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Aims and Scope

Cyprus Journal of Medical Sciences (Cyprus J Med Sci) is the scientific, peer reviewed, open access international publication organ of Cyprus Turkish Medical Association. The journal is published three times a year, in April, August, and December. As of 2020, the journal has become a quarterly publication, publishing in March, June, September, and December. The journal's publication language is English.

The aim of the journal is to publish original research papers of the highest scientific and clinical value in all medical fields. Cyprus Journal of Medical Sciences also publishes reviews, rare case report and letters to the editors.

The target audience of the journal includes healthcare professionals physicians, and researchers who are interested or working in in all fields of medicine.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Cyprus Journal of Medical Sciences is indexed in Web of Science-Emerging Sources Citation Index, TUBITAK ULAKBIM TR Index, EBSCO, and Gale.

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at www.cyprusjmedsci.com. The journal guidelines, technical information, and the required forms are available on the journal's web page.

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Instruction to Authors

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Editorial And Publication Process
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Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

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Manuscripts submitted to Cyprus Journal of Medical Sciences will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

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Cyprus Journal of Medical Sciences is extremely sensitive about plagiarism. All submissions are screened by a similarity detection software (iThenticate by CrossCheck) at any point during the peer-review and/or production process. Even if you are the author of the phrases or sentences, the text should not have unacceptable similarity with the previously published data.

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Each person listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors (ICMJE - www.icmje.org). The ICMJE recommends that authorship is based on the following four criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. Also, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged in the title page of the manuscript.

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Cyprus Journal of Medical Sciences requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. The journal's Editorial Board resolves cases of a potential conflict of interest of the editors, authors, or reviewers within the scope of COPE and ICMJE guidelines.

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Manuscript Preparation
The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2019 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, and TREND guidelines for non-randomized public behavior.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at www.cyprusjmedsci.com. Manuscripts submitted via any other medium and submissions by anyone other than one of the authors will not be evaluated.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following:

- Copyright Agreement and Acknowledgement of Authorship Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) during the initial submission. These forms are available for download at www.cyprusjmedsci.com.

Preparation of the Manuscript
Title page: A separate title page should be submitted with all submissions and this page should include:

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, highest academic degree(s), and ORCID IDs of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number), and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

Abstract: An abstract should be submitted with all submissions except for Letters to the Editor. The abstract of Original Articles should be structured with subheadings (Background/Aims, Material and Methods, Results and Conclusion). Please check Table I below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of five keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).

Main Points: All submissions except letters to the editor should be accompanied by 3 to 5 "main points" which should emphasize the most noteworthy results of the study and underline the principle message that is addressed to the reader. This section should be structured as itemized to give a general overview of the article. Since "Main Points" targeting the experts and specialists of the field, each item should be written as plain and straightforward as possible.

Manuscript Types
Original Articles: This is the most important type of article since it provides new information based on original research. Acceptance of original papers will be based upon the originality and importance of the investigation. The main text of original articles should be structured with Introduction, Material and Methods, Results, and Discussion subheadings. An original article can be signed by maximum 6 authors unless it is a multi-center study or that it required extensive labour. Please check Table I for the limitations for Original Articles.

Clinical Trials
Cyprus Journal of Medical Sciences adopts the ICMJE's clinical trial registration policy, which requires that clinical trials must be registered in a publicly accessible registry that is a primary register of the WHO International Trials Registry Platform (ICTRP) or in ClinicalTrials.gov.

Instructions for the clinical trials are listed below.

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- Clinical trial registry is only required for the prospective research projects that study the relationship between a health-related intervention and an outcome by assigning people.
- To have their manuscript evaluated in the journal, author should register their research to a public registry at or before the time of first patient enrollment.
- Based on most up to date ICMJE recommendations, Cyprus Journal of Medical Sciences accepts public registries that include minimum acceptable 24-item trial registration dataset.
- Authors are required to state a data sharing plan for the clinical trial registration. Please see details under "Data Sharing" section.
- For further details, please check ICMJE Clinical Trial Policy at http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html

Data Sharing

As of 1 January 2019, a data sharing statement is required for the registration of clinical trials. Authors are required to provide a data sharing statement for the articles that reports the results of a clinical trial. The data sharing statement should indicate the items below according to the ICMJE data sharing policy:

- Whether individual deidentified participant data will be shared
- What data in particular will be shared
- Whether additional, related documents will be available
- When the data will be available and for how long
- By what access criteria will be shared

Authors are recommended to check the ICMJE data sharing examples at http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html

While submitting a clinical trial to Cyprus Journal of Medical Sciences;

- Authors are required to make registration to a publicly accessible registry according to ICMJE recommendations and the instructions above.
- The name of the registry and the registration number should be provided in the Title Page during the initial submission.
- Data sharing statement should also be stated in the Title Page even the authors do not plan to share it.

Clinical trial and data sharing policy of the journal will be valid for the articles submitted from 1 February 2020.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

Editorial Comments: Invited brief editorial comments on selected articles are published in The Cyprus Journal of Medical Sciences. Editorials should not be longer than 1000 words excluding references. Editorial comments aim to provide a brief critical commentary by reviewers with expertise or with high reputation in the topic of the research article published in the journal. Authors are selected and invited by the journal to provide such comments. Abstract, Keywords, and Tables, Figures, Images, and other media are not included.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	4000	250 (Structured)	35	6	5 or total of 10 images
Review Article	5000	250	50	6	10 or total of 15 images
Case Report	1200	200	15	No tables	4 or total of 8 images
Letter to the Editor	400	No abstract	5	No tables	No media

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with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in clinical practice and should guide future studies. The subheadings of the review articles should be planned by the authors. However, each review article should include an "Introduction" and a "Conclusion" section. Please check Table I for the limitations for Review Articles.

Case Reports: There is limited space for case reports in the journal and reports on rare cases or conditions that constitute challenges in diagnosis and treatment, those offering new therapies or revealing knowledge not included in the literature, and interesting and educative case reports are accepted for publication. The text should include Introduction, Case Presentation, and Discussion with an unstructured abstract. Please check Table I for the limitations for Case Reports.

Letters to the Editor: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Letter to the Editor." Readers can also present their comments on the published manuscripts in the form of a "Letter to the Editor." Abstract, Keywords, and Tables, Figures, Images, and other media should not be included. The text should be unstructured. The manuscript that is being commented on must be properly cited within this manuscript.

Tables
Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends
Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References
Both in-text citations and the references must be prepared according to the Vancouver style.
While citing publications, preference should be given to the latest, most up-to-date publications. Authors are responsible for the accuracy of references If an ahead-of-print publication is cited, the DOI number should be provided. Journal titles should be abbreviated in accordance with

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the journal abbreviations in Index Medicus/MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article: Yazıcı A. The efficacy of endoscopic ventilation tube insertion in pediatric populations. Cyprus J Med Sci 2019; 4(2): 73-6.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the complete drug reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki öğrencilerin beslenme durumları, fiziksel aktiviteleri ve beden kitle indeksleri kan lipidleri arasındaki ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodIEID/cid.htm.

Revisions

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

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Original Article

DOI: 10.5152/cjms.2020.1406

Hidden Danger: Superbug *Escherichia coli* Isolated from Urine Isolates of Outpatient Women with Uncomplicated Urinary Tract Infection

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Cite this article as: Özdemir S, Aydoğan O, Özbek A, Çiftçi İH, Köksal Çakırlar F. Hidden Danger: Superbug *Escherichia coli* Isolated from Urine Isolates of Outpatient Women with Uncomplicated Urinary Tract Infection. Cyprus J Med Sci 2020; 5(2): 101-6.

BACKGROUND/AIMS
Escherichia coli (E. coli) is responsible for the vast majority of uncomplicated bacterial urinary tract infection (UTI) cases in women. The high ability of the isolates to develop antimicrobial resistance makes the treatment difficult. In this study, we investigated the presence of plasmid-mediated quinolone resistance (PMQR) genes in *E. coli* isolates and their relationship with extended-spectrum beta-lactamases (ESBL).

MATERIAL and METHODS
A total of 300 *E. coli* isolates from urine specimens of women, including 108 ESBL producers and 192 non-ESBL producers, were analyzed. The ESBL production was examined using the E-test ESBL strips, and the carbapenemase activity was examined using the CarbaNP test. The presence of PMQR genes *(qnrA, qnrB, qnrS, and aac (6´)-Ib)* among urine isolates was investigated using polymerase chain reaction. Conjugation experiments were performed to detect the horizontal transferability of the PMQR-positive plasmid.

RESULTS
Among the ESBL-EC isolates, ciprofloxacin resistance was determined at 69%. Eight isolates were resistant to carbapenems. The *aac(6')-Ib-cr* variant was found in 40% of ciprofloxacin-resistant *E. coli* isolates. None of the isolates harbored the *qnrA, qnrB, or qnrS* gene. The transferability was 14% for *aac(6')-Ib-cr*. The MICs of transconjugants showed increased resistance to fluoroquinolones compared with the recipient *E. coli* J53AzR.

CONCLUSION: This study showed that the frequency of PMQR genes in ESBL-producing superbug *E. coli* isolates reduced therapeutic options for treating community-acquired UTIs in affected women and that a careful use of antibiotics is very important.

Keywords: ESBL-producing *Escherichia coli,* superbug, PMQR genes, *aac(6')-Ib-cr,* female patients with UTI, fosfomycin

INTRODUCTION

All over the world, in outpatient practice, uncomplicated bacterial urinary tract infections (UTIs) are one of the most common community-acquired diseases. *Escherichia coli (E. coli)* is responsible for the vast majority of UTIs, and especially women suffer from UTIs because of the proximity of the urethra to the vagina and the rectum, changes in genital microflora, hormonal influences, and other anatomical and physiological characteristics (1). *E. coli* is a part of the normal flora in the intestinal tract of a healthy human. Uropathogenic *E. coli* is generally acquired from sexual partners, household members, pets, food, toilet, and during travel. However, the high ability of the isolates to develop antimicrobial resistance makes the treatment difficult. These bacteria can transfer the resistance genes to other *E. coli* isolates and different Gram-negative bacteria. Therefore, multidrug-resistant *E. coli* deserves a superbug label. Furthermore, antimicrobial options in treatment are limited due to multidrug resistance (2). Quinolones are the first choice for the treatment of UTIs caused by extended-spectrum-beta-lactamase (ESBL)-producing *E. coli*. However, the widespread use of quinolones for therapeutic and non-therapeutic purposes has led to the rapid spread of quinolone-resistant *E. coli* isolates worldwide (3). The resistance to quinolones usually occurs as a result of "DNA target mutations, overexpression of efflux pumps, loss of porins and mobile genetic elements encoded on plasmids, known as plasmid-mediated quinolone resistance

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(PMQR) genes, namely *qnr, aac (6')-Ib-cr, and qepA"* (4, 5). A series of PMQR determinants within the last 10 years further reveal a new issue about the resistance to quinolones. PMQR genes play an essential role in the development of low-level quinolone resistance and facilitate the emergence of high-level resistance in the presence of quinolones at treatment levels (6). PMQR genes, *qnr (qnrA, qnrB, and qnrS),* which protects the DNA gyrase and Type IV topoisomerase enzymes from quinolone inhibition, and *aac(6')-Ib-cr,* which acetylates quinolones, and efflux by QepA and OqxAB have been reported in clinical isolates of *Enterobacteriaceae,* including *E. coli* (3, 6-8). In many studies, PMQR genes have frequently been shown to be associated with genes encoding ESBL and aminoglycosides on the same plasmid (8, 9). Today, plasmids carrying *qnr* and ESBL determinants represent a concern worldwide. Carbapenems are often the last-choice agents used for the treatment of patients with severe infections. However, carbapenemase-producing *E. coli* has been increasingly reported, especially in clinical settings (2, 10). Fosfomycin, known for over 40 years, has recently become attractive as an alternative agent for the treatment of UTIs (11). "The Infectious Diseases Society of America (IDSA) recommends that physicians obtain information on local resistance rates, the appropriateness of empirical therapy proposals and that ongoing surveillance has been conducted to monitor changes in the susceptibility of uropathogens" (12, 13). This study aimed to investigate the presence of PMQR *(qnrA, qnrB, qnrS, and aac(6')-Ib-cr)* genes, and also their relationship to ESBL among *E. coli* strains isolated from urine samples of outpatient Turkish women, with community-acquired UTI.

MATERIAL and METHODS

Methodology

A total of 300 *E. coli* isolates were obtained from urine samples of outpatient Turkish women with symptoms suggestive of community-acquired UTIs in İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine Hospital. In the study, the patients were aged 16-85 years. Patients who were pregnant, with functional, or structural anomalies of the urinary tract, and suffering from an immunocompromized illness, and using immunosuppressants, and who were discharged from the hospital 10–15 days before were excluded from the study. The identification and antimicrobial susceptibility were determined using the BD Phoenix automated identification and susceptibility testing system (BD Diagnostic Systems, Sparks, MD). The isolates resistant or moderately susceptible to tested antibiotics were confirmed using the E-test (bioMerieux, France) method. The susceptibility of ciprofloxacin, carbapenems, tigecycline, colistin, and fosfomycin was determined by E-test (bioMerieux, La Balme-les-Grottes, France) method according to manufacturer's instructions. The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (14). The ESBL production was examined using the double-ended E-test ESBL strips (AB Biodisk, Solna, Sweden) containing gradients of cefotaxime (CT) or ceftazidime (TZ) or cefepime (FEP) at one end and cefotaxime or ceftazidime or cefepime plus clavulanic acid (CTL, TZL, and FEL) at the other, according to the manufacturer's instructions. The carbapenemase activity was investigated using the Carba NP test (RAPIDEC CARBA NP (bioMerieux, La Balmeles-grottes, France) (15). Quinolone-resistant isolates were screened for the presence of PMQR *(qnrA, qnrB, qnrS, and aac(6')-Ib)* genes by the polymerase chain reaction (PCR). DNA was extracted from the fresh culture of *E. coli* colonies according to the protocol performed using the GeneJET Genomic DNA Purification kit (Thermo Scientific, USA). The determination of PMQR *(qnrA, qnrB, qnrS, and aac(6')-Ib)* genes was performed using PCR. All *aac (6')-Ib* positive amplicons were investigated by digestion with BseGI (Fermantas, USA) restriction enzyme to determine the *aac (6')-Ib-cr* variant. The amplification of *qnrA, qnrB, qnrS,* and *aac(6')-Ib* genes was performed using the primers presented in Table I (4, 9, 16-18).

Conjugation Assays Used to Detect PMQR Transferability

Conjugation experiments were performed to detect whether the quinolone resistance could be transferred horizontally to a plasmid-free *E. coli* strain from *E. coli* urine isolates carrying PMQR-positive plasmids. A plasmid-free, sodium azide-resistant *E. coli* J53 (AzR) was used as the recipient, as described previously (19). The recipient (J53) and donor urine isolates were inoculated into the Luria-Bertani (LB) broth (Difco) and grown overnight at 37°C. The equal volumes of the donor and recipient cultures were mixed and incubated overnight at 37°C. The serial dilutions were homogeneously spread onto trypticase soy agar (Oxoid) plates supplemented with sodium azide (150 µg/mL, Sigma-Aldrich) and ciprofloxacin (0.25 µg/mL, Sigma-Aldrich). The transconjugants were selected and collected on the plates. PCR was performed to determine the presence of PMQR determinants (20). Plasmid DNAs of transconjugants and donor isolates were extracted using the GenElute Plasmid Miniprep Kit (Sigma-Aldrich, Vienna, Austria) according to the manufacturer's instructions. The size of plasmid was estimated by electrophoresis using a 0.7 % (w/v) agarose gel, comparing

TABLE I. Primers used for the detection of *qnr* and *aac (6')-Ib-cr* genes

Target gene		Primer sequence (5'→ 3')	Gene size	Genebank accession No.	Predicting the size of amplicon (bp)
qnrA	F	TCAGCAAGAGGATTTCTCA	657	KC493127.1	627
	R	GGCAGCACTATTACTCCCA			
qnrB	F	ATGACGCCATTACTGTATAA	681	EF634464.1	562
	R	GATCGCAATGTGTGAAGTTC			
qnrS	F	ACGACATTCGTCAACTGCAA	656	EU391634.1	417
	R	TAAATTGGCACCCTGTAGGC			
aac(6')-Ib-cr	F	TTGCGATGCTCTATGAGTGGCTA	519	Q2143161	482
	R	CTCGAATGCCTGGCGTGTTT			

the known plasmid molecular size markers of *E. coli* V517 harboring plasmids of 54.4, 7.1, 5.6, 5.2, 3.0, 2.7, and 2.1 kb, as previously described (19). MICs of ciprofloxacin were determined for the PMQR gene-positive donors, recipients, and transconjugants using the E-test.

Quality control was performed using standard strains of *E. coli* ATCC 25922, ATCC 35218, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853.

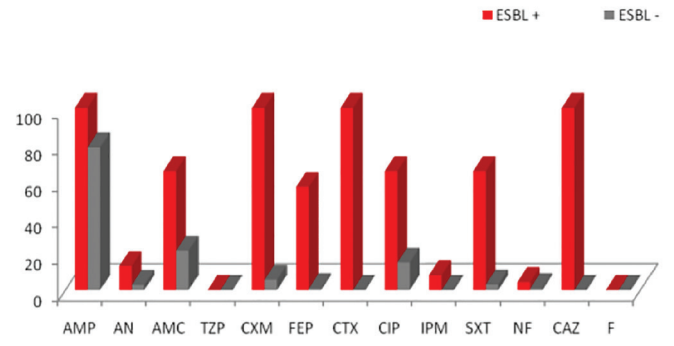

FIGURE 1. Antimicrobial resistance rates of ESBL-EC and non-ESBL-EC isolates
AMP: Ampicillin; AN: Amikacin; AMC: Amoxicillin/Clavulanic Acid; TZP: Piperacillin-Tazobactam; CXM: Cefuroxime; FEP: Cefepime; CTX: Cefotaxime; IMP: Imipenem; SXT: Trimethoprim-Sulfamethoxazole; F: Fosfomycin; NF: Nitrofurantoin; CAZ: Ceftazidime.

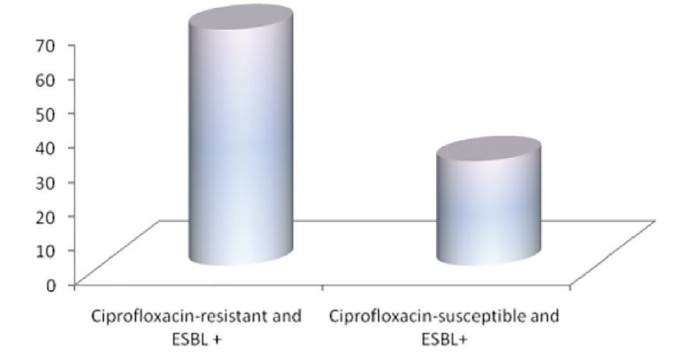

FIGURE 2. Percentage of ESBL production among CREC isolates
ESBL production was significantly more frequent among ciprofloxacin-resistant E. coli isolates than among ciprofloxacin-susceptible isolates (33/108) (p<0.0001).

Statistical Analysis

The statistical analysis of the data was carried out using Fisher's exact test. A p-value of $p<0.05$ was accepted as statistically significant.

RESULTS

A total of 300 *E. coli* isolates were obtained from urine samples of outpatient women with symptoms suggestive of a UTI. The ESBL production was detected in 36% (108/300) of isolates.

Antimicrobial Susceptibility Test

The antimicrobial resistance rates were significantly higher in ESBL-producing *E. coli* (ESBL-EC) isolates than in non-ESBL-EC isolates ($p<0.05$) (Figure 1). Thirty-five percent (105/300) were resistant to ciprofloxacin. Ciprofloxacin had MIC ranges of 0.008 to ≥32 µg/mL with MIC50 at 0.5 µg/mL and MIC90 at 1 µg/mL. Among the ESBL-EC isolates, ciprofloxacin resistance was determined at 69% (75/108). The ESBL production was significantly more frequent among ciprofloxacin-resistant *E. coli* (CREC) isolates than among ciprofloxacin-susceptible isolates (33/108) ($p<0.0001$) (Figure 2). Sixty-five percent (68/105) of CREC isolates belonged to women aged >40 years. Eight isolates were resistant to carbapenems, and the MICs of the isolates were determined ≥32 µg/mL for imipenem, meropenem, and ertapenem, and their carbapenemase activities were positive. These isolates were both resistant to ciprofloxacin and positive for ESBL production. One of the 8 isolates belonged to a 51-year-old woman, and others belonged to young women (average 25 years old).

ESBL-CREC isolates were highly resistant to ampicillin, cefuroxime, cefotaxime, ceftazidime (100%), amoxicillin/clavulanic acid,

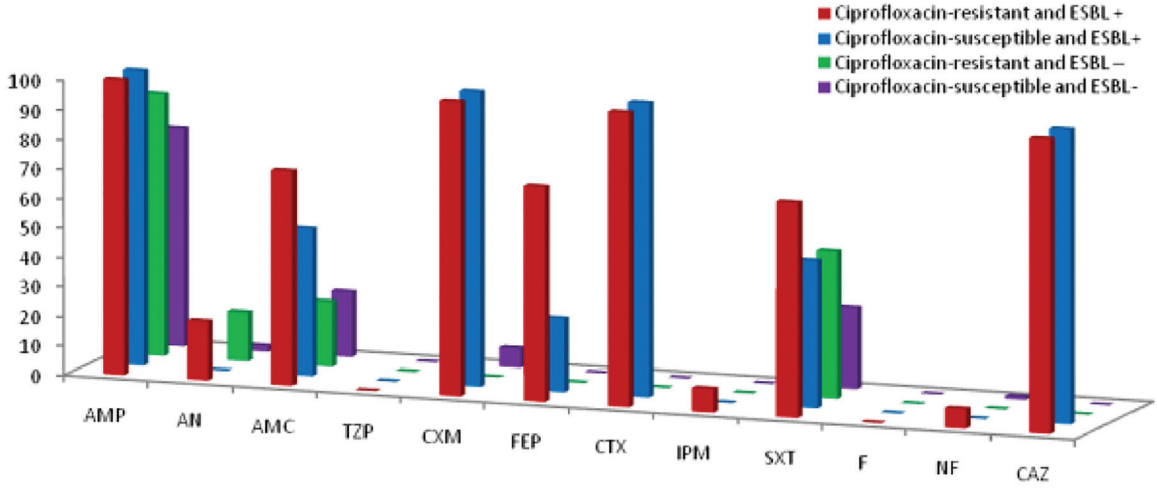

FIGURE 3. Antimicrobial resistance rates of *E. coli* isolates according to the presence or absence of ciprofloxacin resistance and ESBL production
AMP: Ampicillin; AN: Amikacin; AMC: Amoxicillin/Clavulanic Acid; TZP: Piperacillin-Tazobactam; CXM: Cefuroxime; FEP: Cefepime; CTX: Cefotaxime; IMP: Imipenem; SXT: Trimethoprim-Sulfamethoxazole; F: Fosfomycin; NF: Nitrofurantoin; CAZ: Ceftazidime.

FIGURE 4. PCR amplification products of the *aac(6')-Ib-cr* gene in CREC isolates

cefepime, and trimethoprim–sulfamethoxazole (73.3%). The resistance was lower to amikacin (20%) and nitrofurantoin (6.6%) (Figure 3). The combined resistance to third-generation cephalosporins, carbapenems, ciprofloxacin, amikacin, trimethoprim–sulfamethoxazole, and nitrofurantoin was detected in 2.6% (8/300) of urine isolates. Fosfomycin, tigecycline, and colistin resistance was not detected in any of the isolates.

Prevalence of PMQR Genes

Forty percent (42/105) of CREC isolates were positive for *aac(6')-Ib-cr* variant, of which 50% (21/42) were ESBL producers, and 28% (6/21) of these isolates were also resistant to carbapenems. None of the isolates harbored *qnrA, qnrB, and qnrS* genes.

Conjugation Experiments

Transconjugants (with plasmid sizes 54-100 kb) were successfully obtained from 6 of 42 *aac(6')-Ib-cr* gene-positive *E. coli* isolates used as donors. The *aac(6')-Ib-cr* gene was successfully transferred from 6 *aac(6')*-Ib-cr gene-positive *E. coli* urine isolates to their transconjugants. Transferability was 14% (6/42) for *aac(6')-Ib-cr. E. coli* isolates that harbored *aac(6')-Ib-cr* were resistant to ciprofloxacin (MICs 32-256 µg/mL). The MICs of ciprofloxacin for the 6 transconjugants ranged from 0.25 to 1 µg/mL, or were 31- to 125-fold higher than that for the recipient *E. coli* J53AzR (MIC 0.008µg/mL).

The PCR amplification products of *aac(6')-Ib-cr* gene in CREC isolates are shown in Figure 4.

DISCUSSION

UTIs are the most common infections in women, and over 50% of women experience UTI at least once in their lifetime. UTI can significantly affect the quality of life. *E. coli* is the most common causative agent in the UTIs of women. These bacteria can easily become resistant. Many reports have shown that the prevalence of multidrug-resistant *E. coli* isolates is increasing worldwide because of the dissemination of mobile genetic elements (21-24). A surveillance study conducted in Europe between 2004 and 2010, including Turkey, reported that the ESBL production is positive in the mean 15% of *E. coli* isolates from different samples, and Turkey has the highest percentage with 25% (23). In the present study, the ESBL production was 36% among the urine isolates of *E. coli* from outpatient women patients.

An increase in quinolone resistance among ESBL-EC isolates has been reported all over the world. In an antimicrobial resistance surveillance study report on ECDC in 2012, the average percentage of resistance to quinolone was 22%, and it was predominant in Italy and Cyprus (42%), and Slovakia (41%) (21), and in Turkey (52%) (22). In a study conducted in our hospital in 2009, the rate of ciprofloxacin resistance among ESBL-EC blood isolates was 57.6% (24). In the present study, ciprofloxacin resistance was determined as 69% (75/108) in ESBL-EC urine isolates. The ESBL production was significantly more frequent among our CREC isolates than among ciprofloxacin-susceptible isolates (p<0.0001).

PMQR genes may facilitate the spreading and increase the prevalence of quinolone-resistant isolates. The *aac(6')-Ib-cr* encodes a bifunctional aminoglycoside 6'-N-acetyltransferase capable of acetylating both aminoglycosides and fluoroquinolones (25). In the many studies conducted on *E. coli* in different countries, the frequency rates of the *qnr* gene were reported at rates 11%-75% (26-28). In studies conducted in Turkey, the most prevalent PMQR determinant was *aac(6')-Ib-cr* (at rates 46%–60%) (29-31). In the present study, we observed that the frequency of *aac(6')-Ib-cr* was 40% (42/105) in CREC isolates. Several studies demonstrated the association between *aac(6')-Ib-cr* and the ESBL (31-33). Similarly, we determined that 50% of *E. coli* isolates harboring *aac(6')-Ib-cr* were ESBL producers. The conjugation experiments demonstrated that *aac(6')-Ib-cr* was transferable. The MICs of ciprofloxacin for transconjugants harboring *aac(6')-Ib-cr* were 31- to 125- fold higher than the MIC for the recipient *E. coli* J53AzR. In conjugation experiments, we showed the possibility that the *aac(6')-Ib-cr* gene could be transferred horizontally among isolates of *E. coli,* causing uncomplicated community-acquired UTI in Turkish women.

Several reports of *E. coli* resistant to aminoglycosides are increasing worldwide (21, 22, 24). The resistance to amikacin was reported as 11% for ESBL-EC in the United States (34). In the EARSS Annual Report, the resistance to aminoglycosides in *E. coli* isolated from different samples was 35% in Turkey (22). In the current study, amikacin resistance was detected at 20% in urine isolates.

Carbapenems are also considered among the last-resort antibiotics in the treatment of serious infections caused by multidrug-resistant members of the *Enterobacteriaceae,* including *E. coli.* However, because of the global increase of carbapenem resistance, these bacteria have become a worldwide problem (21). In the present study, 8 isolates were resistant to carbapen-

ems (MICs>32 µg/mL). These isolates were both resistant to ciprofloxacin, and ESBL was positive.

The percentage of combined resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides was 4% in Europe (21). In the current study, the percentage of combined resistance, including carbapenems and nitrofurantoin among the ESBL-EC isolates in urine samples of women with symptoms suggestive of a community-acquired UTIs was 2.6%.

Tigecycline was approved by the Food and Drug Administration (FDA) in 2005, and it, like "old" antibiotics, phosphomycin and colistin, is among the remaining options in clinical use for the treatment of UTIs caused by multidrug-resistant *E. coli* isolates (35). In the last decade, colistin has been increased for the treatment of multidrug-resistant Gram-negative bacilli, especially in combination with other drugs (35).

In the 2013 report by IDSA, ESBL-EC was listed among the 6 drug-resistant microbes urgently needed in new treatments (36). These data led to reconsidering nontraditional antibiotics such as fosfomycin, a phosphonic acid derivative approved by the FDA for the treatment of uncomplicated UTIs in women. Recent reports have shown that it has *in vitro* activity against multidrug-resistant pathogens, including ESBL-CREC (37, 38). As it was also seen in the present study, fosfomycin has shown good *in vitro* activity against ESBL-CREC isolates. It may be a promising treatment option. However, clinical data regarding the use of fosfomycin in the treatment of UTIs caused by multidrug-resistant pathogens are still limited, and concerns about the widespread use of fosfomycin include tolerability, cost, and resistance (39). A recent analysis reports a fosfomycin resistance rate of 0.5% in community-acquired *E. coli* UTI in women in the United Kingdom (11). A systematic review of data, mainly from Europe and Asia, showed that 97% of ESBL-EC was susceptible to fosfomycin. Data from both *in vitro* and clinical studies are suggesting that fosfomycin should be used with caution in infections caused by ESBL-EC. In these studies, it is emphasized that the reason for the emergence of resistance to fosfomycin in ESBL-EC may be related to the increased use of this agent (37).

The ability of *E. coli* to transfer resistance genes to other bacteria causes the spread of antimicrobial resistance. This situation threatens the effectiveness of existing antibiotics. High rates of recurrent UTIs suggest that antibiotics are not an effective therapy for all UTIs, and UTIs are resulting in billions of dollars in health care costs annually.

In conclusion, our findings indicate that the rates of ciprofloxacin resistance among urine isolates of *E. coli* in women are high, that CREC isolates carry a transferable *aac(6')-Ib-cr* gene, and that they have a combined drug resistance (2.6%), including carbapenems of ESBL-EC urine isolates. These data point out that the multidrug resistance has the potential to spread among *E. coli* isolates from urine samples of outpatient women with community-acquired UTIs. We observed it had a low resistance for nitrofurantoin (6.6%). None of our multidrug-resistant *E. coli* urine isolates showed resistance to fosfomycin, tigecycline, or colistin. There is a need for accurate epidemiological data for appropriate empirical treatment in patients with both the community and the hospital hospital-acquired infections in each country. Therefore, it is crucial to apply antimicrobial resistance prevention and control strategies to reduce morbidity, mortality, and health care costs; limit the potential spread of resistance genes; and ensure careful antibiotic use in UTIs caused by *E. coli* with superbug potency.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer review: Externally peer reviewed

Author Contributions: Concept - S.Ö., O.A., A.Ö., İ.H.Ç., F.K.Ç.; Design - S.Ö., O.A., A.Ö., İ.H.Ç., F.K.Ç.; Supervision - S.Ö., O.A., A.Ö., İ.H.Ç., F.K.Ç.; Data Collection and/or Processing - S.Ö., O.A.; Analysis and/or Interpretation - S.Ö., O.A.; Literature Search - A.Ö., İ.H.Ç., F.K.Ç.; Writing Manuscript - A.Ö., İ.H.Ç., F.K.Ç.; Critical Review - S.Ö., O.A., A.Ö., İ.H.Ç., F.K.Ç.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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HPV: Obvious but Not Necessary Cause of Cervical Cancer

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BACKGROUND/AIMS
Cervical cancer is one of the most common cancers worldwide. Human papilomavirus (HPV) has been proposed to be one of the main players in the development of cervical cancer. The aim of this study was to investigate the association of HPV DNA and cervical cytology.

MATERIAL and METHODS
A total of 201 women undergoing routine gynaecological check up in Hospital were recruited. HPV genotyping and cervical Pap test were analysed.

RESULTS
Overall, 42% (85/201) of women analysed were tested positive for at least one of the HPV types tested. HPV DNA prevalence was the highest in women younger than 30 years old (59%, 50/85). Eighty four percent (71/85) of the HPV positive women had low to high grade cytological anomalies.

CONCLUSION
The presence of HPV DNA was strongly associated with the cytological anomalies, especially with specific HPV types. This study showed that detection of multiple HPV types is rather important in understanding the possible crosstalk among HPVs during the initiation and progression of cervical lesions. In conclusion, HPV still remains to be the most prevalent marker for cervical cancer and thus regular check up should be evaluated as a preventative policy for cervical cancer.

Keywords: Cervical cancer, cytological pathology, HPV

INTRODUCTION

Cervical cancer is the third most common cancer affecting almost half a million women worldwide. Human papilomavirus (HPV) poses a substantial risk for the development of cervical cancer and genital HPV infections are associated with more than 99% of all cervical cancers (1, 2). HPVs are small, non-enveloped viruses with double stranded circular DNA compromising almost 8000 nucleotide base pairs (3, 4). Up to date, over 180 different types of HPVs have been completely sequenced (5, 6) and all these HPVs are shown to infect the epithelial cells usually either the cutaneous or the mucosal surfaces. Neoplastic changes are mainly caused by HPVs with high oncogenic potential (high risk, HR-HPV) (7). Women with HPV16 (61%) and HPV18 (10%) were shown to have 200-fold higher risks for cervical cancer (3, 8). The prevalence of other HPV types are less observed in cervical cancer cases, in such HPV45 was observed in 6%, HPV31 in 4%, HPV52 in 3%, HPV35 in 2% and HPV58 in 2% of cervical cancer cases (9). HPVs, mainly HR-HPVs, have also been observed in women with intermediate grade cervical cytological abnormalities that may progress to cervical cancer, such as atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous epithelial lesions (LSIL) (2, 10, 11).

The association between the oncogenic HPV types and the subsequent development of cervical cancer has introduced the detection of HPV DNA as an alternative or supplementary screening and early detection strategy (12). Therefore, with a well-designed screening programme involving HPV genotyping and cytological analysis, unnecessary operations, such

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as colposcopies, can be prevented. Moreover, with an established policy, early detection of cervical cancer will improve the mortality and morbidity. Hence, in this study we aimed to assess the prevalence of HPV infection in relation to cytological diagnosis in women undergoing routine gynaecological check up. In this way, the association between the presence of HPV DNA and development of cervical abnormalities can be predicted and appropriate treatment can be applied in advance.

MATERIAL and METHODS

Study Population: Physical Examination and Specimen Collection

Ethical approval conforming to the provisions of the Declaration of Helsinki was granted and written consensus was obtained from each patient. Women between the ages of 19-57 years old undergoing routine gynaecological check-up in Hospital were recruited into this prospective study investigating the correlation between the prevalence of HPV DNA and the cytological findings. Each participant had a pelvic examination at enrolment and exfoliated cervical cells were obtained for cytological analysis and for HPV DNA testing. Informed consent was received from each patient.

Cytological Assessment

The cervical cells were spread on microscope slide and fixed for the cytological assessment. The samples were stained with Papanicolaou (Pap) stain (BD Sure Path liquid based Pap Test; Papnicolou's solution, Merck) within the Laboratory of Pathology, Hospital. Pap smear samples were analysed by trained cytopathologists and the cytological smears were classified following 2014 Bethesda system (13, 14). In brief, the samples were classified as; no epithelial cell anomaly, atypical squamous cell of undetermined significance (ASC-US), atypical squamous cells with high grade squamous intraepithelial lesion (ASC-H), low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL). The presence of fungal or bacterial infection as well as inflammation was also reported.

HPV DNA Testing

HPV DNA testing of all the participants was performed on cervical cell samples at the Medical Genetics and Cancer Diagnosis-Research Centre, Hospital. DNA was extracted using GeneAll® Ribo_spin vRD™ kit (Gene All, Gambio) and DNA was tested using Seeplex® HPV4A ACE Screening kit (Seeplex, Seegene) following manufacturer's instructions. This kit uses the principle of "Dual Priming Oligonucleotide (DPO) technology" maximising PCR specificity and sensitivity by fundamentally blocking non-specific priming. Internal control and positive control provided within the Seeplex® HPV4A ACE Screening kit were included for each reaction. DNA was tested for the simultaneous genotyping of the high-risk HPV types, HPV16 and HPV18, and screening of 16 additional high-risk HPV types (HRC) including HPV26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82 and low risk HPV types, HPV6/11. The presence of HPV DNA was assessed by observing the band with appropriate product size on etihidium bromide stained (2%/1xtris borate EDTA) agarose gel electrophoresis. The presence of HPV16 was detected with a band at 588bp, HPV18 at 230bp, HRC at 465bp, and HPV6/11 at 302bp with an internal control at 1000bp.

Main Points:

- Detection of multiple HPV types is rather important.
- There is a possible crosstalk among HPVs during the initiation and progression of cervical lesions.
- HPV still remains to be the most prevalent marker for cervical cancer.

Statistical Analysis

The prevalence of HPV DNA was correlated according to the women's age. Two-tailed student's t-test was used for the analysis of distribution of high-grade cytological findings among HPV positive and negative women. A $p<0.05$ was considered statistically significant. All statistical analysis was conducted using GrapPad prims v6 (California, USA.)

RESULTS

HPV DNA Detection

The study population was composed of 201 women aged between 19 and 57 years old with the median age of 30. Of these women, 45% (85/201) were tested positive for at least one of the HPV types tested. HPV DNA prevalence was the highest in women younger than 30 years old (59%, 50/85) though these values were not statistically significant compared to women older than the age of 30 years old ($p>0.05$, Table 1). Only six women had two sexual partners and two of these women were tested positive for HPV.

Overall, the presence of HPV16 was detected in 10.9% (22/201) of the women tested, HPV18 in 2.9% (6/201), HPV6/11 in 10.9% (22/201) and at least one of the HRC group of HPVs in 28.9% (58/201; Table 2).

Among HPV positive women 92% (185/201) were tested positive for one HPV type (excluding the analysis of HRC) whereas the rest of the women had multiple HPV types detected. All of these women were positive for at least one type of high-risk HPV and 65% (11/17) have multiple high-risk HPVs detected. Thirty six percent (8/22) of HPV16 positive women were also screened positive for HRC and 18% (4/22) for HPV6/11.

