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Journal's History

Journal's Previous Name	Parallel Title	ISSN	Years
Mustafa Kemal Üniversitesi Tıp Fakültesi Dergisi	Medical Journal of Mustafa Kemal University	1308-7185	2010-2014
Mustafa Kemal Üniversitesi Tıp Dergisi	Medical Journal of Mustafa Kemal University	2149-3103	2015-2022
Interdisciplinary Medical Journal	Interdisciplinary Medical Journal	2980-1915	2023-Present

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Türkish Medline

Turkish Citation Index

DOAJ

Index Copernicus World of Journals

The Journal is committed to practice the publication ethics and takes all possible measures against any publication malpractices.

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

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

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Volume 14, Issue 48, 2023

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INTERDISCIPLINARY MEDICAL JOURNAL

Interdiscip Med J 2023;14(48)

ABOUT

Interdisciplinary Medical Journal is an open access scientific journal, which publishes original contributions in clinical disciplines pertaining to human medicine. In this context, the Journal publishes original research, case reports and reviews based on clinical studies having interdisciplinary approach on medicine. The Journal is official publication of Hatay Mustafa Kemal University, Faculty of Medicine. The manuscript evaluation is based on the principles of blind peer-review process. It is published online three times a year on April, August, and December. The communication, review and publication language of the Journal is English. Manuscripts submitted for publication in the journal should be prepared in accordance with research and publication ethics. All manuscripts should be submitted by online system of the Journal. All manuscripts submitted to the Journal are screened in terms of originality.

Focus & Scope

Interdisciplinary medicine can be defined as "an interdisciplinary approach that relies on health professionals from different disciplines, along with the patient, working collaboratively as a team. The most effective teams share responsibilities and promote role interdependence while respecting individual members' experience and autonomy.

By supporting the interdisciplinary research on medicine, The Journal aims to;

Publish original contributions from different scientific disciplines through the advisory board covering a wide range of clinical medical disciplines,

Offer all its content freely available without charge to the user or his/her institution, to make research freely available to the public, and to support a greater global exchange of knowledge,

By permitting authors to enter into separate, additional contractual arrangements for the non-exclusive distribution of the journal's published version of the work (e.g., post it to an institutional repository or publish it in a book), to lead productive exchanges, as well as earlier and greater citation of published work.

Subject areas include, but are not restricted to the **clinical studies** of the following fields:

First Aid and Emergency Medicine

Family Medicine

Public Health and Preventive Medicine

Internal Diseases

General Surgery

Gynecology and Obstetrics

Ear, Nose and Throat Diseases

Eye Diseases

Orthopedics and Traumatology

Radiology and Radiodiagnostics

Anesthesia and Intensive Care Medicine

Adolescent Diseases

Childhood Diseases

Multisystem Diseases

Physical Medicine and Rehabilitation

Forensic Medicine

Mental Health and Diseases

Cardiovascular System Diseases

Nervous System Diseases

Neurosurgery

Respiratory System Diseases

Infectious Diseases

Occupational Diseases

Nuclear Medicine

Oncological Diseases

Sports Medicine

Genetic Diseases

Medical Pathology

The journal covers all relevant branches in **clinical medicine** specialties of the topics mentioned above.

Audience

Academicians, specialist physicians and research assistants in surgical and non-surgical medical disciplines and general practitioners.

Abstracting and Indexing

Interdisciplinary Medical Journal is indexed by TÜBITAK TR Index, Turkish Medline, Turkish Citation Index, DOAJ and Index Copernicus World of Journals

Editorial Policy

The editorial and publication processes of the journal are shaped in accordance with the guidelines of The International Council of Medical Journal Editors (ICMJE), The World Association of Medical Editors (WAME), The Council of Science Editors (CSE), The Committee on Publication Ethics (COPE), The European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the Principles of

Transparency and Best Practice in Scholarly Publishing (https://doaj.org/bestpractice).

Peer-Review Policy

Initial Manuscript Evaluation

For consideration, all manuscripts should be submitted by online system of The Journal at https://dergipark.org.tr/en/pub/interdiscip. The whole editorial process of The Journal is done via online submission system and can be documented in case of a conflict or objection.

The suitability of papers for publication in The Journal is decided by the editorial policy of the editorial board.

Editor-in-Chief is fully authorized for reviewing process, which is processed in the order of submission to The Journal.

Editor-in-Chief assigns either one of the Co-Editors or himself in order to perform initial assessment. Then, the assignee conducts initial pre-refereeing checks to ensure the article is legible, complete, correctly formatted, original, within the scope of The Journal, in the style of a scientific article and written in clear language.

Ethical Declaration

The Journal is committed to practice the publication ethics and takes all possible measures against any publication malpractices.

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

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.Identifying information, including names, initials, or autopsy numbers of the patients/deceased should not be exposed in written descriptions or photographs in no ways. Identifying details should be omitted if they are not essential. Informed consent should be obtained in human studies and it should be stated in the manuscript.

Human and Animal Rights: For the experimental, clinical and drug human studies, approval by ethical committee and a statement on the adherence of the study protocol to the international agreements (World Medical Association Association of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended October 2013, www.wma.net) are required. In experimental animal studies, the authors should indicate that the procedures followed were by animal rights (Guide for the care and use of laboratory animals, https://nap.nationalacademies.org/catalog/5140/guide-for-the-care-and-use-of-laboratory-animals), and they should obtain animal ethics committee approval. The Ethics Committee approval document should be submitted to the The Bulletin of Legal Medicine together with the manuscript.

The approval of the ethics committee, statement on the adherence to international guidelines mentioned above and that the patient's informed consent is obtained should be indicated in the Method section and is required for case reports whenever data/media used could reveal the identity of the patient. The declaration of the conflict of interest between authors, institutions, acknowledgement of any financial or material support, aid is mandatory for authors submitting a manuscript, and the statement should appear at the end of the manuscript. Reviewers are required to report if any potential conflict of interest exists between the reviewer and authors, institutions.

For persons under 18 years of age, please provide a consent form that includes both parents' signatures or of the person's legal guardian or supervisor.

When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2013. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Check for Plagiarism

The Journal is a member of Crosscheck, and all manuscripts are screened by Crosscheck in terms of originality. If serious issues are identified concerning the manuscript, the assignee will notify the corresponding author as part of our standard procedure. No action is required from you unless specifically requested for very serious concerns.

Any article that has problems with any of the above criteria may be rejected at this stage. Manuscripts rejected at this stage typically are not in line with the focus and scopes of the journal. Essays that pass through this stage are then entered into the peer review process.

Manuscript Files

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit; a blinded manuscript, and a separate title page file.

You may download full manuscript and blinded manuscript templates by following the links on Journal's homepage.

1) Blinded Manuscript File: This is the blinded manuscript file that will be presented to the reviewers. The main text of the article, beginning from Abstract till References (including tables, figures or diagrams) should be in this file. The file must not contain any mention of the authors' names or

initials or the institution at which the study was done, ethical committee or acknowledgements. Self-identifying citations and references in the article text should be avoided.

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After assigned, the reviewers are invited to participate the review process via a notification email. The email invitation to reviewers will contain all necessary information about the manuscript. The reviewers are asked to log into the journal web site to indicate whether they will undertake the review or not, as well as to access the submission and to record their review and recommendation.

Automated email reminders are sent to the reviewer when the reviewer does not respond to a review request within 15 days. The typical period of time allowed for reviews is 4 weeks but note that it can be modified during the editorial process.

