diagnosed as arrhythmogenic right ventricular dysplasia (ARVD). None of the patients had Brugada pattern on electrocardiogram (ECG). Most of the patients could not tolerate VT hemodynamically and initial procedural step was substrate mapping and ablation in 80.6% of the patients. Non-inducibility of VT was achieved in 88.9% of patients at the end of procedure. There were no major procedural complications such as systemic embolization, pulmonary edema or death. However, one patient with ischemic cardiomyopathy died after 48 hours following the procedure because of post-ablation cardiogenic shock. Another patient with unsuccessful VT ablation died during hospitalization due to electrical storm.

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Table I: Demographic and procedural findings and outcome of patients who had undergone VT ablation

Number	36
Age (years)	62.8 ± 13.2
Male gender	31 (86.1%)
Ischemic cardiomyopathy	27 (25.0%)
Nonischemic cardiomyopathy	9 (75.0%)
Ejection fraction (%)	36.1 ± 13.0
Initial step: Substrate ablation	29 (80.6%)
Initial step: Activation mapping and isthmus ablation	7 (19.4%)
Endocardial ablation	23 (63.9%)
Epicardial (or combined) ablation	13 (36.1%)
Success (VT non-inducible at the end of procedure)	32 (88.9%)

There was no significant difference between patients with ischemic and nonischemic cardiomyopathy regarding rate of substrate ablation or procedural success (Table II). However, the frequency of combined endocardial and epicardial ablation was significantly high in patients with nonischemic cardiomyopathy (Table II). Mean ejection fraction was significantly lower in patients with ischemic cardiomyopathy (Table II).

Table II: Comparison of procedural findings and outcome of patients with ischemic and nonischemic cardiomyopathy

	Ischemic cardiomyopathy (n:27)	Nonischemic cardiomyopathy(n: 9)	p value
Ejection fraction (%)	32.8 ± 10.3	46.1 ± 15.8	0.006
Male gender	25 (92.6%)	6 (66.7%)	0.08
Initial step: Substrate ablation	22 (81.5%)	7 (77.8%)	0.58
Epicardial (or combined) ablation	7 (25.9%)	6 (66.7%)	0.037
Success (VT non-inducible at the end of procedure)	24 (88.9%)	8(88.9)	0.74

DISCUSSION

Present findings that reflect our experiences with VT ablation in structural heart disease suggest the effectiveness and safety of VT ablation procedure in patients with ischemic and nonischemic cardiomyopathy. The success rate was defined as non-inducibility of clinical tachycardia that was 88.9% with no procedural complications aside from one patient who developed hemodynamic instability after the procedure and died during hospitalization.

Catheter ablation of VT in structural heart disease is increasingly being used as adjunctive therapy to implantable cardioverter-defibrillator (ICD) [2]. Multicenter randomized trials have shown significantly decreased ICD interventions in patients who had undergone VT ablation [2-6], and one multicenter study has suggested mortality benefit [7]. Activation mapping and entrainment mapping may be used to identify critical isthmus site that is responsible for initiation and maintenance of reentry in patients with hemodynamically tolerated VT. However, it is difficult to get activation mapping in most of VT cases due to hemodynamic intolerance. Three dimensional electroanatomical systems have enabled substrate mapping in sinus rhythm and targeting fractionated/late potentials and LAVA which reflect arrhythmogenic myocardium [2]. In the Ablation of Clinical Ventricular Tachycardia Versus Addition of Substrate on the Long Term Success Rate of VT Ablation (VISTA) trial an extensive substrate-based ablation approach was found to be superior to ablation targeting only clinical and stable VTs in patients with ischemic cardiomyopathy presenting with tolerated VT [8]. We performed activation mapping and entrainment mapping in patients with hemodynamically tolerated VT to delineate critical isthmus for catheter ablation. However, most of the patients could not tolerate VT in our patient population that precluded activation mapping and our initial step was substrate mapping in 80.6% of the patients.

There are no randomized studies related with VT ablation in nonsichemic cardiomyopathy patients [9]. Several studies have reported inferior outcome after ablation compared to patients with ischemic cardiomyopathy which is probably due to patchy and diffuse substrate with frequent intramural or subepicardial location [9]. Our ablation strategy for patients with nonischemic cardiomyopathy was similar to our strategy for patients with ischemic cardiomyopathy. However, epicardial or combined endocardial and epicardial ablation was significantly performed in a high percentage of our patients with nonsichemic cardiomyopathy which was in agreement with previous reports. Success rate of ablation was similar between ischemic and nonischemic cardiomyopathy groups, however, it was difficult to reach a firm conclusion because of the small number of patients in nonischemic cardiomyopathy group.

Conclusion

Our experience confirms the effectiveness and safety of VT ablation in patients with structural heart disease who are resistant to medical therapy and/or who receive recurrent implantable cardioverter-defibrillator shocks. There was no difference between patients with ischemic and nonischemic cardiomyopathy regarding ablation strategy and success rates, however, epicardial ablation had to be performed more frequently in patients with nonischemic cardiomyopathy.

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Ethical Committee Approval

This study was approved by the Kartal Kosuyolu Higher Education Heart and Vascular Diseases Training and Research Hospital Ethics Committee (approval number: 5-206). Informed consent was obtained from all patients before the procedure.

Conflict of Interest

The authors declare that they have no no conflict of interest.

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The most common health care services needed by university students and employees

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ABSTRACT

Objectives: The identification of the health needs of university students and staff may be the first step in improving public health approaches. The aim of this study was to investigate the most needed health care services by the university students and employees.

Materials and Methods: A cross-sectional study was carried out with 864 representative individuals on Marmara University Göztepe Campus, Istanbul, Turkey. All participants were given a questionnaire including questions about the sociodemographic characteristics and health status, healthy lifestyle behaviours and health needs.

Results: The mean age of the participants was 24±7.9 years. The number of participants who had periodic health maintenance/check-up was 11.4%. Smoking and alcohol use prevalence was similar for students and employees (30.9% and 30.2%). Depression screening was found to be positive in 67.4% of the participants (58.7% of the employees, 69.7% of the students).The most commonly requested information was on healthy nutrition (42.4%), followed by screening for diseases (32.3%), emergency situations/first aid (31.2%), exercise counselling (28.6%) and prevention of chronic diseases (27.8%).

Conclusion: Our findings show that the most needed health care services are psychological counselling, periodic health controls, health promotion counselling and first aid training.

Keywords: Prevention and control, Primary health care, Occupational health, Depression

INTRODUCTION

In the World Health Organization's reports, "the needs and expectations of individuals and society should be met" is emphasized as one of the main features of the health systems [1]. In order to meet the health needs and expectations of populations, the needs should be determined first. An assessment of health needs provides the opportunity to review the health problems society faces and to identify and solve the new health issues. At the same time, by giving service providers an idea about how to define priorities, such an assessment offers an additional opportunity to achieve the effective use of resources and reduce inequalities in health service delivery [2]. The assessment of health needs is useful for health care planners in terms of evaluating the changing health parameters of the population [2]. When the needs and expectations of health care recipients are reviewed, it is also ensured that service recipients participate in the decision-making process [2]. Participation in decision-making processes is one of the characteristics that a quality health service should have. Although, the provision of rare and expensive services has been perceived as a mark of high quality in the past, nowadays, meeting the expectations and needs of the health care recipient is defined as a quality indicator [3, 4]. Therefore, taking patient views into consideration is increasingly becoming of great importance in the evaluation of services and in ensuring service continuity.