Cytological Diagnosis

Cytological assessments of cervical cells revealed that 14% (12/85) of the HPV positive women did not have any cytological abnormalities (Table 3). The rest of the women have at least one kind of abnormal cervical cytology ranging from epithelial cell

TABLE 1. HPV DNA positive according to age groups

	HPV DNA positive		HPV DNA negative	
Age groups	Number of women	% (Percentage)	Number of women	% (Percentage)
<30	50	59	41	35
30-40	22	26	41	35
41-55	12	14	31	26
>55	1	1	3	3
Total	85		116	

HPV: human papilomavirus

anomalies to LSIL and HSIL. Overall, abnormal cytological outcome was observed to be more common in HPV positive women (90%, 76/85, of women) compared to HPV negative women (76%, 89/116, Table 3). In HPV positive women low grade cervical abnormalities, such as LSIL (22%) were detected. Additionally, bacterial vajinozis within the vaginal flora (3%, 5/85), fungal infection morphologically similar to candida (6%, 5/85) and inflammation (23%, 20/85), were also detected in HPV positive women. Thirty six percent (32/85) of HPV positive women were diagnosed to have epithelial cell anomalies, 17% (15/85) with ASCUS, 1% (1/85) with ASC-H, 22% (1/85) with LSIL, 8% (6/85) with HSIL (Table 3).

TABLE 2. Distribution of HPV types

HPV type	Number of women	Overall percentage (%)	Percentage among HPV positive women (%)
HPV16	22	10.9	29
HPV18	6	2.9	7
HPV6/11	19	10.9	22
HRC	58	28.9	68

HPV: human papillomavirus; HRC: high-risk HPV types

Association of HPV Types with Cytological Diagnosis

Overall, 23% (5/22) of the HPV16 positive women did not present any cytological anomalies and the rest had at least one aberrant cytological diagnosis ranging from low, intermediate and high-grade cervical anomalies. The findings of cytological diagnoses are summarised in table 4. The low-grade cervical anomalies of HPV16 positive women included bacterial vajinozis, fungal infection, warts, inflammation as well as epithelial cell abnormalities. Intermediate and high-grade cervical lesions for HPV16 positive women included ASCUS (18%, 4/22), LSIL (18%, 4/22) and HSIL (9%, 2/22) (Figure 1). The number of women tested positive for HPV18 is low; however, half these women were diagnosed for ASCUS, 33% (2/6) for LSIL and 17% (1/6) for HSIL. The highest detection of HSIL (26%, 5/19) was reported in HPV6/11 positive women. Presence of warts and ASCUS were detected in 26% (5/19) of the HPV6/11 positive women (Table 4, Figure 1). For the HRC positive women, epithelial cell abnormalities were detected in 38% (22/58) followed by 24% (14/58) LSIL and 21% (12/58) ASCUS.

The risk of having LSIL was considerably increased ($p<0.05$) when the women were HPV16 positive as well as HRC positive, in such cytological diagnosis for half of the HPV16 and HRC positive women revealed that they have LSIL. Although the rate of

TABLE 3. Association of HPV with cytological diagnosis

Cytological diagnosis	Number of women (Total HPV positive: 85)	% (Percentage)	Number of women (Total HPV negative: 116)	% (Percentage)
Negative for intraepithelial lesion or malignancy	12	14	27	23
Bacterial Vaginosis	5	3	2	3
Fungal infection	5	6	2	2
Non-specific Inflammation	20	23	22	19
ASCUS	15	17	17	15
ASC-H	1	1	0	0
LSIL	19	22	5	4
HSIL	6	8	0	0

ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; LSIL: low grade squamous intraepithelial lesion; HSIL: high grade squamous intraepithelial lesion

TABLE 4. Association of HPV prevalence with cytological outcome

Cytological diagnosis	Percentage of women with HPV16	Percentage of women with HPV18	Percentage of women with HPV6/11	Percentage of women with HRC
No abnormalities	23	0	16	14
Epithelial cell abnormalities	27	2	21	38
Bacterial vajinozis	5	0	5	3
Fungal infection	5	0	5	2
Warts	14	0	26	7
Inflammation	23	33	32	3
ASCUS	18	50	26	21
ASC-H	0	0	0	2
LSIL	18	33	21	24
HSIL	9	17	26	2

HPV: human papillomavirus; HRC: high-risk HPV types; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; LSIL: low grade squamous intraepithelial lesion; HSIL: high grade squamous intraepithelial lesion

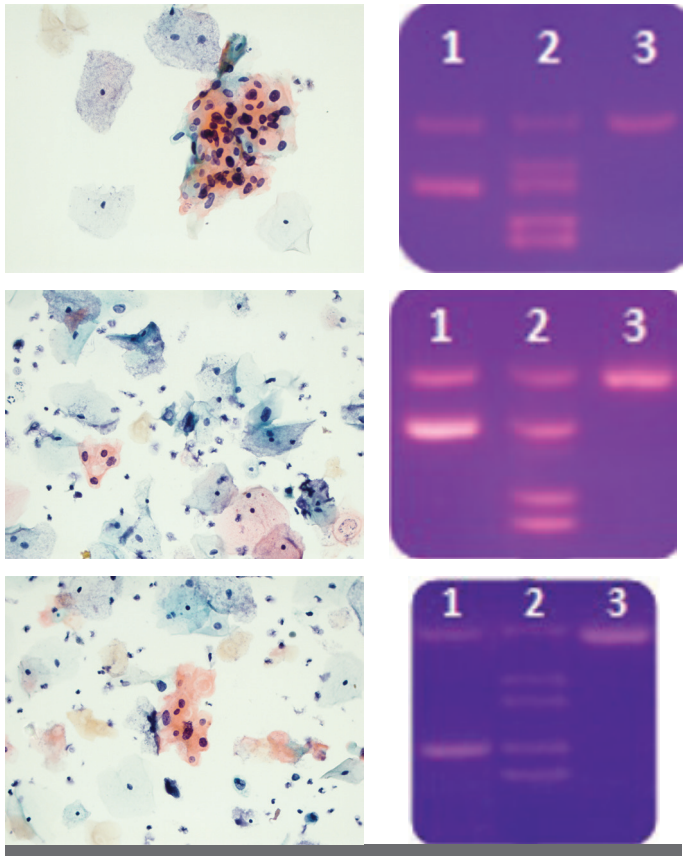

FIGURE I. Diagram showing the pathology and HPV genotyping results
Results of pathological analysis are shown in (a) and the HPV DNA genotyping is shown in (b). Lane 1 represents the patient's DNA, lane 2 represents the marker with a band at 1000bp for internal control, 588bp for HPV16, 302bp for HPV6/11 and 230bp for HPV18. Lane 3 represents the negative control with only a band at 1000bp for the internal control. a) Results of a patient diagnosed for i. ASCUS following pathological analysis and ii. HPV6/11 following HPV DNA genotyping at lane 1. b) Results of a patient diagnosed for i. LSIL following pathological analysis and ii. HPV16 following HPV DNA genotyping at lane 1. c) Results of a patient diagnosed for i. HSIL following pathological analysis and ii. HRC following HPV DNA genotyping at lane 1. Abbreviations: Aypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL).

having HSIL is supposed to be higher when women are positive for more than one HR-HPVs, this study showed that the rates were almost similar in developing HSIL for woman tested HPV16 and HRC positive.

DISCUSSION

High risk HPV types are well known to cause cervical cancer and HPV DNA is detected in approximately 99.7% of cervical cancer tissue specimens (1). Therefore, HPV genotyping is important to investigate the natural history of the infections and whether the infection of HPV is involved in the initiation and progression of cervical cancer lesions (15, 16). With the use of HPV DNA detection strategies, early detection and screening provided tremendous improvement for cervical cancer diagnosis (17). In this study, we present the type specific prevalence of HPVs providing a picture of the frequency of genital infections caused by these mucosal HPVs and estimating the risk of cervical cancer development.

HPV DNA was detected in 42% of 201 women. Worldwide, the prevalence of HPV infection among women was estimated to range between 2% and 44% (18). In general, high risk HPV types are observed at a higher rate in women younger than 30 years (19, 20) which is in agreement with this study, though there are some controversies showing increased prevalence of HPV in the older age group (21), in such high risk HPVs (HPV18 and HPV51) were associated with HSIL (22). All these variabilities among different outcomes including the present study could be due to different techniques used or population differences such as analysis of both normal and abnormal cytologies that may lower the rates of HPV prevalence.

Twelve percent (14/85) of the HPV positive women had normal cytology which is in accordance with the current literature. In confirmation for the carcinogenic role of the high-risk HPV types, cytological diagnosis of the women including low, intermediate and high-grade cervical anomalies were compared to women with normal cytology. The prevalence of epithelial cell anomalies, LSIL and HSIL were shown to be statistically significant in HR-HPV positive women compared to HPV negative women ($p<0.05$). The prevalence of the HRC types followed by HPV16 was higher in women with epithelial cell anomalies. The prevalence of HPV16 and HPV18 was also relatively high in women diagnosed for LSIL and ASCUS as also reported previously (23). The prevalence of different HPV type differs depending on the ethnic group, such as in Botswana women HPV was associated most with HSIL (24). Similar to our findings, HPV positive women were shown to have increased cytological anomalies of LSIL (50%), HSIL (100%) and invasive cervical cancer (11, 19). In this study, HSIL in addition to genital warts and inflammation were mostly detected in HPV6/11 positive women. HPV6 and HPV11 have been mostly associated with genital warts and condyloma acuminatum, whereas HPV16 and HPV18 to be the main cause of cervical cancer development (25-27).

Among HPV positive women, 20% to 40% have been reported to harbor at least two HPV types (8, 12, 28-30). The idea of the presence of one HPV type increasing the likelihood of attaining another HPV type is still controversial. Although it is suspected that presence of multiple HPV types increases the risk of the persistency of HPV and cervical lesions, it remains to be one of the debates due to the unknown biological activity of these HPVs and the viral load (31, 32). In this study, 8% (17/201) of the women were infected with multiple HPV types and in this study, women tested positive for more than one HPV type did not have an increased rate of having cytological anomalies. Since coinfections are shown to be more frequent among women with cytological abnormalities or impaired immune response (33-35), development of cytological anomalies could be due to the patient history profile as well.

One of the other factors affecting the prevalence of genital HPV infection is the number of sexual partners. In this study only 2 of 6 women with two sexual partners were tested positive for HPV; hence having more than one partner did not seem to have the main reason of HPV infection. Although the main factors increasing the HPV infections accounts for multiple sexual partners, other factors increases the rate of HPV infection including the presence of other sexually transmitted infections, smoking, immunosupression, use of oral contraceptives, hormonal thera-

py, age of the first sexual intercourse and sexual behaviours of the male partner (36-42).

One of the main limitations of this study involves the cross-sectional design and the lack of analysis of temporal relationship between the HPV detection and the cytological outcome that may have developed at a later stage. Therefore, prospective studies play a crucial role in understanding the association of the presence of HPV DNA, the persistency of the infection and the risk of cervical lesion formation. The types of HPVs analysed in this study is also limited since genotyping is performed for the high-risk HPV types HPV16 and HPV18 and screening is performed for the other high risk HPV types including HPV26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82 and low risk HPV types HPV6 and HPV11.

In conclusion, detection of multiple HPV types is rather important in understanding the possible crosstalk among HPVs during the initiation and progression of cervical lesions. Our study provides information on the association and distribution of different HPV types with cytological assessment enabling better outcome measures and early detection and screening for cervical cancer. Overall, this study highlights the importance of identifying the prevalence of HPV types that may define the causality of individual HPV types with cervical cancer and form the basis for secondary prevention measures for cervical cancer and the need of longitudinal studies on the natural history of HPV. In the light of the literature and as it is seen with our results, HPV still remains to be the most prevalent marker for cervical cancer and thus regular check up should be evaluated as a preventative policy for cervical cancer.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Original Article

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In-vivo and In-Vitro Examination of the Effect of *Lucilia Sericata* Larvae and Secretions on the Bacteria in Open Wounds

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BACKGROUND/AIMS
Maggot Therapy is an old method used to contribute to the debridement, disinfection and healing of chronic wounds. In this study, we examined the antimicrobial effect of *Lucilia sericata* larvae and secretion on the bacteria in open wounds both in *in-vivo* and *in-vitro* manner.

MATERIAL and METHODS
Samples were taken from 25 wounds belonging to 23 patients and were tested with bacteria cultures made to observe the bacterial variety before and after the Maggot Debridement Therapy (MDT). In addition, in in-vitro conditions the *Lucilia sericata* larvae secretion was examined against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Pseudomonas aeuroginosa* bacteria.

RESULTS
In the in-vivo section of our study in which we compared the bacterial variety before and after applying *L. sericata* larvae, we observed that there were reductions in bacterial load on the infected wounds especially the gram-positive bacteria. The data in the in-vitro section of our study in which we used solid and liquid broth media showed that the anti-bacterial effect changed according to the characteristics of the broth medium.

CONCLUSION
Maggot Therapy may be used in an efficient way in eliminating the pathogen bacteria in infected wounds with the help of its antibacterial activity.

Keywords: Antibacterial activity, larval therapy, *Lucilia sericata*, Maggot therapy, Methicillin-resistant, *Staphylococcus aureus*

INTRODUCTION

Maggot Therapy (sometimes called larval therapy) is the application of the live fly larvae to the wounds of the patient to help the debridement, disinfection, and eventually, to the healing. As a matter of fact, this method is a therapeutic wound myiasis whose reliability and efficiency is controlled in clinical conditions (1).

The *Lucilia sericata* larvae, which are used commonly in the treatment of open wounds, produce plenty of proteolytic enzymes, substances with antibacterial characteristics and different substances that ensure the granulation of the tissue (2, 3). Many clinical reports provide us with the proof on the important effects of the larvae therapy used in the debridement, cleaning, and eliminating infection in many wounds that do not heal with traditional treatment methods (4-6).

The beneficial effects of using larvae in treating open wounds were first mentioned in 1557 (4). The larvae therapy which lately named as Maggot Debridement Therapy (MDT), was used in treating open wounds in 1930 for the first time and became more popular in time. It was used extensively until 1940 in the treatment of chronic and infected wounds. In 1940, the use of it decreased with the discovery of antibiotics and due to some difficulties in using it; and was ignored by the medical community at a great deal. However, the interest in MDT increased again because of the antibiotics being inad-

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equate in the treatment of infected chronic wounds because of the increasing antibiotic resistance incidence as of late 1990s (7).

Studies conducted in recent years showed that the secretions of the larvae contain at least two substances that have antibacterial characteristics. One of these substances is a hydrophobic peptide-like substance whose molecular weight is 3-10 kDa, and the other one is a hydrophilic substance of <1 kDa. These substances shown to eliminate the infection by killing and stopping the growth of the microorganisms those cause infection in wounds (2).

The antibacterial efficiency of larvae secretions was investigated by many authors in in-vitro conditions, and their strong efficiency against many pathogenic bacteria was revealed (2,3). In addition to this, it was seen that the studies conducted on the effects of the MDT for various microorganisms that infect chronic wounds were insufficient.

Based on these findings, the materials from 25 wounds of 23 patients were evaluated to observe the bacterial variety before and after the MDT. In addition, the effect of the MDT against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Pseudomonas aeuroginosa* bacteria was also investigated in in-vitro conditions.

MATERIAL AND METHODS

The study was approved by the ethics committee of Istanbul University Cerrahpaşa School of Medicine (34256/2013). Signed consent forms were obtained from all patients.

Preparing the Sterile *L. sericata* Larvae

A piece of liver was placed on the fly cages in which there were adult *L. sericata* colonies. After 3-4 hours, the liver was taken from the cage and the eggs on liver were collected. The eggs were separated and sterilized; and were then transferred to sterile liver agar. The agars containing eggs were incubated for 36-40 hours at 25-30°C. Within this time period, the larvae, which evolved to the 3rd instar from the 2nd instar, were taken into sterile containers to be used (2, 7).

Obtaining Larvae Secretion

The 4000 pcs of II.-III. instar sterile larvae produced in the laboratory were taken into a sterile 1 Lt beaker and 2 mL distilled water was added for 4 times in total with 1-hour intervals. Five hours later the last distilled water was added, the accumulated larvae secretion was taken and filtered through 0.45 µm injector filters to purify the possible bacteria contamination.

The in-vitro antibacterial effect of the larvae secretions

The antibacterial efficiency of sterile larvae secretion was investigated on two *S. aureus* origins that are resistant and sensitive to methicillin and one *P. aeuroginosa* bacteria. For bacterial cultures, enrichment was performed overnight at 37°C in 5 mL Tryptic Soy Broth (TSB). From each bacteria dilution that was prepared as having 10^2 and 10^4 cultural density, 0.1 mL was taken and added to the tubes that had 2 mL TSB and 2 mL larvae secretion. For positive growth control, 2 mL %0.9 NaCl and 0.1 mL bacteria dilution were added instead of larvae secretion. For negative control, 4 mL 0.9% NaCl and 0.1 mL bacteria dilution were added.

The samples that were prepared were incubated overnight at 37°C and 0.1 mL was spread to chocolate agar for the purpose of counting colonies.

The Selection of the Patients

A total of 23 patients who were sent to our unit from various hospitals and clinics with MDT demand (7 females, 16 males; mean age 55.7 years; range 29 to 77 years) were treated with larvae therapy. The clinical characteristics of the patients are given in Table 1.

Applying Sterile Larvae to the Wounds

In the I. instar, the larvae of the *L. sericata* fly was applied to the wounds of the patients in our study group. In superficial wounds, the larvae were applied to 1 cm^2 area to contain 8-10 larvae; and in deep wounds, more larvae were placed on the wound area directly. The larvae were covered with sterile sponge and it was recommended to the patient to change it frequently to enable necrotic drainage. After the larvae were kept on the wound for 48-72 hours, they were removed.

Definition of the Bacteria in the Samples Taken from the Wound Tissues

Before and after each MDT application, swab samples were taken from the open wounds of the patients; and were evaluated in bacteriological terms. In addition, the antibiotic sensitivity

Main Points:

- Maggot debridement therapy (MDT) has been shown to be an effective method for cleaning chronic wounds and granulation formation.
- *Lucilia sericata* larvae and their secretions may be used in an efficient way in eliminating the pathogen bacteria in infected wounds.
- *Lucilia sericata* larvae and their secretions also has the advantage of eliminating the active bacteria in wounds with their antibacterial effects against increasing resistance.
- It is important to encourage the widespread use of the *Lucilia sericata* larvae, which may play an active role in healing problematic wounds.

TABLE 1. The clinical characteristics of the patients

	Number	(%)
Underlying disease		
Diabetes	16	(69.56)
Venous stasis	1	(4.35)
Buerger	1	(4.35)
Vulva cancer	1	(4.35)
No disease	3	(13.04)
Osteomyelitis + diabetes	1	(4.35)
Wound area		
Feet	22	(88)
Other (perineum, abdomen, armpit)	3	(12)

tests of the isolated origins were investigated in line with the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

Statistical Analysis

The statistical analysis of the results of the study was made with the IBM Statistical Package for the Social Sciences 21.0 version (IBM Corp.; Armong, NY, ABD) and with Chi-Square tests. The frequency, percentage, average and median values were computed for definitive statistics.

RESULTS

In the in-vitro section of our study, the effect of larvae secretion on MRSA, MSSA and *P. aeuroginosa* bacteria was tested in Mueller Hinton agar and positive results were not obtained. When the same trial was performed in tryptic soy broth, it was determined that the colony numbers of MRSA, MSSA and *P. aeuroginosa* decreased at a rate of 50%.

In in-vivo section of our study, when we consider the bacteriological examination of the samples taken from all the wounds before the sterile larvae application, among the isolated bacteria we detected that there were seven different Gram-negative bacteria origins and four different Gram-positive bacteria origins (Table 2).

In our study, after the MDT, no bacteria reproduced in two wounds with MRSA, in four wounds with MSSA and in three wounds with *Enterococcus* spp. It was observed that the number of colonies decreased by 75% in six wounds with MSSA, the number of colonies increased by 50% in one wound and remained unchanged in one wound. In 10 of the wounds in which *Proteus mirabilis* and *P. aeruginosa* reproduced, and in two of the wounds in which *Escherichia coli* and *Klebsiella* spp. reproduced, no additional bacteria reproduction was observed. In four of the wounds in which *P. mirabilis, P. aeruginosa*, *Enterobacter cloaceae* and *Serratia marcescens* reproduced, the number of colonies decreased at a rate of 50%; and in the wounds in which *E. coli* reproduced, the number of colonies decreased at a rate of 25%. In two of the wounds in which *Klebsiella* spp. reproduced, the number of colonies did not change.

TABLE 2. The bacteria isolated form the wounds

Bacteria	Number	(%)
Acinetobacter spp.	1	(2.17)
Enterobacter cloaceae	1	(2.17)
Enterococcus spp.	3	(6.52)
Escherichia coli	3	(6.52)
Klebsiella spp.	3	(6.52)
Coagulase-negative staphylococci	7	(15.21)
Proteus mirabilis	6	(13.04)
Pseudomonas aeruginosa	6	(13.04)
Staphylococcus aureus	14	(30.43)
Serratia marcescens	1	(2.17)
Streptococcus agalactiae	1	(2.17)

DISCUSSION

Larva therapy is used in treating the wounds that are not healed for long years. Larvae ensure that the necrotic tissue is debrided through biochemical and mechanical ways, the inflammation is decreased, and granulation tissue is stimulated (8, 9). In addition to these, many compounds that have antibacterial effects are secreted as well as this complex interaction. Although studies have been conducted to determine what these compounds are, the exact mechanism has not been fully uncovered yet (10, 11).

The use of larvae is gaining importance due to the difficulties in managing chronic wounds infected because of the increasing antibiotic resistance incidence in our present day.

It was determined that *L. sericata* larvae killed the bacteria that have pathogenic properties especially like *S. aureus* and Group A and B streptococci or inhibit their growth in *in-vitro* conditions, and also had some effects against *Pseudomonas* spp.; however, they did not have any effects against *E. coli* and *Proteus* spp. (12). However, Jaklic et al., reported that larvae had very little effects against *Proteus* spp. (13).

Daeschlein et al. (14), used a method to determine the bactericidal effects of *L. sericata* larvae secretions in in-vitro conditions, and reported that larvae secretions had all of the properties of an antiseptic. Bexfield et al. (10), conducted a study and proved that *L. sericata* larvae had antibacterial efficiencies against MRSA in in-vitro conditions. In addition, they also showed that the larvae secretions were influential on some bacteria like *Streptococcus pyogenes, Enterococcus faecalis, Clostridium welchii, P. vulgaris, Streptococcus pneumoniae* and *E. coli* in in-vitro conditions.

Kerridge et al. (3) investigated the antibacterial effects of larvae in their in-vitro study and observed that the reproduction of MRSA and *Streptococcus pyogenes* bacteria was inhibited, and there was a limited effect against *P. aeruginosa*. In addition, they also determined that the antibacterial efficiency of the larvae would change depending on the broth medium used in the trial being solid or liquid.

In the in-vitro section of our study, the effects of larvae secretions were tested on MRSA, MSSA and *P. aeruginosa* in Mueller Hinton agar; however, no positive results were obtained. The same trial was tested with Tryptic Soy broth and it was determined that the number of the colonies of these bacteria reduced at a rate of 50%. The data we obtained in the in-vitro section of our study by using solid and liquid broth medium show that the antibacterial effect varied according to the properties of the broth medium. These findings of our study are consistent with the results of previous studies.

Jaklic et al. (13) investigated the bacterial variety in-vivo conditions as before and after the MDT in 30 patients. According to this study, bactericidal effects were observed against Group C streptococci, Group G streptococci, *Bacteroides fragilis, Citrobacter freundii, Klebsiella* spp., *Peptococcus* spp., *Prevotella bivia, Serratia marcescens* and *Streptococcus agalactiae*, and these bacteria did not reproduce when the treatment was ended. It was also determined that the colony numbers of the coagula negative streptococci, *Citrobacter koseri, Klebsiella oxytoca*, *P. aeruginosa* and *S. aureus* decreased at a serious level;

however, *Enterococcus faecalis, Morganella* spp., *Peptostreptococcus assacharolyticus, Porphyromonas* spp. and *Providencia rettgeri* increased in terms of the colony count when compared with the pre-treatment period.

In our study, after the MDT, no bacteria reproduced in two wounds with MRSA: in four wounds with MSSA and in three wounds with *Enterococcus* spp. It was observed that the number of the colonies decreased at a rate of 75% in six wounds with MSSA; increased in 1 wound at a rate of 50%; and remained the same in one wound. In our study, no bacteria reproduced in 10 of the wounds in which *P. mirabilis* and *P. aeruginosa* reproduced before and in two of the wounds in which *E. coli* and *Klebsiella* spp. reproduced. In four of the wounds in which *P. mirabilis, P. aeruginosa, E. cloaceae* and *S. marcescens* reproduced, the number of the colonies decreased at a rate of 50%; and in two of the wounds in which *E. coli* reproduced, the number of the colonies decreased at a rate of 25%. The number of the colonies did not change in two of the wounds in which *Klebsiella* spp. reproduced.

We may conclude that when the variety in bacterial pathogens in infected wounds is considered, the ever-increasing antibiotic resistance will rank the first among the factors that might affect the wound management. As a result, we believe that the MDT performed with the *L. sericata* larvae will be extremely useful in eliminating the active bacteria in wounds with their antibacterial effects against this increasing resistance.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul University Cerrahpaşa School of Medicine Ethics Committee of Clinical Research (34256/2013).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Effects of Prolotherapy with Organic Silicon in Temporomandibular Joint in Rabbits

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BACKGROUND/AIMS
Temporomandibular joint (TMJ) hypermobility is characterized by condyle hypertranslation, which moves anteriorly to the articular eminence as the mouth opens. This study investigated the efficacy of organic silicon prolotherapy for treating TMJ hypermobility.

MATERIAL and METHODS
The study sample consisted of six young New Zealand White rabbits. One of the rabbit's TMJ's was injected with organic silicon while the other joint was injected with isotonic saline. Isotonic saline (1.5 mL) was bilaterally administered into the upper joint space (1 mL) and pericapsular tissue (0.5 mL). The rabbits were sacrificed using high-dose anesthetics one month after the follow-up. The sample sections were stained with Mallory-Azan dye in order to identify collagen fibers. The samples were analyzed in terms of fibrosis and tissue reactions. Statistical analysis was performed using the Mann-Whitney U test.

RESULTS
There was a significant difference in the severity of fibrosis amongst the study groups (p=0.00512). It was noted that more collagen fibers and adipose tissue were produced from organic silicon than saline in the retrodiscal ligament.

CONCLUSION
The study concluded that organic silicon could be used as an alternative to dextrose injection for prolotherapy. Future studies and clinical trials are necessary to gain further insight.

Keywords: Organic silicon, prolotherapy, TMJ hypermobility

INTRODUCTION

Temporomandibular joint (TMJ) hypermobility is defined as the hypertranslation of the condyle. The condylar head moves anteriorly to the articular eminence as the mouth opens (1). The terms ''subluxation,'' ''luxation,''' and "dislocation" have been used to describe this phenomenon (2). TMJ dislocation can be an acute or a recurrent problem. It occurs when the condyle moves outside the glenoid fossa, locks anteriorly to the articular eminence, and cannot be self-reduced (3, 4).

The cause of TMJ hypermobility is linked to the morphology of the mandibular condyle–glenoid fossa–articular eminence and generalized joint laxity (5). Some actions that contribute to TMJ hypermobility include yawning, trauma, wide biting, intubation with general anesthesia, connective tissue disorders, tooth extraction, occlusal discrepancies, and lost vertical dimension which increases capsule weakness and ligament laxity (6).

TMJ hypermobility can be treated using surgical or conservative techniques. Surgical procedures may include condylectomy, lateral pterygoid myotomy, capsular plication, and augmentation or reduction of the articular eminence (1, 7-9). Other conservative treatment approaches include physiotherapy, prolotherapy occlusal splints, intermaxillary fixation, intramuscular injection of botulinum toxin or sclerosing solutions, and intra-articular autologous blood injections (10, 11).

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Prolotherapy, or "proliferation treatment," is also known as "regenerative injection therapy" and "growth factor stimulation injection therapy." Since 1937, it has been used to strengthen and repair chronic ligaments, capsules, joints, and tendinous injuries through the stimulation of collagen proliferation at the fibro-osseous junctions, which promotes the repair of soft tissues and relieves pain (12). Prolotherapy primarily stimulates a small inflammatory response that promotes healing and/or viable scar tissue formation which leads to stronger fibrous tissue at the TMJ capsule and ligaments. Consequently, it inhibits the condyle from locking in an abnormal position in front of the articular eminence (13).

Prolotherapy involves the use of various agents such as pumice flour, phenol, sodium morrhuate (14), combinations of dextrose–glycerin–phenol, psyllium seed oil (15), and various concentrations of dextrose (16).

Organic silicon is one of the most widely used drugs in mesotherapy. It is a structural component of connective tissue and is an important part of both elastin and collagen. It controls the increase in fibroblast numbers and promotes the regeneration of elastin and collagen fibers. Silicon also stimulates and regulates the mitosis of fibroblasts. The mechanism of action that is used in organic silicon is similar to that of prolotherapy (17).

The most common substance used in prolotherapy is dextrose. However, Kılıç et al. showed that dextrose has had no superiority and does not contribute to the treatment of hypermobility when compared with isotonic saline (18). Based on this finding, it is believed that prolotherapy can be performed with a novel material that has not been previously explored. Therefore, proliferation was tested in this study with a component containing organic silicon.

MATERIAL and METHODS

Gazi University, Animal Experiments Local Ethics Committee (G.Ü.ET-16.004/04.02.2016) The bilateral TMJs of six male New Zealand White rabbits (approximately 1-year-old, weighing 2.5–3.0 kg) were assessed. The maximum number of animals that the ethics committee allowed was used in this study to achieve the minimum number of animals needed to generate statistically significant data. The rabbits' health was observed for seven days before the study. The rabbits were housed in standard-sized individual cages with consistent humidity and temperature (approximately 60% and 22 °C, respectively) under a 12-h light/dark cycle. They were fed a standard laboratory diet with water ad libitum.

Main Points:

- Prolotherapy is one of the conservative treatments currently being used in hypermobility of temporomandibular joint.
- In general, dextrose is used in prolotherapy. However, there is currently no standard set for the concentration of dextrose. This uncertainty propels researchers to research different materials that can be used in prolotherapy.
- Organic silicon may be one such material that can achieve the necessary histological results for prolotherapy.

Each rabbit was given both injection groups. One side of the TMJs received an organic silicon (Conjonctyl, sodium monomethyl trisilanol orthohydroxybenzoate, 5 mL, Sedifa Laboratory, France) injection (six TMJs). The contralateral sides of TMJs were injected with an isotonic saline (six TMJs). The rabbits were injected intramuscularly with ketamine hydrochloride (35 mg/kg) and xylazine (5 mg/kg) as anesthesia. First, the preauricular region was shaved and the injection site (as specified by Artuziet et. al (19) was disinfected using povidone–iodine. Then, a 28-gauge needle was used to inject organic silicon into the upper space of the TMJ (1 mL) and the pericapsular tissues (0.5 mL). Similarly, the control group was injected with isotonic saline: 1 mL into the upper space of the TMJ and 0.5 mL into the pericapsular tissues.

Orthodontic brackets were used for one day on the mandibles to prevent the elongation of newly forming fibrous tissues. No postoperative medication was used. The rabbits were sacrificed one month after the experimental period. Ketamine hydrochloride (45 mg/kg) and xylazine (5 mg/kg) were intramuscularly injected to maintain deep anesthesia. Afterwards, 20 mg xylazine was injected into the pinna vein to sacrifice the rabbits while they were anesthetized. The TMJ regions were then dissected and fixed in a 10% buffered formalin solution for 72 h.

The samples were decalcified with an 8% hydrochloric acid and 8% formic acid solution. The paraffin blocks were sliced to a thickness of 6 µm using a Leica RM 2125RT. The TMJ sections were stained with Mallory–Azan dye to identify collagen fibers for evaluation of fibrosis and tissue reactions in the samples. Routine light microscopy techniques were used.

The guidelines established by Sairyo et al. (20) were used to assess fibrosis severity in retrodiscal ligaments and the lateral capsular ligaments (Grade 0: normal tissue showing no fibrotic regions, Grade I: <25% fibrosis of the entire area, Grade 2: 25%–50% fibrosis, Grade 3: 50%-75% fibrosis, and Grade 4: >75% fibrosis).

The statistical calculations were performed using Statistical Package for the Social Sciences18 software (SPSS Inc., Chicago, IL, USA). The Mann-Whitney U-test was used to estimate the differences in fibrosis severity among the study groups. Statistical significance was accepted as $p<0.05$.

RESULTS

The rabbits in the study underwent the procedure with no complications. Two independent observers, who were unaware of the treatment groups, performed the histopathological examination.

When the articular fibrous disk between the temporal and mandibular bones and the subsequent retrodiscal ligament were examined, it was noted that there was an increase in inflammation of the organic silicon group, especially in the region where the ligament was attached to the posterior part of the temporal bone. It was also observed that there was proliferation of chondrocytes, increased amount of chondral tissue, and cell activation in the area where the temporal bone adhered to the ligament and also on the surface facing the disk. Inflammatory and adipocyte cells, as well as collagen fibers, were also commonly observed in the fibrous articular disk (Figure 1). Thin bundles of collagen fibers, adipocytes, and fibrin accumulation were observed in collateral ligaments in the isotonic saline group. Inflammation was not detected (Figure 1).

TABLE I. Severity of fibrosis in areas of the temporomandibular joint injected with organic silicon and isotonic saline

Treatment Groups	n	Grade 0	Grade I	Grade 2	Grade 3	Grade 4	Mann Whitney U test p
Saline-injected	6	6	0	0	0	0	0.00512
Organic silicon-injected	6	0	2	3	1	0	

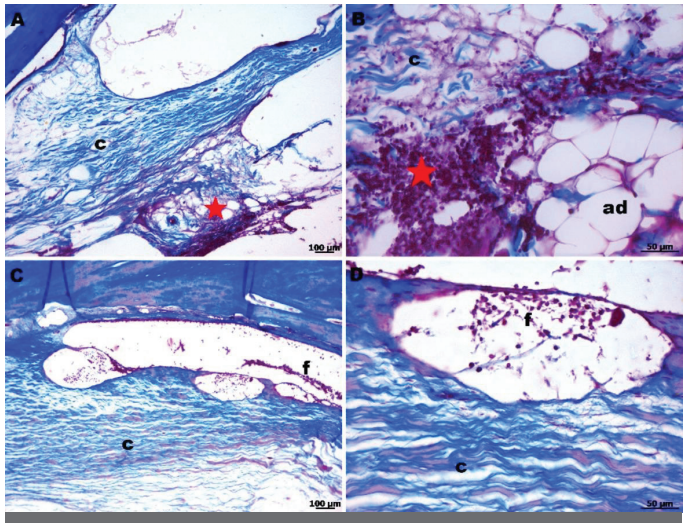

FIGURE I. Histological aspects of groups. (A and B) Organic silicon group. (C and D) Isotonic saline group. ad, Adipocyte; f, fibrin; red star, area of inflammation; c, connective tissue (Mallory–Azan staining: A–C, original magnification × 10; B-D, original magnification × 40).

Fibrosis severity was significantly different amongst the study groups (p=0.00512). Within the organic silicon group, Grade I fibrotic sites were seen in two TMJs (33.3%), grade 2 in three TMJs (50%), and grade 3 in one TMJ (16.7%). Grade 0 fibrotic areas were observed in all of the joints injected with isotonic saline (Table I).

DISCUSSION

This study investigated the efficacy of organic silicon prolotherapy for treating TMJ hypermobility. It was hypothesized that organic silicon prolotherapy would produce superior results to the saline control.

TMJ hypermobility can be treated using both surgical and conservative techniques. Considering the neurological complications and difficulties associated with inappropriate applications of surgical techniques (21), both doctors and patients tend to prefer conservative treatment methods for treating TMJ hypermobility.

Dextrose is commonly used in prolotherapy as it is readily available, inexpensive, and safe to use. There are currently various dextrose concentrations (10%, 12.5%, 15%, 25%, and 50%) that are used in the treatment of TMJ hypermobility (6, 12, 13, 22). However, the number of sessions and amounts injected may vary. This may be confusing for clinicians because differing reasons for its application are related to varying concentrations of dextrose but it has shown no superiority to saline as cited by some studies (18, 23).

Refai (24) stated that 10% dextrose prolotherapy was sufficient for significant recovery of symptoms related to TMJ hypermobility. Tomographic views have shown no morphological effect on the bony components of the joint or the condylar position. However, Ungor et al. (13) mentioned that the maximum mouth opening decreased with 10% dextrose prolotherapy, but the decrease was not statistically significant.

A 12-week follow-up study by Refai et al. (6) reported that 10% dextrose can effectively decrease the maximum mouth opening more than in the placebo group. In the study by Kilic et al. (18), 30% dextrose was not superior to isotonic saline in treating TMJ hypermobility. Both treatments resulted in similarly significant improvements in all visual analog scale measures. However, this study did not find an increase in fibrotic area for TMJs injected with isotonic saline.

Rawand et al. (23) reported that there was no significant difference between different concentrations (10%, 20%, and 30%) of dextrose during TMJ prolotherapy. All concentrations were effective in improving clinical symptoms related to TMJ hypermobility. It was specified that 10% dextrose may be just as adequate in treating TMJ hypermobility as higher concentrations. In one instance, although the patient recovered clinically in the early period following administration of injectable agents, it was observed that the disease recurred in the long term. It is a known fact that examining the histological changes of the agents used produces more objective results than examining the clinical improvements only (25).

Conjoctyl (monomethyl silanetriol salicylate, organic silicon) is an antioxidant used in mesotherapy. Silicon is a component of the structure of elastic connective tissue. It serves as a coenzyme for macromolecular synthesis in the interstitial matrix and increases collagen production (17, 26). Since the mechanism of action of the drug and the mechanism of prolotherapy treatment are generally the same, it is thought that conjoctyl may be a possible new alternative to prolotherapy with dextrose. The present study highlights that the number of inflammatory cells and collagen fibers increased in histological images. Furthermore, a clear and significant increase in the fibrotic area was observed. This reflects the accuracy of the hypothesis proposed in the beginning of this study.