Peer Review Reports

Reviewers make one of the following recommendations to the Editors: accept submission, minor revision, major revision, decline submission. Additionally, reviewers are asked to provide significant commentary for authors and are also provided space to make comments intended solely for the editors. Reviewers are not asked or expected to make any copyediting comments.

If both reviewers agree on acceptance or rejection, the decision stands.

Occasionally, we receive contradicting Reviewers' reports and need to ask for a third reviewer, which does delay the process. When their opinions are poles apart, then the Associate Editor takes a further process to decide acceptance or rejection of that paper. For the further process, Associate Editors request an emergency review by another advisory board member. Associate Editors may take a further process for the manuscript of which reviewing process is overdue.

If a paper is not suitable for publication it will be returned to the author with a statement of reasons for rejection. The author may appeal if he or she believes an erroneous or unfair judgment has been made. A letter to the Editor-in-Chief presenting reasons why the decision should be reconsidered will be subjected to due consideration.

After review, the author is notified by email for revision in accord with suggestions made by the reviewers and the Editor.

At the completion of each round of the peer review process, the submitter receives a formal letter from the Editor that includes notes from the Peer Reviewers.

When authors make revisions to their article in response to the referees' comments, they are asked to submit a list of changes and any replies for transmission to the referees. The author must upload the revised manuscript to the online system within 4 weeks; otherwise, the author will be notified that the paper will be considered withdrawn.

The revised version is usually returned to at least one of the original referees who is then asked whether the revisions are satisfactory. If the referees remain dissatisfied, the paper can be referred to the advisory board of the journal for further consideration.

The assignee then, will check if the manuscript is revised as suggested by editorial members and proceed to the next step. If the assignee finds the revisions satisfying, then he or she will record the decision to accomplish the review process and reach final decision.

Decision Making Process

Based upon two or more reviewers' reports, the Co-Editors are responsible for making the decision regarding acceptance or rejection each essay and for communicating this decision to the submitter. At the completion of the peer review process, each submitter receives a formal letter from the Editors that includes notes from the Peer Reviewers.

The final decision by Editor-in-Chief is usually completed within 2 months from the time of the paper submission.

Authors are notified by e-mail when a manuscript has or has not been accepted for publication. Proofs of accepted manuscripts are sent to the author for correction within 4 weeks after acceptance. They should be proofread carefully according to the instructions attached and returned within the considered period. Authors will be charged for major corrections to their proofs.

For 2020, average days required to complete the review process is 120 days, whereas average days that pass till publication is 180 days.

Publication Frequency

Interdisciplinary Medical Journal publishes three issues online in April, August and December. The communication, review and publication language of the Journal is English.

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Author Guide

Dear author,

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Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

2. Where available, DOIs for the references list have been provided.

3. During the online submission, I undertake to enter the names of all the authors in the manuscript, their positions, ORCID ID*** information and the contact details of the responsible author.

*** The publication of ORCID ID information of all authors is mandatory by TUBITAK ULAKBIM Index.

4. By 2020, in accordance with the TR Directory Rules, the authors have to submit an official letter with an Ethical Commission report or equivalent for the works requiring ethics committee permission.

5. Blinded manuscript file to be sent to the reviewer,

6. Title page, A separate title page file containing article title; academic titles, areas of expertise, affiliations, and e-mail addresses each author; contact address of the corresponding author, disclaimer, financial resources of the study, information on whether there is a conflict of interest, article title, short title, thanks and authorship contribution.

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8. Blinded manuscript file and title page file are in the Microsoft Word file format.

Writing Rules

In order to prevent waste of effort and time, and to evaluate the manuscript drafts you send to Interdisciplinary Medical Journal, you must use Interdisciplinary Medical Journal templates, which you can download to your computer using the links on the journal homepage, and which contain explanations for the writing rules in the comments section.

The Journal is official publication of Hatay Mustafa Kemal University, Faculty of Medicine. It is an open access scientific journal, being published three times a year and peer reviewed. The Journal aims to publish original contributions based on clinical studies having interdisciplinary approach on medicine. The publication language of the journal is English.

Subject areas include, but are not restricted to the **clinical studies** of the following fields: first aid and emergency medicine, family medicine, public health and preventive medicine, internal diseases, general surgery, gynecology and obstetrics, ear, nose and throat diseases, eye diseases, orthopedics and traumatology, radiology and radiodiagnostics, anesthesia and intensive care medicine, adolescent diseases, childhood diseases, multisystem diseases, physical medicine and rehabilitation, forensic medicine, mental health and diseases, cardiovascular system diseases, nervous system diseases, neurosurgery, respiratory system diseases, infectious diseases, occupational diseases, nuclear medicine, oncological diseases, sports medicine, genetic diseases, medical pathology.

The journal covers all relevant branches in **clinical medicine** specialties of the topics mentioned above.

Audience

Academicians, specialist physicians and research assistants in surgical and non-surgical medical disciplines and general practitioners.

Manuscript Preparation

All manuscripts which will be published in the journal must be in accordance with research and publication ethics. All authors should have contributed to the article directly either academically or scientifically. Presentations at congresses or in symposia are accepted only if they were not published in whole in congress or symposium booklets and should be mentioned as a footnote.

Manuscripts are received with the explicit understanding that they have not been published in whole or in part elsewhere, that they are not under simultaneous consideration by any other publication. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by written permission for their use from the copyright owner and authors. All articles are subject to review by the editors and referees.

Process of Peer Review

The journal utilizes a standard online site (https://dergipark.org.tr/en/pub/interdiscip), supported by Tubitak Ulakbim, for the process of both manuscript submission and manuscript peer review. Upon receiving a manuscript submitted for consideration of publication to the journal, the journal manager and editorial staff review the submission to assure all required components as outlined in this Guide for Authors are included. The manuscript is then assigned to one of the co-editors (either the editor in chief or an associate editor) who directs and oversees the peer-review process. The co-editor then reviews the submission for relevance, content and quality. Those submissions deemed appropriate for consideration of publication are then assigned to at least two peer reviewers. In order for a manuscript to be considered for publication, it must be original and significant, providing a contribution to research and importance to field. In general, there should be no flaws in the specific procedures used in performance of the study, or in the logic used for the interpretation of the data. It is important that the results of the study support its conclusions, and that there are no errors in reference to prior work (or no exclusions of pertinent references). Where appropriate, confirmation of regulatory review (such as institutional review board approval) must be present. The validity of the statistics used (often including a justification of a sample size) to analyze data is necessary, and the data presented in the figures and tables should be reflective of the results presented and adequate to justify the study conclusions. In general, the manuscript length and quality of the writing are important to ensure its quality.

When the editor has a full complement of reviews completed, the editor reviews the comments and recommendations, and a decision regarding the suitability for publication of the manuscript is made. Acceptance is based on significance, and originality of the material submitted. If the article is accepted for publication, it may be subject to editorial revisions to aid clarity and understanding without changing the data presented.

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

About the scientific language to be used in writing your manuscript

In line with the recommendation of the international directories we applied to increase the scientific effectiveness of our journal and enrich its content, our Editorial Board has decided that the studies to be published in English. So, the manuscripts sent to our journal are subject to English language control and revision.

Our experience from previous articles has shown that most of the articles prepared in English need to be improved in terms of fluent readability and intelligibility, as well as scientific and technical examination. Most of the manuscripts should undergo a comprehensive review and revision process in terms of language, before they were included in the review stage.

Therefore, we recommend that you receive professional English editing and proofreading services before submitting your manuscript to our journal, although it is not mandatory.

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Use of first person

In addition, it is necessary to make the necessary checks and revisions in terms of language of your work and to ensure integrity in terms of language and time use throughout the entire article.