Most researches conducted on the university campuses related to expected and offered health services focus only on students [5, 6]. Knowing the health needs of young university students, the competent adults of the future, is important in terms of planning the initiatives to protect and promote the health of individuals and the community. However, there are also academicians and other employees working at the universities, and there are only limited research available on the health needs and expectations of these groups [7]. In Turkey, units called "Medico-Social" or "Health Counseling" Centers have been organized to provide primary health care services for students and employees on university campuses. After the government's Reorganization of Primary Care, some

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Medico-Social Centers in Turkey were transformed into Family Medicine Centers, but some continue to serve actively, providing services under the same name and conditions as on Marmara University Goztepe Campus. It has been observed, however, that the number of physicians employed in these health centers is quite low when compared to the entire campus population. So, an assessment of the health needs of students and employees in a university is of vital importance in terms of determining the content and priorities of health service delivery.

The aim of this study was to investigate the most needed health care services by university students and employees.

MATERIALS and METHODS

Our research is a descriptive cross-sectional study that was conducted between January 1, 2016 and February 10, 2016. The population of the study consisted of students enrolled in daytime education and employees working at Marmara University Göztepe Campus. According to the information received from the Rectorate of Marmara University, the total number of these groups stood at 29,575 on the date of the survey. Out of this, 93.61% were students (n = 27,684), 6.39% were employees (n = 1,891). The academic staff among the employees constituted 3.95% of the entire population (n = 1,167); those who work in administrative or service jobs represented 2.44% (n = 724). These figures show that the population of Marmara University Göztepe Campus is greater than most university and residential populations in Turkey.

The number of samples targeted was calculated to be 652 individuals at a 99% confidence interval and a 5% margin of error. It was predicted that 20% of the data collection form would not be filled out, and it was planned that 782 individuals would be reached.

Seventeen units (faculties and academies, administrative units, libraries, etc.) on campus were evaluated as strata and the proportional stratified sampling method was used in order to reach the sampling that would reflect the students and staff in each stratum. The subunits in each stratum were determined by drawing lots. If a subunit was not suitable for the survey, an alternative unit was chosen by re-drawing. In the subunits determined, a re-draw determined which class of students was to be sampled.

The drawing process was repeated until the sample size reached the determined number for that stratum. In each stratum, an attempt was made to reach the number of students / employees according to their ratio in the universe. Permission for the students' participation was obtained from the head of the departments and the lecturers responsible for the course, and a questionnaire was administered in the classes on a voluntary basis. Due to the low number of academic and administrative staff in the sampling (6.4% of the sample: 782x6.4/100 = 50 persons), the researchers planned to collect data from all willing workers who were present on the day of the visit paid to the unit chosen at the drawing.

The inclusion criteria were as below:

- Voluntary participation in the study
- Being enrolled in daytime education, for students
- Being on the staff of Marmara University Göztepe Campus, for employees
- Have no problems with language comprehension and expression (spoken and written)

Those who left more than half of the questionnaire empty were excluded from the study.

Data collection tools

The participants were asked to respond to a self-administered questionnaire that included questions on sociodemographic characteristics such as age, sex, marital status, place of residence, social security, and household monthly income, as well as questions evaluating health status [current diseases, medications, prescription or over-the-counter medications, thoughts about weight problems, health perception, the utilization of health services and the accessibility of certain preventive health services (frequency of doctor visits in the past year, health institutions, periodic examinations or check-up, cancer and other health screening), and healthy lifestyle behaviors (being on a diet for weight loss, exercise frequency, fast food consumption, number of meals, smoking, alcohol use, prevention of sexually transmitted diseases (STD)] and their expectations and needs from a primary health center. In addition, questions on health needs and expectations were open-ended, and similar answers were grouped under one heading.

The perception of general health was evaluated on a 5-point Likert scale, ranging from "very good" to "very bad." A 2-item short depression screening test was used for depression screening [8, 9]. Those who answered "Yes" to any of the questions were evaluated as "positive" in terms of depression screening. This short test, also called the Whooley Questions, has a sensitivity of 0.96 (0.89-0.98) and a specificity of 0.57 (0.52-0.61) [9]. Also, women were queried on any history of unplanned pregnancies.

This study was approved by the Marmara University, School of Medicine Ethics Committee (approval number: 002). The participants who met the inclusion criteria were informed about the study and their consents were obtained.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) 16 computer package program was used to evaluate the data. Categorical variables were compared with the chi-square or Fisher test, and continuous variables were compared with the independent Student's t-test. When parametric test hypotheses were not met, non-parametric equivalents of these tests were used. The value $p<0.05$ was considered statistically significant.

RESULTS

Sociodemographic characteristics of the participants

A total of 864 people, 505 women (58.4%) and 359 men (41.6%), who completed more than half of the questionnaire participated in the research. The average age of the participants was 24 ± 7.9 years (min:18-max:68). Of the participants (n = 696), 80.5% were students, 8.8% (n = 76) were academicians, 10.7% (n = 92) were administrative or service personnel.

The sociodemographic characteristics of all participants are shown in Table I.

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Table I. Sociodemographic characteristics of the participants