In a study by Sangho et al. (27), changes in the collagen structure in rabbits were investigated in the 1st, 2nd, 4th, 8th, and 12th weeks after prolotherapy. Changes in the collagen structure were evident in the fourth week following dextrose prolotherapy. A similar 4-week evaluation was performed in the present study. Multiple sessions of dextrose injection were used in the clinical applications of prolotherapy. It was shown that more fibrotic area can be obtained with multiple sessions of organic silicon injections. Furthermore, it can be safely applied to TMJ prolotherapy in a clinical manner because it is already practiced in many areas of medicine such as in dermatology and ophthalmology (17, 28).

There were a few limitations present in the study. First, it is not ethical to restrict jaw movements for a long period of time in animal studies. Jaw movement was restricted in the rabbits for one day in this study. Second, the sample size was limited as we were restricted in the number of animals allowed by the ethics committees.

In conclusion, dextrose is used at different concentrations in prolotherapy. Many studies emphasized the superiority of using dextrose for prolotherapy, and each dose was shown to be successful in treating TMJ hypermobility. However, some other studies reported no difference between using dextrose or saline for prolotherapy. This study suggests that organic silicon may limit hypermobility of the TMJ. Further studies and clinical trials are needed to shed insight on this matter.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gazi University, Ethical Committee for Experimental Research on Animals (G.Ü.ET-16.004 / 04.02.2016)

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Original Article

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Evaluation of the Epidemiology, Prognostic Factors and Results of the Patients Hospitalized in the Intensive Care Unit Due to Thoracic Trauma

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BACKGROUND/AIMS
We aimed to evaluate patients with thoracic trauma who were followed in intensive care unit in terms of epidemiologic, admission scoring systems, follow-up processes and prognosis.

MATERIAL and METHODS
Patients with thoracic trauma over 18 years of age who were followed up and treated in the intensive care unit between January 1, 2013 and June 1, 2018 were evaluated retrospectively. Patients were evaluated in terms of age, gender, history of trauma, Glasgow Coma Score (GCS), Acute Physiology and Chronic Health Evaluation (APACHE) II score, blood gas values, intubation requirement, mechanical ventilator requirement, length of stay, survival and factors affecting prognosis.

RESULTS
Of the 30 patients included in the study, 28 (93.3%) were male and 2 (6.7%) were female. Twenty-eight (60%) patients had blunt thorax trauma and 12 (40%) had penetrating thorax trauma. Intubation was required in 20 patients and blood transfusion was required in 10 patients. The median follow-up of the trauma patients in the intensive care unit was 120 hours and the median duration of stay in the mechanical ventilator was 84 hours. A statistically significant correlation was found between APACHE II scores and duration of mechanical ventilator stay (p=0.024) and pH and intensive care unit stay (p=0.013). When the patients were evaluated in terms of survival, there was a statistically significant relationship between GCS and APACHE II scores and survival (p=0.001).

CONCLUSION
GCS and APACHE II scoring systems play an important role in predicting mortality in patients admitted to intensive care unit due to trauma.

Keywords: Intensive care unit, mortality, thorax, trauma

INTRODUCTION

Trauma stands out as one of the most common causes of deaths, especially among the young population, and it accounts for about 10% of all deaths in the world. However, it also leads to a high cost with the intensive care treatment requirements and morbidity it creates (1-4). Thoracic traumas can be in isolated forms; however, in patients with multi-traumas, they often accompany traumas of other systems, and approximately 25% of trauma-related deaths occur due to thoracic trauma (5, 6).

Various scoring methods are used to evaluate the general condition of patients followed up in intensive care units and to predict trauma-related prognosis. These scoring systems can be grouped in 2 groups as prognostic scoring systems and scoring systems for evaluating morbidity. Scoring systems for evaluating the prognosis are performed at the time of hospitalization of the patient or by evaluating the patient's data within the first 24 hours, and the aim is to determine the risk of mortality that may occur in the patient. Among these, Acute Physiology and Chronic Health Evaluation 2 (APACHE II)

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and Glasgow Coma Score (GCS) are the two most used scoring methods (2, 7).

In our study, we aimed to investigate the epidemiology of trauma, GCS and APACHE II scores of patients, intensive care follow-up processes, and the relationship between mortality and morbidity in patients with thoracic trauma hospitalized in the intensive care unit due to trauma.

MATERIAL AND METHODS

In the study, the records of patients who received inpatient treatment in Intensive Care Unit between January 1, 2013 and June 1, 2018 were evaluated retrospectively. Patients over 18 years old and with thoracic trauma were included in the study. The patients included in the study were evaluated in terms of age, gender, history of trauma, GCS, APACHE II score, blood gas values, intubation need, mechanical ventilator need, duration of hospitalization, survival, and factors affecting prognosis.

The normal distribution assumption of the data obtained was checked with the Kolmogorov-Smirnov test. The mean standard deviation values of the parametric variables and the median, 25 and 75 percentage of non-parametric data, and the percentages of the categorical variables were calculated as descriptive statistics. Mann-Whitney U test was used for group mean comparisons. Chi-square test and Fisher's exact test were used to analyze the relationship between categorical variables. Those with a p value below 0.05 were considered statistically significant. For statistical analysis, Statistical Package for the Social Sciences 22 (IBM Corp.; Armonk, NY, USA) statistics program was used.

Since our study is a retrospective study, examinations were made retrospectively on files. Therefore, informed consent forms were not obtained from the patients. In the study, Clinical Research Ethics Committee approval (Decision No: 2018/151) was obtained, and the biggest limitation of the study was the low number and diversity of the patients.

RESULTS

Of the 30 patients included in the study, 28 (93.3%) were male, 2 (6.7%) were female, and their mean age was 47.7±20.89. 18 (60%) patients had blunt thoracic trauma, 12 (40%) had penetrating thoracic trauma, and only 3 (10%) had isolated thoracic trauma. The most common additional traumatic pathology observed in the patients was head trauma with 43.3% (Table 1).

Main Points:

- We evaluated the relationship between the APACHE 2 and GCS scores of the thoracic trauma patients and the length of hospital stay, the need for mechanical ventilation and survival.
- We have shown that scoring systems are very important in determining the patient's mortality risk in trauma patients.
- The mortality risk is very high when the GKS value is below 11 and the APACHE 2 value is above 14 in trauma patients hospitalized in the intensive care unit, and its specificity is above 75%.

While 20 of the patients included in the study needed intubation, there was no statistically significant relationship between traumatic pathology and intubation need. While 10 of the trauma patients required blood transfusion, statistical analysis revealed that blood transfusion was statistically significantly necessary for the patients diagnosed with hemothorax (p=0.017) (Table 2).

The median follow-up time in the intensive care unit of the trauma patients included in the study was 120 hours. When the intubation need of the patients and the duration of stay in intensive care unit were compared, it was found that there was no significant difference (p=0.812), while there was a correlation between the duration of being connected to mechanical ventilator and the duration of stay in the intensive care unit in patients supported with mechanical ventilator(r=0.462, p=0.01). The median duration of stay in the mechanical ventilator was determined to be 84 hours in patients with mechanical ventilator support.

GCS, APACHE II scoring, blood gas and biochemical tests were used to take the patients into intensive care and to determine the need for mechanical ventilator and follow-up. When the data obtained were evaluated statistically, it was determined that hematocrit was lower in patients with hemothorax (p=0.020, U=55.5), while in patients with pneumothorax, pO_2 and SaO_2 mean values were lower (p=0.017, U=54 and p=0.043, U=2.5), and pCO_2 values were higher (p=0.004, U=176.5). A statistically significant correlation was found between the APACHE II scores of the patients at the time of admittance to the hos-

TABLE I. Distribution of traumatic pathologies by type of trauma

		BLUNT TRAUMA	PENETRAN TRAUMA	p
Heamothorax	+	5	8	0.035
	-	13	4	
Pneumothorax	+	9	4	0.367
	-	9	8	
Pulmonary contusion	+	11	1	0.007*
	-	7	11	
Rib fracture	+	9	5	0.654
	-	9	7	
Sternum fracture	+	1	2	0.320
	-	17	10	
Diaphragmatic laceration	+	0	4	0.018*
	-	18	8	
Head injury	+	11	2	0.016
	-	7	10	
Abdominal trauma	+	0	7	0.001*
	-	18	5	
Cardiovascular trauma	+	0	4	0.018*
	-	18	8	
Bone fracture	+	7	1	0.099
	-	11	11	
Facial trauma	+	2	1	1
	-	16	11	

TABLE 2. Relationship between traumatic pathologies and intubation and transfusion requirement

	n	%	Intubation requirement relationship	Transfusion requirement relationship
Thorax pathology				
Heamothorax	13	43	0.554	0.017*
Pneumothorax	13	43	0.259	0.794
Pulmonary contusion	12	40	0.656	0.114
Rib fracture	14	46.7	0.450	0.605
Sternum fracture	3	10	0.749	0.197
Diaphragmatic laceration	4	13.3	0.407	0.129
Additional traumatic pathology				
Head injury	13	43.3	0.074	0.602
Abdominal trauma	7	23.3	0.429	0.222
Cardiovascular trauma	4	13.3	0.177	0.129
Bone fracture	8	26.7	0.452	0.770
Facial trauma	1	3	0.749	0.197

TABLE 3. Relationship between arrival values of patients and duration of stay in mechanical ventilator, intensive care stay and survival

	Median (25th Percent-75th Percent)	Residence time in mechanical ventilator (p)	Residence time in Intensive Care Unit (p)	Survival(p)
GKS	11 (5.75-14.25)	0.086	0.130	0.004***
APACHE II	18(11-18)	0.024*	0.155	0.001***
pH	7.32(7.24-7.36)	0.566	0.013**	0.805
pO_2	65(55-89.5)	0.084	0.821	0.072
pCO_2	44.5(39.75-50)	0.712	0.711	0.869
SaO_2	96(88.25-99)	0.328	0.173	0.198
Hct	38.5(29.75-42)	0.441	0.405	0.157

*Spearman Rho:0.412; **Speraman Rho:0.448; ***Mann-Whitney U test

pital and the duration of their stay in the mechanical ventilator (p=0.024, r=0.412) and between pH and the duration of their stay in the ICU (p=0.013, r=0.448). When the patients were evaluated in terms of survival, a statistically significant relationship was found between the GCS and APACHE II scores and survival (p=0.001 U=27.5; p=0.004 U=178) (Table 3).

When the relationship between GCS and survival was analyzed through ROC analysis, it was observed that survival was negatively affected in patients whose GCS scores fell below 11.5, with 72% sensitivity and 76% specificity (AUC=0.805, p=0.005). As for the relationship between APACHE II score and survival, it was found that survival was affected more negatively when the APACHE II score increased over 14.5, with 94% sensitivity and 76% specificity. (AUC=0.876, p=0.001). When the duration of stay in the mechanical ventilator was longer than 18 hours, it was found that the survival rate was worse, with 82% sensitivity, 85% specificity (AUC=0.919, p=0.001).

DISCUSSION

Traumas are one of the main causes of deaths, particularly in the young population in the world. Considering all age groups, although there are regional differences, traumas are one of the most common causes of death after cardiovascular diseases and cancer (8, 9). According to the 2013 data of Turkey Statistics Institute (TSI), traumas rank fifth with a 5% share among the deaths in all age groups (10). In the study conducted by Akturan et al. (11), in which they used the data of TSI between 2009-2016, traumatic deaths were reported to be in the sixth place with 4.63% in all age groups.

Thoracic traumas account for approximately 6% of all traumas and are the direct causes of death in 25% of trauma-related deaths and the concomitant cause of death in 25%. Other systemic traumas accompanying thoracic traumas further increase the risk of death, and patients exposed to trauma often require follow-up and treatment in the intensive care unit (12-14).

In the study conducted by Emircan et al. (5), in which they retrospectively examined patients with thoracic trauma, the majority of patients were blunt thoracic trauma patients and the most common trauma was traffic accident related with 50%. The majority of the patients were female, and 62.2% were young adults between the ages of 20-49. In the study conducted by Pogorzelski et al. (15) which included trauma patients hospitalized in intensive care unit, the most common cause of trauma was traffic accidents with 60.3%, and the mean age of the patients was 33.3. In the study carried out by Abolfotouh et al. (16), which included a series of 3786 diseases, the majority of trauma patients were males. In the trauma patients with an average age of 29.6

years, the cause of trauma was often blunt trauma, and the most common reason was traffic accidents. In our study, 93.3% of our patients were male, and their mean age was 47.7. 60% of patients were exposed to blunt thoracic trauma.

In their study which involved 228 thoracic trauma patients, Moon et al. (17) determined that the most common pathology was pulmonary contusion with 96.5%, while hemothorax, traumatic flail chest and pneumothorax were the other most common pathologies. The average number of rib fractures in patients participating in their study was found to be 10. The study of Lin et al. (12) included 1333 patients with thoracic trauma, and 484 of these patients were treated in intensive care unit. They detected rib or sternum fractures in 79.1%, hemothorax in 56.1%, pneumothorax in 27.3%, and pulmonary contusion in 4.1% of the patients treated in the intensive care unit. They reported the most common additional traumatic pathology in this patient group as head trauma with 52.9%. In his study, Okabe (18) stated that 691 of 4317 blunt trauma patients had rib fractures due to blunt chest trauma, and 206 of these patients needed mechanical ventilation. In our study, the most common pathology was rib fracture, while the most common additional pathology was head trauma.

Various scoring systems are used to predict the prognosis of inpatients in intensive care units. Among these scoring systems, The Therapeutic Intervention Scoring System (TISS) is the first scoring system defined in 1974. In this system, where 57 parameters were evaluated when it was first defined, evaluation has been made with 19 parameters since 1997 (7, 19, 20). The APACHE scoring system, calculated from 34 different variables of seven organs, was defined in 1981. The APACHE II scoring system, which is an edited version of this complex system, was defined in 1985 and is still one of the most frequently used scoring systems. GCS is also the most commonly used scoring system in intensive care unit or emergency department, especially in evaluating the neurological status of head trauma patients (7, 21).

In their study in which they examined trauma patients followed up in intensive care unit, Ünlü et al. (22) found the APACHE II median score as 13, GCS median score as 9, and they determined that the rate of mechanical ventilation need and mortality rates increased in patients as the APACHE II score increased and GCS decreased. In 125 patients who developed mortality, they found the median value of APACHE II score as 21 and GCS median value as 7. In their study, Yıldırım et al. (2) found the average of APACHE II scores in trauma patients with exitus status as 15 and showed that this was statistically significant. In their studies, Emircan et al. (5) and Kara et al. (1) demonstrated that GCS was statistically significantly lower in patients with exitus status than in other traumatic patients. Dur et al. (23) also found the mortality rate to be 57.6% in patients with GCS below 8 in their study. In our study, we determined that there was a statistically significant relationship between GCS and APACHE II score and the duration of stay in the mechanical ventilator and mortality of patients.

In his study, Okabe (1) analyzed the patients in 2 groups according to their duration of stay in mechanical ventilation, and defined those who stayed less than 7 days as short-term ventilation group and those who stayed longer than 7 days as long-term ventilation group. He showed that the number of rib fractures was statistically significant in patients in the long-term mechanical ventilation group, that GCS was below 8 and mortality rates were higher. In our study, we showed that the risk of mortality increases statistically significantly as the duration of stay in the mechanical ventilator increases.

In conclusion, trauma is an important health problem because it ranks first among youth deaths. Thoracic traumas occupy a significant place in deaths due to trauma. APACHE II and GCS systems have an important role in predicting mortality in patients treated in intensive care unit due to trauma. Therefore, intensive care follow-ups and treatments should be planned accordingly by considering the high risk of mortality in patients with high APACHE II score and low GCS score.

Ethics Committee Approval: Ethics committee approval was received for this study from Balıkesir University School of Medicine Clinical Research Ethics Committee (2018/151).

Informed Consent: Due to the retrospective design of the study, informed consent was not taken.

Peer-review: Externally peer-reviewed.

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Original Article

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Cardiac Repolarization Properties in Children with Inflammatory Bowel Disease

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BACKGROUND/AIMS
Inflammatory bowel disease (IBD) (Crohn's Disease-CD and Ulcerative Colitis-UC) is a chronic autoimmune inflammatory disease. Cardiac involvement and electrophysiologic abnormalities has an important place in terms of morbidity and mortality among extraintestinal involvement. In this study, we investigated p-wave and QTc dispersion which may cause ventricular and supraventricular rhythm disorders if prolonged.

MATERIAL and METHODS
Twenty five IBD patient in remission period and 20 control patient were enrolled to the study. Twelve lead electrocardiogram were evaluated in all patients and p wave dispersion, QT and QTc dispersion was calculated manually.

RESULTS
QTcmax value of IBD patients were higher than control patients (p=0.05). UC patients had higher QTc max value and QTc dispersion than control patients (p=0.042).

CONCLUSION
UC patients are under risk of ventricular arrhytmias. Follow up with regular ECG in these patients and QTc calculation will be useful in monitoring of IBD patients.

Keywords: Children, inflammatory bowel disease, p-wave dispersion, QTc dispersion

INTRODUCTION

Crohn's Disease (CD) and Ulcerative Colitis (UC) are chronic, autoimmune and inflammatory diseases that mainly affect the gastrointestinal tract and progress with acute exacerbations and remissions. Systems beyond the gastrointestinal tract can be affected in both UC and CD. Extraintestinal system involvement can be seen at any time in the course of the disease and even symptoms of extraintestinal involvement may appear as the first sign of the disease. Cardiovascular involvement like thrombotic events, valvulitis, myocarditis, pericarditis, electromechanical changes are common in IBD (1-5). Subclinic myocardial involvement was shown in IBD patients, even in remission periods (6). Arrhytmia and conduction disorders are seen in IBD especially in UC patients (7, 8). In the course of disease, cardiac conduction defects, like atrioventricular block, first degree AV block may be seen (8-10). Beyond these electromechanic changes supraventicular tachyardia, mesalamine induced sinus bradycardia are reported previously in patients with IBD (10, 11).

It has also been shown that in IBD patients, corrected QT (QTc) dispersion leading to ventricular tachyarrhythmia has increased p-wave dispersion, which can lead to atrial fibrillation (12-14). Similar to subclinical myocardial mechanical involvement demonstrated by strain echocardiographic studies, electromechanical changes may be observed in IBD patients without obvious conduction disturbances during remission periods (15). From this perpective, we aimed to evaluate QTc and p wave disperison in pediatric IBD patients in remission period.

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MATERIAL and METHODS

Thirty eight IBD patients in remission (at least 1 year or more) who were followed in the tertiary pediatric gastroenterology clinic were evaluated. Thirteen patients were excluded from the study (the family of 4 patients did not want to be included in the study, 4 patients were under the age of 5, 2 patients were using medication that affect Qtc duration and 3 patients had additional rheumotological disease). Twenty-five IBD patients (UC/CD: 15/10, male/female 13/12; 5-18 years of age, mean age 12.7±2.9;) and 20 control patients (male/female; 11/14, 6-18 years of age, mean age 12.1±3.7) were included to the study. All medical records of patients diagnosed with IBD based on clinical symptoms, laboratory, radiological, endoscopic and histopathological features were examined and current clinical and laboratory findings were evaluated. All of the patients were in remission, and none of the patients had active complaints. Thirteen of the patients were treated with Mesalamine and Azathiopurine, 6 of them were taking Mesalamine treatment, three patient were under Infliximab and Mesalamine therapy and three patients were taking Infliximab, Mesalamine and Azathiopurine medication.

Healthy children who referred to pediatric cardiology clinic with noncardiac chest pain or innocent murmur were the control patients. Written consents were taken from patients' family. Local ethics committie in Umraniye Research and Training Hospital approved the study.

Echocardiographic evaluation was performed in all patients. Blood pressure of patients was measured with sphygmomanometry. 12-lead electrocardiograms (ECG) were obtained at a velocity of 50mm/s and at an amplitude of 20mm/mV from all patients. Manual ECG analyses were performed by a pediatric cardiologist who was blinded to patient and control subjects. QT-intervals were measured manually in all leads. the interval from the beginning of the QRS complex to the end of downslope of the T wave that crossing the isoelectric line, which was defined as the QT-interval.

The QTc interval was calculated by using Bazett (17) formula QTc=QT / √RR. In all leads QT and RR intervals were measured in 3 consecutive cardiac cycles. QT-dispersion was defined as the difference between the maximum and minimum corrected QT-interval. The duration between the beginning of the P wave (first deflection of the p wave) and the end of the P wave was defined as the P wave. P wave dispersion was defined as the difference between maximum and minimum P-wave durations (6).

Statistical Analysis

Statistical Package for the Social Sciences 22 software package (IBM Corp., Armonk, NY, USA) was used for stastical analysis. Kolmogorov- Simirnov test was used to determine the distribution of normality. Data were expressed as mean ± standard deviation, categorical data were expressed as number and percentages (%). Mann Whitney U test was used for comparison of groups. $p<0.05$ was used for statistical significance.

Main Points:

- QTc prolongation of QTc dispersion in systemic inflammatory diseases is a risk factor for life-threatening arrythmias
- In our study, QTc dispersion is higher in IBD group, especially in UC patients than control patients.
- We found p wave dispersion above 40 ms which is a risk factor atrial arrythmias.

RESULTS

There were no statistical significant difference in terms of age, sex, weight, height, BMI and systolic and diastolic blood pressure between IBD and control group. Demographic data is shown in Table I.

The study patients were all in remission period (PUCAI score was <10 for UC patients. PCDAI score was <12.5 for CD).

There were no significant difference statistically in terms of heart rate between IBD and control. Heart rate was 92.4±22.8 per minute in IBD group and 95.2±11.2 per minute in control group ($p=0.33$).

QTcmax value of IBD patients and control patients were 526.7±46.5 ms and 452±37.4 ms respectively ($p=0.05$) (Table 2). QTc dispersion (150.4±42.5) was higher in IBD group than control patients (79.1±37.6) but did not reach statistical significance.

TABLE I. Demographic and electrocardiographic features of study and control patients

	Demographic Features of Study Patients		
	IBD	**Control**	**p**
Gender (M/F)	13/17	14/11	0.56
Age (years)	11.8±4.72	10.4±3.87	0.24
Weight (kg)	40.5±23.9	37.1±20.4	0.25
Height (cm)	142.3±24.7	140.1±23.5	0.32
BMI	18.4±5.26	17.4±3.37	0.08
SBP (mmHg)	104.7±14	101±11.9	0.27
DBP (mmHg)	63.7±9.16	58±11	0.27
	Electrocardiographic Measurements		
HR	92.4±22.8	95.2±11.2	0.33
HR (min)	88.5±20.4	92.7±16	0.61
HR (max)	97.2±17.9	104.7±16	0.18
P (min)	75.7±16.7	4.34±0.86	0.23
P (max)	101.4±3.72	60±23	0.53
P dispersion	37.1±14.9	35.7±27.6	0.95
QT (min)	314.2±42.7	300±30.5	0.61
QT (max)	357.1±73.4	317.1±43.8	0.28
QT dispersion	43.8±16.8	37.4±29.5	0.86
QTc (min)	396.5±57	372±16	0.74
QTc (max)	526.7±46.5	452±37.4	0.05
QTc dispersion	150.4±42.5	79.1±37.6	0.095

IBD: Inflammatory bowel disease; BMI: Body mass index; SBP:Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; HRmin: Heart rate minimum; HRmax: Heart rate maximum; P(min): P wave duration minimum; P(max): P wave duration maximum; QTmin: QT minimum; QTmax: QT maximum; QTcmin: corrrected QT minimum; QTcmax: corrected QT maximum

TABLE 2. Electrocardiographic features of Crohn's Disease and Ulcerative Colitis and control patients

	CD	UC	Control	p*	p†	p°
HR	79.3±18.5	107.5±22.7	95.2±11.2	0.78	0.11	0.87
HRmin	75.6±21.3	98±26.3	92.7±16	0.57	0.51	0.87
HRmax	85±14.7	106.5±15.2	104.7±16	0.25	0.067	0.63
P wave (min)	63.3±25.1	85±25.1	160±23	0.39	0.83	0.10
P wave (max)	93.3±11.5	107.5±18.9	85.7±27.6	0.57	0.66	0.63
Pwave dispersion	30±17.3	42.5±12.5	37.1±17.8	0.57	0.66	0.63
QTmin	320±40	310±50.3	300±30.5	0.78	0.51	0.87
QTmax	360±69.2	355±86.9	317.1±43.8	0.78	0.6	0.53
QTdispersion	42.6±26.1	45±24.1	38.4±29.5	0.99	0.83	0.98
QTc min	373±61.9	414±54.5	372.8±16	0.41	0.93	0.62
QTc max	498±2.64	548±53.7	452±37.4	0.41	0.73	0.048
QTc dispersion	136±41.7	161.2±45.7	79.1±37.6	0.42	0.51	0.042

IBD: Inflammatory bowel disease; CD: Crohn's Disease; UC: Ulserative Colitis; HR: Heart rate; HRmin: Heart rate minimum; HRmax: Heart rate maximum; P(min): P wave duration minimum; P(max): P wave duration maximum; QTmin: QT minimum; QTmax: QT maximum; QTcmin: corrrected QT minimum; QTcmax: corrected QT maximum
P*: comparison between CH vs UC
p†: comparison between CH vs Control
p°: comparison between UC vs Control

Mean, minimum and maximum heart rate was higher in UC patients than control patients but did not reached statistical significance. Heart rate was 79.3±18.5 per minute in CD patients, 107.5±22.7 per minute in UC patients and 95.2±11.2 per minute in control group.

QTc max and QTc dispersion were higher in UC patients than control patients. In UC patients QTc max was 548±53.7 and 452±37.4 in control patients (p=0.048). QTc dispersion was 161.2±45.7 in UC and 79.1±37.6 in control patients (p=0.042) (Table 2).

DISCUSSION

Cardiac involvement is not rare in IBD. Besides subclinic myocardial involvement in IBD, there are obvious conditions affecting cardiovascular system such as myocarditis, pericarditis, thromboembolic events and vasculitis (1-5). However, although electrophysiologic changes were shown in IBD, there is not enough emphasis on QTc prolongation and its the consequences. QTc prolongation is the prolongation of ventricular repolarization that can predispose to ventricular arrhythmias and cause sudden death. It may be acquired or congenital. The QTc interval can be affected by many factors. Drugs, toxins, electrolyte imbalance, heart failure, myocarditis, rheumatic heart disease, endocrinologic reasons such as hypothyroidism, diabetes mellitus, increase in intracranial pressure, end stage liver disease are some of the reasons for QTc prolongation (16). Besides QTc prolongation in systemic inflammatory diseases and autoimmune diseases is a prominent finding recently. Regardless of the cause, QTc prolongation can cause life-threatening arrythmias. Studies based on general population showed that increased QTc and QTc dispersion are serious risk factors for malignant ventricular tachyarrythmias and sudden death. Prolongation in QT interval which means prolongation in ventricular repolarization causes early after depolarizations and generate malignant ventricular arryhtmias such as Torsades de pointes which progress to ventricular fibrilation rapidly and it can lead to sudden death (17).

Recent studies showed that QTc prolongation is correlated to inflammation (18, 19). A correlation has been shown between inflammatory cytokines and Qtc prolongation (20). QTc shortening is obtained with anti-inflammatuar treatment in chronic inflammatory processes such as rheumatic arthritis and connective tissue diseases (16). Also in chronic autoimmune inflammatory diseases QTc prolongation is found to be correlated to disease severity and CRP (21).

Activation of central sympathetic system by inflammatory cytokines causes cathecolamine release leads to the release of proinflammatory cytokines from circulating monocytes and lenfocytes via B2 adrenergic receptors. With this vicuous cycle, electrophysiologic changes occur in the heart (18).

Increased sympathetic activity may be a direct cause of arrythmia or indirectly affects the heart through the Ca and K channels resulting in prolongation of the QT interval (16). In addition, proinflammatory cytokines, particularly TNF alfa, IL-1 and IL-6, act on spesific ion channels on cardiomyocites, thereby prolonging the action potential duration resulting in increased excitability.

P wave dispersion is defined as the difference between maximum and minimum duration of p wave. It is an ECG marker that expresses the inhomogeneous spread of sinus stimulation in the atrial myocardium. The increase in p wave dispersion means an increase in the irregularity of atrial refractory period. This is a risk factor for the development of atrial fibrillation by re-entry mechanism (22). As in the QTc dispersion, p wave dispersion has been shown to correlate with systemic inflammation and CRP elevation in different diseases (23-27).

Although the mechanism of QTc prolongation in autoimmune diseases is not fully understood, it is thought that autoantibodies interact with the ion channels of the cardiomyocites and

prolongs the action potential duration (18). This may affect the exitability of the myocardium and it can trigger the development of arrhythmia.

In spondyloarthirits and chronic arthirits new-onset ventricular tachyarrhythmias and QT prolongation were reported (27). In a study with adult patients diagnosed with ankylosing spondylitis, QTc was prolonged due to inflammation, and QTc duration was shortened by controlling inflammation with Infliximab treatment. (28). In our study, only 6 of our patients were receiving Infliximab therapy. The presence of prolongation of Qtc dispersion in these patients who are in the remission period may be interpreted subclinical inflammation. In one study a case of azothiopurine induced acute myocarditis was reported (29). We did not find any signs of myocarditis in any of our patients.

Altough we did not find a statistically significant difference in p wave dispersion between IBD patients and control patients, in our study UC patients had 42.5±12.5 ms p wave dispersion. P wave dispersion value for atrial arrhytmias is >40 ms (30). We have received close monitoring of these patients from atrial arrhythmias.

There is no certain cut off QT dispersion values but according to a few studies >40 ms QT dispersion is a risk factor for ventricular arryhtmias (31). In our study QT dispersion of IBD patients were above this limit.

In our study QTc dispersion in IBD was higher than control patients and this was attributed to UC patients. UC patients had higher both QTc maximum and QTc dispersion than control patients. There are a few studies investigating QT values in IBD. There is only one pediatric study found prolonged p wave dispersion and QT and QTc dispersion (13). Our study is the first study to reveal that UC patients are at risk for ventricular arrhythmias. Therefore, in addition to routine pediatric gastroenterology follow-up, these patients should be monitored regularly by pediatric cardiology. Our study also showed that these patients should be regularly monitored for ECG. With regular follow-up, control of inflammation should be ensured and possible gastrointestinal and extra-intestinal complications can be prevented or detected early.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Umraniye Research and Training Hospital (26.12.2019/54132726-000-272233).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Author Contributions: Concept – E.E.; Design – E.E; Supervision – E.P.; Resources – E.E; Materials – E.P.; Data Collection and/or Processing – E.E., E.P.; Analysis and/or Interpretation – E.E., E.P; Literature Search – E.P.; Writing Manuscript – E.E.; Critical Review –E.P.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Nursing Students' Levels of Tendency to Commit Medical Errors

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BACKGROUND/AIMS
The determination of nursing students' tendency to commit medical errors and having knowledge about the erroes before they affect patients may ensure taking necessary precautions. The aim of this study was to determine the nursing students' tendency to commit medical errors and the variables that affected it.

MATERIAL and METHODS
This was a cross-sectional study. The sample of the study consisted of 167 nursing students who voluntarily accepted to participate in the study. The Student Identification Form and the Medical Error Tendency in Nursing Scale were used to collect the data. Descriptive statistics, number, percentage, independent t test and ANOVA test were used for statistical analysis by Statistical Package For Social Science 22.0 (IBM Corp.; Armonk, NY, USA) package program.

RESULTS
The mean age of the students was 21.23±2.48 years. 65.3% of them were female, 37.1% of them were second year students. The mean score of the nursing students on the whole Medical Error Tendency in Nursing Scale was 214.71±25.58. Lower dimension score average of the scale was found to be 79.39±9.94 in the "Medication and Transfusion Applications" lower dimension, 53.10±7.07 in the "Prevention of Infections" lower dimension, 38.56±5.24 in the "Patient Follow-Up and Material-Device Safety" lower dimension, 21.67±3.36 in the "Prevention of Falls" lower dimension and 21.97±3.45 in the "Communication" lower dimension. There were statistically significant differences between the students' mean scores on the whole Medical Error Tendency in Nursing Scale according to their grades, and it was observed that the fourth grade students had a statistically lower tendency to commit medical errors compared to the second grade students.

CONCLUSION
In conclusion, it was found that the nursing students' tendency to commit medical errors was low and their grades affected their tendency to commit medical errors.

Keywords: Clinical practice, medical errors, nursing student, tendency to commit medical errors

INTRODUCTION

Nowadays, medical errors, which constitute an important risk for patient safety, have become a multidimensional problem that causes ethical-legal problems related to accidents and deaths (1, 2). A medical error is defined as an inability to complete a planned task as intended or making an incorrect plan to achieve the goal, and a deviation from the patient care process (3). Medical errors are alternatively defined as the practices cause being hospitalized for a longer period of time, deteriorating health of patients and damaging them, or causing death due to ignorance, inexperience, apathy, or used technologies (1, 4, 5).

Medical errors are a growing problem in the field of health. In the literature, it has been reported that medical errors are common in all countries of the world (3, 6-8). In a study conducted by Landrigan et al. (9); it was reported that 0.6% of hospitalizations executed in a group of hospitals in North Carolina for six months, resulted in fatalties and 63% were caused by medical errors.

Medical errors are not only an issue that should not be ignored by all members of health teams, but are also more important for nurses. Because nurses are the members of a profession which spend the most time with patients. In addition,

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incorrect practices adversely affect patient safety since nurses are directly involved in patient care (5). According to the literature, medical errors experienced by nurses are caused by inadequacy of application of care standards, inadequacy in applications related to patient safety and protection, inability in record keeping, carelessness, imprudence, non-compliance with orders and regulations, excessive workload, working by shifts, professional inexperience, inadequate number of nurses, and lack of communication (3, 5, 10, 11).

On the other hand, it is important that nursing students, who are nurse candidates, increase their knowledge and awareness about medical errors during their education (2). It has been emphasized that the problems that are frequently encountered in the professional life should be addressed during the undergraduate education, educators should work for raising nurses who can become aware of the faulty medical practices, find solutions, and the quality of nursing education should be improved (4). Among studies on relevant topics, Türk et al. (2), in a study with intern nurses, found that their tendency to make medical errors was low. In a study by Bodur et al. (12), it was found that midwifery and nursing students had a high rate of medical errors. Cooper (13) emphasized that students without enough clinical experience were at risk of making medical errors in patient care. Çevik et al. (14) reported that the most frequent medical error committed by students during clinical practice was administering medicine prepared for a different patient. Zieber and Williams (15) found that the experience of committing a medical error was traumatic for nursing students on clinical practice, and that the students lacked the skills to cope with the experience. Koohestani and Baghcheghi (16) also emphasized that drug administration errors by nursing students often went unreported.

Teaching safe patient care is one of the essential elements of nursing education. Inadequate skills training and learning process are the main causes of medical errors (2). Today, almost all universities providing nursing degree courses include in their curricula as either compulsory or elective classes information on medical errors and how they can be prevented. However, it should be noted that students with a lack of clinical experience have a high risk of committing unintended error in patient care (2, 17). In line with this information, the probability of committing medical errors of nursing students may increase when they spend most of their time with patients and undertake treatment responsibilities. Therefore, the determination of nursing students' tendency to commit medical errors and having knowledge about the erroes before they affect patients may ensure taking necessary precautions. In the light of this information, the objective of this study was to determine the nursing students' tendency to commit medical errors and the variables that affected it.

Main Points:

- Mean score of the senior students on the Medical Error Tendency in Nursing Scale was significantly higher than that of the second year students.
- There are statistically significant differences between the students' mean scores on the whole METNS according to their grades.
- There is no statistically significant differences between the students mean scores' on the METNS according to gender and high school type variables.

MATERIAL AND METHODS

The universe of this descriptive study consisted of 180 second, third and fourth year students in the Nursing Department of Batman University's School of Health between November and December 2019. First year students were excluded from the study because they did not have any clinical practice experience at the time of the study. The sample of the study consisted of 167 nursing students who voluntarily accepted to participate in the study. Participation in the study was 92.77%. The Student Identification Form and the Medical Error Tendency in Nursing Scale were used to collect the data.

Student Identification Form

This form was created in accordance with the literature (2, 3, 6, 12, 18) by the researchers, and included items on the descriptive characteristics of the students such as age, education year, gender and the type of high school they graduated from.

Medical Error Tendency in Nursing Scale (METNS)

The Medical Error Tendency in Nursing Scale was developed by Özata and Altunkan (18) and its validity and reliability were analyzed by same researchers. It contains 5 sub-scales on the activities of nurses and doctors in the treatment and care process (49 items in total) as the subscales of Medication and Transfusion Applications (18 items), Prevention of Infections (12 items), Patient Follow-up and Material-Device Safety (9 items), Prevention of Falls (5 items), and Communication (5 items). It is a 5-point Likert type scale and the items are scored as 1: never, 2: very rare, 3: occasionally, 4: usually, 5: always. The highest and lowest scores can be obtained from the scale are 245 and 49, respectively. As mean score on the whole scale increases, the tendency of nurses to commit medical errors is interpreted as low (18). In the validity and reliability study of the scale, the Cronbach's alpha coefficient was found to be 0.95. In this study, the Cronbach's alpha coefficient of the scale was calculated as 0.97.

The students informed that all of the collected data would be used for scientific purposes and their answers would not affect their course grades in any way. The questionnaire forms were distributed to the students and collected from the students who completed their answers and then evaluated.

Ethical Considerations

In order to conduct the study, a written permission was obtained from the researcher who developed the scale, and the ethical approval (Decision No. 2019-217) was obtained from the Non-Interventional Clinical Research Ethics Committee of Batman Regional State Hospital. In addition, required legal permissions and informed consents were obtained from the institution and students, respectively.

Statistical Analysis

Descriptive statistics, number, percentage, independent t test and ANOVA test were used for statistical analysis by by Statistical Package For Social Science 22.0 (IBM Corp.; Armonk, NY, USA) package program.

RESULTS

The mean age of the students was 21.23±2.48 years. 65.3% of them were female, 37.1% of them were second year students and 65.9% of them were Anatolian High School graduates (Table 1).

The mean score of the nursing students on the whole METNS was 214.71±25.58. Examining the students' mean MENTS sub-dimension scores, it was seen that their highest mean scores were on "Prevention of Infections", and their lowest mean scores were on "Patient Follow Up and Material-Device Safety" (Table 2).

The descriptive characteristics of the students and their mean scores on the whole METNS are given in Table 3. According to this, there were statistically significant differences between the students' mean scores on the whole METNS according to their grades, and it was observed that the mean score of the senior students on the METNS was significantly higher than that of the second year students ($p<0.05$, Table 3). On the other hand, there was no statistically significant differences between the students mean scores' on the METNS according to gender and high school type variables ($p>0.05$, Table 3).