Expressions such as ... "Our study, in our study, we, we did, we found, we aimed, I did, I found, I think ... etc." should be revised as follows;

- In this study, ... it was found/determined/... or
- In this study ... it was aimed to ...

Names made up of single word should not be abbreviated.

Instead of,

- Hypertension (HT) is one of the most ...

Throughout the manuscript, you should use;

- Hypertension is one of the most ...

Instead of,

- Rituximab (RTX) is an IgG1 kappa chimeric monoclonal

Throughout the manuscript, you should use;

• Rituximab is an ...

Numbers should always be used to indicate statistics, age and measurements (including time as in the 3 weeks example). In specifying the others, only the numbers one to nine should be written in letters. (Numbers between 1-10 should be written with letters, except for the date and number of cases)

For example;

• In 2 studies, ...

Should be replaced with;

• In two studies ...

For example;

• ... perivascular lymphotic infiltration in only 10 percent and fibrosis in 7 percent of the patients,

Should be replaced with;

• ... perivascular lymphotic infiltration in only 10% of patients ... in 7% of patients ...

Prejudiced expressions should be avoided in expressions other than classical textbook knowledge, which has been verified by dozens of studies and has become the industry standard in the literature.

• determined to be high

Should be replaced with;

• ... was found to be high.

Or throughout the entire manuscript;

• found to be significantly higher ...

If diametrically opposite findings are mentioned among the studies mentioned in the Discussion section, it should be stated as "... a significant relationship was found / observed / reported", rather than "a significant relationship was determined" etc.

• While no significant relationship was determined between blood pressure and disease severity (26,27), a strong relationship was determined in some studies (28,29).

Should be replaced with;

While no significant relationship was observed between blood pressure and disease severity (26,27), it was reported that a strong relationship was found in some studies (28,29).

General Principles

The text of articles reporting original research should be divided into Introduction, Method, Results, and Discussion sections. This so-called "IMRAD" structure is not an arbitrary publication format but a reflection of the process of scientific discovery. Articles often need subheadings within these sections to further organize their content. Other types of articles, such as meta-analyses, may require different formats, while case reports, narrative reviews, and editorials may have less structured or unstructured formats.

Electronic formats have created opportunities for adding details or sections, layering information, cross-linking, or extracting portions of articles in electronic versions. Supplementary electronic-only material should be submitted and sent for peer review simultaneously with the primary manuscript.

Sections of the manuscript

Article title

The title provides a distilled description of the complete article and should include information that, along with the Abstract, will make electronic retrieval of the article sensitive and specific. Information about the study design could be a part of the title (particularly important for randomized trials and systematic reviews and meta-analyses). Please avoid capitalizing all letters of the title and capitalize only the capital letter of first word of the title, proper nouns, proper adjectives. Other words and conjunctions (e.g., and, but, both, or, either, neither, nor, besides, however, nevertheless, otherwise, so, therefore, still, yet, though etc.) should be in small letters. No abbreviations or acronyms should be used within the titles.

Short title

You should add a running title not exceeding 40 characters to be placed at the header of the inner pages.

Abstract

Original research, systematic reviews, and meta-analyses require structured abstracts. The abstract should provide the context or background for the study and should state the study's purpose, basic procedures (selection of study participants, settings, measurements, analytical method), main findings (giving specific effect sizes and their statistical and clinical significance, if possible), and principal conclusions. It should emphasize new and important aspects of the study or observations, note important limitations, and not overinterpret findings. Please, do not cite figures, tables or references in the abstract.

Because abstracts are the only substantive portion of the article indexed in many electronic databases, and the only portion many readers read, authors need to ensure that they accurately reflect the content of the article. All the articles submitted to the journal require to include abstract in English. Abstracts of original articles should not exceed 250 words.

Keywords

Three to six words or determinative groups of words should be written below the abstract. Abbreviations should not be used as keywords. Keywords in English should be chosen from MESH (Medical Subject Headings http://www.nlm.nih.gov/mesh) index. Abbreviations cannot be used as keywords, but instead they should be written explicitly. Letters that do not exist in Latin alphabet (e.g., alpha, beta, delta etc.) should be used with their pronunciation.

Examples: carbon monoxide, firearms, sexual abuse, oral mucosa

Introduction

Provide a context or background for the study (that is, the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation. Cite only directly pertinent references, and do not include data or conclusions from the work being reported.

Method

The guiding principle of the Method section should be clarity about how and why a study was done in a particular way. The Method section should aim to be sufficiently detailed such that others with access to the data would be able to reproduce the results.

The authors should clearly describe the selection of observational or experimental participants (healthy individuals or patients, including controls), autopsied persons, including eligibility and exclusion criteria and a description of the source population.

In general, the section should include only information that was available at the time the plan or protocol for the study was being written; all information obtained during the study belongs in the Results section. If an organization was paid or otherwise contracted to help conduct the research (examples include data collection and management), then this should be detailed in the method section.

The Method section should include a statement indicating that the research was approved or exempted from the need for review by the responsible review committee (institutional or national). If no formal ethics committee is available, a statement indicating that the research was conducted according to the principles of the Declaration of Helsinki should be included.

Identifying information, including names, initials, or autopsy numbers of the patients/deceased should not be exposed in written descriptions or photographs in no ways. Identifying details should be omitted if they are not essential.

Informed consent should be obtained in human studies, and it should be stated in the manuscript.

When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Statistical Analysis

The authors should describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to judge its appropriateness for the study and to verify the reported results. They should define statistical terms, abbreviations, symbols and should specify the statistical software package(s) and versions used.

Results

You should present your results in logical sequence in the text, tables, and figures, giving the main or most important findings first. Please, do not repeat all the data in the tables or figures in the text; emphasize or summarize only the most important observations. Provide data on all primary and secondary outcomes identified in the Method Section. Extra or supplementary materials and technical details can be placed in an appendix where they will be accessible but will not interrupt the flow of the text, or they can be published solely in the electronic version of the journal.

You should give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical significance attached to them, if any. You should restrict tables and figures to those needed to explain the argument of the paper and to assess supporting data. Please, use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as “random” (which implies a randomizing device), “normal,” “significant,” “correlations,” and “sample.” Separate reporting of data by demographic variables, such as age and sex, facilitate pooling of data for subgroups across studies and should be routine, unless there are compelling reasons not to stratify reporting, which should be explained.

Discussion

It is useful to begin the discussion by briefly summarizing the main findings and explore possible mechanisms or explanations for these findings. Emphasize the new and important aspects of your study and put your findings in the context of the totality of the relevant evidence. State the limitations of your study and explore the implications of your findings for future research and for clinical practice or policy. Do not repeat in detail data or other information given in other parts of the manuscript, such as in the Introduction or the Results section.

Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data. In particular,

distinguish between clinical and statistical significance, and avoid making statements on economic benefits and costs unless the manuscript includes the appropriate economic data and analyses. Avoid claiming priority or alluding to work that has not been completed. State new hypotheses when warranted but label them clearly.

In-text Citations and References

Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Although references to review articles can be an efficient way to guide readers to a body of literature, review articles do not always reflect original work accurately. On the other hand, extensive lists of references to original work on a topic can use excessive space. Fewer references to key original papers often serve as well as more exhaustive lists, particularly since references can now be added to the electronic version of published papers, and since electronic literature searching allows readers to retrieve published literature efficiently.

Do not use conference abstracts as references: they can be cited in the text, in parentheses, but not as page footnotes. References to papers accepted but not yet published should be designated as "in press". Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source.