	Students n = 696	Academic Staff n = 76	Other Staff n = 92	Total n = 864
Age				
Mean ± SD	20.9 ± 2.5	35.4 ± 9.2	38.8 ± 9.6	24 ± 7.9
Median (Min-Max)	21 (18-50)	33 (23-68)	38 (20-59)	21 (18-68)
Marital status				
Married	17 (2.5%)	42 (55.3%)	58 (64.5%)	117 (13.7%)
Single	671 (97.1%)	34 (44.7%)	30 (33.3%)	735 (85.7%)
Widow	3 (0.4%)	-	2 (2.2%)	5 (0.6%)
	n = 690	n = 76	n = 90	n = 857
Education				
Middle school and lower	-	-	7 (7.6%)	9 (1.0%)
High School	686 (98.6%)	-	14 (15.2%)	740 (85.7%)
University	-	4 (5.2%)	62 (67.4%)	25 (2.9%)
Post-graduate degree	10 (1.4%)	72 (94.8%)	9 (9.8%)	90 (10.4%)
	n = 696	n = 76	n = 92	n = 864
Residence (longest lived)				
City	560 (81.5%)	66 (86.8%)	80 (87.9%)	706 (82.7%)
Town	81 (11.8%)	9 (11.8%)	7 (7.7%)	97 (11.3%)
Village	46 (6.7%)	1 (1.3%)	4 (4.4%)	51 (6.0%)
	n = 687	n = 76	n = 91	n = 854
Place of residence				
House	549 (82.2%)	75 (100%)	84 (91.3%)	708 (85.6%)
Private dorm	64 (9.6%)	-	-	64 (7.7%)
State dorm	47 (7.0%)	-	-	47 (557%)
Other	8 (1.2%)	-	-	8 (1.0%)
	n = 668	n = 75	n = 84	n = 827
Reside with				
Alone	369 (53.9%)	11 (14.7%)	6 (6.7%)	71 (8.3%)
Family	154 (22.5%)	13 (17.3%)	18 (20.0%)	400 (47.0%)
Friends	93 (13.5%)	6 (8.0%)	5 (5.6%)	165 (19.4%)
Spouse	54 (7.9%)	15 (20.0%)	10 (11.1%)	36 (4.2%)
Spouse and children	11 (1.6%)	27 (36.0%)	46 (51.1%)	78 (9.2%)
Other*	4 (0.6%)	3 (4.0%)	5 (5.5%)	101 (11.9%)
	n = 685	n = 77	n = 90	n = 851
Social insurance				
Yes	538 (79.1%)	74 (98.7%)	90 (98.9%)	704 (83.15)
No	142 (20.9%)	1 (1.3%)	1 (1.1%)	143 (16.9%)
	n = 680	n = 75	n = 91	n = 847
Perception of income				
Low	390 (56.9%)	1 (1.3%)	63 (69.2%)	99 (11.6%)
Moderate	196 (28.6%)	35 (46.7%)	14 (15.4%)	489 (57.3%)
Good	15 (2.2%)	37 (49.3%)	13 (14.3%)	247 (29.0%)
Very good	85 (12.4%)	2 (2.7%)	1 (1.1%)	18 (2.1%)
	n = 686	n = 75	n = 91	n = 853

Percentages were calculated for the respondents of each group
* Other: siblings, relatives, etc.

Health status of the participants

The data on the health status of the voluntary participants in the study are shown in Table II. Accordingly, it was seen that approximately more than half of the participants (59.2%) had no known disease, while the three most common diseases were anemia (9.6%), allergic rhinitis (7.6%), and asthma (4.2%). The number of individuals who had diseases was greater among the staff rather than the students. Of the participants, 68.8% described their general health status as "good" or "very good". Fifteen participants (1.9%) declared that they had a physical disability and 4 of them reported their physical disability as being advanced myopia and visual impairment.

Table II. Health status of the participants

	Total n = 864	Students n = 696	Staff n = 168	p
Any disease (n = 701)	n (%)	n (%)	n (%)	
No	415 (59.2%)	355 (63.6%)	60 (42.0%)	N/S
Yes	286 (40.8%)	203 (36.4%)	83 (58.0%)	<0.001
Physical disability (n=791)	15 (1.9%)	11 (1.8%)	4 (2.4%)	N/S
General health perception (n = 784)				
Good-Very good	539 (68.8%)	444 (69.5%)	95 (65.5%)	N/S
Moderate	233 (29.7%)	186 (29.1%)	47 (32.4%)	N/S
Bad-Very bad	12 (1.5%)	9 (1.4%)	3 (2.1%)	N/S
Prescribed medication use (n=846)	110 (13.%)	69 (10.1)	41 (25.0%)	<0.001
Over-the-counter medication use (n=830)	256 (30.8%)	198 (29.6)	58 (35.8)	N/S
Self-perceived weight status (n=801)	257 (32.1%)	184 (29.0)	73 (44.0)	<0.001
Positive for depression screening (n=814)	549 (67.4%)	451 (69.7%)	98 (58.7%)	p = 0.007

Percentages were calculated for the respondents of each group.

Among the responders, (n = 814) 67.4% (n = 549) responded positively to at least one question of the two-item depression screening questions. Depression screening was found to be positive in 74.6% of the women (n = 350) and in 58.9% of the men (n = 199) (p<0.001).

The frequency of regularly taking at least one prescribed medication was 13% and this frequency was more common among women than men (16.0%, 8.9%; p = 0.003). Among the employees, the number of those who regularly took prescribed medications was more than the number of the students (25.0%, 10.1%; p<0.001). Those who were reported to be taking at least one over-the-counter medication in the last year represented 30.8% and the majority of these over-the-counter medications were pain killers and cold/flu drugs.

The frequency of the participants' thinking that they had a weight problem was 32.1% and 13.4% were on a special weight loss diet. While there was no significant difference with regard to gender among those who thought that they had a weight problem, the frequency of following a weight loss diet among the women was significantly higher than among the men (17.2%, 8.2%; p<0.001). Among the employees, the number of those who thought that they had weight problems was greater than the number of students (44.0%, 29.0%; p<0.001).

Utilizing some health services

Of the participants, 18.2% reported that they had never applied to a doctor for any reason in the last one year. Among the applicants, the average number of applications to the doctor in the last year was 3.2 ± 3.1 (median: 2; min 1-max 30; IQR 1-3). The frequency was higher among the women than the men (3.06 ± 3.38 for women; median = 2; IQR:1-4; for men 2.08 ± 2.42, median=2; IQR:1-3; p<0.001).

The most frequently utilized health institutions are public hospitals at a rate of 63.2%, family health centers at 40.5% and private hospitals at 33%. The frequency of applicants who applied to a Family Physician for any reason in the last 1 year was 71.4%, and this rate is higher among women than men (75.9%; 64.8%; p = 0.001). The frequency of those who previously obtained services from the Medico-Social Center of the University was 37.4%. Of those who were obtaining service from the Medico-Social Center, 58% stated that they were "satisfied" or "very satisfied" with the service they received. Among the employees, the number of those who obtained services from the medico-social health center were greater than the number of students (85.5%, 24.9%; p<0.001). Among those who did not receive any service from the Medico-Social centers (n = 183), in responding to the open-ended question asking why they did not receive any services, 94.53% (n = 173) stated that they did not need it, or they had no information about the services provided; 10 individuals (5.47%) reported that they were not satisfied with the services they received and did not use the medico-social center because they felt insecure there.

Table III shows situations in which some preventive health care had been previously obtained. The number of people who declared that they had periodic health maintenance or check-ups and had no previous complaint represented 11.4% (n = 92) and this rate was higher in women (13.5%) than the rate of men (8.6%) (p = 0.033). Among the employees, the number of those who had periodic controls/check-ups was greater than the number of students (24.6%, 8.0%; p<0.001). To the open-ended question about "check-up content," the participants' most common responses were blood tests, eye examinations, cardiology examinations, and breast examination. The most frequently reported scans performed were blood pressure (30.1%) and fasting glucose control (27.3%). There were some significant differences between the participants' scans performed when compared by gender, namely that more women than men reported that they had cholesterol, blood pressure and fasting glucose check-ups (p = 0.002; p = 0.001; p = 0.000, respectively). Among women over the age of 40, the frequency rate of mammogram screening was 65%.