TABLE 1. Distribution of the descriptive characteristics of the nursing students (n=167)

Characteristics	n	%
Gender		
Female	109	65.3
Male	58	34.7
Grade		
2. year	62	37.1
3. year	47	27.5
4. year	58	35.3
Alma Mater (Type of High School)		
Common High School	32	19.2
Anatolian – Science High School	110	65.9
Other*	25	15.1

*Vocational High School, Religious Vocational High School

TABLE 2. The distribution of the mean scores of the students on the Medical Error Tendency in Nursing Scale (n=167)

METNS Subscales	Min-Max X±SD	Mean Scores X±SD	Item Mean Scores
Medication and Transfusion Applications	48-90	79.39±9.94	4.41±0.55
Prevention of Infections	26-60	53.10±7.07	4.42±0.58
Patient Follow Up and Material-Device Safety	18-45	38.56±5.24	4.28±0.58
Prevention of Falls	10-25	21.67±3.36	4.33±0.67
Communication	9-25	21.97±3.45	4.39±0.69
Total METNS	123-245	214.71±25.58	4.38±0.52

METNS: Medical Error Tendency in Nursing Scale; X: mean; SD: standard deviation

DISCUSSION

The knowledge and experience gained during nursing education affects the competence of students to provide and improve patient safety for care practices (2). It is very important to determine the tendency of nursing students to commit medical errors and to determine the errors before they affect patients. This study was conducted in order to determine the level of tendency of nursing students to commit medical errors and the variables that affect this. In conclusion, it has been seen that the students' tendency level to commit medical errors was low. The low tendency of the students participating in our study to make medical errors is a very welcome finding with regard to developing patient safety procedures. It suggests that students are taking care to avoid medical errors when performing nursing care, and that this may have had an effect on this result. In similar studies conducted by Türk et al. (2) and Güneş et al. (6) with intern nursing students, it was similarly found that the students' tendency to commit medical errors was low.

Similar results have been obtained in most of other studies on nursing students in the literature (14, 19-21). It is a promising finding that the nursing students participating in the study were attentive and careful to ensure patient safety during clinical practice in order to avoid medical errors. However, another study conducted with nursing students reported that one out of five students made a medical error (15). In a study on nursing and midwifery senior students, 37% of midwife and nurse candidates indicated that they made medical mistakes during their training period and more than half of these mistakes (59%) were medication errors (12). Attree et al. (22) reported that all student nurses commit medical errors or encounter errors that were prevented at the last moment. Altuntaş et al. (23) reported that nursing students had low levels of medical error perceptions. In a study conducted by Zaybak et al. (24) on senior students, it was concluded that the students were more likely to commit drug administration errors. Our results are not consistent with these findings. This difference between the results may be due to the differences between the independent variables related to the students in the samples of the studies such as grade, age, curriculum, etc.

TABLE 3. Comparison of the mean scores of the students on the Medical Error Tendency in Nursing Scale according to their descriptive characteristics

Characteristics	METNS X±SD	Statistical Value
Gender		
Female	217.47±20.40	t=1.930
Male	209.51±32.79	p=0.055
Grade		
2. year	207.12±31.06	F=5.576
3. year	215.28±26.75	p=0.005
4. year	222.71±13.58	
Alma Mater (High School Type)		
Common High School	216.12±21.83	F=1.570
Anatolian – Science High School	216.20±23.74	p=0.210
Other	206.36±35.58	

METNS: Medical Error Tendency in Nursing Scale; X: mean; SD: standard deviation; t: Independent t test; F: One-Way ANOVA

Examining the mean scores of nursing department students on the sub-dimensions of MENTS in the conclusion of the study, it was seen that their highest mean score was on the sub-dimension of "Prevention of Infections", and their lowest was on "Patient Follow Up and Material-Device Safety" (Table 2). It is seen from this finding that the tendency to make medical erros was lowest in the sub-dimension of "Prevention of Infections". Similar results were found in studies by Türk et al. (2) and Güneş et al. (6) conducted with nursing department intern students. In a study by Kahriman et al. (19) conducted with nursing students, it was found that the students had the highest scores in the field of infection. It is seen that the results of our study support the results of this study in the literature.

It was found that the fourth grade students had a lower tendency to commit medical errors compared to the other grade students (Table 3). According to this result, we can suggest that as the grade level increases, students' tendency to commit medical errors decreases. The senior students had a lower tendency to commit medical errors, it is an expected finding because senior students are near the completion of the education process and have more experience in terms of clinical practice compared to lower grade students. However, Altuntaş et al. (23) found that the senior students' perceptions of medical errors were lower than the students with lower grades and stated that this finding was surprising and needs to be emphasized. Our results are not similar to the findings of their study.

According to the results, it was found that the variables of gender and high school type did not affect the tendency of the students to commit medical errors (Table 3). Similarly, Güneş et al. (6) and Altuntaş et al. (23) found that the variable of gender did not affect the tendency levels of the students to commit medical errors. Parallel results were also found in similar studies conducted with nurses (3, 20). Our results are consistent with the results of the studies listed above. However, in the study of Türk et al. (2), it was found that gender variable significantly affected the students' tendency to commit medical errors. The results of that study are not similar to the findings of us. It can be suggested that this difference between the results may be due to the difference between the samples because Türk et al. (2) included only senior students in their study. On the other hand, it has been estimated that the students' high school type did not affect their tendency to commit medical errors because most of the students participating in the study graduated from Anatolian and Science High Schools and thus the curriculum differences between their high schools were low.

In conclusion, it was found that the nursing students' tendency to commit medical errors was low and their grades affected their tendency to commit medical errors. It was also observed that gender and type of high school graduated did not affect the level of tendency to commit medical errors. In line with these results, it can be suggested that the knowledge and skills of nursing students on the prevention medical errors should be updated and this study should be repeated in other universities by considering different variables.

There are some limitations in this study. The research was conducted at a single center, the answers to the questions on the forms depended on the students' own statements, and data were collected after a certain time interval. These all constitute limitations to the study.

Ethics Committee Approval: Ethics committee approval was received for this study from Non-Interventional Clinical Research Ethics Committee of Batman Regional State Hospital (2019-217).

Informed Consent: Oral informed consent was obtained from students who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.A., D.Y.; Design - S.A., D.Y.; Supervision - S.A., D.Y.; Resources - D.Y.; Materials - S.A., D.Y.; Data Collection and/or Processing - S.A.; Analysis and/or Interpretation - S.A.; Literature Search - S.A., D.Y.; .; Writing Manuscript - S.A., D.Y.; Critical Review - D.Y.

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Platelet Indices: Impact of Helicobacter Pylori Infection

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BACKGROUND/AIMS
Platelets act as reactive immune cells in inflammatory conditions. Mean platelet volume (MPV) and platelet distribution width (PDW) is an indicator of platelet activity and function. We aimed to investigate the effect of helicobacter pylori eradication on platelet markers in patients who were infected by H. Pylori and eradicated.

MATERIAL and METHODS
Fifty-two patients with H. Pylori positivity confirmed by esophagogastroduodenoscopy and biopsy were included in the study. Platelet parameters (platelet count, MPV-mean platelet volume, PDW-platelet distribution range, MPV-mean platelet volume) of the patients were evaluated before and after treatment.

RESULTS
The mean age of the patients was 11.8±4.4. The mean height was 138.6±25.7 cm and the mean weight was 36.4±17.3 kg. The number of platelets was 297673±60409 before treatment and 288514±74108 after treatment (p=0.48). PDW was 15.9±0.66 before treatment and 15±0.32 after treatment (p=0.050). MPV was 9.39±1.41 before treatment and 9.67±1.21 after treatment (p=0.12). The severity of Helicobacter pylori infection was not correlated with platelet count, MPV, PDW.

CONCLUSION
The results from this study demonstrate that eradication of H. Pylori infection has an impact on the platelet indices.

Keywords: Children, Helicobacter pylori infection, platelet functions 19

INTRODUCTION

Helicobacter Pylori (H. Pylori), which is primarily colonized in the human gastric mucosa, is a gram-negative, spiral-shaped, bacterium with flagella (1, 2). H.Pylori is reported to be the most common infectious agent in the world (3). According to meta-analyses, the global prevalence of H. pylori was reported to be 33% and 44.3% (4, 5). The rate is reported to be 80% in developing countries and 10% in developed countries (4,5). As a primary colonization site, H. pylori increases toxic mucosal pH by displaying toxic effects on gastric epithelium and disrupting gastric epithelial functions and mucus secretion that generally protects gastric epithelium.

It is possible that H. pylori infection may be completely asymptomatic. Chronic gastritis, duodenal ulcers, gastric ulcers, gastric cancers, and MALT (mucosa-associated lymphoid tissue) lymphomas have been previously associated with H.Pylori infection (6). Without peptic ulcer disease, H. pylori-associated gastritis rarely presents as a symptom during childhood but may lead to more serious complications in the future. Detection of H.Pylori in biopsy specimens taken from the antrum and corpus (with Sydney score) is currently the gold standard for diagnosis in patients undergoing upper gastrointestinal endoscopic evaluation for organic causes of abdominal pain. In the case of a positive finding, eradication success should be evaluated by urea breath test (C13) or stool test following standard eradication therapy (at least 15 days after discontinuation of proton pump inhibitor-PPI and at least 1 month after discontinuation of antibiotic therapy) (6).

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H.pylori has been shown to trigger a local and systemic immune response. It is also known to play a role in the etiopathogenesis of some diseases with extra-gastric location. These include iron deficiency anemia, coronary heart disease, immune thrombocytopenic purpura (ITP), dermatological diseases (Acne rosacea, prurigo pigmentosa, prurigo chronica multiformis, chronic idiopathic urticaria), neurodegenerative diseases and autoimmune diseases (7).

Platelets play a primary role in hemostasis and coagulation. Inflammation is known to be an important stimulus for platelets. The presence of inflammation causes the release of cytokines and chemokines from the platelet membrane. MPV and PDW are both indicators of platelet function and activation. In the presence of thrombopoietic stress, an increase in MPV occurs through stimulation of the megakaryocytic series. It has been stated before that MPV is a useful indicator in evaluation of platelet function and activation and may help in early diagnosis of some diseases through changes in platelet structure and number. (8). It is a well known fact that platelet count increases with H. pylori treatment in chronic ITP patients (9). Based on this information, we planned to investigate the effect of treatment on platelet markers in patients with H.Pylori detected in esophagogastroduodenoscopy due to treatment- resistant dyspepticcomplaints.

MATERIAL and METHODS

Ethics committee approval was received from Umraniye Research and Traning Hospital. Fifty-two patients who tested positive for H.Pylori and had an esophagogastroduodenoscopy performed for treatment-resistant dyspepsia in a pediatric gastroenterology clinic in two different centers were included in the study. Platelet parameters included platelet count, MPV-mean platelet volume, PDW-platelet distribution range, and MPV-mean platelet volume of the patients and were evaluated before treatment. First-line eradication therapy (Amoxicillin + Clarithromycin + Lansoprazole) was given to patients whose biopsies were positive for H. pylori. H. pylori eradication status was evaluated by stool H. pylori antigen test one month after the end of the treatment. The blood counts of the patients who were successful in eradication treatment were repeated three months after the end of the treatment and platelet parameters were re-evaluated subsequently. Patients who were not successful during the first line eradication treatment and who subsequently needed a secondary eradication treatment were excluded from the study.

RESULTS

The mean age of the patients was 11.8±4.4 (30 girls, 22 boys). The mean height was 138.6±25.7 cm and the mean weight was 36.4±17.3 kg. The number of platelets was 297673 ± 60409 before treatment and 288514±74108 after treatment (p=0.48). PDW was 15.9±0.66 before treatment and 15±0.32 after treatment (p=0.050). MPV was 9.39±1.41 before treatment and 9.67±1.21 after treatment (p=0.12). H. pylori infection severity was not correlated with platelet count, MPV, PDW.

Main Points:

- Bone marrow can be affected by inflammatory conditions.
- Changes in platelet parameters (platelet count, mean platelet volume and platelet distribution width) in H.pylori infection is to be expected.
- Platelet parameters can be used for the evaluation of H. pylori infection eradication.

DISCUSSION

H. Pylori infection has an effect on platelet count and bone marrow. Studies have shown a decrease in platelet count after H. Pylori eradication in non-ITP patients (10). Peripheral platelet counts have been shown to increase rapidly in H. Pylori positive patients with bone marrow transplantation. It has also been shown that H. Pylori infection increases IL-6 levels and this then has a positive effect on bone marrow platelet production (11). In the last decade, platelets have been reported to act as immune cells. Platelets increase cytokines IL-6 and TNFalpha release against H. Pylori IL-6 causes an increase in the number of platelets, while TNFalpha has an adverse effect on the number of platelets. This dual effect prevents an excessive decrease in platelet number after H. Pylori eradication (12). In this study, a decrease in the number of platelets was observed after eradication of H. Pylori but it was not statistically significant.

Mean platelet volume (MPV), a widely used platelet function marker, is influenced by production, activation, and finally sequestration. which is a marker of platelet functionis influenced by platelet activation, production and sequestration. MPV is reported to have been involved in many diseases. As an important component of the immune system, MPV is known to associate with inflammatory conditions. Platelets with high MVP have been proven to be more active and more susceptible to aggregation. Additionally, MPV is considered to be higher in H.pylori positive patients than compared to H.pylori negative patients (2, 13, 14). There was no significant difference found in the study. PDW is a measure of platelet size variability. It shows heterogeneity in platelet morphology and increases with platelet activation. PDW is also considered to be a more specific indicator of platelet reactivity than MPV (15). In this study, a significant decrease in PDW was observed with eradication of H.pylori, indicating that platelet activation decreased.

The results from this study indicate that there is a relationship between platelet indices and H. Pylori infection. The relationship between H. Pylori infection and MPV platelet count was demonstrated in previous studies. However, this study provided new information about the impact of H. Pylori infection on PDW, which is another platelet function parameter. In the future, it is possible that platelet parameters may be used to determine eradication success of H.pylori infection.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ümraniye Research and Traning Hospital (18.12.2019/B.10.1.TKH.4.34.H.GP.0.01/237).

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Original Article

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Use of Red Blood Cell Distribution Width, Platelet Distribution Width, and Mean Platelet Volume Values as Diagnostic Markers in Patients with Recurrent Aphthous Stomatitis

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BACKGROUND/AIMS
To evaluate the use of red blood cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) as diagnostic markers in patients with recurrent aphthous stomatitis (RAS).

MATERIAL and METHODS
The retrospective case-control study included a patient group of 51 RAS patients a control group of 51 age- and gender-matched healthy individuals. RAS and control groups were determined as two groups. Complete blood counts were registered from patients' medical records. RDW, MPV, PDW, NLR, and PLR were recorded for each subject and were compared between the two groups.

RESULTS
Mean RDW was 15.66±2.03 in the RAS group and 14.86±1.44 in the control group (p=0.026). Mean PDW was 15.44±2.86 in the RAS group and 14.42±1.69 in the control group (p=0.032). Mean MPV was 8.82±0.87 in the RAS group and 8.42±0.56 in the control group (p=0.007). Mean NLR was 1.94±0.74 in the RAS group and 1.80±0.80 in the control group (p=0.374). Mean PLR was 119.49±36.58 in the RAS group and 121.98±32.96 in the control group (p=0.718). Only RDW, PDW, and MPV values were significantly higher in the RAS group compared to the control group.

CONCLUSION
The results indicated that both NLR and PLR cannot be considered as valuable parameters for routine diagnosis and in the prediction of prognosis in RAS patients. Increased RDW, PDW, and MPV values could have a diagnostic value in RAS patients. Accordingly, it is wise to consider that inflammation, thrombosis and acute hypoxic ischemia should be prioritized in the etiology of RAS.

Keywords: Erythrocyte, lymphocyte, mean platelet volume, neutrophil, recurrent aphthous stomatitis

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is an inflammatory disease that develops most frequently on the buccal mucosa and less frequently on the undersurface of the tongue and floor of the mouth, characterized by recurrent episodes of solitary, painful, and round or ovoid ulcers with a necrotic center surrounded by erythematous halo (1). RAS occurs in the absence of systemic diseases and typically heals within 7-10 days without scarring (2). With a prevalence ranging between 0.9-78% across countries, RAS affects almost 20% of the general population (1). RAS is the most common disease of the oral mucosa and is associated with chronic inflammation and endothelial dysfunction (1, 2), and is mostly seen children and adolescents (3).

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are peripheral blood markers of systemic inflammation, calculated simply from complete blood count (CBC) test. Additionally, mean platelet volume (MPV), red blood cell distribution width (RDW), and platelet distribution width (PDW) are also calculated from CBC test. PDW assesses variability in platelet size, changes with platelet activation, and indicates the heterogeneity in platelet morphology (4). MPV is a platelet function index used for the determination of platelet size, which directly reflects platelet function

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and activity and indirectly reflects platelet production and stimulation. Platelets play a key role in immune and/or inflammatory events (5). RDW reflects variability in red blood cell size and plays a role in the detection of false-negative mean corpuscular volume results in the assessment of morphological alterations in erythrocytes (6).

The present study aims at evaluating the use of CBC parameters including RDW, PDW, MPV, NLR, and PLR as diagnostic markers in patients with RAS. Additionally, the present study, to our knowledge, is the first of its kind to evaluate the effects of all these five parameters in RAS through a holistic approach. With this study, we aim to compare the RAS patients in the world with the conditions of such patients in our region. In RAS, it is aimed to take necessary measures in line with the underlying causes and to plan the treatments for these reasons.

MATERIAL AND METHODS

The retrospective case-control study included a RAS group of 51 patients that presented to Ear-Nose-Throat (ENT) department between October 2018 and May 2019 with at least three episodes of active RAS within the previous one year and a control group of 51 age- and gender-matched healthy volunteers. This study was accepted by Scientific Research Ethic Board. It was performed in accordance with the Helsinki Declaration.

A detailed ENT examination was performed in each patient and the patients' CBC results were retrieved from their medical records.

CBC test was performed in all subjects using a hematology analyzer (Sysmex XE-2100; Sysmex, Kobe, Japan), and the parameters including white blood count (WBC), absolute neutrophil, lymphocyte, and platelet counts, RDW, MPV, and PDW were recorded for each subject. NLR and PLR were calculated as follows: NLR=absolute neutrophil count/absolute lymphocyte count; PLR=absolute platelet count/absolute lymphocyte count.

Inclusion criteria included a history of RAS for at least two years, a diagnosis of minor or major RAS, and no history of smoking. Exclusion criteria were as follows: a known diagnosis of cardiovascular and endocrine diseases, metabolic syndrome, Behçet's disease, recurrent herpetic ulcers, malignancies, acute and chronic inflammatory diseases, vertigo, tinnitus, obesity, facial palsy, platelet and neutrophil disorders, a history of drug or alcohol abuse, and a history of drug use including steroids, oral contraceptives, aspirin, iron supplementation drugs, vitamins, and immunosuppressive drugs.

Main Points:

- NLR and PLR cannot be considered as valuable parameters for routine diagnosis and in the prediction of prognosis in RAS patients.
- Increased RDW, PDW, and MPV values could have a diagnostic value in RAS patients.
- Inflammation, thrombosis and acute hypoxic ischemia should be prioritized in the etiology of RAS.

Statistical Analysis

Data were examined using IBM Statistical Package for the Social Sciences Standard Concurrent User V 25 (IBM Corp., Armonk, NY, USA). Descriptives were expressed as frequencies, mean, and standard deviation (SD). The two groups were compared by Independent-samples t-test for parametric data. Categorical variables were evaluated using Chi-square test. The two groups were compared regarding RDW, PDW, MPV, NLR, and PLR values. P value of <0.05 was considered significant.

RESULTS

The patient group comprised 29 (56.9%) women and 22 (43.1%) men with a mean age of 44.35±15.43 years. The control group comprised 26 (51.0%) women and 25 (49.0%) men with a mean age of 46.58±17.62 years. No significant difference was found between the two groups with regard to age and gender (p>0.05) (Table 1).

Mean RDW was 15.66±2.03% in the RAS group and 14.86±1.44% in the control group (p=0.026). Mean PDW was 15.44±2.86% in the RAS group and 14.42±1.69% in the control group (p=0.032). Mean MPV was 8.82±0.87 fL in the RAS group and 8.42±0.56 fL in the control group (p=0.007). Mean NLR was 1.94±0.74 in the

TABLE I. Distribution of demographic and complete blood count values between recurrent aphthous stomatitis (RAS) and control groups (Independent sample test and chi-square test were applied between groups)

Variable	RAS Group (n=51) Mean±SD	Control Group (n=51) Mean±SD	p
Age	44.35±15.43	46.58±17.62	0.497
Gender			
Female	29 (56.9%)	26 (51%)	
Male	22 (43.1%)	25 (49%)	0.556
White Blood Cell (WBC) Count	6.96±1.99 $10^3/mm^3$	6.65±1.83 $10^3/mm^3$	0.419
Absolute Neutrophil Count	4.10±1.47 $10^3/mm^3$	3.78±1.43 $10^3/mm^3$	0.268
Absolute Lymphocyte Count	2.21±0.71 $10^3/mm^3$	2.19±0.59 $10^3/mm^3$	0.846
Absolute Platelet Count	246.92±59.36 $10^3/mm^3$	255.75±56.43 $10^3/mm^3$	0.444
Neutrophil to lymphocyte r atio (NLR)	1.94±0.74	1.80±0.80	0.374
Platelet to lymphocyte ratio (PLR)	119.49±36.58	121.98±32.96	0.718
Mean platelet volume (MPV)	8.82±0.87 fL	8.42±0.56 fL	0.007*
Red cell distribution width (RDW)	15.66±2.03%	14.86±1.44%	0.026*
Platelet distribution width (PDW)	15.44±2.86%	14.42±1.69%	0.032*

SD: Standard Deviation
* Statistically significant

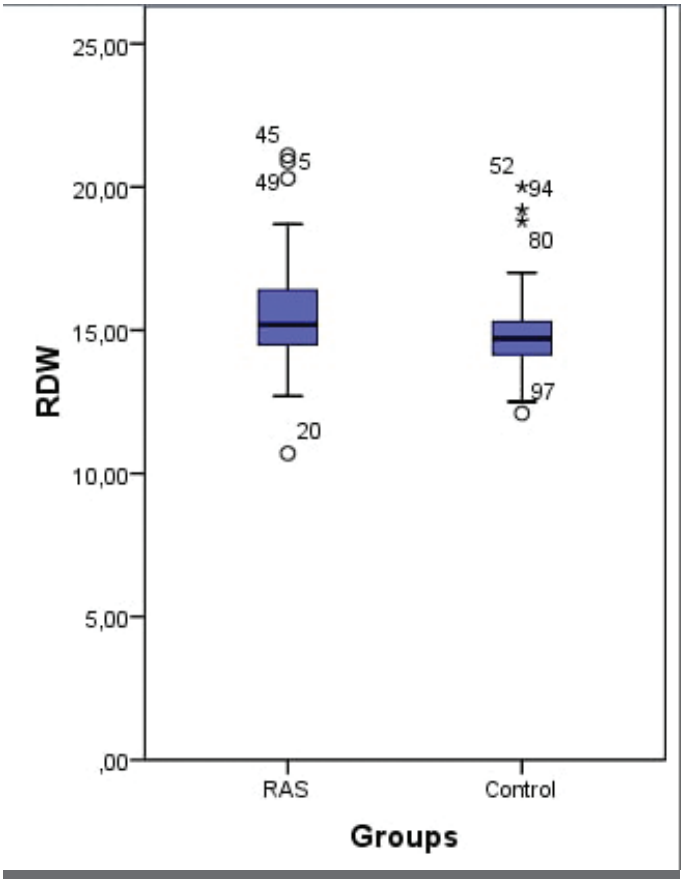

FIGURE 1. Distribution of red blood cell distribution width (RDW) values between RAS and control groups

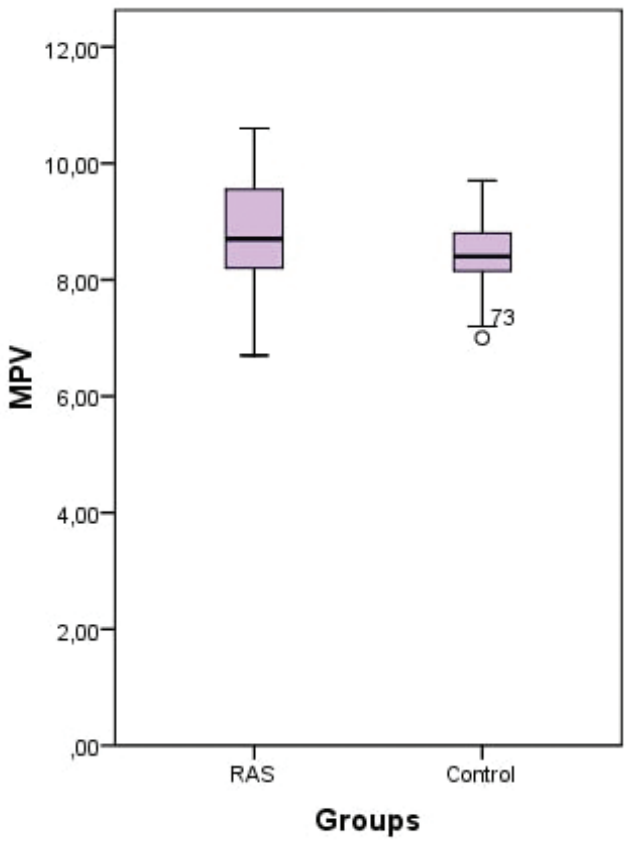

FIGURE 3. Distribution of mean platelet volume (MPV) values between RAS and control groups

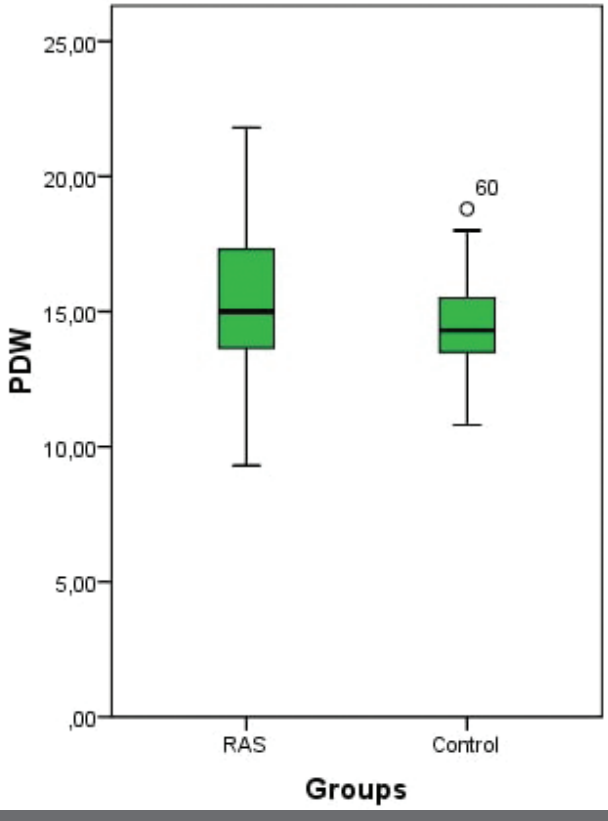

FIGURE 2. Distribution of platelet distribution width (PDW) values between RAS and control groups

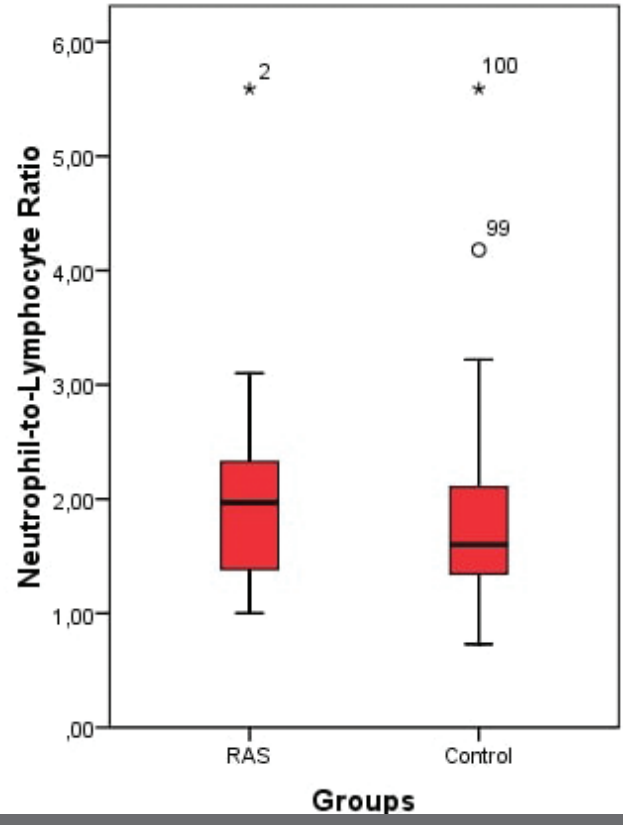

FIGURE 4. Distribution of neutrophil-to-lymphocyte ratio (NLR) values between RAS and control groups

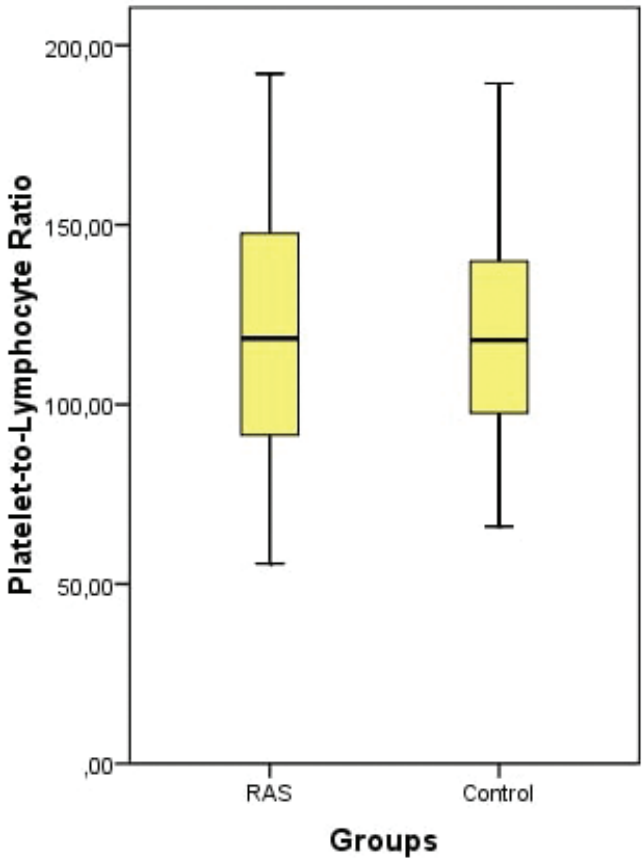

FIGURE 5. Distribution of platelet-to-lymphocyte ratio (PLR) values between RAS and control groups

RAS group and 1.80±0.80 in the control group (p=0.374). Mean PLR was 119.49±36.58 in the RAS group and 121.98±32.96 in the control group (p=0.718). The RDW, PDW, and MPV values were significantly higher in the RAS group compared to the control group (Figure 1, 2, and 3). Although NLR was significantly higher in the RAS group compared to the control group, no significant difference similar to that of PLR was found (Figure 4 and 5). On the other hand, no significant difference was found between the RAS and control groups with regard to WBC and neutrophil, lymphocyte, and platelet count (p>0.05) (Table 1).

DISCUSSION

The exact etiology and pathogenesis of RAS remains unknown although it has been attributed to chronic inflammation. The formation of RAS is multifactorial. These are positive family history, smoking cessation, immune system disorder, excessive food sensitivity, genetic, allergic, microbial factors, immunosuppressive drugs and psychological stress (1, 7). The etiology of RAS is largely unknown and racial/ethnic differences may help explain disparities in RAS presentation. The differential diagnosis of RAS includes PFAPA (Periodic fever - aphthous stomatitis-pharyngitis - adenopathy) syndrome, Behçet's disease, Crohn's disease, celiac disease, acquired immunodeficiency syndrome (AIDS), nutrition disorders, and immune and neutrophil disorders. Moreover, numerous other factors have been blamed in the formation of RAS, including relevant family history (genetic predisposition), food hypersensitivity, cessation of smoking, psychological stress, vascular abnormalities, oxidative stress, endocrine disorders, mechanical trauma, viral and bacterial infections, vitamin and mineral deficiencies, immune system disorders, and anxiety (1, 2).

RAS is typically diagnosed by family history and physical examination. However, there are no hematological or biochemical inflammatory markers used for the diagnosis of RAS. Typical histological findings of RAS include vascular dilatation, inflammatory cell infiltrate, and epithelial ulceration. RAS is classified into three types based on clinical manifestation – minor, major, and herpetiform. Of these, the minor type is the most common form detected in 80-90% of all cases, in which the lesion size is often <5 mm (8).

Both NLR and PLR are new biomarkers of subclinical inflammation that have emerged as popular markers in the determination of the severity of inflammation and the diagnosis and prognosis of various diseases (9, 10). In RAS patients, the increased production of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-2, and IL-12 and the decreased production of antiinflammatory cytokines such as IL-10 implicate the role of inflammation in RAS (11). Additionally, NLR is a valuable diagnostic tool in the prediction of long-term mortality and poor prognosis in various malignant diseases (12).

Increased NLR has been reported in numerous clinical conditions including vascular pathologies such as ischemic cerebrovascular events and acute coronary syndrome, as well as inflammatory diseases including head and neck squamous cell carcinoma (HNSCC), sudden hearing loss, peripheral facial palsy, vertigo, ulcerative colitis, and appendicitis (12-18). Acartürk et al. (19) used NLR as an inflammatory marker for the assessment of the severity inflammatory bowel disease and found a significant correlation between NLR and disease severity. Seçkin et al. (20) evaluated CBC parameters in a total of 60 RAS patients both before and three months after the colchicine treatment and found a significant decrease in NLR, WBC, and RDW values while they found no significant change in the MPV, PLR, and hemoglobin values. Similarly, Terzi et al. (21) evaluated a cohort of 80 RAS patients and found significantly increased NLR values, which confirmed the role of inflammation in the pathogenesis of RAS. However, the authors found no significant difference with regard to PLR values between the patient and control groups.

PLR is a novel marker that reflects chronic inflammation and has been associated with atherosclerosis and peripheral arterial occlusive disease (POAD) (22). Additionally, there are some studies suggesting that PLR is a better marker than NLR in patients with soft tissue carcinomas and end-stage renal disease (23, 24). In our study, however, both NLR and PLR established no significant difference between the RAS and control groups.

MPV is an inexpensive and practical inflammatory marker of chronic diseases. Increased MPV values reflect increased platelet activation (*i.e.* platelet dysfunction), which, in turn, may aggravate inflammation. MPV has been shown to increase in various conditions including cardiovascular and cerebrovascular diseases, atherosclerosis, venous/arterial thrombosis, and thromboembolism (5). In ENT practice, increased MPV values have been reported particularly in patients with hypoxic conditions, idiopathic sudden hearing loss, and subjective tinnitus, and MPV has been shown to be a significant marker (25, 26). In

a similar way to our study, a study by Şereflican et al. suggested that MPV could be a diagnostic marker in RAS patients (3). Another study found a significant relationship between increased MPV values and Behçet's disease with thrombotic tendency while no significant relationship was established between NLR and the disease (27). Similarly, Ekiz et al. (28) found significantly increased MPV values in patients with RAS and Behçet's disease compared to healthy controls. Moreover, the authors proposed that increased MPV values can be indicative of inflammation, as was revealed in our study.

RDW is a potential marker of acute hypoxemia which can increase in anemia, hematological diseases, and myelodysplastic syndromes and may lead to peripheral microvascular diseases (29). Felker et al. (30) postulated that RDW could be an ischemic marker in the prediction of coronary artery disease and cardiac failure. Based on these notions, the present study investigated the ischemic relationship between RDW and RAS. The results indicated that RDW was significantly increased in RAS patients compared to healthy controls, thus supporting the existence of an ischemic relationship between RDW and RAS.

Ischemic and hypoxic tissues stimulate the production of reticulated platelets from bone marrow, thereby promoting platelet production, and ultimately leading to increased PDW values. The increased PDW values, in turn, may trigger thrombosis (4).

According to our knowledge, literature reviews indicate that there have never been study reporting on a relationship between RDW and PDW in RAS patients. The present study revealed that PDW was significantly increased in RAS patients. The limitations of study can be counted meticulous selection of patients and control subjects based on the exclusion criteria of the study, small sample size, and heterogeneity of the RAS group.

The results indicated that both NLR and PLR cannot be considered as valuable parameters for routine diagnosis and in the prediction of prognosis in RAS patients, and that the use of these parameters as inflammatory markers remains controversial. Nonetheless, it was revealed that increased RDW, PDW, and MPV values could have a diagnostic value in RAS patients. Accordingly, it is wise to consider that inflammation, thrombosis and acute hypoxic ischemia should be prioritized in the etiology of RAS. Future studies with larger patient series and long-term follow-ups are needed to substantiate our findings.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Nevsehir Hacı Bektaş Veli University Scientific Research Ethics Committee (26.04.2019/2019.06.62).

Informed Consent: Due to the retrospective design of the study, informed consent was not taken.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Author has no conflict of interest to declare.

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Original Article

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Clinical Value of Hemoglobin and Albumin Levels and Lymphocyte And Platelet Count (HALP) Combination in Predicting Postoperative Complications, Lymph Node Positivity and Prognosis in Gastric Cancer Patients Who Underwent Curative Surgical Resection

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BACKGROUND/AIMS

In this study, we aimed to determine the clinical value of the Hemoglobin and Albumin Levels and Lymphocyte and Platelet (HALP) score in predicting postoperative complications, lymph node positivity and prognosis in patients with gastric cancer undergoing curative surgical resection.

MATERIAL and METHODS

Patients who underwent total gastrectomy for gastric adenocarcinoma between 2015-2018 were included in the study. Two groups, Group1 (lowHALP) and Group2 (highHALP), were formed. Demographic and clinical characteristics and mean survival were compared. The value of HALP score in predicting lymph node positivity and postoperative complications was evaluated at the determined cut-off value.