Laws (e.g., penal code), statutes and regulations are not scientific writings. In addition to being published on the official gazette, since it is published on various internet sites, a reference number should not be given to laws, statutes and regulations. If it is to be cited within the text, the law could be cited by specifying the number of the law, the date and number of publications in the official gazette (e.g., A Review of Article 5 of the Turkish Criminal Penal Code No. 5237). They should not be numbered within the text, or in the reference list.

To minimize citation errors, references can be verified using either an electronic bibliographic source, such as PubMed, or print copies from original sources. Reference list should be numbered consecutively in the order in which they are first mentioned in the text. Roman numerals should be avoided. Identify references in text, tables, and legends by Arabic numerals (1, 2, 3 ... 9, 0) in parentheses. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. The titles of journals should be abbreviated according to the style used for MEDLINE (www.ncbi.nlm.nih.gov/nlmcatalog/journals).

If you refer to a work more than once, use the first number also for the second and following references. References to more than one source in the same phrase may be entered like this: (2-4), i.e., references 2 through 4 in the reference list, and (2-4, 8), i.e. the references 2 through 4, plus reference no 8 in the list of references.

Sample for in-text citation:

In a clinical research in healthy individuals, Ellis (25) has studied the sciatic nerve excursion using ultrasound technique.

Wright and Ellis (10) has investigated the excursion of nerves around the elbow joint.

In another and similar cadaveric study by Wright et al (13), the radial nerve median excursion values were 4.1, 8.8, and 0.2, 0.1 mm with motions of shoulder, elbow, wrist and fingers respectively.

Suicide is a major public health problem and globally the second leading cause of death among young adults (1). Studies focusing on how mental health risk factors impact on youth suicidal behaviors suggest that psychopathological symptoms are associated with suicidal behavior (3,4). Adverse effects of H2S on human health vary from local irritation to immediate death depending on the form, concentration, duration and route of exposure (9, 13-15).

Reference Style

The Vancouver system, also known as Vancouver reference style or the author–number system, is a citation style that uses numbers within the text that refer to numbered entries in the reference list. Vancouver style is used by MEDLINE and PubMed. The names "Vancouver system" or "Vancouver style" have existed since 1978. The latest version of the latter is Citing Medicine, per the References > Style and Format section of the ICMJE Recommendations. In 1978, a committee of editors from various medical journals, the International Committee of Medical Journal Editors (ICMJE), met in Vancouver, BC, Canada to agree to a unified set of requirements for the articles of such journals. This meeting led to the establishment of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URMs). Part of the URMs is the reference style, for which the ICMJE selected the long-established author–number principle.

Since the early to mid-2000s, the United States National Library of Medicine (which runs MEDLINE and PubMed) has hosted the ICMJE's "Sample References" pages. Around 2007, the NLM created Citing Medicine, its style guide for citation style, as a new home for the style's details. The ICMJE Recommendations now point to Citing Medicine as the home for the formatting details of Vancouver style.

Interdisciplinary Medical Journal, since the first day of its publication uses the PubMed/NLM reference style. Thus, references list should follow the standards summarized in the NLM's International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals: Samples of Formatted References for Authors of Journal Articles web page and detailed in the NLM's Citing Medicine, 2nd edition.

According to the Vancouver rules, you can only refer to the literature you have read yourself. If you find anything interesting in a text where it is referred to another text, you must read and refer to the original.

References List

The references list should be ordered numerically in the order in which the references appear in the text.

The journal's name may be abbreviated, according to the abbreviation rules for journal titles. Records retrieved from a search for the full journal title in the National Library of Medicine's search page include the abbreviated title.

Authors' names should be given as surname followed by initials. There should be a space between surname and initials. A maximum of two initials are allowed for each author, they should be entered without spaces or punctuation. Different authors should be separated by a space and a comma. A period (.) should follow the last author's name. If six or more authors, list the first six authors followed by et al.

Only capital letter of the first word of the title, proper nouns, proper adjectives, acronyms, and initialisms should be capitalized.

The most reliable method for calculating the impact factor of our journal and number of citations of articles published in our journal or calculating the number of times your own article is cited in a healthy way, is to add DOIs to the references section. In order to give the DOIs to the articles published in Interdisciplinary Medical Journal, the CrossRef membership application has been completed and all the research articles, case reports, and reviews are being assigned DOIs. For this reason, DOIs need to be added to the References section if available for those references. We hope that the Simple Text Query Form will be helpful in referencing articles published in our journal.

With the help of the Simple Text Query Form web page, which has a link in the full-text template, DOI records need to be added to the sources.

https://apps.crossref.org/SimpleTextQuery

Note: Please, **do not insert Pubmed ID (PMID) or Pubmed Central ID (PMCID) records** to the reference list since they are useless in determining the citation counts.

We place great importance to the addition of DOIs to the references list.

Sample for Journal Article without DOI

Dokgöz H, Kar H, Bilgin NG, Toros F. Forensic Approach to Teenage Mothers Concept: 3 Case Reports. Turkiye Klinikleri J Foren Med 2008;5(2):80-4

Kaufman DM, Mann KV, Miujtjens AM, Van der Vleuten CP. A comparison of standard setting procedures for an OSCE in undergraduate medical education. Academic Medicine 2000;75:267–71.

Sample for Journal Article with DOI

Koçak U, Alpaslan AH, Yağan M, Özer E. Suicide by Homemade Hydrogen Sulfide in Turkey a Case Report. Bull Leg Med. 2016;21(3):189-192. https://doi.org/10.17986/blm.2016323754

Article not in English

Kar H, Dokgöz H, Gamsız Bilgin N, Albayrak B, Kaya Tİ. Lazer Epilasyona Bağlı Cilt Lezyonlarının Malpraktis Açısından Değerlendirilmesi. Bull Leg Med. 2016;21(3):153-158. https://doi.org/10.17986/blm.2016323748

Books and Other Monographs

Personal author(s)

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Author(s) and editor(s)

Breedlove GK, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services; 2001.

Chapter in a book

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113

Emmerson BT. Gout and renal disease. In: Massry SG, Glassock RJ (Editors). Textbook of Nephrology 1. Baskı, Baltimore: Williams and Wilkins; 1989. p. 756–760.

Conference proceedings

Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.

Article published on the Internet ahead of the print version:

Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Part of a homepage/Web site [Edited 28 Dec 2016]

American Medical Association [Internet]. Chicago: The Association; c1995-2016 [cited 2016 Dec 27]. Office of International Medicine; [about 2 screens]. Available from: https://www.ama-assn.org/about/office-international-medicine

Thesis

Skrtic L. Hydrogen sulfide, oil and gas, and people's health [Master's of Science Thesis]. Berkeley, CA: University of California; 2006.

Weisbaum LD. Human sexuality of children and adolescents: a comprehensive training guide for social work professionals [master's thesis]. Long Beach (CA): California State University; 2005. 200 p.

For the reference types not listed here, please visit Samples of Formatted References for Authors of Journal Articles available at Medline Web site (https://www.nlm.nih.gov/bsd/uniform_requirements.html).

Tables

Tables capture information concisely and display it efficiently; they also provide information at any desired level of detail and precision. Including data in tables rather than text frequently makes it possible to reduce the length of the text.

It would be appropriate to place the tables at the end of the main text. Number tables consecutively in the order of their first citation in the text and supply a title for each. Titles in tables should be short but self-explanatory, containing information that allows readers to understand the table's content without having to go back to the text. Be sure that each table is cited in the text. Give each column a short or an abbreviated heading. In the tables, case counts (n) and percentages (%) should be specified in separate columns, not in the same cell.