Table III. Some preventive health services previously received

	n	%
Periodic health maintenance or check-ups	92	11.4
Screenings		
Blood pressure measurement	260	30.1[a]
Blood glucose measurement	236	27.3[a]
Cholesterol measurement	95	11.0[a]
Pap smear	28	31.5[b]
Mammogram	26	65.0[c]
Fecal occult blood test	1	4.3[d]
Colonoscopy	4	17.4[d]
Prostate examination	0	0.0[e]
Other blood tests	56	6.5[f]

a: For all participants, b: For women >35 years old (n=89), c: For women ≥ 40 years old (n=40), d: For women > 50 years old (n=23), e: For men ≥ 50 years old (n=23), f: Other blood tests: vitamin level, etc.

Data on lifestyle behaviors

The frequency of participants engaging in physical activity 3 times a week or more was 27.7%. When compared by gender, men (37.2%) performed physical activity more frequently than women (26.4%) (p=.006) (Table IV). In the question about which physical activity the participants engaged in regularly, the 3 most frequent responses were "fitness (gym sports)," "team sports" (e.g., football, basketball, volleyball) and "walking/running."

Of the participants, 30.9% reported that they smoke sometimes or every day, 30.2% are alcohol users and 4.5% have tried drugs/stimulants. The prevalence of smoking, alcohol and drug use among men was higher than among women (36.6%, 26.8%, p = 0.002; 35.6%, 26.5%, p = 0.006; 7.7%, 2.3%; p<0.001). There was no statistically significant difference in the frequency of physical activity, smoking, alcohol and drug use and consuming fast food and missing meals among staff and students.

The most skipped meals of the research group were breakfast (35.1%) and snacks (34.8%), and 56.3% reported consuming fast food once or more a week (Table IV).

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Table IV. Lifestyles of the participants

	Total		Students		Staff		p
	n	%	n	%	n	%	
Frequency of physical activity							
1-2 per week	228	38.1	196	39.0	32	33.0	N/S
< 1 per week	205	34.3	161	32.1	44	45.4	
≥ 3 per week	109	18.2	90	17.9	19	19.6	
Everyday	57	9.5	55	11.0	2	2.1	
	n = 599		n = 502		n = 97		
Smokers*	257	30.9	209	31.2	48	29.3	N/S
	n = 833		n = 669		n = 164		
Alcohol users*	242	30.2	197	30.8	45	28.0	N/S
	n=801		n=640		n=161		
Drug users*	37	4.5	33	5.0	4	2.5	
	n = 820		n = 658		n = 162		
On diet to reduce weight	106	13.4	80	12.7	26	16.1	
	n = 791		n = 630		n = 161		
Frequency of consuming fast food	52	6.6	24	3.8	28	17.1	
Never							
Rarely	346	43.7	252	40.1	94	57.3	N/S
1 per week	202	25.5	170	27.1	32	19.5	
3-4 per week	141	17.8	132	21.0	9	5.5	
Everyday	51	6.4	50	8.0	1	0.6	
	n = 792		n = 628		n = 164		
Missing meals							
Breakfast	303	35.1	267	38.4	36	28.9	N/S
Lunch	161	18.6	131	18.9	30	22.4	
Dinner	46	5.4	39	5.6	7	2.6	
Snacks	301	34.8	240	34.5	61	42.1	
None	143	16.6	88	12.7	55	23.7	
			n = 641		n = 166		

*: Every day or sometimes

Expectations and needs regarding Primary Health Care

The subjects that the participants wanted to know regarding their own health are shown in Table V. The subjects that the participants wanted to have information mostly were healthy nutrition (42.4%), screening for diseases (32.3%), emergency situations/first aid (31.2%), exercise counselling (28.6%), prevention from chronic diseases (27.8%), respectively. The number of staff, who wanted to have information on health problems of family members, management of current chronic diseases, screening for diseases, protection against chronic diseases, emergency case/first aid, were higher than the number of students (p =0.006, p =0.006, p<0.001, p = 0.001, p = 0.001, respectively). When compared according to gender, the information on disease screening, exercise counselling, contraception, healthy nutrition, emergency situations/first aid were more demanded by women (p = 0.004, p<0.001, p = 0.03, p = 0.002, p = 0.001, respectively).

Table V. The subjects that the participants wanted to know regarding their own health

	Total		Students	Staff	p
	n	%			
Healthy nutrition	366	42.4	42.1%	43.5%	N/S
Screening for diseases	279	32.3	28.2%	48.8%	<0.001
Emergency case/first aid	270	31.2	28.6%	42.3%	0.001
Exercise counselling	247	28.6	28.7%	28.0%	N/S
Protection against chronic diseases	240	27.8	25.0%	37.5%	0.001
Prevention of communicable diseases	151	17.5	16.2%	22.6%	N/S
Health problems of family members	118	13.7	10.2%	17.9%	0.006
Management of chronic diseases	101	11.7	12.1%	20.2%	0.006
Family planning methods	40	4.6	4.3%	6%	N/S
Others*	23	2.7	-	-	-

*Other subjects: weight loss diet, sleep disorders, etc.

In the case of a new family health center on the campus, the services participants expect to benefit from are shown in Table VI. When compared by gender, the expectations of "counselling/education about sexually transmitted diseases," "treatment of sexually transmitted diseases," "smoking cessation counselling," "alcohol /substance abuse counseling" was more common among men than women (p = 0.014, p = 0.026, p = 0.019, p = 0.01, respectively). However, the expectations of "wound care, dressing, injections," "psychological counselling," "sleep regulation," "exercise counseling," "nutrition counseling," "pregnancy follow-ups" and "regularly health checkups" were more common among women compared to men (p = 0.006, p<0.001, p = 0.044, p<0.001, p<0.001, p = 0.017, p = 0.010, respectively).

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Table VI. In the case of a new family health center on campus, the services expected by the participants

Expected Services	Total	Students	Staff	p
General health assessment	454 (52.55)	341(49%)	113 (67.3%)	<0.001
Oral health	391 (45.3%)	288(41.4%)	103 (61.3%)	<0.001
Nutrition counselling	375 (43.4%)	291(41.8%)	84 (50%)	N/S
Psychological counselling	360 (41.7%)	291(41.8%)	69 (41.1%)	N/S
Exercise counseling	327 (37.8%)	255 (36.6%)	72 (42.9%)	N/S
Wound care, dressing, injections	311 (36.0%)	234 (33.6%)	77 (45.8%)	0.003
Request to receive a medical report	295 (34.1%)	228(32.8%)	67 (39.9%)	N/S
Sleep regulation	292 (33.8%)	240 (34.5%)	52 (31%)	N/S
Weight management	222 (25.7%)	165(23.7%)	57 (33.9%)	0.007
Vaccination	194 (22.5%)	141(20.3%)	53(31.5%)	0.002
Smoking cessation counselling	188 (21.8%)	150 (21.6%)	38 (22.6%)	N/S
Management of new onset health problems	152 (17.6%)	115(16.5%)	37 (22%)	N/S
Alcohol, and drug counselling	136 (15.7%)	117(16.8%)	19 (11.3%)	N/S
Management of chronic diseases	130 (15.05)	92 (13.2%)	38 (22.6%)	0.002
Management of family members' health problems	128 (14.8%)	68 (9.8%)	60 (35.7%)	<0.001
Counseling/education about sexually transmitted diseases	96 (11.1%)	76 (10.9%)	20 (11.9%)	N/S
Family planning methods	90 (10.4%)	63 (9.1%)	27 (16.1%)	0.008
Treatment of sexually transmitted diseases	81 (9.4%)	66 (9.5%)	15 (8.9%)	N/S
Pregnancy follow-ups	71 (8.2%)	44(6.3%)	27 (16.1%)	<0.001
Others*	18 (2.2%)	-	-	-

*Other subjects: Eye examinations, solving orthopedic problems, etc.