RESULTS

Patients were divided into two groups according to the cut-off value of 14.98. Group 1 consisted of 20 patients and Group 2 consisted of 62 patients. The average age in Group 1 was higher than Group 2 (65vs57) (p=0.046). Female sex was higher in Group 2 than Group 1 (38.7%vs15%) (p=0.042). Total survival time was higher in Group 1 (41vs28) (p=0.02). We did not find HALP score as a risk factor for survival in multivariate analysis (HR=0.247, 95% CI=0.113-0.485, p=0.061). According to the cut-off value, if the HALP value was above 14.98, it was seen that ClavienDindo 2 and more complications developed with 84.09% sensitivity and 33.33% specificity. It is assumed that the person's lymph node is positive with a HALP value 9.14 and below, with sensitivity of 20.00% and specificity of 96.97%.

CONCLUSION

Our findings showed that HALP is closely related to clinicopathological features but it is not an independent prognostic factor for survival. Its value in predicting the risk of complication development and lymph node positivity is limited.

Keywords: Gastric cancer, HALP score postoperative complications, prognosis

INTRODUCTION

Gastric cancer is the fourth most common cancer and the second most common cause of cancer-related deaths (1). The incidence in our country is approximately 6.3-14.2 per hundred thousand. It ranks 2nd in cancer related deaths in men and 4th in women in the world (2). Gastric cancer is still an important health problem in terms of incidence and prognosis. Knowledge of prognostic factors in gastric cancer is important for determining survival and optimal treatment strategies.

Tumor, nodes, and metastases (TNM) stage and distant metastasis of tumor have been accepted as the primary factor in determining prognosis (3). The disadvantage of TNM staging in determining prognosis is that it only reflects the characteristics of cancer. In particular, the outcomes of some patients at the same stage may be completely different. Therefore, other factors should be considered in predicting prognosis in patients with gastric cancer. Recently, various

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predictions have been defined and applied to predict the short- and long-term outcomes of gastric cancer patients. These predictions include cancer-related factors, host-related factors, surgical-related factors, and systemic inflammatory response markers (4-9).

Systemic inflammation and nutritional status play an important role in the prognosis of cancer patients (10). Hemoglobin and albumin levels are commonly used markers to assess nutritional status and performance of the patient. Anemia and low albumin were associated with poor prognosis in cancer (11, 12). In previous studies, increased preoperative platelet count was associated with increased recurrence, serosal invasion and advanced stage of gastric cancer (13). Lymphocytes play an important role in defense against cancer by inducing cytotoxic cell death, inhibiting cancer cell proliferation and migration (14). Based on this evidence, several inflammatory index combinations such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) HALP combination and systemic immune-inflammation index have been used to predict prognosis (15-19).

In recent studies, a new composite index named HALP, calculated as Hemoglobin (g/L) × Albumin (g/L) × Lymphocyte (/L) / Platelet (/L) was reported to be related to survival in gastric cancer (19), colorectal cancer (20), bladder cancer (21), and renal cancer (22) patients. Currently, there is no study on the relationship between HALP and postoperative complications in gastric cancer and its ability to predict lymph node positivity.

In this study, we aimed to determine the relationship between preoperative hemoglobin, albumin level and lymphocyte and platelet count (HALP) combination with prognosis, postoperative complications and lymph node positivity in patients with gastric cancer undergoing curative resection.

MATERIAL AND METHODS

Study Population and Data Collection

120 patients who underwent curative surgery for gastric cancer in Çukurova Medical Faculty General Surgery Clinic, between January 2015 and December 2018, were included in the study. Permission was obtained from Çukurova University Faculty of Medicine Ethics Committee dated 04.09.2019 and numbered 91/26. Patient files and hospital information system records were examined prospectively, and a database was created. Patients were analyzed retrospectively using this database. Patients with stage IV gastric cancer, patients under eighteen years of age, pregnant patients, patients with chronic inflammatory disease (Tuberculosis, Sarcoidosis, etc.), patients who underwent palliative surgery, patients with autoimmune disease, patients with hematologic disease, who were using steroids for any reason and whose records could not be accessed were excluded from the study. The remaining 82 patients were included in the study.

Main Points:

- Nutritional and immune status is important to the prognosis of patients with gastric carcinoma.
- HALP score is closely related to clinicopathological features but it is not an independent prognostic factor for survival.
- We did not find any correlation between HALP score and risk of postoperative complications.
- HALP score is closely related to clinicopathological features.

After the cut-off value was determined by ROC curves, the patients were divided into two groups according to the cut-off value as Group 1 (low HALP) and Group 2 (high HALP). Demographic characteristics, Body Mass Index (BMI), comorbidities, American Society of Anesthesiologists (ASA) score, neoadjuvant treatment status, type and nature of the operation, tumor localization and tumor pathologic stage were recorded. Pathological stage of the tumor, total and metastatic lymph node number, operation duration, mean blood loss, conversion rate in laparoscopic cases, mean time to start oral intake, postoperative complication status according to Clavien Dindo classification (23), rate of anastomosis leakage, postoperative hospital stay, 30-day mortality, unplanned re-admission rate and total survival rate in the postoperative 30-day period were recorded and this information was compared between the two groups. The clinical value of the HALP score in predicting postoperative complications and lymph node positivity was calculated.

Tumor-node-metastasis (TNM) 2016 system was used for tumor staging.

Anastomosis leakage was defined as a deterioration of anastomosis integrity determined by combination of clinical, radiological and operative tools.

Wound infection was defined as superficial or deep incisional surgical site infection in the surgical wound according to the definition of the Centers for Disease Control (CDC) (24).

Tumor invasion depth was evaluated preoperatively by endoscopic ultrasound in suspected cases. Contrast-enhanced thorax, upper and lower abdominal computed tomography were performed for staging and Positron Emition Tomography (PET-CT) was performed for metastasis screening.

The total blood count was measured by an automated hematology analyzer (Roche Hitachi *Cobas® 8000* Roche Diagnostics, Indianapolis, IN, USA). While calculating the HALP index, hemoglobin (g/L), albumin (g/L), lymphocytes (/L), platelets (/L) units conversion was performed in normal value units.

Statistical Analyses

Data were analyzed using IBM Statistical Package for the Social Sciences for Windows, version 24 (IBM Corp.; Armonk, NY, USA). When evaluating the data of the study, in addition to descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum), Student's t test was used for the comparison of quantitative data and Mann Whitney U test was used for the evaluation of neutrophil/lymphocyte ratio which did not show normal distribution. Pearson's Chi-squared test and Fisher's Exact test were used to compare qualitative data, and logistic regression was used for multivariate evaluations. The patients were divided into two groups according to survival and cut off value was found by ROC analysis. The cut-

off value for lymph node positivity was calculated by dividing the cases into lymph node positive and negative groups. Diagnostic accuracy was evaluated using receiver operating characteristic (ROC) curve analysis Appropriate cut-off values were identified, and sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were calculated for parameters with an area under the curve (AUC) of above 0.600. To assess the association of HALP with gastric cancer overall survival, multivariate Cox's proportional hazard model was conducted to estimate Hazard ratios (HRs) and their 95% confidence intervals (CIs). Kaplan-Meier and Log Rank tests were used for survival analysis. A p value of <0.05 was considered statistically significant.

RESULTS

In order to create a cut-off value for HALP, ROC analysis and ROC curve were created. As a result of ROC analysis, the area under the ROC curve was calculated as 50.3%. The obtained cut-off value gives a correct answer at a rate of 50.3%. According to the cut-off value, if the HALP value was above 14.98, it was seen that Clavien Dindo 2 and more complications developed with 84.09% sensitivity and 33.33% specificity. The findings of this evaluation are shown in Table I and Figure I.

In order to create a cut-off value for HALP, ROC analysis and ROC curve were created. As a result of ROC analysis, the area under the ROC curve was calculated as 50.3%. The obtained cut-off value gives a correct answer at a rate of 50.3%. According to the cut off value obtained, the lymph node positivity is assumed to be positive for a HALP value of 9.14 and below, with a sensitivity of 20.00% and specificity of 96.97%. It is shown in Table 2 and Figure 2.

Patients were divided into two groups according to the cut-off value of 14.98. Group I consisted of 20 patients and Group 2 consisted of 62 patients. The average age in Group I was higher than

TABLE I. Proposed cut-off values for significant parameters in postoperative complications, Clavien Dindo 2 and above

	HALP
AUC	0.503
Cut-off	>14.98
Specifity	33.33
95%-CI (%)	19.1-50.2
Sensitive (%)	84.09
95%-CI (%)	69.9-93.4
PPV	58.7
NPV	65.0
+LR	1.26
-LR	0.48
p	0.958

AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; OR: Odds ratio; +LR: Positive likelihood ratio; -LR: Negative likelihood ratio; HALP: Hemoglobin and Albumin Levels and Lymphocyte and Platelet

TABLE 2. Proposed cut-off values for significant parameters in lymph node positivity

	HALP
AUC	0.503
Cut-off	<9.14
Specificity	96.97
95%-CI (%)	84.2-99.9
Sensitive (%)	20.0
95%-CI (%)	10.0-33.7
PPV	90.9
NPV	44.4
+LR	6.60
-LR	0.83
p	0.962

AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; OR: Odds ratio; +LR: Positive likelihood ratio; -LR: Negative likelihood ratio; HALP: Hemoglobin and Albumin Levels and Lymphocyte and Platelet

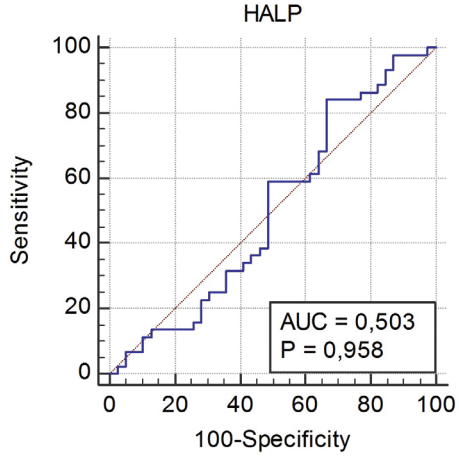

FIGURE I. Receiver operating characteristic (ROC) curve analyses for postoperatif complications

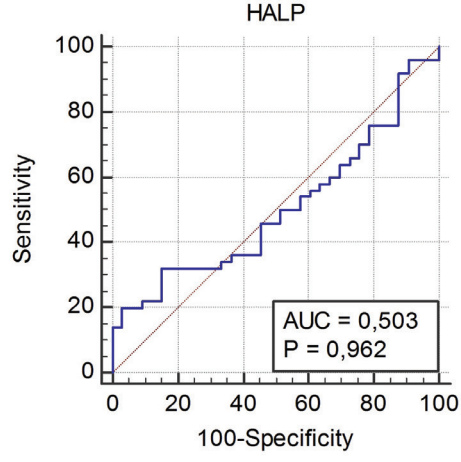

FIGURE 2. Receiver operating characteristic (ROC) curve analyses for lymph node positivity

Group 2 (65 vs 57) (p=0.046). Female sex was higher in Group 2 than Group I (38.7% vs 15%) (p=0.042). Body mass index was higher in Group 2 than Group I (25.4 vs 22.91) (p=0.020). No statistically significant difference was found between the groups in terms of ASA scores and neoadjuvant treatment (p>0.05). Demographic characteristics and preoperative findings of the patients are shown in Table 3.

In both groups, conventional surgical operations were performed more frequently (95% vs 82.3%) than other surgical techniques (p=0.149). Duration of operation was similar in groups (2188 vs 232 min, p=0.385). Postoperative complication rates were similar among the groups according to the Clavien Dindo classification (p=0.298). Anastomotic leakage rates were similar in both groups (p=0.692). Postoperative mortality rates were similar in both groups (20% vs 8.1%; p=0.142). Postoperative hospital stay was similar among the groups (p=0.157). The most common reason for admission to the hospital within 30 days after discharge was wound infection (10% vs 9.7%, p=0.134). Intraoperative and postoperative outcomes are shown in Table 4.

Tumor localization was most commonly in the antrum in both groups (p=0.646). The total number of dissected lymph nodes was similar in the groups (30 vs 29) (p=0.876). The number of positive lymph nodes was significantly higher in Group I than Group 2 (14 vs 5; p=0.002). The rate of lymph node positivity was similar in the groups (p=0.089). The pathological stage was similar in the groups (p=0.110). The rate of well-differentiated tumors was lower in Group I (5% vs 29%; p=0.047). The pathological features of the tumors are shown in Table 5.

TABLE 3. Characteristics of patients

		Group I Low HALP	Group2 High HALP	p*
Age (min-max)		65,30+17,82 (14-89)	57.53+13.91 (28-85)	0.046
Sex	Female	3 (15.0)	24 (38.7)	0.042
	Male	17 (85.0)	38 (61.3)	
ASA score	I	12 (60.0)	33 (53.2)	0.449
	2	4 (20.0)	21 (33.9)	
	3	4 (20.0)	8 (12.9)	
BMI (min-max)		22,91+2,41 (19-28.6)	25.4±4.47 (16-40.3)	0.020
Neoadjuvant Chemotherapy	No	16 (80.0)	43 (69.4)	0.268
	Yes	4 (20.0)	19 (30.6)	

* p<0.05; BMI: Body Mass Index; ASA: American Society of Anesthesiologists score; HALP: Hemoglobin and Albumin Levels and Lymphocyte And Platelet

TABLE 4. Intraoperative and Postoperative outcomes

		Group I Low HALP	Group2 High HALP	p*
Operation type	Open	19 (95.0)	51 (82.3)	0.149
	Laparoscopic	1 (5.0)	11 (17.7)	
Operation duration (min)		218.25±29.03 (180-310)	232.50±70.76 (170-500)	0.385
Complication (Clavien Dindo)	I	1 (5.0)	14 (22.6)	0.298
	2	12 (60.0)	34 (54.8)	
	3A	3 (15.0)	9 (14.5)	
	3B	1 (5.0)	2 (3.2)	
	5	3 (15.0)	3 (4.8)	
Anastomosis leakage	None	17 (85.0)	55 (88.7)	0.692
	Stump leak	1 (5.0)	4 (6.5)	
	Esophagojejunostomy	2 (10.0)	3 (4.8)	
Postoperative mortality	Yes	4 (20.0)	5 (8.1)	0.142
	No	16 (80.0)	57 (91.9)	
Postoperative duration of hospitalization (day)	14.0+11.88 (5-45)	10.79+7.48 (2-46.0)	0.157	
30-day readmission to the hospital	None	15 (75.0)	55 (88.7)	0.123
	Ileus	1 (5.0)	1 (1.6)	
	Oral intake disorder	1 (5.0)	0 (0.0)	
	Pneumonia	1 (5.0)	0 (0.0)	
	Wound site infection	2 (10.0)	6 (9.7)	

HALP: Hemoglobin and Albumin Levels and Lymphocyte And Platelet

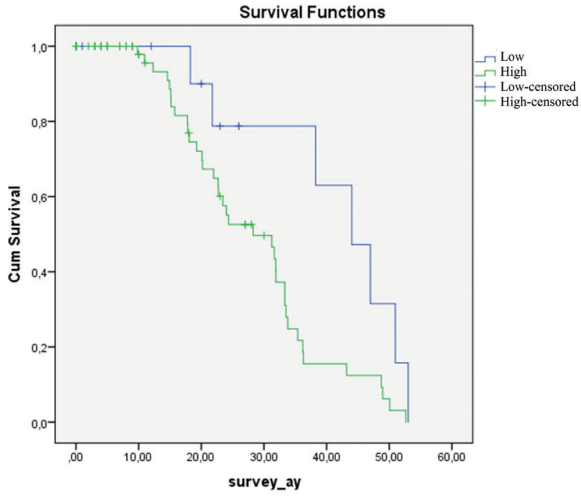

FIGURE 3. Total survival time according to HALP groups

Total survival time was higher in Group I (41 vs 28; p=0.02). It is shown in Table 6 and Figure 3.

There were statistically significant differences in univariate and multivariate analyzes in terms of age and pathological grade groups (p <0.01). There was no statistically significant difference between the patients in terms of sex, pathological stage, HALP group, lymph node positivity and tumor localization (p>0.05). Univariate and multivariate analyzes of the variables of age, sex, pathological grade, pathological stage, HALP score level, lymph node positivity and tumor localization are shown in Table 7.

DISCUSSION

Proper prognostic evaluation is necessary for the optimal treatment of gastric cancer. TNM staging system plays an important role in the prognostic evaluation of gastric cancer in routine clinical practice. However, clinical outcomes may vary between pa-

TABLE 5. Characteristics of tumor

		Group I Low HALP	Group2 High HALP	p*
Tumor localization	Antrum	6 (30.0)	25 (40.3)	0.646
	Cardia	1 (5.0)	4 (6.5)	
	Corpus	7 (35.0)	19 (30.6)	
	Small curvature	3 (15.0)	9 (14.5)	
	Linitis Plastica	3 (15.0)	3 (4.8)	
	EGJ	0 (0.0)	2 (3.2)	
Total dissected lymph node number (mean) (min-max)		30.35+15.85 (3-62)	29.79+13.16 (7-63)	0.876
Positive lymph node number (mean) (min-max)		14.05+15.94 (0-47)	5.54+7.77 (0-38)	0.002
Lymph node positivity	Negative	5 (25.0)	28 (45.2)	0.089
	Positive	15 (75.0)	34 (54.8)	
pSTAGE	IA	0 (0.0)	12 (19.4)	0.110
	IB	1 (5.0)	5 (8.1)	
	2A	1 (5.0)	4 (6.5)	
	2B	4 (20.0)	16 (25.8)	
	3A	1 (5.0)	7 (11.3)	
	3B	3 (15.0)	3 (4.8)	
	3C	10 (50.0)	15 (24.2)	
Pathological grade	Non-differentiated	3 (15.0)	11 (17.7)	0.047
	Poorly differentiated	9 (45.0)	25 (40.3)	
	Mildly differentiated	7 (35.0)	8 (12.9)	
	Well differentiated	1 (5.0)	18 (29.0)	

* p<0.05; EGJ: Esophagogastric junction; HALP: Hemoglobin and Albumin Levels and Lymphocyte and Platelet

TABLE 6. Total survival time according to HALP groups

		Mean (Mean+sd (Min-Max))	Median (Mean+sd (Min-Max))	p
HALP Group	Low HALP	41.0+4.32	44.02+5.27	
		32.54-49.47	33.68-54.37	0.020
	High HALP	28.44+1.86	28.26+5.15	
		24.8-32.09	18.16-38.37	

HALP: Hemoglobin and Albumin Levels and Lymphocyte and Platelet

tients with the same staging process, and this is evidence that the TNM staging system is not sufficient to predict prognosis (25-27). Due to the insufficiency of the TNM staging system in determining the prognosis, researchers have begun to work on new scoring systems.

It is widely accepted that inflammatory response and nutritional status are associated with the prognosis of cancer patients (4). Serum albumin is one of the most commonly used indicators to predict the nutritional status of the patient. Serum albumin level is one of the parameters used to evaluate cancer progression and prognosis. Low albumin levels in cancer patients are correlated with low survival rates (28). Anemia is a common finding in cancer patients and is considered a negative prognostic factor (11). Lymphopenia is also common in advanced cancer patients and is a warning for cancer progression. Metastasis formation is associated with platelet stimulation. Platelets are thought to protect cancer cells from immunological attacks (29-31). HALP is an integration of these four hematological and biochemical parameters and has been shown to have a prognostic value in patients with gastric cancer (19).

In this study, the HALP score was calculated based on preoperative hemoglobin, albumin, lymphocyte and platelet values and its importance in the prognostic evaluation of gastric cancer was evaluated.

In this study, the clinical significance of the HALP score in predicting cases with a score of 2 or more according to the Clavien Dindo scoring system evaluating postoperative complications was investigated. At a 14.98 cut off value, the sensitivity in detecting complications was as high as 84%, while the specificity was very low at 33%. When used to estimate lymph node positivity, the specificity of detecting lymph node positivity at a cut-off value of 9.14 was very high (96.7%), whereas its sensitivity was low (20%).

Chen XL et al. (19) found the HALP score to be closely related to many clinicopathological features such as tumor diameter, T and N stage, and vascular invasion. They found the HALP score as a risk factor for survival in multivariate analyzes, and the median survival time and overall survival rates for 1, 2, 3 years were higher in the high HALP group. In our study, it was associated with age, sex, and body mass index. We did not find any relationship

TABLE 7. M Univariate and multivariable analysis of factors associated with overall survival in gastric cancer

		Univariate	Multivariate	
Measurements		**p**	**HR (95% - CI)**	**p**
Age group	<59	0.006	1.00	0.003
	>59		0.258 (0.076-0.441)	
Sex	Male	0.051	1.00	0.058
	Female		0.198 (0.110-0.396)	
Pathological grade	Poorly differentiated	0.046	1.00	0.039
	Non-differentiated			
	Well differentiated			
	Mildly differentiated		0.414 (0.126- 0.702)	
Pathological stage	IA	0.108	1.00	0.225
	IB			
	2A			
	2B			
	3A		0.233 (-0.147- 0.613)	
	3B			
	3C			
HALP	<14.98	0.055	1.00	0.061
	>14.98		0.247 (0.113-0.485)	
Lymph node positivity	Negative	0.113	1.00	0.148
	Positive		0.155 (-0.037-0.346)	
Tumor localization	Antrum	0.664	1.00	0.545
	Cardia			
	Corpus			
	Small curvature		0.194 (-0.828-0.441)	
	Linitis Plastica			
	EGJ			

EGJ: Esophagogastric junction; HR: Hazard ratios; CI: confidence intervals; HALP: Hemoglobin and Albumin Levels and Lymphocyte and Platelet

between intraoperative and postoperative outcomes. Contrary to the findings of Chen XL et al. (19), it was not associated with pathological tumor stage. However, the rate of positive lymph nodes was high, especially in the group with low HALP scores (14 vs 5; p=0.002). The pathological grade had a higher proportion of well differentiated tumors in the group with high HALP (29% vs 5%; p=0.047).

We did not find the HALP score as a risk factor for survival in the multivariate analysis. The mean total survival time was higher in the low HALP group, contrary to expectations (41 months vs 28 months; p=0.020).

The most important limitation of our study was its retrospective evaluation and single-centeredness. However, we believe that it provides comprehensive data and contributes to valuable reference data in terms of HALP score's value in predicting lymph node positivity in gastric cancer. Multicenter prospective studies are needed to confirm our findings.

In our study contrary to what I expected to have a low Halp score, we did not find it related to poor prognosis. Preoperative high HALP score was found to be associated with poor prognosis. We did not find any correlation between HALP score and risk of postoperative complications. The HALP score is an easy-to-access and inexpensive biomarker. However, it cannot be used as a prognostic factor alone in gastric cancer. The. Prognostic tools are needed to create personalized cancer treatment programs.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Çukurova University Faculty of Medicine (04.09.2019/91/26).

Informed Consent: Due to the retrospective design of the study, informed consent was not taken.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.Y., U.T., A.G.Ü., A.G.S.; Design - O.Y., U.T., A.G.Ü.; Supervision - O.Y.; Resources - O.Y., U.T., A.G.Ü., A.G.S.; Materials - O.Y., U.T., A.G.Ü., A.G.S.; Data Collection and/or Processing - U.T., A.G.Ü.; Analysis and/or Interpretation - O.Y., U.T.; Literature Search - O.Y., U.T., A.G.Ü., A.G.S.; Writing Manuscript - O.Y., U.T.; Critical Review - O.Y., U.T.

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Original Article

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Genistein-Induced Apoptosis Affects Human Telomerase Reverse Transcriptase Activity in Acute Promyelocytic Leukemia

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BACKGROUND/AIMS
The purpose of this study was to research the effects of genistein on telomerase activity and apoptosis in an acute promyelocytic leukemia cell line (HL-60).

MATERIAL and METHODS
In HL-60 cells, the cytotoxic effect of commercially available genistein was evaluated by the XTT method. The XTT method is a Cell Proliferation Assay. The Annexin V-EGFP method was used to determine apoptosis. The human telomerase reverse transcriptase (hTERT) is a marker of telomerase activity. hTERT gene expression analysis was performed by LightCycler real-time RT-PCR.

RESULTS
In HL-60 cells, the IC50 of genistein was found to be 50 µM at 72 hours. It was observed that the induction of apoptosis was 4.25-fold higher compared to the genistein untreated cells used as the control group. Compared to the control group, hTERT activity was found to decrease by 5.16, 3.81 and 5.04-fold at 24, 48 and 72 hours, respectively.

CONCLUSION
Induced apoptosis of HL-60 cells by the reduction of human telomerase reverse transcriptase activity may be a beneficial parameter in leukemia patients.

Keywords: Apoptosis, Genistein, HL-60, hTERT

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic malignancies characterized by the proliferation and maturation of myeloid blasts in bone marrow and blood (1).

Acute promyelocytic leukemia (APL) is characterized by translocation t (15;17). This translocation is responsible for the production of promyelocytic leukemia / retinoic acid receptor α (PML/RARα) fusion protein, which leads to inhibition of the differentiation of myeloid cells (2). Telomerase consists of two main components (hTERT and human telomerase RNA (hTR) in a ribonucleoprotein structure. High telomerase activity is seen in most malignant cells. Specific expression of telomerase hTERT in most tumor types provides good separation between cancerous and normal cells. hTERT expression is associated with human cells (3). Inhibition of hTERT expression is shown as a therapeutic target to terminate telomerase activity in cancer cells. Several studies have been conducted to inhibit telomerase activity in promyelocytic leukemia with arsenic and retinoid. Expression of hTERT in cultured human primary cells has been shown to allow for reactivation of telomerase activity and to promote immortal cell proliferation (4).

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Genistein, an isoflavonoid compound derived from soybeans, has been shown to benefit several cells. Most of the flavonoids inhibit Topoisomerase I and Topoisomerase II (5). Studies are showing that genistein induces apoptosis, anti-fiber proliferation, and inactivation of the MAPK pathway. Researches have also been performed on leukemia cells (6).

Genistein is a potent tyrosine kinase inhibitor that exhibits estrogen receptor binding activation and DNA topoisomerase II inhibition. Also, down-regulation of the PI3K/Akt and NF-kB signaling pathways leads to inhibition or activation of MAP kinase in the G2/M arrest, produced by the change of cell cycle regulatory proteins (7).

Genistein has an inhibitory effect on c-Myc oncogene. This inhibition is possible by the inhibition of tyrosine kinase activity. It affects tyrosine kinase activity and suppresses c-Myc activity and stops cell cycle. (8). Genistein suppresses the G2/M cell cycle and expresses markers of differentiation in human acute myeloid leukemia cell lines; genistein has been shown to increase differentiation by cooperating with ATRA (7). In this study, the effect of genistein on apoptosis and Human Telomerase Reverse Transcriptase Activity was investigated in acute promyelocytic cell lines.

MATERIAL and METHODS

Cell Culture and Chemicals

Genistein (Sigma Chemical Co., St. Louis Missouri) and the HL 60 cell line (ATCC, USA) are commercially available. The chemicals were diluted with 0.1% dimethylsulphoxide (DMSO) (Sigma -Aldrich). The HL-60 cell line was reproduced using RPMI-1640 (Sigma -Aldrich) medium containing 10000 U/mL penicillin, 10mg/mL streptomycin and 10% inactive fetal bovine serum (FBS) (Sigma -Aldrich), 1% L-glutamine (Sigma -Aldrich). It was incubated at 37ºC and in a 95% humidity, 5% CO_2 incubator. Our study was conducted according to the Helsinki Declaration.

XTT Assay

HL-60 cells (2×10^5 cells/mL) were plated on 96-well plates and incubated for 24 hours. At 24 h, cell viability was measured with the trypan blue dye exclusion test. HL-60 cells treated with genistein at concentrations ranging from 0 to 100 µM and were incubated for 24, 48 and 72 hours. Cytotoxicity of genistein was determined according to the kit protocol with the XTT kit (Cell Proliferation Kit, Roche). Cells without genistein treated were used as control. The optical density (OD) of each well was read in the microplate reader (Multiskan FC, Thermo) with 450 and 620-nm reference wavelength. Cell viability was determined by proportioning the OD values according to the control.

Main Points:

- Genistein has anti-proliferative effect in acute promyelocytic leukemia cell line.
- In acute promyelocytic leukemia cells, genistein was very effective on the hTERT gene expression.
- Genistein and hTERT activity may play a role in the treatment of acute promyelocytic leukemia.

Apoptosis Assay

The apoptotic effect of genistein's IC_{50} dose was investigated in the HL-60 cell line. HL-60 cells ($5x10^5$ cells/mL) were placed in 6 wells and treated with genistein's IC_{50} dose for hours. Acridine orange/ethidium bromide apoptosis assay was performed as described previously (8). The number of apoptotic, necrotic and live cells (Figure 1) were counted using a fluorescence microscope (BX-50, Olympus). The percentage of apoptosis was calculated as the total number of apoptotic cells divided by the total number of cells multiplied by 100.

Isolation of tRNA and hTERT Gene Expression Analysis

Total RNA (High Pure RNA Isolation Kit, Roche, Germany) was isolated from the cells exposed to doses of IC_{50} at 24, 48, and 72 hours. cDNA synthesis was performed using a RT2 first Strand Kit (Sigma, Germany). hTERT gene expression analysis was performed with a LightCycler real-time RT-PCR using a Lightcycler TeloTAGGG hTERT Quantification Kit (Roche, Germany). and was normalized to Porphobilinogen deaminase gene expression. Changes in hTERT gene expressions (fold change) were calculated by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The mean and standard deviation was calculated by using Excel 2016 (Microsoft). The IC_{50} value was calculated using the log (inhibitor) vs normalized response - variable slope model in Prism (GraphPad). A comparison of the two groups was analyzed using the *Student's t* test in Prism (GraphPad). Gene expression data were analyzed by the $2^{-\Delta\Delta CT}$ method, according to the Light Cycler 480 Quantification Software program (Roche, Germany). The change in hTERT expression of ≥±2-fold change according to the control group and p values <0.05 were considered significant.

RESULTS

The IC50 value of genistein was found to be 50 µM in a time- and dose-dependent manner in the HL-60 cells (Figure 2). We determined that the induction of apoptosis was significantly increased by 4.25-fold in the genistein-treated HL-60 cells ($p<0.05$, Figure 3). The activity of hTERT in the genistein-treat-

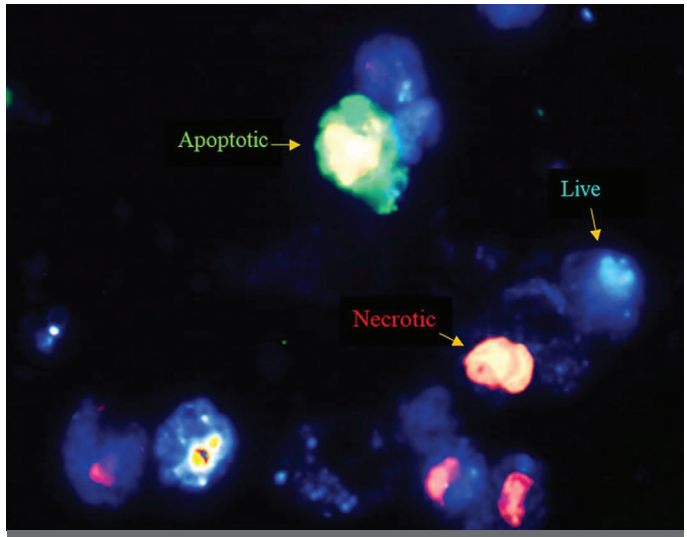

FIGURE 1. Apoptotic necrotic and live cells were visualized by fluorescence microscopy (40×)

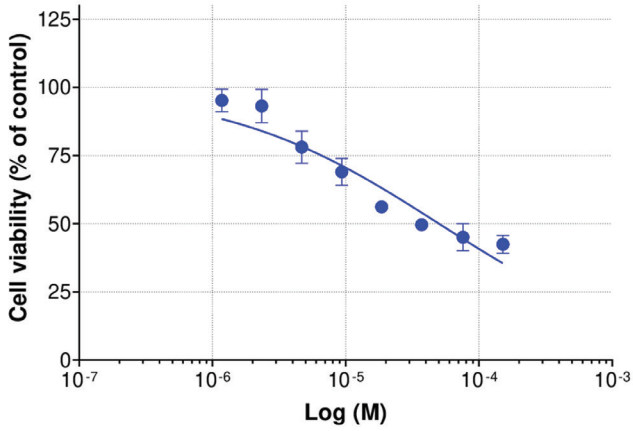

FIGURE 2. IC_{50} dose of genistein in HL-60 cell line

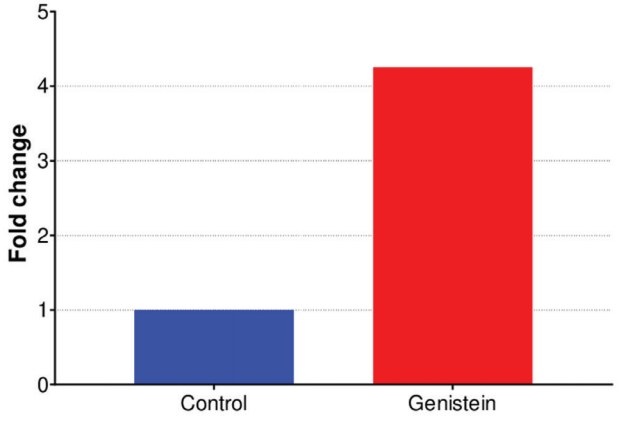

FIGURE 3. Apoptotic effect of Genistein at IC_{50} Dose

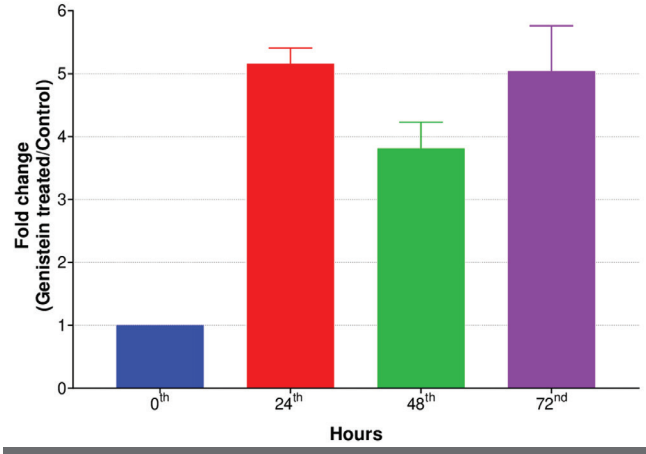

FIGURE 4. Fold change of hTERT gene expression in Genistein-treated HL-60 Cells. hTERT expressions were normalized to Porphobilinogen deaminase

ed HL-60 cells compared to the control group was found to be significantly 5.16, 3.81 and 5.04-fold down-regulation at 24th, 48th and 72nd hours respectively ($p<0.05$, Figure 4).

DISCUSSION

Many studies have been conducted on the discovery of anti-cancer drugs from natural agents that can induce apoptosis in cancer cells. It has shown that genistein exhibits anti-cancer effects that involve the inhibition of cell proliferation and induce apoptotic cell death in various cancer cells. The results obtained with Annex V/PI double staining showed that when compared to the control group, genistein increased significant apoptosis in HL-60 cells over time and is dose-dependent (6). Genistein has been shown to induce apoptosis in hematological malignant cells through multiple mechanisms while maintaining normal cells from toxicity (9). In our study, we found that genistein increased apoptosis by approximately 4.25-fold in HL-60 cells. Yamasaki et al. (10) found that genistein (30 µM) inhibited cell proliferation by inhibition of the G2/M cell cycle in adult T-cell leukemia cells. Some naturally occurring flavonoids, including genistein (dose range 0-50µm), have been shown to inhibit human promyelocytic leukemia cell lines (11).

Genistein has been found to suppress telomerase activity and hTERT gene expression levels in prostate cancer and brain tumor cells (12, 13). In the prostate cancer cell line (LNCAP), the anticancer effect of genistein has been investigated, and genistein has been shown to reduce hTERT and telomerase activity (13). It has effects such as anti-telomerase, anti-cancer, anti-inflammatory, anti-proliferative on various cancer cells such as breast cancer, prostate, melanoma (12). Genistein has been shown to inhibit hTERT transcription, the catalytic subunit of human telomerase activity in the MCF-7 cancer cell line depending on time and dose (14). Telomerase is expressed at high levels in malignant gliomas. Ferrandon et al. (15) found that the imetelstat telomerase inhibitor decreased cell proliferation and increased resistance to radiotherapy in both in vivo and in vitro studies. Telomere and telomerase enzyme have particularly been studied in chronic myeloid cells. Shorter telomere lengths were detected in chronic myeloid leukemia patients compared to healthy control groups (16). The synergistic effect of doxorubicin with telomerase inhibitor MST132 was found to induce apoptosis in pre-B ALL cells by down-regulation of c-Myc and telomerase-associated hTERT genes and up-regulation of Bax/Bcl-2 expressions and also to increase cell proliferation inhibition (17). In another study, thymoquinone and genistein have been found to induce apoptosis in anaplastic thyroid cancer cells, and decreased levels of hTERT mRNA expression after combined therapy (12). Malloy et al. (18) were showed that doses of genistein (50 µM and 100 µM) about 50% decrease in hTERT expression for endometrial cancer cell. Genistein is known to inhibit telomerase activity and cause telomere shortening. In their study with brain cells, genistein showed that growth inhibition associated with telomerase inhibition by inhibition of TR- and TERT mRNA (19). Jagadeesh et al. (20) showed that genistein reduced hTERT expression and transcriptional activity dose-dependent. In our study, we found that whether genistein suppresses hTERT gene expression. The hTERT gene is active in cancer cells and telomerase activity is high. The length of the telomere is thus preserved and the cancer cells are immortalized. Reduction of hTERT expression may also prevent the proliferation of cancer cells.

In conclusion, genistein can reduce hTERT activity and could be effective on apoptotic cells, and that this component can be used as an anti-cancer agent. One of the important limitations is that only a cell line has been studied in acute promyelocytic leukemia. The other is the counting of the cells with the eye in apoptosis assay.

Ethics Committee Approval: Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

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The Use of Dietary Supplements Among Public High School Students in North Cyprus

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BACKGROUND/AIMS
The use of dietary supplements is common among adolescents in many countries. This study aimed to determine the prevalence and underlying reasons of usage and sources of information about dietary supplements among public high school students in North Cyprus.

MATERIAL and METHODS
A structured survey measuring behaviors about dietary supplements was administered to 1131 students (514 male and 617 female, aged from 14 to 18). Statistical differences between sex, age group and sports participation were done by using a Chi-square analysis. Differences were considered significant at a p-value <0.05.