Authors should place explanatory matter in footnotes, not in the heading. Explain all nonstandard abbreviations in footnotes and use symbols to explain information if needed. Symbols may be as alphabet letters or such symbols as *, p t> T §). Please, identify statistical measures of variations, such as standard deviation and standard error of the mean.

Illustrations (Figures)

The lexical meaning of figure constitutes a number symbol (numeral, digit), a written or printed character, a diagram or pictorial illustration of textual matter, arithmetical calculation or digits representing an amount when plural. While definition of picture includes a design or representation made by various means (as painting, drawing, or photography), illustration means a picture or diagram that helps make something clear or attractive. Although these terms bear distinctive meanings, they are too often used interchangeably. Thus, we meant them in the same way without distinction.

Digital images

The 300 DPI Story

In the ancient times when digital cameras have not been invented, the photos taken by analogue cameras were used to be printed on photo papers. In order to transfer these photos to the digital environment, they had to be scanned by optical devices called scanners. On the same dates, desktop publishing and printing technology was far beyond the digital photography, and many years had passed since the invention of laser printing technology. Here, several technical terms should be explained to make the concept clearer. DPI is used to describe the resolution number of dots per inch in a digital print and the printing resolution of a hard copy print dot gain, which is the increase in the size of the halftone dots during printing. A dot matrix printer, for example, applies ink via tiny rods striking an ink ribbon, and has a relatively low resolution, typically in the range of 60 to 90 DPI (420 to 280 µm). An inkjet printer sprays ink through tiny nozzles and is typically capable of 300–720 DPI. A laser printer applies toner through a controlled electrostatic charge and may be in the range of 600 to 2,400 DPI. Along with the cheaper memory chips, 1200 dpi printers have been widely available in the consumer market since 2008. Monitors do not have dots but do have pixels. The closely related concept for monitors and images is pixels per inch or PPI. Old CRT type video displays were almost universally rated in dot pitch, which refers to the spacing between the sub-pixel red, green and blue dots which made up the pixels themselves. The DP measurement of a printer often needs to be considerably higher than the pixels per inch (PPI) measurement of a video display in order to produce similar-quality output. This dithered printing process could require a region of four to six dots (measured across each side) in order to faithfully reproduce the color in a single pixel. An image that is 100 pixels wide may need to be 400 to 600 dots in width in the printed output; if a 100×100-pixel image is to be printed in a one-inch square; the printer must be capable of 400 to 600 dots per inch to reproduce the image. The dpi of early model laser printers was 300 to 360, thus scanning images at 300 DPI was a common practice at that time.

In printing, DPI (dots per inch) refers to the output resolution of a printer or imagesetter, and PPI (pixels per inch) refers to the input resolution of a photograph or image. DPI refers to the physical dot density of an image when it is reproduced as a real physical entity, for example printed onto paper. A digitally stored image has no inherent physical dimensions, measured in inches or centimeters. Some digital file formats record a DPI value, or more commonly a PPI (pixels per inch) value, which is to be used when printing the image. This number lets the printer or software know the intended size of the image, or in the case of scanned images, the size of the original scanned object. For example, a bitmap image may measure 1,000 × 1,000 pixels, a resolution of 1 megapixel. If it is labeled as 250 PPI, that is an instruction to the printer to print it at a size of 4 × 4 inches. Changing the PPI to 100 in an image editing program would tell the printer to print it at a size of 10×10 inches. However, changing the PPI value would not change the size of the image in pixels which would still be 1,000 × 1,000. An image may also be resampled to change the number of pixels and therefore the size or resolution of the image, but this is quite different from simply setting a new PPI for the file.

Therefore, an image that is 2048 pixels in width and 1536 pixels in height has a total of 2048×1536 = 3,145,728 pixels or 3.1 megapixels. One could refer to it as 2048 by 1536 or a 3.1-megapixel image. Or you can think of it as a very low-quality image (72 ppi) if printed at about 28.5 inches wide, or a very good quality (300 ppi) image if printed at about 7 inches wide.

Since the 1980s, the Microsoft Windows operating system has set the default display "DPI" to 96 PPI, while Apple/Macintosh computers have used a default of 72 PPI. The choice of 72 PPI by Macintosh for their displays arose from the convenient fact that the official 72 points per inch mirrored the 72 pixels per inch that appeared on their display screens. (Points are a physical

unit of measure in typography, dating from the days of printing presses, where 1 point by the modern definition is 1/72 of the international inch (25.4 mm), which therefore makes 1 point approximately 0.0139 in or 352.8 µm). Thus, the 72 pixels per inch seen on the display had exactly the same physical dimensions as the 72 points per inch later seen on a printout, with 1 pt in printed text equal to 1 px on the display screen. As it is, the Macintosh 128K featured a screen measuring 512 pixels in width by 342 pixels in height, and this corresponded to the width of standard office paper (512 px ÷ 72 px/in ≈ 7.1 in, with a 0.7 in margin down each side when assuming 8.5 in × 11 in North American paper size (in Europe, it's 21 cm x 30 cm - called "A4")).

In computing, an image scanner—often abbreviated to just scanner, is a device that optically scans images, printed text, handwriting or an object and converts it to a digital image. Although the history of digital cameras dates back to the 1970s, they have become widely used in the 2000s. While the resolution of the first digital camera invented by Kodak was as low as 100 by 100 pixels (0.01 megapixels), the first commercially available digital camera, Fujix DS-1P had a resolution of 0.4 megapixels. On the other hand, modern scanners are considered the successors of early telephotography and fax input devices. The pantelegraph was an early form of facsimile machine transmitting over normal telegraph lines developed by Giovanni Caselli, used commercially in the 1860s, that was the first such device to enter practical service. The history of the first image scanner developed for use with a computer goes back to 1957. Color scanners typically read RGB (red-green-blue color) data from the array. This data is then processed with some proprietary algorithm to correct for different exposure conditions and sent to the computer via the device's input/output interface. Color depth varies depending on the scanning array characteristics but is usually at least 24 bits. High quality models have 36-48 bits of color depth. Another qualifying parameter for a scanner is its optical resolution, measured in pixels per inch (ppi), sometimes more accurately referred to as samples per inch (spi).

Images in web pages, video, and slide shows can be as low as 72 PPI for a static image or 150 PPI if we are going to focus in on the image. For printing, the DPI needs to be larger, with images scanned in at least 300 DPI. The DPI standard for and images to be printed within journals and books is 300 DPI and for museum exhibits, it's 600 DPI.

The most important factors determining image quality of digital images can be considered as pixel dimensions and color depth. Increasing the dpi value of an image by resampling in Photo Editors (e.g., Adobe Photoshop) has no improving effect on its quality, but it lets us to determine target printing size.

For vector images, there is no equivalent of resampling an image when it is resized, and there is no PPI in the file because it is resolution independent (prints equally well at all sizes). However, there is still a target printing size. Some image formats, such as Photoshop format, can contain both bitmap and vector data in the same file. Adjusting the PPI in a Photoshop file will change the intended printing size of the bitmap portion of the data and also change the intended printing size of the vector data to match. This way the vector and bitmap data maintain a consistent size relationship when the target printing size is changed. Text stored as outline fonts in bitmap image formats is handled in the same way. Other formats, such as PDF, are primarily vector formats which can contain images, potentially at a mixture of resolutions. In these formats the target PPI of the bitmaps is adjusted to match when the target print size of the file is changed. This is the converse of how it works in a primarily bitmap format like Photoshop but has exactly the same result of maintaining the relationship between the vector and bitmap portions of the data.