DISCUSSION

This research is a cross-sectional study conducted to investigate the primary care health needs of students and employees working on a university campus. According to the results of the study, it was determined that the most needed service is regular health checkups, which should be carried out in order to contribute to maintaining the health of healthy individuals through screening tests, physical examinations, counselling and health education. In addition, our findings suggest that the entire study group should be evaluated especially in terms of smoking, healthy eating, obesity and depression.

According to the Ministry of Health's Health Statistics Yearbook 2016 data, the rate of daily or occasional tobacco use among individuals over 15 years of age in Turkey is 30.6% [10]. Similarly, in our study, the prevalence of smoking was found to be 30.9% in the whole population. On the other hand, in our student group with the median age of 21, smoking prevalence was higher than in the data compiled in Turkey for the use of all tobacco products in the country's 15-24 age group (31.3% vs. 21.4%). Our study, however, only evaluated cigarette smoking. Our findings suggest that smoking cessation counseling is a health service that the research group thought should be given priority. The counseling expectations about smoking stated by approximately 21.8% of the respondents also supports this belief. The fact that the use of other tobacco products other than cigarettes was not questioned is one of the limitations of our research since it is known that the use of the hookah or waterpipe is steadily increasing all over the world [11]. Another priority that should be given in counselling is the use of alcohol. This is important because the study showed that both the entire university population and approximately one-third of the student group used alcohol, and this figure is greater than the data for Turkey (12.2% in Turkey overall and 9.3% in the younger age group) [10]. In many studies conducted at universities, the research group consists of only students and therefore, the intervention-targeted group was always young. However, the data of our study shows that employees as well as students, should be targeted for cigarette and alcohol counselling. The importance of this issue will be better understood when it is considered that adults, especially teachers, are role models for young people.

In our study, although the prevalence of chronic disease and regular use of medications was low, it is noteworthy that the use of over-the-counter drugs was high in all groups. Warnings about the use of over-the-counter medication should be one of the services that should be provided. In addition, one of the first studies in this regard should be conducted on the reasons for the prevalent use of over-the-counter medication. The fact that approximately one-third of the respondents wanted to be informed about protection against chronic diseases may be associated with the increasing incidence of chronic diseases in Turkey and all over the world, and thus with increased susceptibility to these conditions. The further demand for nutrition and exercise counselling, and the fact that blood pressure and blood glucose measurements are the most common screenings are elements that support this sensitivity. Our research suggests that interventions to protect against chronic diseases should be one of the services that should be given first priority on university campuses.

Depression is one of the most frequently reported occupational health problems [12]. Because of this, in 2010, the International Labor Organization included "stress and related diseases" in its "list of occupational diseases" and depression has been defined as being among the stress-related diseases [13]. In our study, the positive screening for depression in 67.4% of the study group suggests that there is a great need for psychological counseling and depression management. Positive screening for depression is more common in women than men. Similarly, in the study conducted by İnanç et al., 28% of the students examined at the University Health Center (Medico-Social Center) were diagnosed with depression [14]. Although, depression screening was high in the whole study group, the fact that it is higher in students than in employees may be related to increased feelings

of hopelessness among adolescents due to the effects of factors such as academic problems and the social, cultural and economic changes experienced [15, 16]. However, the factors that may be associated with depression were not examined since they were outside of the purpose of this research. Further research should be carried out on this subject. Whatever the reason, for prevention and successful management of negative effect of depression on success or productivity, strategies must be developed. Providing opportunities to students, the employees of the future, to acquire the skills needed to cope with stress during their university education will contribute to the prevention of stress-related occupational health problems in the future. Some universities are developing unique programs that enable high-risk students to recover on campus. More research is needed to determine how to deliver the best health care to students [17].

According to Turkey 2016 data, the prevalence of obesity in individuals 15 years of age and older is 53.9% [10]. Approximately only one-fifth of the participants reported that they engage in physical activity 3 times or more per week. Although the research group was a young population, the fact that only a small number of people exercise regularly suggests that exercise counseling services should be planned. Furthermore, reducing sedentary behavior and increasing physical activity may promote life satisfaction, happiness and perceived health status among university student population [18].

Although an anthropometric measurement was not performed, 32.1% of the participants thought that they have a weight problem, 13.4% dieted, 43.4% asked for healthy nutrition counselling, and 37.8% wished to have exercise counseling. Such requests for counseling on these issues may facilitate the fight against obesity. Hence, the challenging obesity in the university setting can also contribute to the improvement of community health.

Although the frequency of application to a Family Physician in the last 1 year for any reason was quite high, it is worthy to note that there are many health needs and expectations not yet met. In order to meet these needs, there must be a fully equipped primary health care center and physicians who can be in close contact with both students and employees and are aware of expectations and needs on campus. On the other hand, the most common problems making it difficult to receive health services were reported as "indifference/lack of interest" and long waiting times; these issues should be considered.

It is significant that about a third of the respondents wanted to have information about emergency situations/first aid and this frequency was higher among employees than students. Considering that the campus is a workplace for both students and employees, attempts to meet this are of great importance for a healthy environment. In particular, a proactive approach would facilitate the prevention and early intervention of possible accidents during student training (e.g., prevention of accidents in the laboratory and when using devices, radiation protection).

Our research has a weak aspect as it was conducted at a single university, yet it has a strong aspect as it represents the students and employees on the Marmara University Göztepe campus, which has many faculties, institutes and units with a very large population. In addition, besides the students, the inclusion of academic and administrative staff as well as service workers in our research, is the other strong aspect of our study because similar studies in the literature, focus only on students [5-7]. With the inclusion of employees in the study, it was also revealed that students and employees had different health needs. This suggests that, among the preventive health services, prevention of chronic diseases, screening, health problems of family members, first aid and reproductive health services should not be ignored. Other weakness of this study is that the hazards of the participants were not asked. A research on the occupational hazards of each faculty, institution or unit in the university and the interventions may be the subject of another study. Another limitation is that fewer students than the sample required were reached. An attempt had been made at the data collection stage to reach the number of students determined in each strata constituting the sample, but the study was ultimately completed with fewer students due to the fact that in some units there were no current courses, meaning that fewer students participated and some students did not fill out more than half the questionnaire.