RESULTS
Dietary supplements use prevalence was 6,4 % among public high school students, with a higher rate in males (10.7 %) compared to females (2.8 %), and athletes (9.3 %) compared to non-athletes (3.0 %). The most common reason for using dietary supplements was 'building muscle' (61.8 %) for males and 'burning fat' (29.4 %) for females. The most common source of information was trainers (52.8 %), followed by internet (29.2 %) and other athletes (23.6 %).

CONCLUSION
According to our results, although the rate of dietary supplement use among adolescents in North Cyprus was much less compared to other countries, the behaviors of adolescents about dietary supplements were similar. Therefore, education of the adolescents should be the priority for the intervention programs and the legal regulations must also be done to protect the adolescents from excess, unnecessary and inappropriate use of dietary supplements.

Keywords: Adolescent, athletes, nutritional supplements, motivation

INTRODUCTION

The US Food and Drug Administration (FDA) definition of a dietary supplement was included in the Dietary Supplement Health and Education Act (DSHEA) of 1994 (1). A dietary supplement is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet. The DSHEA places dietary supplements, whatever their physical form, in a special category under the general umbrella of "foods," not drugs, and requires that every supplement be labelled a dietary supplement.

Dietary supplements are prevalent all over the world and have a market value of more than US$100 billion annually. It is reported that up to 50 % of adults and about one-third of children in economically advanced countries are using supplements (2).

Dietary supplements companies have a large variety of claims for their products, including improvements in overall health, improved cognitive or physical performance and energy, weight loss, increased lean body mass, pain management and many other favorable effects (3).

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There are many studies that have been showing increased amount of dietary supplements use in many countries such as the United States (4, 5), European countries (6, 7), Australia (8) and Japan (9, 10). Studies mostly focus on the adult population while recent research on the adolescents is limited. Existing literature surveys mainly focused on the prevalence of dietary supplements use, source of information, awareness and knowledge about these products, the types of supplements used and finally if gender, age or exercise status influences supplements use (10-14).

Although the increasing usage all over the world, acute and chronic potential side effects and drug interactions with active ingredients are still unknown. This situation is becoming a public health issue. Although all the recommended manufacturers' dosage is arranged for adults, it seems that the recommended doses are often exceeded (12) and never defined for adolescents.

Moreover, most of the time, this consumption is not prescribed or supervised by medical professionals but as a result of suggestions from classmates, teammates, magazines, websites, coaches, and friends attending gyms. The aim of this study was to determine the prevalence and underlying reasons of usage and sources of information about dietary supplements among public high school students in North Cyprus. In our knowledge, this is the first study conducted in North Cyprus regarding dietary supplements consumption among adolescents.

MATERIAL and METHODS

Sample

We conducted a self-administered questionnaire survey with 1131 students from 15 public high schools in North Cyprus. The sample size was calculated based on the total population size (6610) according to the reports obtained from the Ministry of Education, TRNC. From these 15 public schools, calculated sample size was allocated proportional to the reported school and class sizes provided by Ministry of Education. Also, within each class, female and male students were represented proportional to their numbers. Afterwards, class lists were used as sampling frame and selection was randomly performed. For a confidence level of 95 % and a confidence interval of 2.5 %; the required sample size was calculated as 1123. The study covered more students to ensure the statistical power to remain over 80 %. Calculations were performed with G*Power (for Mac Version 3.1.9.3). Informed consents were obtained from the subjects and their parents prior to the questionnaire being applied. The study was conducted according to the Declaration of Helsinki and approved by the Near East University Scientific Researches, Evaluation and Ethics Commission (YDU/2018/57-553).

Main Points:

- The use of dietary supplements among adolescents is increasing all over the world, especially in developed countries.
- Acute and chronic potential side effects and drug interactions with active ingredients are still unknown.
- There is no defined dosage for adolescents and even the recommended doses for adults are often exceeded.
- Adolescents are likely to believe unsubstantiated information from coaches, internet and friends about supplements instead of health care professionals.
- In the light of these facts, the regulation and control on dietary supplements must be improved in order to regulate the sale of supplements to adolescents.

Study Survey

A questionnaire was developed based on the questionnaires used in previous studies to collect data about dietary supplements (10, 12, 13). The questionnaire was self-administered to all the participants and answered anonymously. The questionnaire was exploring the following domains: use of dietary supplements, commonly used dietary supplements, from where or from whom information about supplements was obtained, the motivations behind the use of dietary supplements and from whom or where dietary supplements were purchased. To conduct the survey in all public high schools, an official permission (TTD.0.00.03-12-16/383) was received from the Directorate of Secondary Education. School administrations were called up and an appointment was requested for the proper time and date. The survey was conducted in 15 high schools, and a total of 1131 students completed the survey accurately.

Statistical Analysis

The prevalence of dietary supplements use was reported by sex, age group, and sports participation. The characteristics of the participants were reported as supplements users and non-users among male and female students. Statistical differences between sex, age group, and sports participation were done using a Chi-square analysis. Differences were considered significant at a $p<0.05$. Throughout the text, data for all subjects were presented as mean ± standard deviation (± SD), percentages and frequencies. Questionnaires with missing values were excluded from the present analysis. Statistical analysis was performed using Statistical Package for the Social Sciences 18.0 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

We investigated the dietary supplements use; the motivation behind the use; most commonly used products; source of information and purchase for the supplements in public high school students in North Cyprus. A total of 1131 students (male: 514, 45.4%) (female: 617, 54.6%) aged between 14-18 (16.2±1.1) contributed in the research. 72 (6.4%) subjects declared that they were using dietary supplements and 1059 (93.6%) subjects stated that they were not using dietary supplements.

The use of dietary supplements according to age groups, gender and sports participation was shown in Table 1.

There was no statistically significant difference between age groups 14-16 (7.6%) and 17-18 (5.9%). Dietary supplements use was found higher in males (10.7%) compared to females (2.8%) ($p<0.001$) and higher in subjects who were participating in sports (9.3%) compared to non-participants (3.0%) ($p<0.001$).

TABLE I. The use of dietary supplements according to age groups, gender and sports participation

		Not Using	Using	Total
Age Group	14-16	767 (94.1%)	48 (5.9%)	815 (100%)
	17-18	292 (92.4%)	24 (7.6%)	316 (100%)
Gender	Male	459 (89.3%)	*55 (10.7%)	514 (100%)
	Female	600 (97.2%)	*17 (2.8%)	617 (100%)
Sports Participation	Participate	547 (90.7%)	*56 (9.3%)	603 (100%)
	Does Not Participate	512 (97%)	*16 (3%)	528 (100%)
Total		1059 (93.6%)	72 (6.4%)	1131 (100%)

* <0,001

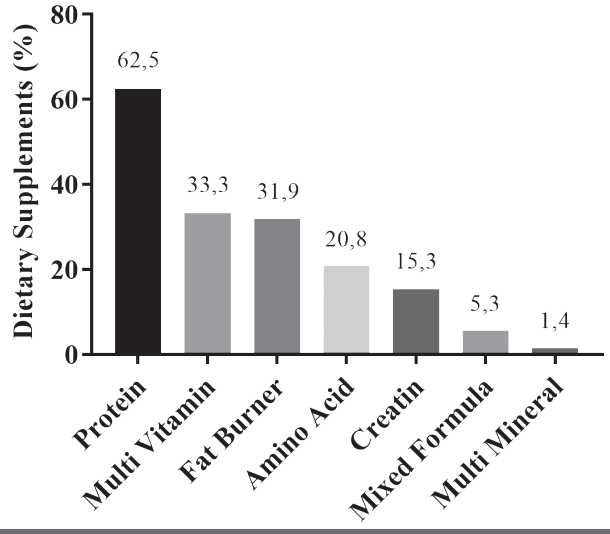

FIGURE I. Frequently used dietary supplements

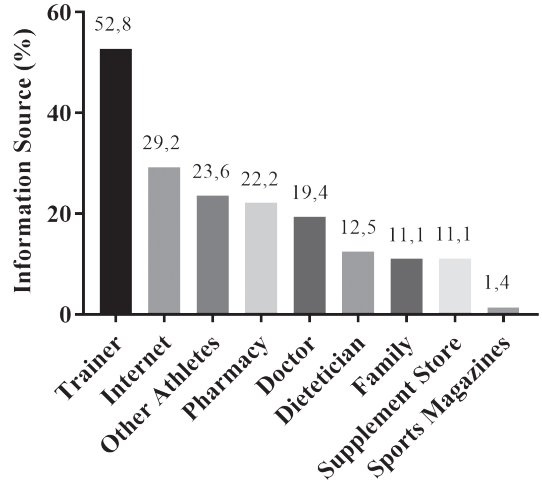

FIGURE 3. Sources for gathering information about dietary supplements

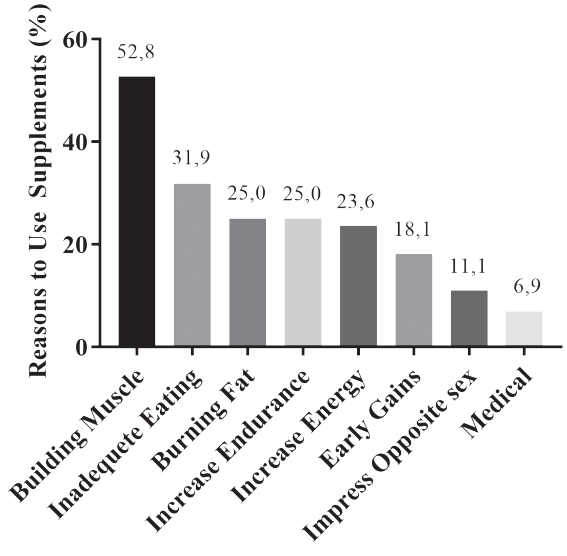

FIGURE 2. Reasons for using dietary supplements

Figure I shows the frequently used dietary supplements according to their frequency of use. Most commonly used supplements were found as proteins (62.5%) followed by multi-vitamins (33.3%), fat burners (31.9%), amino acids (20.8%), creatine (15.3%), mixed formulas (5.6%) and multi-minerals (1.4%).

According to the gender of the participants; proteins were found as the most frequently used dietary supplements (74.5%) followed by multi-vitamins (30.9%), amino acids (25.5%), fat burners (29.1%), creatine (20%), mixed formulas (7.3%) and multi-minerals (1.8%) in male students. In female students, fat burners (41.2%) and multi-vitamins (41.2%) were found as the most frequently used dietary supplements. Proteins (23.5%) and amino acids (5.9%) followed this. There was no creatine, mixed formula and multi-mineral consumption stated by the female students.

Figure 2 shows the reasons for using dietary supplements. The most common reason was building muscle (52.8%) followed by inadequate nutrition (3.9%), burning fat (25%), increasing endurance (25%), increasing energy (23.6%), early gains (18.1%), impressing opposite sex (11.1%) and medical reasons (6.9%).

Building muscle (61.8%) was found as the most frequent reason for dietary supplements use for male students. Inadequate nutrition (36.4%), increasing endurance (27.3%), increasing energy (23.6%), burning fat (23.6%), early gains (18.2%) and impressing opposite sex (14.5%) followed this. For the female students burning fat (29.4%) was found as the most frequent reason for dietary supplements use. Increase energy (23.5%), building muscle (23.5%), inadequate eating (17.6%), increase endurance (17.6%), early gains (17.6%) and medical reasons (17.6%) followed this. Female population stated that they were not using dietary supplements for impressing the opposite sex.

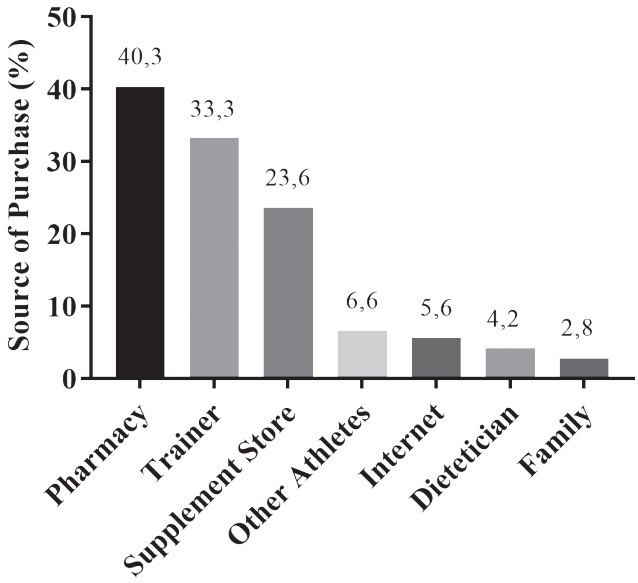

FIGURE 4. Source of purchase for dietary supplements

Figure 3 shows the sources for gathering information about dietary supplements. Trainers (52.8%) were identified as the mostly used sources of information and followed by internet (29.2%), other athletes (23.6%), pharmacies (22.2%), doctors (19.4%), dieticians (12.5%), supplements stores (11.1%), families (11.1%) and sports magazines (1.4%).

Figure 4 shows the source of purchase for dietary supplements. Pharmacies (40.3%) were found as the most common source of purchase of dietary supplements. Trainers (33.3%), supplements stores (23.6%),other athletes (6.6%), internet (5.6%), dieticians (4.2%) and family members (2.8%) followed this.

DISCUSSION

This is the first study investigating dietary supplements use in Turkish Cypriot adolescent population. We investigated the prevalence of dietary supplements use among public high school students. We found that 6.4 % of public high school students were using dietary supplements. This rate was lower compared to other similar surveys conducted in Korea (31%), the United States of America (27.4% to 32.4%), Italy (35%) and Japan (16.8%) (9, 11). The low rates might be due to the method used to assess supplement use in high school students. Although it was clearly explained that the questionnaire was for a scientific study and all answers would be anonymous, students might have felt that it was more of an interrogation rather than a survey. This might have resulted in the under-reporting of supplements used by the students. However, it is still possible that the reported prevalence is the real representation of supplements use in adolescents.

Besides, it is a well-known fact that socio-economic status and income levels are important determinants of supplements use (13, 15, 16) and most of the data on supplements use are from developed and high-income countries whereas North Cyprus is an unrecognized country with a relatively low-income level. Moreover, as a limitation of our study, it was conducted in public high schools, and private high schools were excluded due to the difficulty of obtaining permission from the school administrations. Private high schools are quite expensive while public high schools are free. Hence, children of low income families mostly attend public high schools.

There are many studies showing that the use of dietary supplements increases with age to maintain body weight, building muscle, enhance performance and health purposes (4, 10, 13, 17, 18). Evans et al. (17) stated that dietary supplements use increases with age for those who started sports early because of the performance expectations. These expectations forces them to use different methods, one of which being the use of dietary supplements. In our study dietary supplement use increased with age, but it was not statistically significant. The low number of dietary supplement users might have concealed the increase with age.

There are many studies in the literature showing that the use of dietary supplements use is higher in males compared to females (14, 15, 19, 20). Our results are consistent with these studies. Kotnik et al. (21) explained this difference with the eager nature of the men to reach their goals as immediate as possible. Similarly, Kotnik et al. (21) stated that male Slovenian adolescents tend to use dietary supplements in order to benefit from sports performance-enhancing effects of dietary supplements.

Sports participation is a well-known factor affecting dietary supplement use. We have found that the use of dietary supplements was higher in sports participants compared to non-participant adolescents. Male and female athletes tend to use dietary supplements because they believe that their regular diet is not sufficient and therefore need supplements to cover their additional needs arising from sports (14). Grm et al. (11) reported that the prevalence of nutritional supplements use was significantly higher for sports participant adolescents (24.6%) than non-participants (16.2%). They emphasised that the athletes might be more susceptible to advertising or encouragement to engage in the use of nutritional supplements. Similarly, Kotnik et al. (21) stated that adolescent athletes could be pressured by their coaches and/or teammates to use dietary supplements and this may explain an increase in dietary supplements use in adolescent athletes.

Results of our study revealed that the most commonly used supplement among all adolescents was proteins. The choice for supplements use is influenced by the motivations behind. For this study, the most common motivation for supplements use was for the building of muscles. Manufacturers of protein supplements mainly claim increased muscle mass and strength with their products. The most commonly used supplements were different for male and female subjects. Proteins were the most common supplements among male subjects while fat burners were the most common among females. The reason might be that female adolescents give more importance to their appearance and weight while male adolescents prefer to have a muscular physique. There are many studies in the literature showing that the adolescent males who are willing to build muscle and increase muscle strength tend to use protein supplements(3, 10, 17, 22-25) while the female adolescents tend to use fat burners for losing weight(10, 20).

Sources of information regarding food supplements are significant because it affects the subjects decision for using and choosing the specific supplement. In our study, the most common source of information was found as trainers (52.8%) followed by internet (29.2%) and other athletes (23.6%). Most trainers are not educated about supplements, and their knowledge about the supplements are mostly anecdotal or coming from their personal experience. This situation is also valid for athletes as a common source of information. Moreover, information gathered from internet makes this situation even more complicated because it may give inadequate or false information about dietary supplements. Most of the time there are no filter or control as to whether the information is correct or misleading.

Additionally, many web sites or blogs are commercial and advertise their merchandise. As a result, much of the information may be inaccurate, incorrect, or indeed, potentially harmful. Lieberman et al. (13) noted that the information on dietary supplements from media sources such as websites, TV or printed media is widely available, but the information is often inconsistent and confusing.

According to Balzo et al. (12), the most common sources of information about dietary supplements are coaches and other athletes. This situation is worrying as dietary supplementation should be started under the prescription and the supervision of health care professionals (e.g. physician, pharmacist, nutritionist). We also found that the health care professionals were much less consulted for information about supplements compared to coaches, internet and other athletes. Unfortunately, it is a well know fact that adolescents are likely to believe unsubstantiated information about supplements instead of expert opinions (19, 21).

Interestingly pharmacies were the most common suppliers for dietary supplements (40,3 %) followed by trainers (33.3%) and supplement stores (23.6%). It shows that adolescents are choosing to buy the supplements from pharmacies but they do not consult to pharmacists as much. Trainers were the most common source of information and the second most common suppliers. In this case, trainers may encourage the athletes to use the supplements that they sell, and that is not only unethical but also potentially harmful. Unfortunately there are not enough regulation and control on dietary supplements, and it is straightforward to reach any type of supplements even for minors. This is another concern which must be addressed by health authorities in order to regulate or limit the sale of supplements to adolescent population.

As a preliminary study in North Cyprus there were some limitations of our study. The main limitation was the limited number of variables. There was no socio economic variable such as income status. Moreover, it was conducted in public high schools, and private high schools were excluded due to the difficulty of obtaining permission from the school administrations. This might have understated the real presentation of supplement use in general adolescent population.

In conclusion, it is crucial to understand the motivation for using supplements and to specify the source of information about supplements for developing prevention and public health intervention strategies to target specific groups. According to the results of this study, education of the adolescent athletes and their coaches should be the priority for the intervention programs. The legal regulations must also be done to protect the adolescents from excess, unnecessary and inappropriate use of dietary supplements.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Near East University Scientific Researches, Evaluation and Ethics Commission (YDU/2018/57-553).

Informed Consent: Written informed consent was obtained from students who participated in this study.

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Original Article

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Human Milk Fortifier with Higher Energy for Preterms: Beneficial or Not?

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BACKGROUND/AIMS
Human milk(HM) is considered to be the best nutrient for premature infants, it is usually inadequate to support the appropriate growth rate and it is frequently supplemented with a human milk fortifier to supply more energy and other nutrients. However, in spite of this supplementation, some infants still fall short of the expected growth rate. Additional calories provided by maltodextrin could help such infants to grow better was hypothesised in this research.

MATERIAL and METHODS
Preterms of less than 34 weeks of gestation and weighing less than 1500 g, were evaluated. The infants were randomly placed into two groups while receiving 100 ml/kg human milk. Group 1 infants received human milk mixed with protein and Group 2 infants received human milk mixed with protein plus maltodextrin. This regimen continued minimum 15 and maximum 29 days and the body weight, height and head circumference measurments were recorded.

RESULTS
A total of 187 preterm infants were analysed, 46 infants were excluded from the study, while the remainder were randomly placed into two groups, respectiveley. Their gestational age, birthweight, first day of nutrition, consumption and duration of feeedings were all comparable. There were no significant differences between their weight, height and head circumference gains on the 28th day. There were also no significant differences between their serum blood urea nitrogen, albumin, calcium, phosphorus, alkaline phosphatase, sodium, potassium, chloride, thyroid stimulating hormone and free T4 levels.

CONCLUSION
Although the addition of HMF increases weight gain in premature infants, the added calories do not provide any statistically significant but better growing paterns.

Keywords: Calorie, fortification, growth rate, preterm, protein

INTRODUCTION

Body and Brain development are crucial for newborns, particularly for preterm infants, therefore inadequate feeding during the developmental period can negatively affect the short- and long-term outcomes. Therefore, adequate feeding is essential for preterm infants for optimal growth (1- 4).

The postnatal growth rates of preterm infants should be the same as the intrauterine growth rate (3). The American Academy of Pediatrics (AAP) and the World Health Organization recommend breast milk for all newborns (5, 6). However, the inadequate protein and mineral intake from breast milk by preterm infants has previously been documented, and most of the time it is not sufficient to maintain the appropriate growth rate (7, 8). Therefore, breast milk is frequently enriched with a breast milk fortifier to provide more energy and other nutrient supplements. According to recommendations of the AAP and the ESPGAN, the protein intake of preterm babies should be increased to 4–4.5 gr/kg/day (3, 4). In addition to protein intake, calorie requirements are also higher for preterm babies according to the same recommendations (3, 4). Moreover, breast milk calorie intake may be inadequate to meet the required daily amount of 105–135 kcal/kg/day for preterm babies (3, 4).

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Although breast milk fortifiers provide more energy and protein than breast milk alone, they may not provide adequate growth requirements for preterm babies. Increasing the volume of milk to be consumed may also cause hypervolemia in preterm infants. In this study, we investigated whether increasing the calorie intake of preterm infants from 77kcal/100 mL to 99 kcal/100 mL by adding fortifiers in breast milk would result in better growth rates.

MATERIAL and METHODS

This study was conducted at the Neonatal Intensive Care Unit between June 2009 and March 2010. The preterm infants included in the study were all small-for-gestational-age infants, less than 34 weeks of gestational age, and less than 1500 gr in birth weight. One hundred and eighty-seven preterm infants were evaluated. Infants who: had congenital anomalies, suffered perinatal asphyxia, had undergone a major surgical operation, had sepsis, were not feeding exclusively on breast milk were excluded from the study. The study was approved by the Ethics Committee of the Hospital (No:24/04/2009-28) and written informed consent was obtained from the parents of the infants included in the study.

All the participants were weighed daily, while the height and head circumferences (HC) were measured weekly. Participants were monitored for at least 15 days or until they were 29 days old. All participants were fed breast milk only and daily intakes were increased by 20 ml/kg/day until the infant received up to 200 ml/kg. Once the participants were receiving up to 100 ml/kg of breast milk, they were randomly assigned to two groups. Group 1 infants, the control group, received breast milk mixed with HMF (Eoprotin) in a ratio of 1 spoon per 30 mL; while Group 2 infants, the study group, received breast milk mixed with HMF (Eoprotin) in a ratio of 1 spoon per 30 ml in addition to ½ a spoon of maltodextrin powder (Fantomalt) per 30 mL. Following the enrichment of the breast milk in two distinct ways, the caloric intake for Group 1 was 77 kcal/100 mL and 99 kcal/100 mL for Group 2. Blood urea nitrogen (BUN) calcium, magnesium, alkaline phosphatase, sodium, potassium, and chloride levels were monitored on the st day, 15th day, and 29th day. Thyroid-stimulating hormone (TSH) and free T4 levels were checked on the 10th day of life. In addition, gastrointestinal side effects (abdominal distension, gastric residue, vomiting, feeding intolerance, diarrhea), allergy, sepsis, and other problems were noted.

Statistical Analysis

Statistical analysis was performed using the NCSS 2007 software for Windows. Continuous variables were expressed as mean ± standard deviation of the mean (SD). The results of the two groups were compared using Student's t-test for normally distributed data. To determine the relationship between principal variables and the other continuous variables, Pearson correlation or Spearman non-parametric correlation tests were used. When equality of variances was not present, Kruskal Wallis and Mann–Whitney U non-parametric tests were used. Values of $p<0.05$ were considered statistically significant.

Main Points:

- Standard fortification techniques may not be adequate for providing enough energy and protein.
- Higher protein with higher calorie is essential for preterm growth.
- Increasing protein and calorie did not cause any gastrointestinal side effects.

RESULTS

Subjects

The study was initially performed on 187 preterm infants aged 6–16 days. However, 10 infants were excluded from the study for not taking adequate breast milk. Furthermore, 35 infants were excluded because of side effects, while 1 infant was transferred to another hospital. Seventy-one preterm babies were enrolled into Group 1 (51% girls, 49% boys) and 70 preterm babies were enrolled into Group 2 (43% girls, 57% boys). Gestational age, birth weight, sex, onset of enteral feeding, maximum feeding volume, first day of fortification, and duration of fortified feeding in days were not significantly different between groups (Table 1). The mean age at first enteral feeding (in days) was 1.9±0.47 days in Group 1 and 1.95±0.21 days in Group 2, while the first day of fortification was 10.94±4.39 days in Group 1, and 10.4±4.39 days in Group 2. Participants were fed for 23.96±6.62 days in Group 1 and 21.33±7.55 days in Group 2 with fortified breast milk.

The volume of intake of the modified breast milk by participants in both groups was maintained around the target volume of 180-200 mL/kg/day.

Growth

The weight gain of Group 1 participants and Group 2 participants was 31 mg/kg/day and 32 mg/kg/day respectively on the 14th day of fortification; 32 mg/kg/day and 35 mg/kg/day respectively on the 28th day of fortification. Although an increase in weight gain was noted in Group 1 participants, there was no statistical difference between the two groups ($p>0.005$). (Figure 1)

TABLE 1. Demographic and feeding characteristics of patients

	Group 1	Group 2	p
Gestational age (weeks)	29.13±1.93	28.91±1.95	0.574
Birthweight (g)	1157.88±18932	1152.15±178.85	0.878
Girl/boys (n/n)	37/34	30/40	0.404
First enteral feeding (day)	1.9±0.47	1.95±0.61	0.605
Maximum feeding (ml/kg/gün)	196.78±6.66	198.15±3.56	0.249
First day of fortification (day)	10.94±4.96	10.4±4.39	0.584
Duration of fortified feeding (day)	23.96±6.62	21.33±7.15	0.076

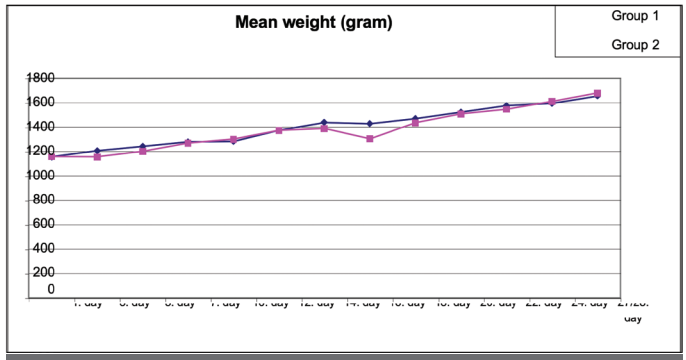

FIGURE 1. Mean weights of groups

Participants' mean height in both groups did not differ on the Ist day of fortification (37.237.2±2.32 cm in Group I and 37.55±2.13 cm in Group 2) (p>0.05). On the 12th day of fortification an increase in the height of participants by 1.55±0.78 cm/day for Group I participants and 1.31±0.72 cm/day for Group 2 participants (p>0.05). At the end of the study, there was no statistically significant difference between the mean height increase of participants in either group (2.44±1.11 cm/day in Group I and 2.16±1.21 cm/day in Group 2) (p>0.05). (Figure 2)

At the end of the study, Group 2 participants had a significantly higher HC. (p=0.014). The mean HC was 27.52±2.15 cm in Group I and 27.34±1.56 cm in Group 2 (p>0.05). An increasing rate in HC was not noted, 1.29±0.63 cm/day in Group I and 1.38±0.7cm/day in Group 2 on the 12th day of fortification. HC measurements were 3.19±0.79 cm/day in Group I and 3.89±1.15 cm/day in Group 2 on the 28th day of fortification (p=0.014). (Figure 3)

TABLE 2. Side effects

Side effect	Group I	Group 2	p
Gastric residual	3 4.23%	0 0.00%	0.179
Abdominal distention	4 5.71%	0 0.00%	0.098
Feeding intolerance	3 4.29%	2 2.86%	0.632
Apnea	4 5.71%	0 0.00%	0.098
Vomiting	3 4.29%	0 0.00%	0.179
Sepsis	5 7.14%	3 4.29%	0.491
NEC	2 2.86%	2 2.86%	0.930
Hypercalcemia	7 9.86%	4 5.71%	0.577
Total cases	26 (36.61%)	20 (28.58%)	0.551

NEC: necrotising enterocolitis

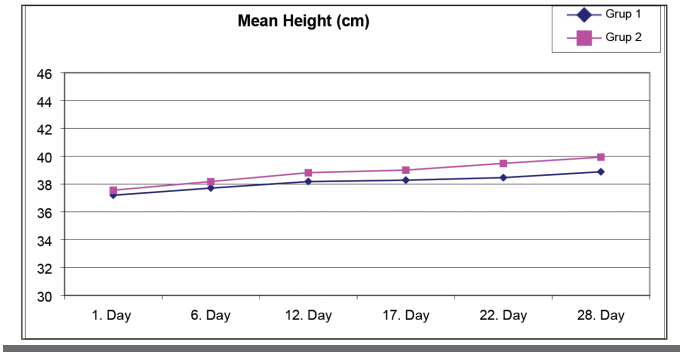

FIGURE 2. Mean heights of groups

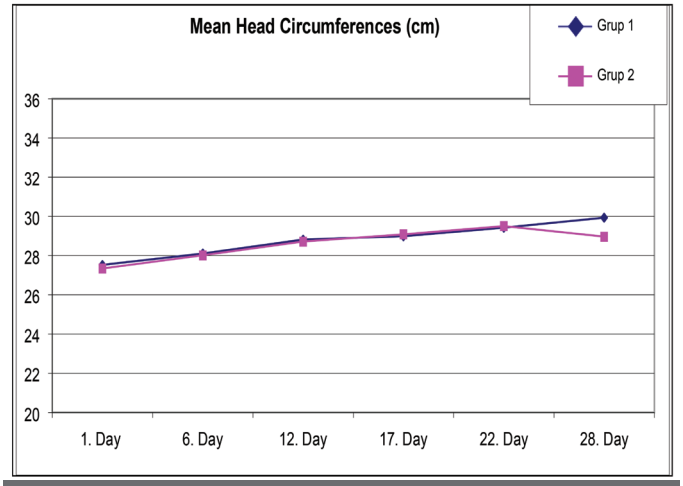

FIGURE 3. Mean head cirfumferences of groups

Serum Biochemical Data

There was no statistically significant difference between the levels of BUN, albumin, TSH, free T4, phosphorus, magnesium, alkaline phosphatase, sodium, chloride, and potassium levels on the Ist, 14th, and 28th day of fortification (p>0.05). However, although calcium levels in Group 2 participants were significantly higher, they were still within the normal ranges for preterm babies (p=0.016).

Feeding Tolerance and Clinical Course

Associated side effects among the participants were not statistically significant between the two groups (Table 2). In fact, there were no statistically significant differences in any of the variables compared between the two groups. Three infants in Group I had gastric residue and 4 had abdominal distention (p>0.05). Three infants from Group I and 2 from Group 2 had feeding intolerance (p>0.05). Vomiting was only noted in 3 participants from Group I (p>0.05).

Apnea was not documented among Group 2 participants but was noted with 4 participants in Group I (p>0.05). Five participants from Group I and 3 from Group 2 had sepsis (p>0.05). Two participants, one from each group, had necrotizing enterocolitis (p>0.05).

DISCUSSION

The AAP and the ESPGAN recommend the fortification of breast milk to increase the growth rate of preterm infants. Different fortification strategies were recommended to correct inadequacies in both the quality and quantity of protein and calorie concentrations of FHM. (3, 4) Standard fortification techniques may not be adequate for providing adequate energy and protein. Increasing the intake of milk may cause hypervolemia problems in preterm infants. Therefore, in order to prevent this, we added an energy provider to standard fortification to increase the caloric amount in HM. In this randomized prospective controlled trial conducted with 187 preterm infants, energy intakes were increased from 77kcal/L to 99 kcal/L; and infants' growing rate, biochemical results, and possible side effects were monitored. Adding an energy provider to HM may increase osmolarity and cause feeding intolerance and Necrotising Enterocolitis (NEC). However, there was no difference between the two groups with regards to gastrointestinal side effects. In addition, the rate of sepsis was similar in both groups. Infants provided with HMF supplemented with an energy provider had an improved growth rate, weight and height gain, and an increase in HC measurements when compared with the infants who were only given HMF.

In a previous study (9), two different protein-containing fortification techniques were compared using preterm infants whose weight was less than 1250 gr and had a weight gain of less than 15/gr/kg/day as the subjects. Daily weight gain was reported to be 17.0±2.0 gr/kg/day in the group given 3.5 gr/kg/day of protein and 11.5±4.8 gr/kg/day in the group given 3 gr/kg/day of protein at the 4th week of fortification. However, in the current study, the infants in Group I were given fortified breast milk at 10.94±4.96 days and Group 2 at 10.4±4.39 days. Growth rates were found to be 33±22 gr/kg/day for the Group I infants and 35±27 gr/kg/day for the Group 2 infants. The difference in the recorded growth rates between

the preterm infants in the current study and the participants in the Brummer study may have resulted from the fact that in the current study protein-rich milk was given at an earlier age.

In a study by Mukhopadhyay et al. (10), 166 preterm infants were examined, and 2 different calorie-containing milk products were compared (66 kcal/100 mL to 79 kcal/100mL). Participants were given 150/mL/kg/day until they weighed 2000 gr. The fortified milk-fed group was followed up to 31.9±16.2 days and the only-breast-milk-fed group was followed up to 29.4±13.2 days. In this study, the reported growth rate was 15.1±6 gr/kg/day in the higher-calorie-given group and 12.9±4 gr/kg/day in the control group. We reported 33±22 gr/kg/day in the control group (Group 1) and 35±27 gr/kg/day in the study group (Group2). Our preterm infants had a higher weight gain because fortification of milk products occurred earlier. In addition, the reported increase in height was 1.04±4 cm/week in the control group and 0.86±0.2 cm/week in the study group. Among the preterm infants in this study, the increase in height was observed to be 0.61±0.78 cm/week in the control group and 0.54±0.30 cm/week in the study groups. The difference in height measurements between the two studies may be due to ethnic differences.

According to the study conducted by Aslanoglu et al. (11), breast milk was fortified in three steps. Initially, 1.9 gr/100 mL during the study in the control group and 1.9 gr/100 mL of breast milk in the 1st week, 2.2 gr/100 mL of breast milk in the 2nd week, and 2.3 gr/100 mL of breast milk in the 3rd week in the study group were added when preterm babies were taking 100 mL/kg/day enteral feeding and excessive breastmilk was given to the babies in the control group. Weight increase rates were reported as 24.8±4.8 gr/day in the control group and 30.1±5.8 gr/day in the study group. In the current study, comparatively, these rates were noted as 38.65±7.56 gr/day in the control group and 41.37±6.29 gr/day in the study group. The difference in weight gain by the preterm infants between the two studies may have resulted from the preterm infants' increased protein consumption, which is 3 gr/kg/100 mL from the 1st day of fortification.

In this prospective, randomized controlled study, differences in either weight gain or rate of increase in height between the two groups of preterm infants were not statistically significant. However, the higher-energy-consuming group (Group 2) displayed an increase in height and weight by the end of the study, in addition to an improved HC increase rate. Side effects, especially gastrointestinal side effects, Necrotizing Enterocolitis, and feeding intolerance rates were comparable in both groups.

In conclusion, despite the better growth pattern of the higher-calorie-given group, the main factor required for the growth of preterm infants is higher protein intake. As a result of this, higher protein with higher-calorie diets are essential to achieve the same growth ratio as in utero growth in the nutritional management of preterm babies. Amino acids are important in the synthesis of insulin, insulin-like factors, and other growth-related hormones and it is reported that the lower-protein-given group shows slower growth (12-16).

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Zeynep Kamil Women Health and Children Diseases Training and Research Hospital (28-24/04/2009).

Informed Consent: Written informed consent was obtained from patient who participated in this study

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Review

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Magnetic Resonance Imaging Features of Tumefactive Perivascular Spaces

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The perivascular spaces (PVSs) are pia-lined potential spaces filled with interstitial fluid (ISF) and form a network throughout the brain, which is a part of glymphatic system and helps to derange of metabolites from cerebral parenchyma. The enlarged PVSs are the most common cystic lesion of the brain and gradually become prominent with age. Literally, tumefactive PVS is the giant enlargement of PVS, measuring 15 mm and more in size, and thought to be result of blocking of the outlet of ISF for any reason. Typically, they tend to from clusters of cysts, but tumefactive forms are usually solitary. They usually have moderate mass effect and can cause obstructive hydrocephalus when located in mesencephalothalamic region. Although they don't contain cerebrospinal fluid (CSF), they have similar signal to CSF on all magnetic resonance imaging (MRI) sequences and do not enhance. These MRI features are very useful for their differential diagnosis from other congenital, infectious, ischemic and tumoral cystic/necrotic lesions. The precise diagnosis of PVS is critical to prevent patients with "leave-me-alone" lesions, from unnecessary surgical interventions.

Keywords: Perivascular Spaces, Virchow-Robin Spaces, Glymphatic System, Magnetic Resonance Imaging

INTRODUCTION

From the historical perspective, the perivascular spaces (PVSs) were first described by the German pathologist Rudolf Virchow and French anatomist Charles Philippe Robin, so they were also called as Virchow-Robin spaces (1, 2). Until to the advent of magnetic resonance imaging (MRI) to neuroradiology, they have been reported as incidental findings at autopsies and classified as normal anatomical variant and/or result of aging brain, in literature (3-5). Following the widespread usage of MRI, they became more visible, because they were usually invisible on computed tomography (CT) images (6). They were also considered as normal variant in radiology literature (3, 4, 6-8). Following the recognition of cerebral glymphatic system and the excretory role of PVSs in this system, they were started to be evaluated in more detail (9-11).