Long story short, it is not technically possible to talk about DPI value for images that were taken by digital cameras or any type of digital images that were transferred to the computer's storage media. The DPI value stored within exif information of images is just a virtual value just to guide the photo editing software and the graphic artist to determine the target printing size of that image.

Requirements for Digital Media

Figures and Figure Legends

Dear author, since the Journal has decision of publishing online, there is no need to upload the photos, pictures, drawings or shapes in the article as a separate file. However, to avoid blurring of images in the pdf of the article, you should add the photos or other images (X-ray, BT, MR etc.) in your Microsoft Word program as follows.

Insert menu - Pictures - Related image file in your computer

You must add the related image file on your computer and add the picture width to 16 cm. Since the need to upload each image (photo, X-ray, BT, MR or other images) is eliminated, please do not upload it to the system during submission. Place only at the end of full text and blind text.

Due to the reasons explained above, images should be taken by a digital camera of 5 megapixels or more in JPEG, RAW, or TIFF format, and should be inserted in their original form as JPEG, PNG or TIFF files.

Paper-printed images or documents should be scanned at 300 DPI resolution and should be inserted as TIFF, PNG or JPEG files.

Each vector graphic software has its own built-in settings and may have been preset at 72 dpi. So, the document should be created enough big to obtain the image in the desired dimensions. The vector graphics should be exported to a rasterized image format and inserted such as JPEG, PNG or TIFF files.

For X-ray films, CT scans, and other diagnostic images, as well as pictures of pathology specimens or photomicrographs, you should insert high-resolution photographic image files. Since blots are used as primary evidence in many scientific articles, we may require deposition of the original photographs of blots on the journal website.

Letters, numbers, and symbols on figures should therefore be clear and consistent throughout, and large enough to remain legible when the figure is reduced for publication.

Figures should be made as self-explanatory as possible. Titles and detailed explanations belong in the legends— not on the illustrations themselves.

Figures should be numbered consecutively according to the order in which they have been cited in the text.

In the manuscript, legends for illustrations should be in Arabic numerals corresponding to the illustrations. Roman numerals should be avoided. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, you should identify and explain each one clearly in the legend.

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Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Authors must consult the International System of Units (SI).

Authors should add alternative or non-SI units, when SI units are not available for that particular measurement. Drug concentrations may be reported in either SI or mass units, but the alternative should be provided in parentheses where appropriate.

Abbreviations and Symbols

Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. The spelled-out abbreviation followed by the abbreviation in parenthesis should be used on first mention unless the abbreviation is a standard unit of measurement.

Types of paper

Interdisciplinary Medical Journal publishes the following types of articles.

1. Original Articles: Original prospective or retrospective studies **clinical research** in areas relevant to medicine.

The manuscript should contain English abstract, a maximum of 250 words, and the structured abstract should contain the following sections: objective, method, results, and conclusion. Three to six words or determinative groups of words should be written as keywords below the abstract.

The text of articles reporting original research might contain up to 5000 words (excluding abstract, references list and tables) and should be divided into Introduction, Method, Results, and Discussion sections. References list should also be included so that their number does not exceed 50. This so-called "IMRAD" structure is not an arbitrary publication format but a reflection of the process of scientific discovery. Articles need subheadings within these sections to further organize their content.

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Some authors claim, the influence of the pharmaceutical industry on medical research has been a major cause for concern. In contrast to this viewpoint, some authors emphasize the importance of pharmaceutical industry-physician interactions for the development of novel treatments and argued that moral outrage over industry malfeasance had unjustifiably led many to overemphasize the problems created by financial conflicts of interest.

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Acknowledgement

The Acknowledgements section immediately precedes the Reference list. All contributors who do not meet the criteria for authorship should be listed in an 'Acknowledgements' section. Additionally, if the article has been submitted on behalf of a consortium, all author names and affiliations should be listed at the end of the article in the Acknowledgements section. Authors should also disclose whether they had any writing assistance.

Authorship contribution: please indicate which part of the article each author contributed .

Article Format

The submitted file must be in Microsoft Word Document format.

The page size must be 210 mm × 297 mm (A4 size). All margins must be

set to 2.5 cm. If you are using Microsoft Word 2007 or later, you can easily set the margin by choosing "Normal" setting from Margins menu within Layout tab. The text layout should consist of single column.

Do not capitalize diseases or syndromes unless they include a name or proper noun. Note that the words "syndrome" and "disease" are never capitalized; for example, Down syndrome, Hodgkin disease.

The authors should turn off automatic hyphenation. Do not use hyphens with common prefixes unless the word looks confusing when closed up or unless the prefix precedes a proper noun, some other capitalized word, or an abbreviation. Common prefixes that should be "closed up" include ante, anti, hi, co, contra, counter, de, extra, infra, inter, intra, micro, mid, neo, non, over, post, pre, pro, pseudo, re, semi, sub, super, supra, trans, tri, ultra, un, and under.

Use italics sparingly for emphasis in the text.

Spell out Greek letters or use the "Insert, Symbol" feature in Microsoft Word. Do not create your own symbols.

Do not use italics for common expressions, such asin vivo, in utero, en face, aide-mémoire, or in situ.

Use bold type sparingly in text because it competes with headings for the reader's attention.

Always use numerals for statistics, ages, and measurements (including time, for example, 3 weeks). For other uses, spell out numbers from one to nine only.

Spell out abbreviations at first mention in the manuscript, with the abbreviation following in parentheses (except for units of measure, which are always abbreviated following numerals).

Manuscripts including tables, references list and figure legends, must be typewritten with a Unicode font (e.g., Times New Roman, Arial, etc.) that is available both for Windows and Mac Os operating systems. Please avoid using a mixture of fonts or non-Unicode fonts that do not support accented characters. The recommended font size is 12 points, but it may be adjusted for entries in a table. Authors should use true superscripts and subscripts and not "raised/lowered" characters. For symbols, please use the standard "Symbol" fonts on Windows or Macintosh.

Use the TAB key once for paragraph indents, not consecutive spaces. The pages should be numbered consecutively, beginning with the first page of the blinded article file. The pages should include title and abstract in English, the main text, tables, figures or diagrams-if exists- and reference list.

The title of the article should be centered at the top of the main text page, with the abstract below, and followed by Keywords. The capital letter of the first word of title should start with upper case letter. Please avoid capitalizing all letters of the title and conjunctions. The title, abstract, and keywords should be present in English and must be organized respectively. In order to start the Introduction section in a new page, a page break could be inserted at the end of Keywords.

While figure legends should be placed below the figures themselves, table captions should be placed above each table. Characters in figures, photographs, and tables should be uncapitalized in principal.

It would be appropriate to place the figures, tables and photographs at the end of the main text. Please, insert them at the end of main text at appropriate sizes, and order.

Figures and Figure Legends

Dear author, since the Journal has decision of publishing online, there is no need to upload the photos, pictures, drawings or shapes in the article as a separate file. However, to avoid blurring of images in the pdf of the article, you should add the photos or other images (X-ray, BT, MR etc.) in your Microsoft Word program as follows.

Insert menu - Pictures - Related image file in your computer

You must add the related image file on your computer and add the picture width to 16 cm. Since the need to upload each image (photo, X-ray, BT, MR or other images) is eliminated, please do not upload it to the system during submission. Place only at the end of full text and blind text.