In summary, our findings show that psychological counselling, healthy lifestyle counselling, first aid training and periodic health controls, including prevention of diseases, screening examinations and tests, are the most needed services in the university population. Identifying the health needs of university workers and students, who will become employees of the future, may contribute to developing interventions that improve these individuals' physical and psychological health and performance.

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Compliance with Ethical Standards

Ethical approval: The study was reviewed and approved by the Marmara University, School of Medicine Ethical Committee.

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Informed consent: The participants who met the inclusion criteria were informed about the study and their consent was obtained.

Author contributions: Conception and design of the study: CAK and SC, Acquisition of data:SKA, SB and BK, Analysis and interpretation of data: CAK, SKA and EA, Drafting of the manuscript: CAK, SKA and SC, Critical revision:: CAK and SKA.

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Difficulties in indicating appendectomy; diagnosis of appendicitis in patients with normal white blood cell count

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ABSTRACT

Objectives: Even after 130 years have passed since the first appendectomy, diagnosing acute appendicitis can still be challenging. The objective of this study was to investigate clinical, imaging and laboratory findings of patients diagnosed with acute appendicitis in order to determine whether white blood cell count is helpful in the diagnosis.

Materials and Methods: Parameters of Alvarado score, patients' complaints, physical examination and laboratory findings were recorded. Appendiceal diameters of patients detected with ultrasound (US) were also recorded.

Results: Of the 98 patients, 89 patients (91%) had an appendiceal diameter wider than 8 mm and 9 patients (9%) had an appendiceal diameter of 8 mm and smaller in the preoperative US. Pathology was normal in 8 out of 9 patients (89%) with a diameter of 8 mm and below; and 9 out of 89 patients (9%) with a diameter above 8 mm ($p<0.05$). Sensitivity and specificity of the 8-mm cut-off was 99% and 47% respectively. Positive and negative predictive values were 90% and 89% respectively.

Conclusion: Distribution of pathology results with respect to appendiceal-diameters revealed that there was an obvious threshold between normal and pathology-proven appendicitis. This outcome highlights the importance of imaging in the diagnosis.

Keywords: Appendicitis, Diagnostic ultrasound, White blood cell count

INTRODUCTION

In 1880, Robert Lawson Tait performed the first appendectomy for appendicitis in the United Kingdom [1]. Even after 130 years, diagnosing acute appendicitis can still be challenging for surgeons. This is demonstrated by high negative laparotomy rates documented in different literature. In a Swedish study, female and male patients had negative appendectomy rates of 24% and 12%, respectively [2]. In another large North American study, the rate of negative appendectomy was 13% [3]. It is assumed that negative laparotomy rate decreases to approximately 10 by routine use of ultrasonography (US) [4]. The higher sensitivity of computed tomography (CT) seems to have a greater impact on the rate of negative laparotomies, which has reduced the estimated rate by 5-10% [4,5]. In most European countries, most surgeons consider acute appendicitis as a clinical diagnosis and do not routinely perform imaging techniques [6].

Scoring systems are designed to assist the clinical evaluation of patients with acute appendicitis (AA). The Alvarado score, even though there are some disadvantages, is the most common scoring system and its use is validated in several studies [7-9]. The structure was based on a review of patients who were operated after suspicion of appendicitis, whereas the score should be used in all patients with suspected appendicitis. Furthermore, the score does not contain C-reactive protein as a variable, but many studies have demonstrated the importance of C-reactive protein in the evaluation of patients with appendicitis [10].

The recent appendicitis inflammatory response (AIR) score is designed to overcome these disadvantages [11].

The white blood cell count (WBCC) in the diagnosis of AA is neither sensitive nor specific. WBCC is high in almost 70% of the spectrum of etiologies causing right lower abdominal pain [10]. The use of imaging techniques reduced the rate of negative appendectomy. However, it is accepted that WBCC alone is not sufficient [10,12].

In this study, we aimed to investigate the clinical, imaging and laboratory findings of patients diagnosed with AA in order to determine whether WBCC is helpful in the diagnosis of future cases.

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MATERIAL and METHODS

This study included 436 patients (patients with complete records of related medical information) who were operated on in our surgical clinic between January 2015 and September 2018 with the diagnosis of AA. Approval for this study was obtained from Akdeniz University, School of Medicine Clinical Research Ethics Committee (approval number 917) .

We excluded patients with incomplete clinical information.

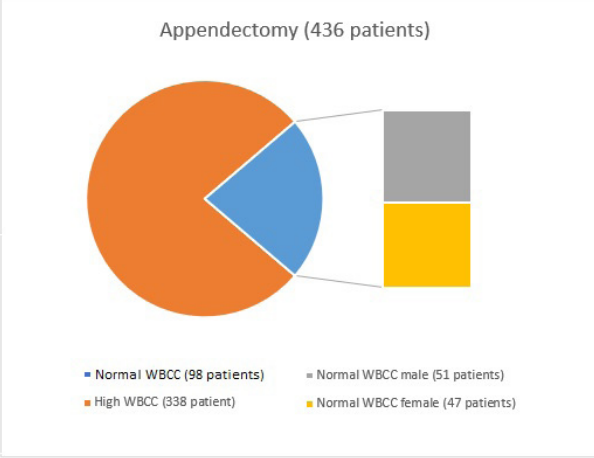

Figure 1. The white blood cell count distribution of patients who underwent appendectomy during the study period.

Complaints and physical examination findings of the patients were obtained from their medical files. Patients with hematological disease, immunosuppression and malignancy were excluded from the study. In addition, the parameters of Alvarado score were evaluated and patients' complaints, physical examination findings and laboratory findings were recorded retrospectively. Appendiceal diameters of patients with normal WBCC were also recorded. These diameters were calculated based on the abdominal images taken before the operation. Whole blood samples were collected in 4 mL K2EDTA tubes and leukocyte count was performed by optical method using an automatic hematology analyzer. Pathology samples were divided into two groups as normal appendix and appendicitis.

Statistics Analysis

In the statistical analysis of data, Statistical Package for Social Sciences for Windows (SPSS Inc., Chicago, Illinois, USA) version 16.0 was used. In addition to the descriptive statistical methods (mean, standard deviation), for the intergroup comparison of normally distributed parameters of quantitative data and for non-normally distributed parameters the Student's t-test and the Mann-Whitney U-test were used. The relationships between numerical data were analyzed by a correlation analysis. Qualitative data were compared using the Chi-square test.

RESULTS

Throughout the study period, out of 436 patients who underwent appendectomy, only 98 patients had a normal WBCC. Of these, 51 (52%) were male and 47 (48%) female (Figure 1). The mean age of the patients was 36 ± 11 (min 18, max 72).