Under normal conditions, the PVSs are not recognizable on MRI in pediatric and adult population but become prominent with age and sometimes formed a cyst in the brain parenchyma. Enlarged PVSs are the most common parenchymal cysts in the human brain and usually considered as a normal variant. By general acceptance, they are "leave me alone" lesions that should not be mistaken for serious disease (6). Besides age, recent studies have also linked enlarged PVSs with lacunar stroke subtype and enlarged PVSs were considered as a potential biomarker of neurovascular and neurodegenerative diseases (3, 4, 12-16).

Histopathology

The PVSs are pia-lined potential spaces filled with interstitial fluid (ISF), not cerebrospinal fluid (CSF) and formed by the invagination of the pia mater into the brain parenchyma along the penetrating cerebral arteries and draining veins (3, 7, 12). PVSs form a complex intraparenchymal network distributed over the whole brain, connecting the cerebral convexities, basal cisterns, and ventricular system but, the PVSs do not communicate directly with the subarachnoid space or CSF. PVSs network plays an important role both in providing drainage routes for cerebral metabolites and in maintaining normal intracranial pressure (6, 9-11). They are also part of the cerebral glymphatic system, in which CSF-ISF exchange occurs within brain parenchyma probably mediated by aquaporin 4 water channels and a substantial amount of ISF and cerebral metabolites, such as amyloid beta, exits the brain via connections between the PVSs and leptomeningeal vessels (9). By this way, the intravenous gadolinium-based contrast agents can also enter the PVSs (10, 11, 17).

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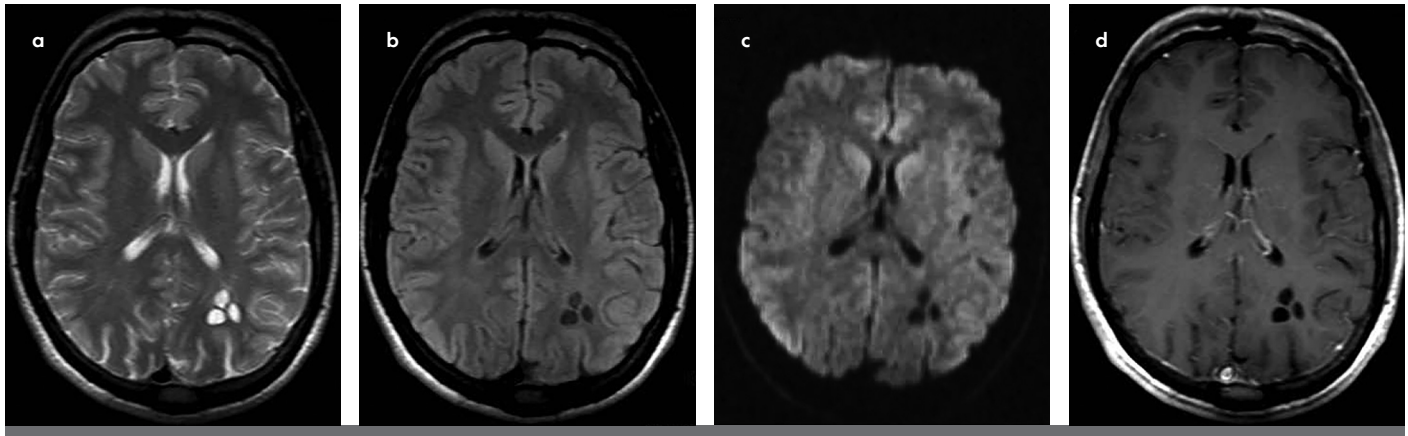

FIGURE I. a-d. Type I PVSs. Axial T2 (a), FLAIR (b), trace DWI (c) and postcontrast TI (d) images reveal the cluster of cysts having similar signal to CSF on all sequences without any prominent enhancement. They are asymmetrically located and follow the vascular distribution at the deep white matter of left occipital lobe.

They can be seen throughout brain wherever vessels are present, but mostly seen in the inferior third of the basal ganglia (clustered around the anterior commissure), subcortical white matter (including the external capsule), ponto-mesencephalic junction, midbrain and dentate nuclei of the cerebellum (3, 6-8). The PVSs are classified into three groups according to their location: type I (along the lenticulostriate arteries entering the basal ganglia through the anterior perforated substance), type 2 (along the perforating medullary arteries entering over high convexities through cortical gray matter), type 3 (along the penetrating collicular arteries in the mesencephalothalamic region) (8). Cortical PVSs (type 2) are lined by a single layer of pia, whereas two layers accompany lenticulostriate (type I) and collicular arteries (type 3) (5).

Type I and 2 tumefactive PVSs are usually asymptomatic or with nonspecific symptoms such as headache, dizziness, migraine, memory impairment, and Parkinson-like symptoms have been reported in some cases, but their relationship to enlarged PVSs is unclear (I3-I6). About 75% of type 3 giant or tumefactive PVSs are symptomatic and can cause obstruction on CSF pathways, which occurs slowly over a long period of time, and can lead to noncommunicating type hydrocephalus (6-8) The patients with osbtructive hydrocephalus, can be treated either by ventriculoperitoneal shunting or endoscopic third ventriculostomy, or by cystoperitoneal shunting (7).

Imaging Findings

MRI is the modality of choice in the imaging of PVS and their detectability increases with higher MR strengths. Compare to I.5 T and less MR imagers, even very small PVSs can easily be detected by high-resolution 3T MRI in nearly all patients, in virtually every location, and at all ages. Additionally, PVSs can also be identified in pediatric population on high-resolution MR scans. But, PVSs are not visible as they pass through cortex, even at 7T MRI (6).

Main Points:

- Tumefactive perivascular spaces are benign non-pathological conditions of cerebral white matter.
- Radiologically they mimic many pathological conditions including ischemic, congenital, infectious or tumoral cerebral cystic lesions.
- Unique MRI features of tumefactive PVSs having similar signal intensity to CSF on all MRI sequences and following the vascular distribution allow their differential diagnosis.

PVSs are usually detected incidentally in routine MR images. The high-resolution volumetric fluid attenuated inversion recovery (FLAIR) or T2 weighted images have the highest diagnostic accuracy in the identification of PVSs. The postcontrast TI weighted images and other advanced MR imaging techniques such as; diffusion (DWI), perfusion (PWI), susceptibility (SWI) weighted images are usually reserved for the differential diagnosis of PVSs from other congenital, infectious, ischemic and tumoral cystic/necrotic lesions.

In MRI, enlarged PVSs may be round, ovoid, linear or tubular in shape but always follow the vascular distribution (Figure I). This is one of the most important differential clues for their diagnosis. They may vary from solitary, unilocular, small, and unobtrusive lesions to multiple, large, bizarre, multilocular CSF-like fluid clusters, which leads to misdiagnosis of multicystic brain tumors. They have smooth and regular contours. Most of them are less than I5 mm in size (5-8, I8, I9). Asymmetric distribution is common. Commonly, they can cause mass effect, and symptoms especially when located in the brainstem. Even though they are filled with ISF, PVSs usually follow CSF on all MRI sequences but, quantitative studies revealed statistically significant difference between PVSs and CSF, which is generally unrecognizable by human eye (20). This is the result of different contents of both spaces and supports the theory that PVSs are pia-lined potential spaces filled with interstitial fluid (ISF), not cerebrospinal fluid (CSF) (3, 5, 6). They always have high apparent diffusion coefficient (ADC) values like CSF on trace DWI images, which helps to differentiate them from the ischemic lesions having restricted diffusion (I9). They don't have any calcification, hemorrhage or high protein content unlike other congenital, infectious or tumoral cerebral cystic lesions. About 75% suppress completely on FLAIR images and 25% have hyperintense rim and 5-I0% have hyperintensity in perilesional white matter (6). Typically, they do not enhance with intravenous gadolinium-based contrast agents.

Extensive enlargement of PVSs is a rare but important matter and is also referred as giant so-called tumefactive PVSs (Figure 2) (4, 7, I8). These are usually bigger than I.5 cm, measuring up to 9 cm in diameter have been reported (4, 6, 7, I8). The precise cause of this cystic enlargement is still unknown. Most investigators believe

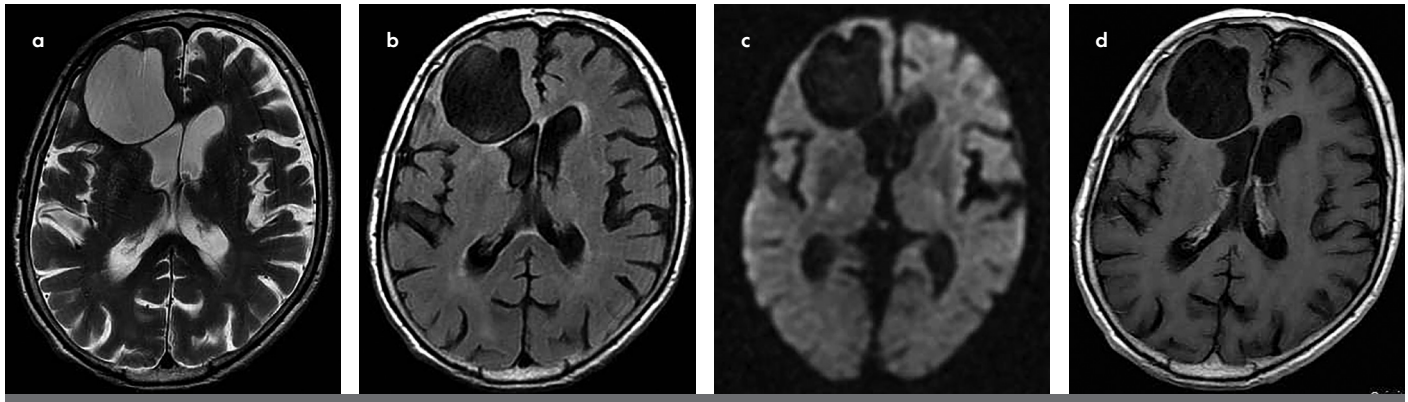

FIGURE 2. a-d. Tumefactive PVS. Axial T2 (a), FLAIR (b), trace DWI (c) and postcontrast TI (d) images reveal right frontal tumefactive PVS, having 45 mm highest diameter and similar signal to CSF on all sequences without any prominent enhancement. It has moderate mass effect to the right frontal ventricular horn.

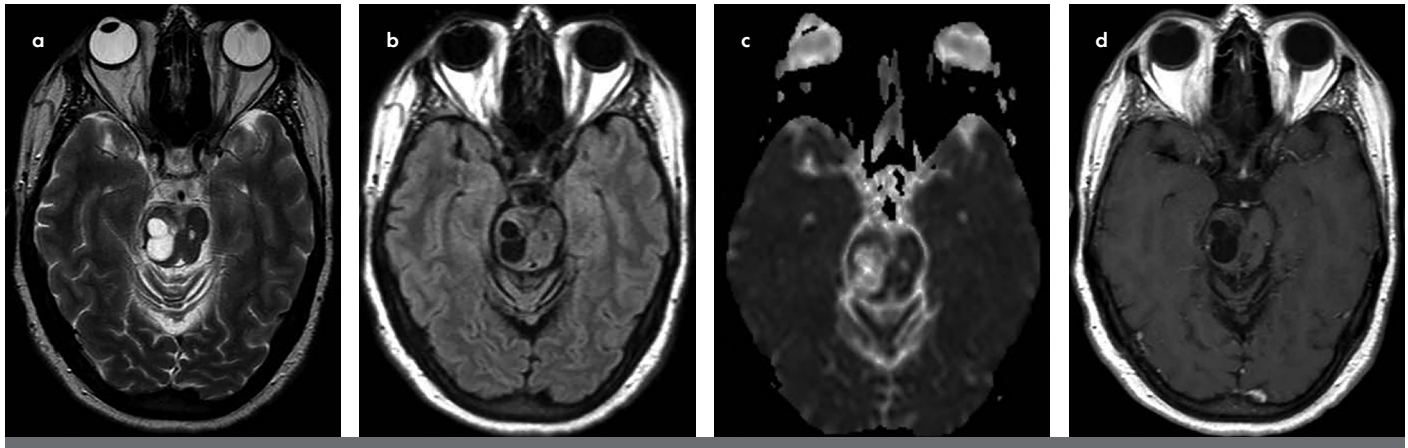

FIGURE 3. a-d. Tumefactive PVS. Axial T2 (a), FLAIR (b), trace ADC (c) and postcontrast TI (d) images reveal multiloculated tumefactive PVS at the right ponto-mesencephalic junction, having 20 mm highest diameter and similar signal to CSF on all sequences without any prominent enhancement. It has moderate mass effect to the brainstem and Sylvian aqueduct but not cause obstructive hydrocephalus yet.

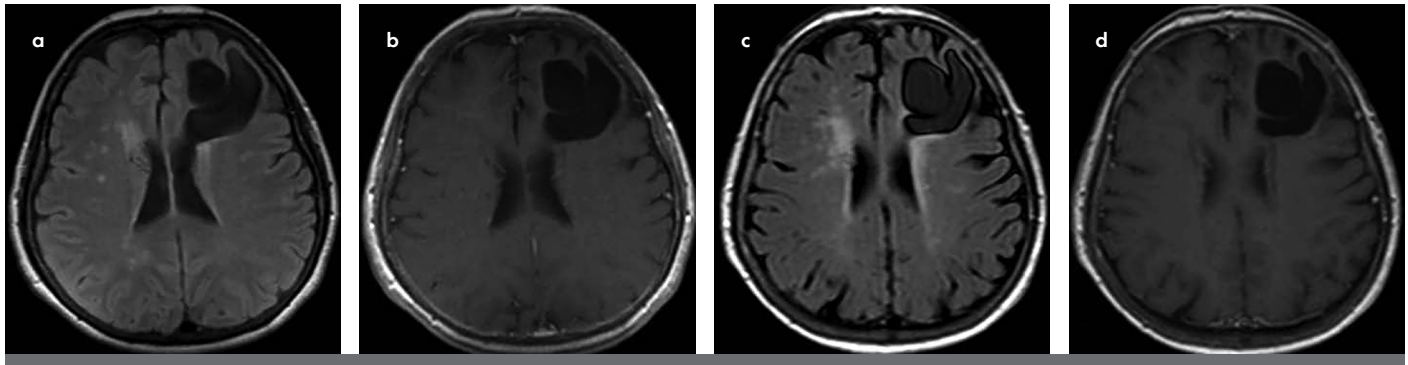

FIGURE 4. a-d. Tumefactive PVS. Axial FLAIR (a), and postcontrast TI (b) images reveal left multiloculated frontal tumefactive PVS, having similar signal to CSF on all sequences without any prominent enhancement but perilesional high signal on FLAIR (a) images resembling gliosis. On follow-up axial FLAIR (c), and postcontrast TI (d) images of the same patient obtained 2 years later, the size, appearance and signal characteristics of the PVS shows no change except mild increase in perilesional high signal on FLAIR (c)

that cystic enlargement of the PVSs is a result of blocking of the outlet of ISF for any reason, such as mechanical trauma due to CSF pulsation or vascular ectasia, increased vascular permeability resulting in fluid exudation into ISF or ischemic injury to perivascular tissue causing a secondary ex vacuo effect (3, 5, 18). Similar to distribution of simple PVSs, tumefactive PVSs are also seen throughout the all cerebral vascular territories, but mostly seen in the mesencephalothalamic region (Figure 3) and frontal lobe (Figure 2) (7). They have also similar MRI features and one third of tumefactive PVSs have surrounding T2/FLAIR hyperintensity, reflecting gliosis or edema (3, 5, 18). When lesion becomes bigger (more than 2 cm), the diagnosis becomes difficult because linear sign is lost and detection of relationship with the vascular distribution becomes harder (21). At this point, detection of similar signal intensity and diffusion characteristic to CSF in all sequences, and the absence of contrast enhancement even in delayed images are very useful for their differential diagnosis from other tumor like lesions (17). The stability in size and appearance of tumefactive PVSs over time is

critical for their diagnosis (Figure 4) although a few cases of progressively enlarging PVSs have been reported but the progression rate was not as fast as tumors had (4, 7, 8, 16).

Differential Diagnosis

The major differential diagnosis is chronic lacunar infarction. Although they often affect the basal ganglia and suppress on FLAIR, lacunar infarcts do not cluster around the anterior commissure, are often irregular in shape, and frequently exhibit hyperintensity in the adjacent brain. In acute stage, infarcts have low ADC values (diffusion restriction) on DWI and relative cerebral blood volume (rCBV) values on PWI, but ADC values gradually increase with chronicity of the lesion, while rCBV values remain low.

Another mimic is the neuroglial cysts, which are fluid-containing cavity buried within the cerebral white matter like enlarged PVSs. The neuroglial cysts are always solitary and located anywhere in the brain but not follow the vascular distribution. They have a similar appearance with enlarged PVSs on MRI but enlarged PVSs are usually multiple and form clusters.

The an intraparenchymal arachnoid cyst is quite rare compare to usual extra-axial location. They have also signal like CSF. It is often difficult to differentiate them from PVSs and neuroglial cysts on solely MRI findings. Contrary to arachnoid cysts, MRI demonstration of the communication of the cyst with the ventricle helps to differential diagnosis of the porencephalic cysts from other mimics.

Infectious cysts are usually small and represents with diffusion restriction and moderate to strong but irregular enhancing rim. In immunocompromised patients, enhancement of maybe faint or incomplete. Although often multiple or multilocular, they typically do not occur in clusters of variably sized cysts as is typical for enlarged PVSs. Diffusion restriction can be identified in the center of the infectious cyst, like in bacterial abscess due to presence of pus, or in the wall, like in tuberculous abscess due to inflammation.

Primary or metastatic cystic tumors may also resemble tumefactive PVSs, but even low-grade, tumors are usually enhanced, and have high perfusion and low diffusion values unlike PVSs. High rCBV values of primary or metastatic tumors on PWI help to differentiate them from other mimics.

CONCLUSION

Tumefactive PVSs are rare but important lesions, which must be differentiated from other ischemic, congenital, infectious or tumoral cerebral cystic lesions. The precise diagnosis of PVSs prevents patients from unnecessary surgical interventions. They have unique MRI features such as; they always follow the similar signal to CSF and the vascular distribution, usually they do not enhance, and sometimes have surrounding high T2/FLAIR signal due to edema or gliosis. The MRI features such as enhancement, diffusion and perfusion characteristics of other cystic lesions allow proper differential diagnosis of tumefactive PVSs, even they have reached to huge dimensions.

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Review

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Non-Operating Room Anesthesia: An Overview

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Today, an increasing number of diagnostic and therapeutic procedures are performed in specialized units outside the classical operating room, such as endoscopy units, interventional radiology, neurology and cardiology laboratories. The main reasons of this global trend are recent advances in medical technology, increased knowledge of disease pathogenesis, and some financial factors. Additionally, number and complexity of these procedures are rapidly raising throughout the world. In parallel, the importance of anesthesia management of such patients, also known as non-operating room anesthesia, has become better understood in recent years. In this review, we aimed to discuss the potential difficulties of non-operating room anesthesia, preprosedural patient preparation, intraoperative anesthesia applications, and postprosedural patient care in the light of the current literature.

Keywords: Complication, interventional procedure, non-operating room anesthesia, management

INTRODUCTION

Thanks to advances in medical technology, increased knowledge of disease pathogenesis, and some financial factors, a significant number of diagnostic and therapeutic procedures are strictly being performed outside the traditional operating room (OR) such as endoscopy suites, cardiac catheterization laboratories, and invasive radiology units (1, 2). Those procedures were previously performed in selected patients and indications whereas potentially risky groups with medically unstable conditions, including geriatric, pediatric, and emergent patients, are now increasingly treated in these non-OR locations. In parallel to this global trend, anesthesia management of the patients undergoing such procedures, namely non-operating room anesthesia (NORA), has become an important key factor for patient safety and the success of the procedure. Several national anesthesia and reanimation societes, such as The American Society of Anesthesiologists (ASA), have published their clinical guidelines in order to make a standardization in routine practice (3, 4). However, more novel procedures are being included daily practice with each passing day. This situation, naturally, poses new challenges for anesthesiologists. Therefore, NORA should be considered as a special subunit of anesthesia practice.

In this review, we aimed to report both avantages and disadvantages of NORA, to discuss periprocedural patient assessment, and to highlight the potential key points of the anesthetic management, relevant with current literature.

Potential Challenges of NORA

In general, many anesthesiologists do not feel themselves safely when they have to work in any places outside the classical OR. Therefore, working in such places are usually not preffered by anesthesiologists. The most important reasons of this situation are unfamiliarity with the enviroment, unavilability of some critical equipments and supplies, and limited help from other anesthesiologists in case of emergent conditions (5-7). Increasing complexity of the procedures and patient expectancy are also among the potential concerns of anesthesiologists (8, 9). In addition, patients scheduled for such procedures are often selected by the severity of their disease, which prevents them from undergoing a major surgical procedure in the traditional OR. These patients are sometimes more medically compromised and less optimized in comparison to OR patients. Thus, anesthetic management of such patients is considered as more difficult or less safely.

Sufficiency of staff personnel in those locations may be another problematic issue. Those personnels may be less familiar with the overall management of anesthetized patients (10, 11). They may even have limited medical background. However, it is a reality that assistance of trained personnel is an important factor at all stages of patient management, particulary for emergency situations (12).

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Preprocedural Evaluation of Patients

Basic data and goals

Preprocedural evaluation of patients is a critical step in the management of these patients. With the growing number of non-OR procedures, a significant number of patients now require assessment before their procedural date. The reality is that many anesthetic evaluations are today performed on the day of the procedure. However, this may be more costly by increasing the risk of procedural cancellations or delays (13). Therefore, a standard protocol for preprocedural evaluation of non-OR patients should be established in all medical centers where non-OR procedures are routinely performed (14). It should be also stated here that this kind of preprocedural screening should be similar with patients scheduled for surgery at OR (12).

There are several basic goals for anesthetic management in non-OR procedures (Table 1). The first goal of the preprocedural screening should include a comprehensive evaluation of patients, which should not be limited to only the past medical, surgical, anesthesia, family, medication and allergic reactions but also include potential procedure-related problems (15). A complete physical examination is also an essential part of the preprocedural assessment. In the evaluation of pediatric population, the anesthesiologist should be attentive to the dynamics of the patient parent/guardian interaction (12). Similar to preanesthesia assessment of classic OR patients, anesthesiologist should be encourge to consult other medical services since these patients may have accompanying diseases.

Preparation and education of the patient, parent or guardian are also among the most important parts of the preprocedural evaluation (16). Patients and/or their relatives should be informed about the process in detail, and their written consent forms must be taken from all. In pediatric population, it has been showed that written information enhances a parent's understanding (17).

System evaluations and diagnostic testing

A complete examination of the patient should be a mandatory strategy to conducting a safe procedure. System evaluations, including airway assessment, pulmonary, cardiac, and neurologic examinations must be performed in all non-OR patients. As a general approach, preprocedural diagnostic testing should be individualized based on patient risk factors and the procedure itself. Routine preoperative tests have rarely been shown to impact patient management (18). These tests should only be ordered if they will impact the care provided. Baseline laboratory tests including blood count, hepatic and renal function parameters, coagulation profile, and blood typing should be drawn for patients with potential bleeding risks or renal injury risk (14). However, it is a reality that classical laboratory tests, chest X-ray, and electrocardiography (ECG) are routinely performed in most medical centers, due to the legal concerns. Several basic rules have been determined by major international guidelines (Table 2).

Airway status should be clearly defined with using standard scoring systems such as mallampati score. Additionally, thorough airway examination with assessment of mouth opening, thyromental distance, dental condition, and neck mobility should be performed and recorded.

Pulmonary complications can contribute significantly to perioperative morbidity and mortality. Hypoxia and desaturation are the most common respiratory events during the periprocedural period. Smoking, lung disease, older age, malnutrition and long duration of anesthesia are the well known respiratory risk factors, especially for patients undergoing general anesthesia (19-21). Chronic obstructive pulmonary disease, higher ASA scores, impaired sensorium, and obstructive sleep apnea are the other medical risk factors. Pulmonary function tests and/or consultation from department of pulmonary diseases may be useful in patients with bronchial asthma or chronic obstructive pulmonary disease. Current medications of the patients who have a pulmonary disease also carry great importance in the management

Main Points:

- Increased number and complexity of interventional procedures at specific places outside the classical operating room in recent years have clearly showed the importance of NORA.
- NORA carries several potential challenges including unfamiliarity of anesthesiologists with the enviroment, unavilability of some critical equipments and supplies, limited help from other anesthesiologists in case of emergent conditions, and sufficiency of staff personnel.
- Design of locations performed NORA procedures, careful preprocedural patient evaluation, appropriate anesthesia techniques, and closed postprocedural patient care play crurical role for the success of these procedures.
- A closed collaboration between anesthesiologists, proceduralists, other staff personnels, and hospital administrators is a mandatory step to minimizing the potential challenges and providing good results.

TABLE I. Basic goals for procedural anesthesia

Minimizing psychological discomfort and anxiety for both the patient and/or family
Selection of accurate anesthetic technique
Application of accurate and adequate medications
Careful monitoring during the anesthetic management
Optimizing pain management
Control movement to improve safety of invasive procedures

TABLE 2. Basic rules of radiological and hematological testing in preprocedural evaluation outside of the OR (18, 19)

ECG is indicated in patients above 50 years, and is valid for six months provided no interval change.
Chest radiography is not mandatory at any age.
Complete blood count and electrolyte panel as indicated by history, and valid for six months.
Blood glucose check for all diabetic patients.
Potassium check for patients with end stage renal disease.
Pregnancy test on the day of the procedure is indicated for women of child-bearing age who had no documented hysterectomy.
An indication should be made for all tests ordered.
Tests already in the record are acceptable.

of possible pulmonary complications. In this context, pulmonary inhalers should not be held on the day of procedure. Contrary, these inhalers should be administered before the procedure to avoid or minimizing the potential pulmonary events (19).

Cardiac evaluation is of great importance in these patients. Using a cardiac risk assessment algorithm is usually recommended during the preprocedural setting (22). Presence of ischemic heart disease, cardiac failure, diabetes, stroke, peripheral vascular disease, and renal insufficiency (creatinine above 2 mg/dl), high-risk surgery, and older age have been determined as risk factors (23, 24). Additional cardiac testing has been recommended for patients with unstable coronary syndrome (myocardial infarction and unstable angina), decompensated heart failure, significant dysrhythmias (high degree or Mobitz II AV block, 3rd degree AV block), and severe valvular disease (19). The current medications, especially anticoagulants, antiplatelets, and antihypertensives, should be also examined in those patients because some drugs should be continued the day of procedure while others should be held on before the procedure. Stopping or continuing such medications should be discussed with the relevant departments during the preprocedural setting. The current trend is that most of these drugs, such as aspirin, should be continued unless there is a patient/procedure-related contraindication. There is a controversy on the preprocedural use of beta-blockers. However, the general approach on this issue is that beta-blocker drugs, especially metoprolol, should not be introduced immediately before the procedure but that beta-blockade should be continued in patients who are chronically receiving these medications. Angiotension converting enzyme inhibitors and angiotension receptor blockers should be held 12-24 hours before the procedure due to the concerns of causing vasoplegia. Diuretics should be also held on the day of the procedure whereas other antihypertensive drugs should be continued during the periprocedural period (19). Finally, endocarditis prophylaxis during the procedure is required for patients with several conditions including prosthetic material used for cardiac valve repair, prior history of infective endocarditis, congenital heart disease, and cardiac transplant patients who develop cardiac vavulopathy (14).

TABLE 3. Fasting status in NORA patients (24)

Type of oral feeding	Patient group	duration
Breastfeeding	Newborns and infants	4 hours
Baby food or solid food	Newborns and infants	6 hours
Clear fluids	Adult and pediatrics	2 hours
Light solid meal	Adult	6 hours
Solid or heavy meal	Adult	8 hours

TABLE 4. Special clinical conditions indicating risky anesthesia management (28)

Emergent procedures
Complex, very long, or risky (ie, bleeding) procedures
Patients under 18 years old
Pregnancy
Uncooperative or patients with agitated or altered mental status
Patients with significant cardiac, respiratory, or systemic comorbidities
ASA class III–IV
Increased risk for airway obstruction (morbid obesity, sleep apnea, mallampati score 3-4, Micrognathia, retrognathia, small mouth opening (<3 cm in an adult)
Intolerance to the medications used for sedation (ie, benzodiazepines and narcotics)
Previous problems with sedation or with anesthesia
History of long-term use/abuse of benzodiazepines, narcotics, alcohol, or neuropsychiatric
medications

TABLE 5. Common potential problems that can occur during NORA*

Respiratory events (ie, hypoxia, desaturation)
Hypothermia
Hypovolemia
Airway difficulties
Aspiration of gastric content,
Side effects of drugs or anaphylaxis
Postoperative nausea and vomiting
Procedure related complications (ie, bleeding, gastrointestinal perforation)
Harm to the anesthesiologist (ie, waste inhalational anesthetics and exposure to radiation)
Death

*Adopted from the study by Melloni C. (29)

Fasting guideline

Anesthesiologists must questioned the fasting status of NORA patients; because, patients, parents of pediatric patients, and even proceduralists may not show sufficient attention to this issue. However, fasting status for NORA patients should be similar to classical OR-patients. In this contex, the standard ASA guideline should be followed to avoid the risk of aspiration, except for emergency procedures (Table 3). In pediatrics, clear fluids, such as water and apple/orange juice without particles, can be taken until two hours prior to procedure. This liberal approach avoids hypoglycemia, possible decreased intravascular volume, and irritation of children and their parents (12).

Choice of anesthetic technique

Although a small number of NORA procedures is still considered to be performed without assistance of anesthesia experts in some countries of the world, the dominant opinion in this issue is the necessity of an anesthesia care for all procedures. In addition, several clinical indications require a special anesthetic attention, due to the potential risky conditions (Table 4). Many anesthetic techniques including sedation, general anesthesia with endotracheal intubation or laryngeal mask, and regional anesthesia can be used in patients undergoing any interventional procedure outside the OR. Several factors such as type and duration of the procedure, associated medical diseases and patients' health status, and traditional habits of the anesthesia clinic can influense the choice of anesthetic technique. It should be noted that the choice of anaesthetic technique is not directly associated with differences in major outcome parameters (12).

Standard monitoring of patients undergoing NORA is similar to OR patients, and includes electrocardiography (ECG), blood

pressure measurement, oxygen saturation, exhaled carbon dioxide and temperature (25). Regular end-tidal carbondioxide ($ETCO_2$) measurement is of great importance in the monitorization of patients undergoing NORA, especially those with cardiopulmonary diseases (15). The ETCO2 monitoring is strongly recommended as an audible alarm for the ventilatory depression associated with the use of propofol to provide deep sedation with or without instrumentation of the airway (4).

Contrary to standard monitorization, procedural medication is different from traditional surgical intervention in terms of many reasons. First of all, most of the procedures are shorter than open surgical interventions. Therefore, anesthetic drugs with rapid onset and offset are generally preffered in NORA procedures (26, 27). Tissue trespass is quite less in non-OR procedures compared with classical OR surgeries, which means that most procedures do not result in extensive perioperative pain. Thus, potent, long-acting opioid agents are rarely required in NORA procedures. Neuromuscular blocking agents are only used in patients with endotracheal intubation. Short-acting neuromuscular blockers are, therefore, the preferred choice (12).

Postprocedural Patient Care

All patients undergoing a non-OR procedure should be closely monitored during the postprocedural period. Anesthesiologists should be actively involved in the management of airway, oxygenation, ventilation, hemodynamic parameters, and pain control. Possible adverse events such as nausea and vomiting should be also followed up carefully (28). Discharging or transfer of the patient to inpatient clinic or intensive care unit should be decided by the anesthesiologist, in communication with proceduralist.

Morbidity and Mortality in NORA

Several potential problems related to NORA procedures have been documented (Table 5). Anesthetic complications associated with OR procedures have extensively analyzed to date whereas NORA-related complications have examined in a limited number of clinical studies (29). Hence, current knowledge on NORA-related morbidity and mortality usually comes from closed claims data (30, 31). In those studies, NORA patients were older, and had more coexisting systemic diseases, in comparison to OR cases. In addition, emergent cases constitued a higher proportion within overall NORA procedures. In parallel, the proportion of claims for mortality was higher in NORA procedures. When evaluated the cases developed mortality, respiratory events were the leading causes. Specifically, respiratory depression as a result of oversedation was found to be the most common mechanism of injury. Difficult or esophageal intubation and aspiration of gastric contents were the other common complications (32, 33). As a result, both loss of airway patency due to airway obstruction and respiratory depression due to a excessive depth of anesthesia/sedation may cause hypoxia and hypercarbia that can result in hypotension and cardiac depression (28, 30, 34). Administering oxygen during procedure is recommended to reduce the incidence of respiratory complication (35).

Some authors also reported similar mortality rates between OR and NORA procedures in their studies (36). However, more healthier patients, a greater number of short procedures, and a lower proportion of emergency procedures were shown as the possible reasons of this situation by the authors. Interestingly, patients aged under two years had higher mortality rates compared to other patient groups. As known, extremes of age (very young and old) are considered as an independent risk factor for perioperative mortality (37).

In general, periprocedural morbidity or mortality in NORA is associated with three major risk categories including patient-related, procedure-related, and anesthesia-related reasons. Among these, comorbidities or general health status of patients seems to be most frequently observed risk factors (36, 38). This data clearly shows the importance of preprocedural evaluation and careful intraprocedural monitoring of the patients undergoing NORA, for minimizing the incidence of unwanted conditions.

CONCLUSION

Frequency and complexity of NORA procedures are rapidly raising throughout the world. In addition, novel NORA procedures are increasingly introducing routine practice. It is a reality that anesthesia management has a key role for the success of all these procedures. This increases the importance of anesthesiologists in the management of such procedures. Therefore, anesthesia experts, in a closed comminication with other clinicans and proceduralists, should be active in all stages of patient management.

NORA should be considered as a subspeciality of anesthesia, due to its own features and increasing volume. The locations where NORA procedures are performed should be designed to meet all anesthesia needs. A proper and sufficient preprocedural patient evaluation is an important and mandatory step that identifies patient/procedure-related risk factors. Risk stratification algorithms should also include anesthesia/location-related factors. The major endpoint of all these strategies is to improve the patient outcomes, and a multidiciplinary effort by anesthesiologists, proceduralists, other staff personnel, and hospital administrators is necessary to achieve this goal.

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Review

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Advancements in 3D Printing Technology: Applications and Options for Prosthetic Dentistry

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The use of additive manufacturing systems in dentistry is becoming a widespread phenomenon. Additive manufacturing technology is defined as the fabrication of a 3D model or prototype by agglomerating the biomaterials layer by layer in a specific pattern dictated by the computer-aided design software. With the aid of this technology; structures with superior biocompatibility are rapidly, precisely, and inexpensively fabricated for direct medical utilization. In contemporary dentistry, manifold additive manufacturing techniques have been developed for the fabrication of fixed prosthetic restorations, removable dentures, surgical guides, individualized implants, custom impression trays, and anatomical models. Of these; stereolithography, selective laser sintering, selective laser melting, fused deposition modeling, and electron beam melting are commonly used. However, scientific data regarding their material options and working principles are still insufficient. Therefore, the aim of this review is to study the current status of common additive manufacturing techniques in prosthetic dentistry.

Keywords: 3D printing, additive manufacturing, computer-aided manufacturing rapid prototyping

INTRODUCTION

Additive manufacturing (AM), which is more colloquially known as either 3-dimensional (3D) printing or rapid prototyping (RP), was first expressed by Charles Hull in the late 1980s (1). The fundamental concept of additive manufacturing is to fabricate a 3D model by depositing biomaterials layer by layer in a specific pattern dictated by the computer-aided design software (1-10). The popularity of AM techniques is deliberately increasing as they allow fast, precise, and cost-effective fabrication of highly customized functional structures for direct medical utilization (2). Moreover, the amount of waste is significantly reduced (1). With all these opportunities, there is a considerable shift from standardized to personalized dentistry as manufacturing of custom structures including craniomaxillofacial implants, surgical guides, root-analogue implants, impression trays, polymer-matrix composites, and anatomical models is feasible through this disruptive innovation (1-3).

To date, numerous novel AM techniques have been developed which can present superior print qualities. The five leading technologies prominent in contemporary dentistry are stereolithography (SLA) (1-5, 7, 8, 10-14), selective laser sintering (SLS) (1-5, 9, 11), selective laser melting (SLM) (2, 3, 5, 9, 11), fused deposition modeling (FDM) (1-5, 7, 10, 11, 15), and electron beam melting (EBM) (2, 3, 9, 11). Each adopts different methods of fabrication. However, the fabrication process through AM technologies generally consists of several mutual stages including data acquisition, processing, segmentation, outputting, and post-processing (not necessary for every system) (3-5, 11). Digital data of the related structure can commonly be acquired via computerized tomography, conic-beam computerized tomography (CBCT), magnetic resonance imaging, and digital scanners (6, 7, 11). During processing, a 3D model is virtually designed by means of computer-aided design software and saved as either standard tessellation language file or as another proprietary formats (4, 6, 11). The process continues with the segmentation of the model into 2D layers (1). Subsequently, during outputting, sliced layers are stacked and fused together, thus processed data are printed out with the aid of additive-based printers (4). Last steps can be post-curing for complete polymerization and post-processing (7).

To the best knowledge of authors, the aforementioned common techniques have not been investigated extensively and therefore, data regarding this issue are scarce. In this review, it was aimed to set out the current status of most employed

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additive manufacturing techniques in prosthetic dentistry by comparing their advantageous and disadvantageous properties. Material options and working principles were also scrutinized.

Am Techniques Extensively Used in Dentistry

Stereolithography

SLA technique allows the solidification of liquid photopolymer by using ultraviolet (UV) laser source. After converting the 3D digital model into 2D cross-sections, a coherent light source is emitted in a particular manner by specific points located in a photosensitive resin-containing platform, thus inducing selective photo-polymerization and forming the very first layer. The platform is then lowered into the vat by a one-layer thickness, allowing the liquid to cover the first layer. The same process is then repeated over and over again, until the intended 3D model is physically manufactured (1-5, 7, 8). Laser scan speed, power, and exposure time can become influential on resolution and curing time (1). The resultant model is then removed from the platform and placed into an UV oven, in order to complete the curing process and thereby to meet the required physical properties (3) (Table I).