The sections (i.e., Introduction, Method, Case, Results, Discussion, and Conclusion) and their subheadings should not be numbered. Paragraphs might be aligned left or justified, but this situation should be consistent throughout the article. Please, use single return after each paragraph. All headings should be typed on a separate line, not run in with the text. There should be no additional spacing before or after lines. Headings and subheadings should not be numbered, and their depth should not exceed three levels. You should not use the "Endnotes" or "Footnotes" feature for your references and remove any Word specific codes. When 'Magic Citations' inserts citations, or formats your manuscript in Microsoft Word, it uses "fields", which you can typically recognize as boxes that turn grey when the insertion point is placed inside one of them. Here is how to remove the fields in a Microsoft Word document:

1. Make a copy of the final manuscript. From the File menu in Word, select the Save As command. Give the file a new name.

2. In the new file, go to the Edit menu and choose Select All.

3. Press Ctrl+Shift+F9 or Cmd+6 to unlink all fields.

Your in-text citations and bibliography will become regular text, without field codes or any hidden links. If you want to do further editing or change citations in any way, make the changes to the original file. When you are ready to submit your manuscript, make another copy of the original file to unlink field codes.

INTERDISCIPLINARY MEDICAL JOURNAL
Interdiscip Med J 2023;14(48)

Reviewer Guide

Dear Reviewer,

Thank you for agreeing to conduct a peer review which will help us decide whether a manuscript is to be published in this journal.

Peer-review is a critical part of the functioning of the scientific community, of quality control, and the self-corrective nature of science. Participating in peer review of scientific publications can be viewed as a responsibility, a burden, and an opportunity all at the same time. Nonetheless, peer review remains a critical component of our profession that helps to ensure the quality, originality, and reliability of scientific findings and claims. Peer review is requested of a colleague with specific interest and expertise in the topic relevant to the manuscript submitted to The Journal. Yet despite the importance of this process in upholding rigorous scientific standards and the integrity of the journal, few if any reviewers receive any formal training or instruction in how to provide a quality manuscript review. This document serves to orient and guide individuals asked to provide peer review for This journal in the process and responsibilities of review and reviewer. In doing so, the hope is to increase scientific quality of the manuscripts and contribution to the medical scientific community.

Process of peer review in The Journal

The journal utilizes a standard online site https://dergipark.org.tr), supported by TUBİTAK, for the process of both manuscript submission and manuscript peer review. Upon receiving a manuscript submitted for consideration of publication to The Journal, the Journal Manager and editorial staff review the submission to assure all required components as outlined in the Guide for Authors are included. The manuscript is then assigned to one of the Co-Editors (either the Editor in Chief or an Associate) Editor who directs and oversees the peer-review process. The Co-Editor then reviews the submission for relevance, content and quality. Those submissions deemed appropriate for consideration of publication are then assigned to at least two peer reviewers. Selection of these reviewers is a key step in the peer review process, as this represents a critical component in ensuring quality of manuscript review and in the overall quality of the Journal. Specifically, the selection of a reviewer with expertise in the topic of the manuscript to be reviewed and without any conflict of interest improves both the timeliness and quality of the review. As such, the designation of an area of interest or expertise by the reviewer (entered at the time of registration into the system (and updated in the change details section of the website, in the subsection areas of expertise) is critical for this component of the process. Reviews are chosen to a great extent from members of the advisory board.

Once the reviewers are selected by the editor, an email is sent requesting the review; 30 days is provided to choose to review (or not review) the manuscript. A lack of response to this request leads to the reviewer being uninvited. Statistics on individual reviewers are maintained and reviewed by the journal editors, including the number of reviews requested (and those accepted, uninvited, and refused). These data help in the process of evaluating the overall quality of a reviewer and are used in the selection of future editorial board members.Before Accepting

Please consider the following:

Does the article you are being asked to review match your expertise?

If you receive a manuscript that covers a topic that does not sufficiently match your area of expertise, please notify the editor as soon as possible. Please feel free to recommend alternate reviewer.

Do you have time to review the paper?

Finished reviews of an article should be completed within four weeks. If you do not think you can complete the review within this time frame, please let the editor know and if possible, suggest an alternate reviewer. If you have agreed to review a paper but will no longer be able to finish the work before the deadline, please contact the editor as soon as possible.

Are there any potential conflicts of interests?

While conflicts of interest will not disqualify you from reviewing the manuscript, it is important to disclose all conflicts of interest to the editors before reviewing. If you have any questions about potential conflicts of interests, please do not hesitate to contact the receiving editorial office.

Finally: Educate yourself on the peer review process through the international guides on how to conduct a good review

Some resources;

https://violentmetaphors.com/2013/12/13/how-to-become-good-at-peer-review-a-guide-for-young-scientists/

https://www.theguardian.com/higher-education-network/blog/2013/sep/27/peer-review-10-tips-research-paper

https://www.degruyter.com/document/doi/10.7556/jaoa.2013.070/html

https://scholar.google.com.tr/scholar?hl=tr&q=good+peer+review&btnG=&lr=

(https://www.google.com.tr/search?num=50&btnG=Ara&q=how+to+write+a+good+peer+review).

Respond to the invitation as soon as you can – delay in your decision slows down the review process, whether you agree to review or not.

General criteria for a peer review

There are a number of general criteria that make for a quality review of a scientific manuscript, and a number of responsibilities that come with being a peer reviewer that further enhances the review process.

The peer reviewer is responsible for critically reading and evaluating a manuscript in their specialty field, and then providing respectful, constructive,

and honest feedback to authors about their submission. It is appropriate for the Peer Reviewer to discuss the strengths and weaknesses of the article, ways to improve the strength and quality of the work, and evaluate the relevance and originality of the manuscript.

Timely – Given the time sensitive nature of many scientific manuscripts, the rapid return of a solicited peer review minimizes the timeline between submission and decision (which helps the authors with resubmission if the manuscript is rejected and helps the journal with a shorter time from submission to publication if accepted). Thus, the reviewer plays a very important role in ensuring expeditious dissemination of data. Peer reviews that cannot be completed on time should not be accepted by the reviewer; every effort should be made to complete those accepted within the time allotted for review.

Fair – A reviewer has a responsibility to both The Journal and the author to provide a review that is thoughtful and complete. While the immediate goal of peer review is providing a decision regarding the suitability for publication in the journal, an additional goal is to provide the author comments that will ultimately improve the science and manuscript and providing it the best chance for publication in a peer-reviewed journal. For manuscripts eventually accepted for publication, quality peer review will ensure that the highest quality science is ultimately published (and will weed out unsound papers). Peer reviews requested in areas outside of the area of expertise of a reviewer should not be accepted; in that case, the review process is facilitated by the reviewer recommending those who could provide a quality review.

Collegial – It is rare for any manuscript to be reviewed without comments or criticisms. However, the responsibility of the reviewers is to provide these critiques constructively and objectively, and in a fashion, that is collegial and respectful. Consider each manuscript as one that was written by a valued colleague when drafting a peer review. Importantly, review the manuscript as you would like your own manuscript reviewed.

Clear – The goal of peer review is to provide an advisory recommendation to the editors as to the suitability of a manuscript for publication in The Journal. As such, the responsibility of the reviewer is to provide a clear signal to the editor regarding the appropriateness and priority for publication of a manuscript. The reviewer is expected to provide comments and criticisms to the editor that clearly justifies their recommendation for disposition of the manuscript. It is also critical that the comments to the editor are consistent with those made to the author (such that the comments of the reviewer justify the recommendation regarding the disposition of the manuscript).