According to pathology reports, 81 patients (83%) had AA and 17 patients (17%) had normal results. Of the 98 patients, 89 patients (91%) had an appendiceal diameter wider than 8 mm US and 9 patients (9%) had an appendiceal diameter of 8 mm and smaller in the preoperative US. Pathology was normal in 8 out of 9 patients (89%) with a diameter of 8 mm and below; and 9 out of 89 patients (9%) with a diameter above 8 mm. This difference was statistically significant ($p<0.05$). Sensitivity and specificity of the 8-mm cut-off was found to be 99% and 47% respectively. Positive and negative predictive values were 90% and 89% respectively. Accuracy diagram of ultrasound guided diagnosis of acute appendicitis revealed a threshold of 8-mm for appendiceal diameter (Figure 2).

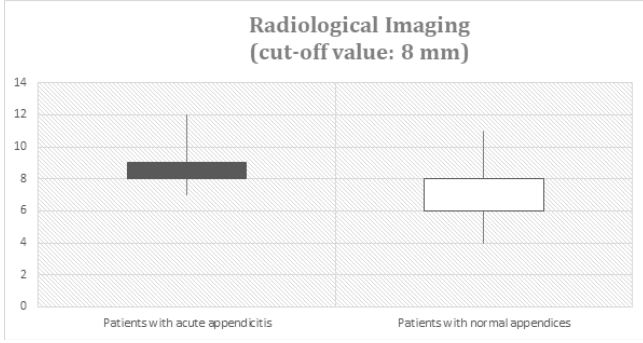

Figure 2. Accuracy diagram of ultrasound guided diagnosis of acute appendicitis revealing the importance of 8-mm threshold for appendiceal diameter ($p<0.05$).

The rate of negative exploration was 17% and all were reported as pathological end-stage lymphoid hyperplasia. In the univariate analysis, neutrophil count, appendix diameter and Alvarado score were statistically significant ($p <0.05$).

DISCUSSION

In this study, distribution of pathology results with respect to appendiceal diameters revealed that there was an obvious cut-off limit between normal pathology and pathology-proven appendicitis. This outcome highlighted the importance of imaging in the diagnosis of acute appendicitis. Radiology's role was central to the management. Conceivably, imaging techniques are strong tools for physicians supporting their diagnosis of AA and they reduce negative appendectomies. Several studies had reported that radiological examination reduced the rate of negative appendectomy from 20% to 2-14% [13]. Our results also indicated that the sensitivity of radiological imaging was over 90%. On the other hand, diameter itself does not prove any pathology. The normal diameter of the appendix

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can be as high as 12.8 mm in adults and majority of normal appendices are larger than 6 mm [14]. When CT findings are equivocal in patients with suspected appendicitis, appendicitis is encountered in about 30% but in the case of the appendix measured less than 9 mm alone, similar to our study findings, the likelihood of appendicitis is much smaller [15]. Yet, strong reinforcements with physical examination and laboratory tests are always should be kept in mind. Ortega-Deballon et al., suggested that patients with a normal WBCC should not undergo appendectomy [16]. Similarly, Atema et al., showed that WBCC and C-reactive protein were important parameters to exclude AA [17].

There are many studies focusing on the optimal criteria for the diagnosis of AA utilizing CT with different types of contrast and routes (e.g. oral, rectal, intravenous) of application. The data from literature highlights the high accuracy of CT in the diagnosis of AA. However, there are convincing results on the high accuracy of CT for the diagnosis of AA. A recent meta-analysis included 9330 patients published in 28 studies reported a significant difference in the negative appendectomy rate (NAR), from 16.7 % when using clinical evaluation without imaging compared to 8.7 % with use of CT [18]. In addition, NAR decreased from the pre-CT era to the post-CT era (21.5 % to 10 %) [18]. In 2011, multi-detector row computed tomography (MDCT) showed a sensitivity of 98.5 % and a specificity of 98 % for the diagnosis of AA in 2871 patients [19]. Another meta-analysis included 4341 patients (children and adults) from 31 studies reported a pooled sensitivity and specificity for diagnosis of AA in children of 88 % and 94 %, respectively, for US studies and 94 % and 95 %, respectively, for CT studies. Pooled sensitivity and specificity for diagnosis of AA in adults were 83 % and 93 %, respectively, for US studies, and 94 % and 94 %, respectively, for CT studies [20].

The studies and reports detailed above give an overview of the persistent difficulties in the clinical diagnosis of AA in pediatric and adult patients, the usefulness of various clinical scores (which are not commonly used in routine practice) and recent developments of modern imaging techniques focusing on US imaging. To date, US imaging for suspected AA is performed world-wide by radiologists and many physicians of other medical subspecialties, with or without the support of sonographers.

The 2011 American College of Radiology Appropriateness Criteria® for right lower quadrant pain-suspected appendicitis state that for adult patients with clinical signs of AA the sensitivity and specificity of CT are greater than those of US, but that in pediatric patients, the sensitivity and specificity of graded-compression US can approach those of CT, without the use of ionizing radiation [21].

Patients with a normal WBCC may be acceptable for observation, but several studies had shown that high WBCC could be very important for the diagnosis of AA [22]. Alder et al. [23] demonstrated that the appendix mucosa might develop secondary to viral infections and that uncomplicated cases of viral appendicitis could pass without antibiotics [24].

As a result, an appendix diameter greater than 8 mm in imaging had a positive predictive value of 90% for AA patients with normal WBCC. Appendectomy is recommended in patients with normal WBCC with an appendix diameter greater than 8 mm.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Akdeniz University School of Medicine Clinical Research Ethics Committee (approval number 917).

Conflict of Interest: The author declares that he has no conflict of interest.

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Case Report

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Immunoglobulin G4 related mastitis: A case report

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ABSTRACT

Immunoglobulin (Ig)G4-related sclerosing disease is a recently recognised condition characterised by mass forming lesions associated with storiform fibrosis, obliterative phlebitis, lymphoplasmacytic infiltrate rich in IgG4 positive plasma cells and elevated serum IgG4 levels. IgG4-related mastitis (IgG4-RM) is exceedingly rare with only thirteen cases reported in the literature to date.

Immunoglobulin G4-RM is diagnosed exclusively on histological analysis. It is a benign chronic inflammatory process that can be treated sufficiently with excision or steroid. However, conservative treatment should be preferred and unnecessary surgery should be avoided as IgG4-RM respond to simple and effective steroid treatment. Herein, we presented a 28-year-old patient with IgG4-RM. She was the youngest patient in the literature at the time of her diagnosis.

Keywords: Mastitis, IgG4, Steroid therapy, Inflammatory pseudotumour

INTRODUCTION

Immunoglobulin (Ig)G4-related sclerosing disease, first described in the pancreas is now recognized as a systemic entity that can involve the liver, salivary gland, lymph nodes, mesentery, breast, and others [1-3]. The milestone of this disease is increased serum concentration of IgG4 levels and tumor-like swelling of organs, infiltration of lymphocytes that are enriched in IgG4-positive plasma cells, and storiform-patterned fibrosis. Differentiating this process from malignancy clinically can be a challenge as the lesions often present with suspicious radiographic features. It is important to recognize this entity as patients often respond well to steroid therapy and can avoid unnecessary surgery. To the best of our knowledge, the IgG4-related sclerosing disease of the breast is exceedingly rare, with 13 cases reported in the literature [4]. The well-known treatment of IgG4-related mastitis (RM) is glucocorticoid therapy. However, most cases undergo excision.