Selective Laser Sintering

SLS technique allows the creation of 3D models by consolidating consecutive layers of powdered materials. In this method, a laser beam (usually carbon dioxide laser or neodymium-doped yttrium aluminum garnet laser) with a controlled path scans the powder to sinter (to partially melt) it by heating (1-5). High power of laser allows the fusion of powder through molecular diffusion (1). After scanning, the powder platform is lowered by a one-layer thickness, typically between 20-100 µm depending on the type of device, and a new layer of powder is sprayed onto the previous one. The process is repeated until the completion of the 3D model (9) (Table I).

Selective Laser Melting

SLM technique can be considered as a variation derived from SLS, as the same steps are applied in both techniques, with the main difference being that SLM completely melts the powder particles with powerful laser beam in order to form fully dense metallic models (2, 3, 5) (Table I).

Fused Deposition Modeling

FDM technique, also known as fusion filament fabrication (FFF), has a widespread use among AM technologies due to its relative inexpensiveness, high speed, and simplicity (1-4). This technique depends on the deposition of material in semi-liquid state through heat-producing nozzle that extrudes material in a specific path to form layer-by-layer a 3D model (7). The extrusion head heats the material. The molten viscosity has to be high enough to exhibit structural support and low enough to allow extrusion (to avoid clogging). In newer models, multiple nozzles that allow the use of multiple materials with different properties are present. Processing parameters such as raster width, layer thickness, and raster angle can become influential on the printing quality (1) (Table I).

Main Points:

- The AM techniques are very popular as they allow fast, precise, and cost-effective fabrication of highly customized functional structures for direct medical utilization.
- Of these AM techniques; stereo-lithography, selective laser sintering, selective laser melting, fused deposition modelling, and electron beam melting are in use in contemporary dentistry.
- These techniques have been developed for the fabrication of fixed prosthetic restorations, removable dentures, surgical guides, individualized implants, custom impression trays, and anatomical models.

Electron Beam Melting

From a technical standpoint, EBM and SLM share the same melting process of consecutive powder layers for fabricating the 3D model. However, EBM uses an electron beam instead of laser beam as a source of energy (2,5) (Table I).

Current Applications in Prosthodontics

Printing of 3D casts

One of the earliest implementations of AM technology into prosthodontics was to acquire 3D printed casts based on digital impressions, either for diagnostic purposes or to obtain definitive cast to manufacture dental prostheses (7). However, these printed casts have to show accuracy levels at least similar to that of conventional ones in order to be beneficial to the dental practice. Several studies exist in the literature that compare the accuracy of 3D printed casts, conventional casts, and the casts produced by subtractive method (10, 12, 13). In this regard, Revilla-León et al. (10) assessed the capability of 4 different RP technologies to duplicate a fully edentulous model including 7 implant analogues and to fabricate definitive casts for implant prostheses. It was highlighted that conventional dental stone casts could be accurately duplicated by using multi-jet printing and direct light processing technologies. Another study by Patzelt et al. (12) concluded that SLA technology was superior for the fabrication of dental casts; although all of the investigated casts (SLA-based and milled) indicated clinically acceptable accuracy. On the other hand, Alshawaf et al. (13) found that 3D printed casts are inferior to their conventional counterparts in terms of surface finish, interproximal space replication, and accuracy.

Fabrication of Surgical Guides

Surgical guides are important during implantation for pinpointing the best location to drill. The placement of dental implants in the right position and at the right angle (surgical navigation) both increases the success rate of the procedure and minimizes the possibility of damage to the surrounding anatomical tissues (2, 3, 14). During fabrication, initially, data of the patient are acquired with CBCT and intraoral scanner. Subsequently, digital processing and virtual planning through a computer-aided design (CAD) software are conducted (Figure 1). Consequently, surgical guide is produced with the aid of a computer-aided manufacturing (CAM) device (8).

Surgical guide systems can be divided into static or dynamic. Stereolithography is the most commonly used technique as it allows the production of high-precision transparent guides which facilitates the visualization of anatomical structures during the surgical procedure. Stereolithographic guides can be referred as static because they do not allow modification of the virtually-planned position during implant surgery (8).

TABLE I. Material options, technical data, and approximate accuracy level of commonly used additive manufacturing modalities

AM technique	Material Options	Advantages	Disadvantages	Accuracy
SLA	• Acrylate photopolymer • Plastic • Ceramics	• High resolution and accuracy • Rapid fabrication and smooth surface finish • Able to create complex parts with fine details • As being nozzle-free technique, nozzle clogging can be avoided.	• High cost of machining • Requirement of post-processing procedures • Possible cytotoxicity of residual photo-activator and uncured resin	≈50-55 µm
SLS	• Wax • Polymers • Polymer/glass composites • Polymer/metal powders • Metals • Ceramics	• No support material is required • Good chemical resistance • Parts possess high strength and stiffness • High accuracy	• Sometimes, the powder-filled tank is preheated to reduce the power consumption by the laser source and to avoid large thermal differences between particles which can lead later to distortion/cracking in the final product • Post-processing is sometimes needed. • In terms of surface roughness, SLS exhibits inferior results than SLA (4). • Parts are porous.	≈45-50 µm
SLM	• Metals and metal alloys o Stainless steel o Cobalt chromium alloy o Nickel chromium alloy o Titanium (Ti-6Al-4V) alloy	• Superb accuracy • Parts present full density and excellent mechanical properties, compared to SLS (5). • Able to create complex parts with fine details.	• High energy is needed to melt powder. particles which makes the process very difficult to control. • Fluctuations in temperature between particles due to rapid laser scanning result in solid-liquid-solid phase transformation. This may cause thermal shock that leads to accumulation of residual stress, distortion, shrinking or cracking. • Depending on material, parts can be porous	≈20-35 µm
FDM	• Polylactic acid (PLA) • Acrylonitrile butadiene styrene (ABS) • Polycarbonate, Polypropylene • Polyesters • Composites	• Relative inexpensiveness • High fabrication speed • Simplicity, multi-material usage • Wide array for material colour • PEEK material can be printed • Parts exhibit high strength	• The surface finish is relatively poor. This may be solved by polishing or sand-blasting. • High variation in temperature may cause delamination. • Composites has to be in a filament form to be extrudable.	≈35-40 µm
EBM	• Metals	• Vacuumed medium avoids impurities or any deflection of electrons by air molecules. • The presence of well-fused powder can become beneficial as it reduces residual stresses in the final product and enhances mechanical properties considerably.	• Vacuumed medium is expensive. • This technology produces X-rays. • The surface finish is relatively poor. This may be solved by sand-blasting the model using the same building powder in order to avoid contamination.	≈40-50 µm

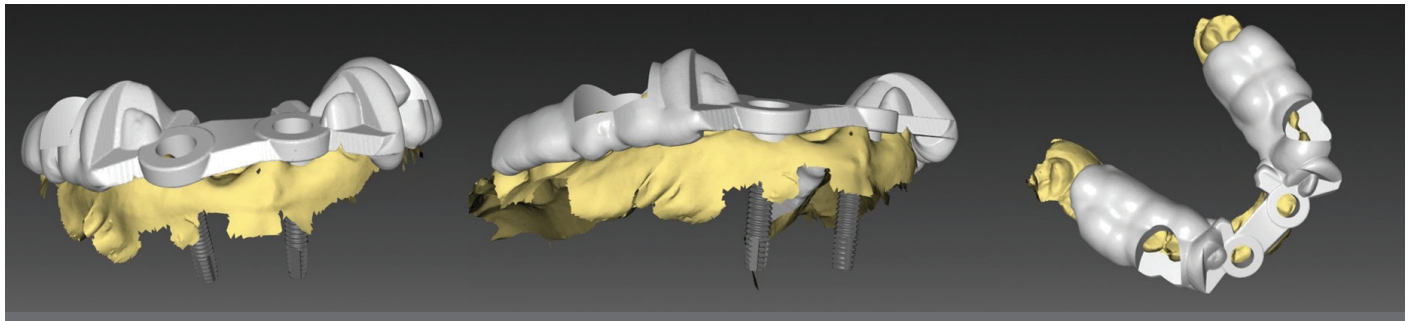

FIGURE I. Virtually designed surgical guide by correlating with CT data of patient (Design was conducted with a CAD software [InLab 15, Sirona Dental Systems, Bensheim, Germany])

Fabrication of Custom Impression Trays

The utilization of 3D polymer modeling technologies in prosthodontics omits some manual, time-consuming processes such as the fabrication of custom trays for taking conventional impressions (Figure 2). Moreover, by digitizing this process, a homogeneous space for the impression material can be achieved (7, 8). The ability of these trays for taking accurate, superior final impressions is also evident in the literature (15). Additionally, the fabrication of custom trays designed especially for maxillofacial prosthetics have also been proven to be feasible (16).

Fabrication of Removable Complete Dentures

Another earliest employment of AM technology is the fabrication of complete dentures in 1994, when Maeda et al. (17) described series of steps to manufacture a complete denture using light-cured resin with the assist of an SLA machine. Since then, there

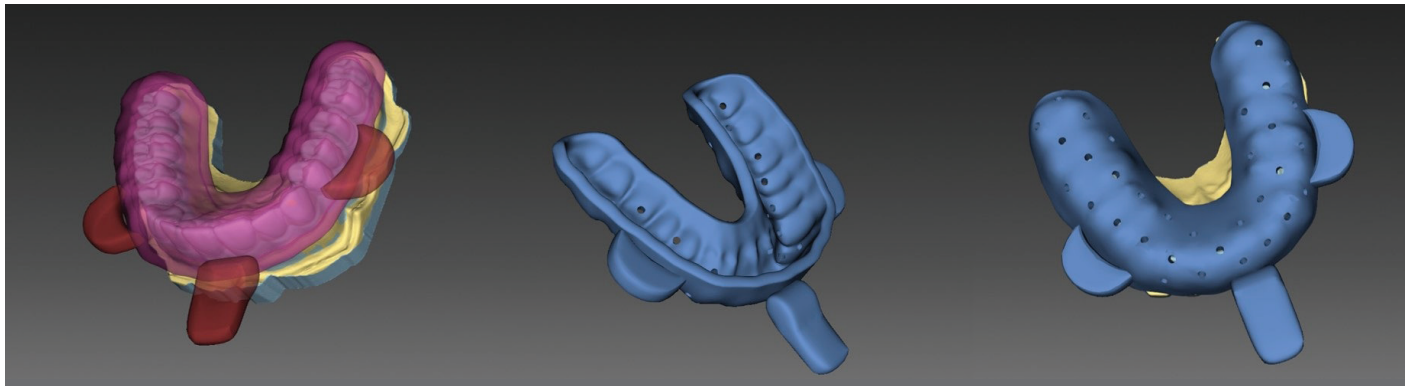

FIGURE 2. Custom tray (Design was conducted with a CAD software [InLab 15, Sirona Dental Systems, Bensheim, Germany])

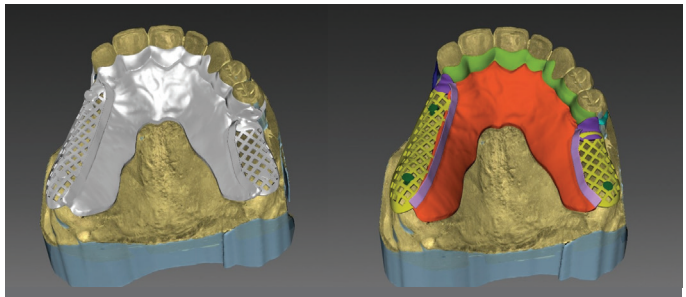

FIGURE 3. Framework design of removable partial denture ready for 3D-printing (Design was conducted with a CAD software [InLab 15, Sirona Dental Systems, Bensheim, Germany])

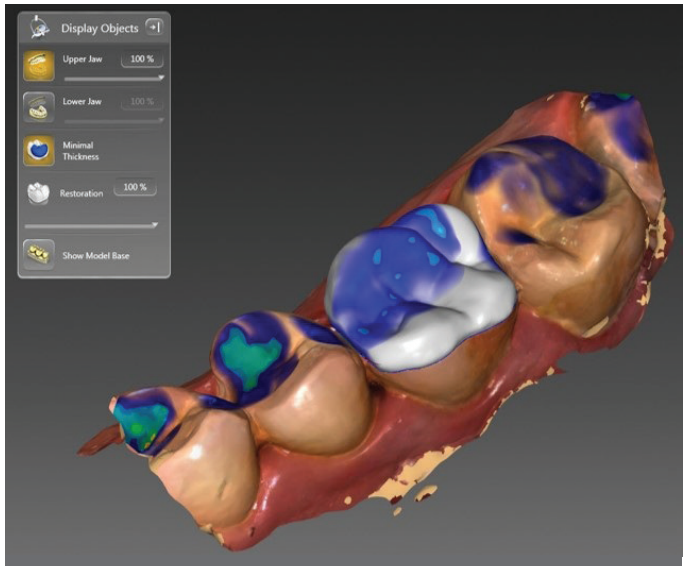

FIGURE 4. Full-ceramic indirect restoration designed for maxillary I. Molar (Design was conducted with a CAD software [InLab 15, Sirona Dental Systems, Bensheim, Germany])

have been several depictions of different methods to incorporate AM technologies into the fabrication of removable complete dentures (7). There are several studies that investigated complete dentures fabricated with additive, subtractive, and conventional manners (18-20). According to Davda et al. (18), AM technique is superior to the conventional methods in terms of precision and accuracy. Inokoshi et al. (19) stated that the use of AM to produce trial wax dentures presents comparable results with conventional technique, and although further improvements are needed; applying RP technique to obtain trial dentures seems to be a promising method. To the best knowledge of authors, manufacture of dentures by using CAD/CAM techniques are considered to be a valid method as these dentures provide equal or better fit, analogous biocompatibility, improved mechanical properties, and high patient/clinician satisfaction. The feasibility of employing AM techniques to manufacture definitive dentures is, however, questionable. A study by Kalberer et al. (20) reinforced this hypothesis by reporting that milled dentures were superior to printed ones in terms of trueness of intaglio surface.

Fabrication of Interim Dental Restorations

Different AM methods to manufacture interim crowns, bridges, or even fixed implant dentures have been described in the literature (8, 21, 22). Additionally, there are several studies that compared 3D printed interim restorations with their milled and conventional counterparts. These studies supported the usability of such interim restorations based on their sufficient mechanical properties and acceptable marginal-internal fit values (23, 24). However, there is a necessity for additional studies regarding the polymers used in AM in terms of biocompatibility and long-term viability (8).

Printing of Castable Patterns

Several commercially available castable polymers are in use for AM technologies. These polymers are shaped with rapid tooling to produce patterns for different restorations which can be casted using conventional methods to obtain metal or pressed lithium disilicate restorations (7). Several descriptions exist in the literature for employments of 3D printed patterns for the fabrication of several types of restorations such as inlays, onlays, crowns and bridges, frameworks for partial dentures, frameworks for implant-supported prostheses, and even maxillofacial prostheses (9, 25-30). Though available, the aforementioned applications of printed patterns have to be investigated in order to verify their viability for replacing with conventional techniques. Inlays and onlays produced from printed patterns were found to have marginal and internal fit values that are clinically acceptable (27, 28). The marginal and internal fit investigations of casted, SLA printed-, and milled-patterns were conducted by Kim et al. (31). They concluded that all test groups have indicated clinically acceptable and comparable marginal-internal fit values, except milled copings. The fit of removable partial denture frameworks has also been investigated in the literature, and despite the lack of sufficient clinical trials, the available evidence supports the fact that printed patterns provide enough fit to the frameworks for clinical applications (32). Regarding the frameworks for implant-supported fixed dentures, Alikhasi et al. (30) have found that although frameworks casted from printed pat-

terns were inferior to the ones produced from milled patterns in terms of retention values; the amount of retention achieved by both groups was clinically acceptable.

Polyetheretherketone (PEEK) is a thermoplastic, semi-crystalline polymer belonging to a family of linear aromatic polymers containing ether and ketone linkages (33, 34). It presents acceptable composition of properties including good biocompatibility, chemical resistance, good mechanical properties, and a low elastic modulus (3-4 GPa) which is analogous to the human cortical bone's (14 GPa) (34). Some of the novel implementations of 3D printed polymers in dentistry is the indirect use of rapid prototyping to produce PEEK frameworks for the partial dentures through thermo-pressing of printed patterns. According to a study by Negm et al. (33), milled PEEK frameworks presented significantly better trueness in comparison to the ones fabricated with indirect AM technique. Nevertheless, both techniques have been found to possess enough fit values from a clinical standpoint.

Fabrication of Dental Implants

The success of dental implants relies heavily on the location of important landmarks (mandibular nerve canal and maxillary sinus) and on the anatomic features of the alveolar bone, mainly the presence of ample bone tissue. Therefore, the idea of manufacturing individualized dental implants with specific dimensions for each patient can improve success ratios in patients with relatively inadequate bone. The aforementioned concept has already become a feasible reality with the advent of AM as the incorporation of rapid manufacturing techniques into implant dentistry allows the manufacturing of highly customized dental implants (2,3,35). The introduction of SLM and EBM to the implant dentistry has unlocked several possibilities for the development of dental implants. Aside from customization, the concepts of osseointegration, titanium alloys, implants with special geometries are all aspects to be exploited thanks to the technologies that rapid manufacturing offers. 3D printed implants have features like micro-roughness, nano-roughness hydrophilic surfaces, and controlled porosity which can all improve the osseointegration process (36). Furthermore, the implementation of 3D printed implants has already yielded good clinical results (37, 38). An additional improvement that AM has to offer in the implantology sector is the use of a new additively manufactured implant material based on Ti-42Nb alloy, as a substitute for the commercially available titanium alloy (Ti-6Al-4V). Schulze et al. (39) proved that the printed implants from this alloy have lower *Young* modulus when compared with standard implant materials, thus improving the elastic compatibility with human bone.

The mixture of above-mentioned characteristics also makes PEEK a viable alternative to titanium and ceramics for applications in implant dentistry (3, 34). Mounir et al. (38) have conducted a study to evaluate highly customized 3D printed titanium and PEEK implants for the rehabilitation of severely atrophic anterior maxilla. The results obtained from a 12-month follow-up showed the success of both titanium and PEEK implants. However, the use of PEEK as an AM material is fairly recent and although it seems promising; the current evidence in the literature that supports the use of 3D printed PEEK in implant dentistry is very scarce.

The production of zirconia implants through AM is also present in the literature (5). It has been demonstrated that printed customized zirconia implants are feasible and can present acceptable dimensional accuracy along with mechanical properties close to the conventionally manufactured ones (40). Additionally, with the aid of advantages that material extrusion techniques can offer, it is possible to create zirconia-based customizable implants. The deposition of two different materials can produce implants in both dense and porous structures, which in turn can reduce the elastic modulus and favour osteointegration thanks to the presence of pores (41).

Fabrication of Metal Frameworks for Fixed Prostheses and Removable Partial Dentures

Lost wax technique and the milling technique are both considered to be the traditional ways to produce metal frameworks for fixed partial restorations, removable partial dentures (Figure 3), and implant-supported dentures. However, with the advent of additive manufacturing, the limitations of the milling technique can be omitted as AM techniques waste minimum amount of material and can produce models with greater accuracy and detail (3, 4, 9, 11).

The mechanical properties, marginal-internal fit, and dimensional accuracy of additively manufactured metal frameworks were all investigated in the literature. The mechanical properties of the printed Cr-Co copings were found to be greater than those produced with milling or conventional techniques (42). Regarding the discrepancy values and dimensional accuracy, Akçin et al. (43) have found that regardless of unit number, implant-supported frameworks fabricated with SLM technique had similar values to the ones fabricated with casting technique and better values than the milled ones. The use of AM to produce metal frameworks for removable partial dentures and for complete dentures has become a useful alternative to the milling and conventional casting techniques as it produces effective prostheses with acceptable clinical results (44, 45). As for implant-supported denture frameworks, several studies have revealed the practicality of AM methods in producing frameworks that have low misfit values and favourable outcomes (46).

Fabrication of Full-Ceramic Fixed Prostheses

The widespread use of ceramic materials in the dental practice can be attributed to a specific set of features that they possess such as excellent biocompatibility, chemical stability, decent mechanical properties, and high aesthetics (Figure 4). However, the brittle nature of ceramics dictates a very strict control over the manufacturing process to acquire dental pieces with convenient mechanical properties. It is because of such properties that ceramics were only lately introduced into additive manufacturing. The high melting point, development of different phases in such high temperatures, and the formation of cracks during the cooling stage due to thermal shocks are all factors that increase the difficulty of processing ceramics through additive techniques (5, 11). The current techniques used for the additive manufacturing of zirconia are material extrusion/jetting and stereolithography for the production of a green body which will be subjected later to post-processing and sintering (5). It has been demonstrated in the literature that by using the aforementioned methods, it is possible to produce zirconia parts with post-sintering densities (ranging between 96.9% and 99%), high dimensional accuracy, and similar mechanical properties to conventionally-manufactured zirconia (11). Problems like anisotropic roughness can be addressed with post-polishing. However, complications like

clogged nozzles that can produce process-related defects, and high abrasion of the machine components are still a cause for concern (47).

In the literature, the rapid manufacturing of alumina ceramics has also been examined. Through techniques like FDM, it is possible to print alumina parts with up to 99% density, homogenous microstructure, and improved mechanical properties. Methods like vacuum infiltration can be used on the green bodies to improve density and strength (48). Dehurtevent et al. (49) have conducted a study that compared stereolithography-manufactured alumina ceramics to the subtractive-manufactured ones. The results indicated the possibility of printing alumina with anisotropic shrinkage, density, and flexural strength similar to those of a subtractive-manufactured ceramic. Wilkes et al. (50) were able to manufacture objects from a mixture containing 41.5 wt.% zirconia and 58.5 wt.% alumina by using SLM technology. The produced models had good mechanical properties and density percentage of almost 100% without the need for any post-processing or sintering. However, they also pointed out some challenges that must be addressed including thermal stresses and surface roughness.

CONCLUSION

AM technology started a new era in the rapid fabrication of net-shaped products by automating stages. As evidenced by the above-mentioned studies, different approaches and different biomaterials have been introduced for precise fabrication of complex-shaped individualized patterns and prototypes with superior print quality in the layer-by-layer manner.

Currently, with the help of this cost-effective innovation in which the amount of residual material is negligible, elaborate dental crowns, removable dentures, surgical guides, individualized implants, custom impression trays, and anatomical models can be manufactured. However, scientific documentation regarding these systems is somewhat scarce and further studies are needed.

The upcoming trends for practitioners will be the use of AM-manufactured root-analogue implants that can be inserted immediately after tooth-extraction and the milling of all restorations (especially zirconia-based ones) by in-house CAD/CAM centres.

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Letter to the Editor

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Some Aspects of Stem Cell Therapy

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This short report is an overview of recent literature on stem cells and cell therapies. Certain papers tend to exaggerate positive effects leaving adverse events out of attention. Therefore, conclusions are partly based here on theoretic considerations. There is a discrepancy between the supposed ability of exogenous stem cells to migrate and engraft in tissues, differentiate along various cell lineages, and the absence of clear morphological evidence in vivo. Some papers discuss rejuvenation, replacement of senescent and damaged cells; others explain reported beneficial effects by paracrine or immunomodulating mechanisms. There are no prima facie reasons to assume that paracrine functions are more developed in morphologically primitive SC than in more mature cells. Stem cells are a promising field of research; however, studies of differentiated cells and cell-free products mimicking paracrine effects of cell therapies may be promising as well. Obviously, therapeutic methods with unproven effects should be applied within the framework of sound research shielded from the funding bias.

Keywords: Cardiology, cell therapy, myocardium, stem cells

It is evident for a reviewer of scientific literature that the quality of argumentation in some areas of medical research has deteriorated since the last decades. Publication series of questionable reliability have been continued without making references to the published criticism. Another tendency is that drugs and treatments without proven efficiency are advertized and corresponding products marketed as evidence-based medications. Scientific concepts are sometimes construed for this purpose or existing ones used arbitrarily (1-4). The conclusions of this report are partly based on theoretic considerations. In conditions when it is difficult to distinguish between reliable and unreliable papers, theoretic considerations gain in importance. Some questions are not entirely clear, so that arguments provided here can induce a constructive discussion.

Last time, a large number of publications on stem cells (SC) and cell therapies have emerged, some of them containing attractive terms such as rejuvenation, anti-aging strategy etc. (5-7). Discussed topics include the differentiation of exogenous SC into various cell lineages, replacement of senescent, dysfunctional and damaged cells. Remarkably, assumptions that SC can differentiate into specialized cellular elements have not been confirmed for such a perfect SC as the fertilized ovum. In the "experiment" performed by the nature - extrauterine pregnancy - no differentiation of pluripotent embryonic cells towards surrounding tissues is observed but an embryo and germinal layers are formed. The implantation of embryonic SC can result in a development of teratoma (8, 9). It is known from general pathology that a focal cell proliferation results in the formation of a nodule rather than migration of individual cells into surrounding tissues. For a pathologist, it is difficult to envisage how SC migrate in tissues such as myocardium, liver or cartilage, arrive at the areas where they are supposed to be needed, and engraft in preexisting structures (10, 11), commented in (12). The survival and engraftment rates of SC are regarded to be poor (13).

The migration of SC into ischemic myocardium or infarct zone was reportedly associated with a scar size reduction, cardiomyogenesis and neovascularization (7, 14-17). However, no cardiac SC therapy has been conclusively proven effective (9). Immunohistochemical analyses revealed neither transdifferentiation of mesenchymal SC into cardiomyocytes nor increased vascularization (15). The participation of SC in myocardial regeneration has been questioned and other mechanisms of the therapeutic action assumed e.g. improved vascularization (16, 18). However, the benefit from such vascularization, if it really occurs, is doubtful because ischemia is usually caused by an obstruction of larger epicardial vessels. Accordingly, ischemia can be alleviated by functioning collaterals but not by a locally enhanced microcirculation (19, 20).

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As mentioned above, it is difficult to envisage how SC migrate in tissues. In osteoarthritis, SC would have to move through the dense matrix of hyaline cartilage. If even SC after an intra-articular injection are homing in superficial defects of the joint cartilage, synovial or meniscal surfaces (21), proliferate there and produce extracellular substance, it remains unclear how the smoothness and congruence of joint surfaces is maintained, why the focal cellular proliferation does not result in excrescences crumbling into the articular cavity causing dysfunction and inflammation. Reproducible protocols to induce chondrogenesis by SC are lacking (22). In publications dedicated to the therapy of liver cirrhosis, a differentiation of mesenchymal and other SC to hepatocytes as well as promotion of hepatocyte proliferation is regarded possible (23, 24). "The ability of mesenchymal SC to differentiate into hepatocyte-like cells makes them an ideal alternative method for treating liver fibrosis" (25). However, potential differentiation along the mesodermal lineage e.g. to fibroblasts is not discussed. The fibroblastic differentiation would possibly accelerate the advancement of fibrosis and cirrhosis of the liver or other organs. The theoretical basis for the cirrhosis therapy with SC is hardly comprehensible as hepatocytes are capable of mitosis and can hyper-regenerate in cirrhosis whereas nodules are formed.

The action mode of SC remains incompletely described; alternative mechanisms have been proposed: immunomodulating, paracrine (anti-inflammatory, anti-apoptotic, anti-fibrotic, angiogenic, mitogenic), activation of precursor cells in the microenvironment etc. (7, 26-28). It was hypothesized that SC secrete anti-aging substances (29). However, there are no prima facie reasons to assume that such special functions would be more developed in morphologically primitive SC or partly differentiated progenitor cells than in more differentiated cells. In any case, experiments with mature cells would be less expensive. The same can be said about cell-free products obtained e.g. from cell culture media and mimicking the paracrine action of cell-based therapies. The latter approach would achieve a better dose standardizing than cell implantations whatever is understood under it (30). Meanwhile, doubts regarding efficiency of cell therapies and concerns about their safety are remaining. Allogeneic transplantations carry the risk of infections and immunologic complications (31). Among others, this is a matter of concern when cell therapies are applied for the treatment of diseases with participation of immune mechanisms. Routes of SC administration or "implantation" include transvenous, transendocardial, intracoronary and transepicardial injections (17, 32-34). In this connection, sources of SC used for intracoronary injections e.g. tissues from induced abortions (32) and their purification from potentially immunogenic components are of importance (35). The infusion of autologous bone marrow cells or fractions of the patient's own blood is sometimes named autotransplantation; it is associated with a lower risk than allotransplantation. However, benefits from such procedures are questionable apart from a restoration of the pool of hemopoietic cells after cytotoxic or immunosuppressive treatments (e.g. of hematological malignancies or multiple sclerosis) or similar applications that have been known long since.

Main Points:

- Genetic instability, tumorigenic and immunogenic potential have limited the clinical application of SC.
- There is increasing evidence that a majority of implanted SC do not survive due to the immune rejection and lack of a favorable microenvironment.
- Alternative action mechanisms of SC have been proposed, including paracrine, immunomodulating and trophic. However, there are no reasons to expect more special functions from morphologically primitive SC than from differentiated cells.
- SC are a promising field of research; studies of differentiated cells and cell-free products mimicking paracrine effects of cell-based therapies are promising as well.
- Therapies with unproven effects should be applied within the framework of high-quality research, shielded from bias and to conflicts of interest.

All said, SC seem to be a promising field of research. However, studies of differentiated cells and cell-free products mimicking paracrine effects of cell-based therapies may be promising as well. Unfortunately, the literature is partly biased, exaggerating positive effects, if there are any. Some patients pay for cell therapies; but the experience is partly lost for the science because some conflicted researchers overestimate positive results leaving adverse effects out of attention. One of the objections to prohibitive measures (36, 37) is that the hope is taken from severely ill patients. Obviously, therapeutic methods with unproven effects must be applied within the framework of sound research shielded from the funding bias. Patients participating in such research should be treated free of charge.

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Letter to the Editor

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COVID-19 and Impairment Due to Inactivity

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COVID-19 has been rapidly spreading worldwide since it was first reported to the WHO by China on 31 December 2019. As of April 3, there are almost one million confirmed cases and about 50 thousand deaths all over the world (1). Governments try to use different policies such as herd immunity, mitigation, suppression or even curfew but any policy has major challenges. They are mainly focused on how to control the pandemic while the scientists are racing to find the best medication and vaccine. The highly recommended strategy is "social distancing" (2). Although it is both protective and preventive for those with chronic diseases and the elderly, it may impair the physical and mental health of the noninfected people as the isolation period extends. Another aspect of the global pandemic is the limitations in mobility, especially results in staying home. It promotes inactivity for the noninfected people by limiting their chance to exercise due to the measures taken. Along with this, people may tend to consume more than their daily requirements because of stress and boredom. Caloric intake may increase due to binge eating, comfort eating, junk food, and/or high-calorie drinks including alcohol. It is currently unpredicted when this pandemic will end but it is simply answered as "not very soon". Optimistic expectations that the outbreak will end and life will get back to normal in the next 12 weeks but the vaccine is 12 to 18 months away. So, staying home for at least 12 weeks will abnormally increase the caloric intake and physical inactivity will also contribute to the gain fat. A hamburger, fries and a milkshake or a can of soda can reach a total of 1500 calories, without bars, nuts, snacks, and extra drinks. Consuming some other traditional meals, containing animal fat, oil, and sugar, may provide higher than this amount of calories. If a person gets extra 1500 calories per day, at the end of the 12-week period, the increase in weight will be almost 14 kgs and this will adversely affect health. To promote physical activity and to inform people about the effects of extra calorie intake will be an appropriate approach to prevent this "delicious and easy" threat. Social media should be used as a means of communication by the governments to warn people about the risk of excess calorie intake and physical inactivity, or the obesity epidemic may worsen in the near future.

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Letter to the Editor

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Spontaneous Renal Artery Dissection with Renal Infarct

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Dear Editor,

A 51-years-old woman was admitted to emergency service with left flank pain and nausea for the last few hours. She denied history of the renal stone disease. Abdominal examination revealed tenderness of left flank region. Vital signs were insignificant. Microscopic haematuria was observed on the urine analysis. Leucocyte count was 9530/mm3. Total triglyceride and cholesterol levels were high than normal values. The abdominal US was normal. Contrast-enhanced abdominal computed tomography (CT) revealed irregular and long segment stenotic left renal artery due to spontaneous dissection and wedge-shaped infarcts at the lower pole of the left kidney (Figure 1). 3D volume rendering reformatted CT image revealed a disconnected focal area on the left renal artery due to dissection (stippled red arrow). The proximal segment of the left renal artery (yellow arrow) were normal on the reformatted images (Figure 2). The patient subsequently underwent endovascular treatment. The patient was discharged on the 3rd day after treatment with no further symptoms or complications. The patient's consent was assessed.

Renal artery dissection usually occurs due to secondary to iatrogenic or traumatic injury. However, spontaneous renal artery dissection (SRAD) is a rare, and it is often unrecognized entity but remains a radiological finding that all clinicians should be aware of. The incidence of SRAD is very rare; approximately 200 cases of SRAD have been published in the English literature, especially in the fourth to the sixth decade with a marked male predominance (1, 2). Approximately 10-15% of all cases have bilaterally renal artery dissection.

Progressive renovascular hypertension, changes in renal function, haematuria and symptoms of kidney infarction are frequent symptoms of SRAD; however, a most frequent symptom is sudden onset flank pain and renal colic symptoms in patients with SRAD. The patient we present had a sudden onset of flank pain and nausea, which was similar to literature. Clinically differential diagnosis of SRAD is difficult and include renal stone disease and pyelonephritis (3). Ultrasound is

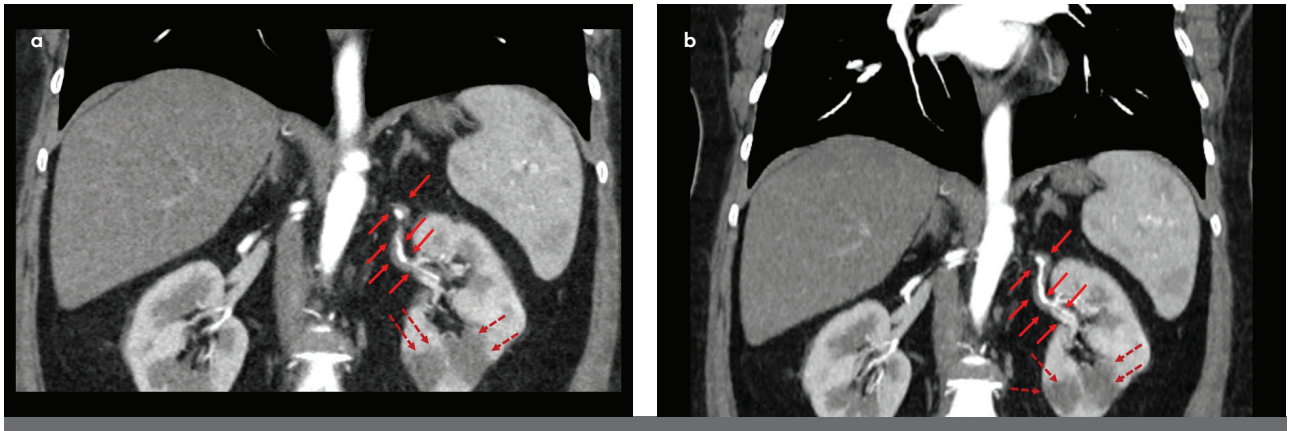

FIGURE 1. Irregular and long segment stenotic left renal artery due to spontaneous dissection and wedge-shaped infarcts

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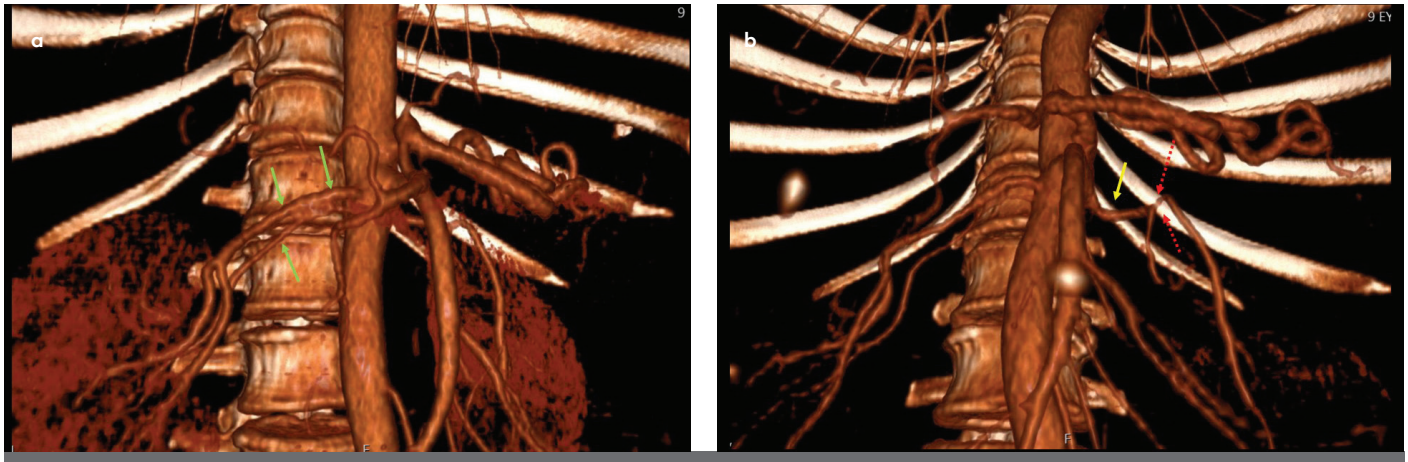

FIGURE 2. Disconnected focal area on the left renal artery due to dissection

the first imaging method for flank pain, whereas it has a limited role for diagnoses in renal artery dissection. IV contrast-enhanced computed tomography (CT) has a diagnostic accuracy rate of up to 100% in renal infarcts and renal artery dissection (4). On CT images, renal infarct areas reveal wedge-shaped hypodense areas on the axial CT images. Peri-renal fat stranding can also observe around the infarcted renal parenchyma. On the contrast-enhanced CT images, diffuse wall thickening of the renal artery due to SRAD and narrowing at the renal artery lumen may also help to establish a final diagnosis (4).

Although the majority of patients with SRAD may be treated conservatively with anticoagulant therapy (5), endovascular treatment (stenting or coiling) is the important non-invasive treatment alternative in the patients who are hemodynamically unstable (6, 7). As a result, SRAD is a very rare vascular disorder and most complicated with renal infarct. Diagnosis is mostly delayed because most patients have insignificant symptoms. Contrast-enhanced abdominal CT is significant for quick and definitive diagnosis.

Informed Consent: Written informed consent was obtained from patient who participated in this study.

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