Herein, we presented a 28-year-old patient with IgG4-RM. She was the youngest patient in the literature.

CASE REPORT

A-28-year-old female applied to our clinic. She suffered from a mass in upper inner quadrant of the right breast with no pain. She has two kids and no additional family history. Her first pregnancy was when she was 21 years old. She never used oral contraceptives. The patient noticed the mass one month ago. Red fluffy painful lesions increased around the abdomen and face, simultaneously. The mass size was 3 cm and palpable with an irregular shape. Preoperative laboratory values were C-reactive protein 1.2 mg / dL, white blood cell 9.3 K / u L and neutrophil 6.0 K / uL.

Malignancy could not be ruled out by examination and radiological findings in our patient, therefore, excisional biopsy was performed The specimen revealed that the lesion infiltrated the ducts around the areas of fibrosis in the breast tissue by invading the normal breast tissue in most areas. In addition, multinuclear giant cells, which eliminated lobular structures, intense lymphoplasmacosmoid inflammation with histiocytes in situ with neutrophils and lymphoid follicle structures were observed. Immunohistochemical examination revealed multiple vascular structures in the area of inflammation with CD34. CK 5/6 (+) in natural-looking breast duct epithelial cells. CD68 showed histiocytes (+) but granuloma structures were not observed. However, CD20 and CD8 focal (+) and fewer lymphocytes; CD3, CD4 with common (+) lymphoid follicles around the ductus were detected. CD138 was detected (+) in plasma cells

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around lymphoid follicles. Plasma cells stained with (+) IgG were detected. The dendritic cell network in lymphoid follicles was not followed by CD21 and CD23. No histopathological findings were found in favor of lymphoma with Ki-67 and Bcl-2 and Skin D1. HHV-8 (-) was detected. Intensive fibrosis was observed around the lymphoid follicles with Masson's trichrome special stain. No specific findings were observed with periodic acid–Schiff (PAS) staining. The inflammation described in the surgical margins showed continuity. Histopathological findings were consistent with IgG4-RM. Findings showed no malignancy.

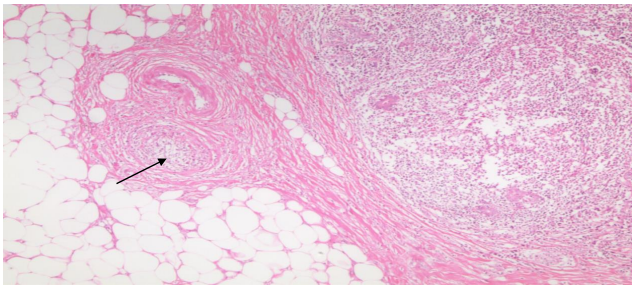
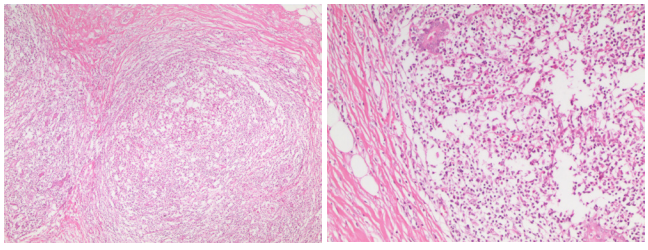


Figure 1. Dense lymphoplasmacytic infiltrate with storiform fibrosis and obliterative phlebitis (arrow) (H&EX40).



Figures 2 and 3. High magnification of nodular lymphoplasmacytic and histiocytic infiltrate between lobular structures (H&EX100 and H&EX200).

When the IgG4-related mastitis was diagnosed the patient was consulted to the Department of Rheumatology in our hospital. Rheumatologists started methotrexate and steroid therapy. IgG4 value was detected as 44 mg/dL two months after the surgery. 7.5 mg methotrexate was used weekly and folic acid was added to the therapy. Prednisolone loading dose 40 mg/day was initiated and gradually tapered to 5 mg/day maintenance dose. The control imaging after treatment was completely normal. Extensive whole-body imaging was not recommended unless symptomatic. The patient had no recurrence 2 years after diagnosis.

DISCUSSION

IgG4-related sclerosing disease of the breast has been described as sclerosing mastitis or inflammatory pseudotumor in the literature [5,6]. In IgG4-RM the lesions can appear as a single lesion or multiple lesions affecting one or both breasts. The lesions are often palpable and painless. Radiologic findings often mimic malignancy. The main diagnostic modality for IgG4-RM is excisional biopsy. In the literature, cases who underwent core needle biopsy or vacuum assisted core needle biopsy were reported [5-11].

In the literature, all patients with IgG4-RM were female except one and their ages ranged between 37 to 66. Our patient was 28 years old at the time of diagnosis and the youngest patient in the literature. Here, we emphasized the importance of conservative treatment as there were no recurrent cases in the literature who received conservative treatment [5-11].

A 51-year-old woman presented with bilaterally swollen eyelids and an elevated serum IgG4 concentration. IgG4-related sclerosing disease of the breast was diagnosed after core needle biopsy and steroid therapy was initiated. The lesion shrank with a 4 week steroid treatment similar to our patient and at 7 months follow-up, the lesion did not show any new growth [7]. Prednisolone treatment was started in a patient after vacuum assisted core needle biopsy. No new lesions were observed in her follow-up [4]. Although, clinically suggesting malignancy, overdiagnosis should be avoided by using the appropriate pathological method [8].

It should be kept in mind that this disease can be a manifestation of a systemic disease. Extensive whole body imaging is generally not recommended unless the patient is symptomatic. [10] Our patient did not have extra manifestation or lymphadenopathy and was asymptomatic.

Exclusion of carcinoma, lymphoma, and other entities that can mimic this disease is essential [11]. Diagnostic criteria of the lgG4-RM (according to the International Concensus Criteria) are histopathologically presence of lymphoplasmacytic infiltration of lgG4 plasma cells, obliterative phlebitis and fibrosis. IgG4 counts and IgG4:IgG ratios are secondary in diagnosis. [12]

We would like to emphasize that IgG4-RM is extremely rare with 13 cases reported in the literature [5-11]. Our patient was the youngest of all cases in the literature.

Conclusion

IgG4-related sclerosing disease of the breast is rare. However, diagnosing this disease is a key point. Clinical and radiological findings of this disease resemble those of a malignant tumor. Therefore, timely diagnosis and appropriate therapy can be effective in majority of patients. We would like to underline that unneeded surgical biopsy should be avoided and conservative therapy should be preferred.

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Conflict of interest

The authors declare that they have no competing interests.

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