Comprehensive – A quality review will include a number of considerations, and may be specific to the manuscript being reviewed. In order for a manuscript to be considered for publication, it must be original and significant, providing a contribution to research and importance to field. In general, there should be no flaws in the specific procedures used in performance of the study, or in the logic used for the interpretation of the data. It is important that the results of the study support its conclusions, and that there are no errors in reference to prior work (or no exclusions of pertinent references). Where appropriate, confirmation of regulatory review (such as institutional review board approval) must be present. A reviewer is expected to comment on the strengths and weaknesses or limitations of the study. The validity of the statistics used (often including a justification of a sample size) to analyze data is necessary, and the data presented in the figures and tables should be reflective of the results presented and adequate to justify the study conclusions. In general, the manuscript length and quality of the writing are important to ensure its quality.

Considerations for a quality peer review of a manuscript

Structure

Is the article clearly laid out? Are all the key elements present: abstract, introduction, methodology, results, conclusions?

Consider each element in turn:

Title: Does it clearly describe the article? This will be used for medical database searches, so it shouldn't try to be "cute".

Abstract: Does it reflect the content of the article? Are the data consistent with the results reported in the manuscript?

Introduction: Does it describe what the author hoped to achieve accurately, and clearly state the problem being investigated? Normally, the introduction is two or three paragraphs long. It should summarize relevant research to provide context, and explain what findings of others, if any, are being challenged or extended. It should describe the experiment, hypothesis; general experimental design or method.

Methodology: Does the author accurately explain how the data were collected? Is the design suitable for answering the question posed? Is there sufficient information present for you to replicate the research? Does the article identify the procedures followed? Are these ordered in a meaningful way? If the methods are new, are they explained in detail? Was the sampling appropriate? Have the equipment and materials been adequately described? Does the article make it clear what type of data was recorded; has the author been precise in describing measurements?

Results: This is where the author should explain in words, tables and figures what was discovered in the research. It should be clearly laid out and in a logical sequence. You will need to consider if the appropriate analysis been conducted. Are the statistics correct? If you are not comfortable with statistics, advise the editor when you submit your report and recommend review by a statistical editor. Any interpretation should not be included in this section.

Conclusion/Discussion: Are the claims in this section supported by the results, do they seem reasonable? Have the authors indicated how the results relate to expectations and to earlier research? Does the article support or contradict previous theories? Does the conclusion explain how the research has moved the body of scientific knowledge forward?

Language: If an article is poorly written due to grammatical errors, while it may make it more difficult to understand the science, you do not need to correct the language. You may wish to bring it to the attention of the editor, however, and we can refer the authors to an language editing service if you feel the paper may be worth publishing.

Finally, on balance, when considering the whole article, do the figures and tables inform the reader, are they an important part of the story? Do the figures describe the data accurately? Are they consistent (are the bars in the charts the same width, are the scales on the axis logical)? Are the legends appropriate?

Previous Research

If the article builds upon previous research, does it reference that work appropriately? Are there any important works that have been omitted? Are the references accurate and up to date?

Reviewer's Suggestions

Once accepted, the reviewer has 4 weeks to complete the review (details of the components of a review are described in more detail below), which is submitted through The Journal site. Failure to complete the review during this time period leads to a reminder email.

It is the responsibility of the reviewer to provide a recommendation to the editor for the disposition of the manuscript. Importantly, the recommendation of the reviewer is advisory to the editor, as it is ultimately the decision of the editor as to the final disposition of the manuscript.

When the editor has a full complement of reviews completed, the editor reviews the comments and recommendations, and a decision regarding the suitability for publication of the manuscript is made.

The recommendations can be categorized into 6 groups.

Accept Submission (without modification)

Minor Revision (Revisions Required): Accept with minor modification (but manuscript requires modifications to improve its quality)

Major Revision (Resubmit for Review): Major modifications required, manuscript is unique, but requires extensive revision and reevaluation prior to potential acceptance

Resubmit Elsewhere: manuscript is unique, but out of the journal scope.

Decline Submission: manuscript is of low quality or low interest to the readership)

The reviewer has two types of comments that can be provided – one to the authors, and one to the editors. It is strongly encouraged that the reviewer utilizes the comments to the editor to provide confidential comments regarding the manuscript under consideration. These comments help assure that the editor understands the true recommendation of the reviewer and provides key assistance to the Editor in determining a manuscript's ultimate disposition. In addition, completing the manuscript rating form is helpful in supporting a reviewer's recommendation for the disposition of a manuscript, and assists the Editor in justifying the final decision.

Review of the reviewer

The editor evaluates the quality of a review upon its receipt. Utilizing the criteria defining a quality review (timely, fair, collegial, clear, and comprehensive), a reviewer is evaluated and scored (from 0-5) on their review. This statistic, in combination with a separate statistic regarding the timeliness of the review, is helpful in assigning subsequent reviews to a reviewer. Reviewers with low scoring or late reviews are not considered highly for subsequent reviews.

Why be a reviewer?

Reviewing requires the investment of time and a certain skillset. Before you decide if you want to become a reviewer, we recommend that you read more about the peer review process and conducting a review.

A reviewer may directly benefit from the peer review process by learning from the work of others prior to publication. Reviewer's insights may also lead to future research ideas, improvements in their own study design and manuscript preparation. In addition, The Council of Higher education supports peer reviewing financially within the context of academic refunds.

As a reviewer, you can;

Establish your expertise in the field and expand your knowledge.

Improve your reputation and increase your exposure to key figures in the field.

Stay up to date with the latest literature, and have advanced access to research results.

Develop critical thinking skills essential to research.

Advance in your career – peer review is an essential role for researchers.

Important Considerations;

* It is important for our Journal that you ***request a revision*** by making criticism, evaluation and comments that will help to enrich the scientific content of the article.

* You can **suggest rejection for outdated or inadequate studies** that are similar to previous studies but do not have significant scientific value, or contain some fundamental mistakes or erroneous judgments.

* In accordance with the TR Index criteria, in all (research) studies that require ethics committee approval, a legible copy of the ethics committee approval is required to be uploaded to the system together with the article files, and the manuscript is not sent to our reviewers for evaluation before this process is fulfilled.

* In accordance with the principles of double-blind review, the information regarding the approval of the center where the study was conducted and the approval of the ethics committee were removed from the article after we reviewed it and will be added again during the copyediting following the end of the review. There is no need for our reviewers to make an examination in this respect.

* Before all studies are sent to the reviewer, while they are in the pre-control stage, they are subjected to "Similarity Check" with iThenticate Crosscheck software and if they are above the tolerable level, the author is requested to make the necessary corrections.

* We ask the authors to use a dot as a decimal separator throughout the article, including the Turkish and English abstracts, so this is not an error.

* Therefore, we would like to inform you that there is **no need for you to request any correction regarding the use of a dot as a decimal separator or not, whether the approval of the ethics committee** has been obtained.

Ethical Principles and Editorial Policy

Ethical Responsibilities of The Editors

The Journal is committed to practice the publication ethics and takes all possible measures against any publication malpractices.

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

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

The Journal requires corresponding authors to submit a signed and scanned version of the Copyrights & Ethics form (available for download through this link) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all responsibility for authorship during the submission and review stages of the manuscript.

The Journal requires and encourages the authors and the individuals involved in the evaluation process of the submitted manuscripts to disclose any existing or potential conflicts of interest, including financial, consultant, and institutional. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal's Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complain t cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to cases that cannot be resolved internally. The Editor-in-Chief is the final authority in the decision- making process for all appeals and complaints.

The Journal is committed to objective and fair double-blind peer-review of the submitted for publication works and to prevent any actual or potential conflict of interests between the editorial and review personnel and the reviewed material. Details on this subject have been explained in the authors guide and reviewers guide respectively.

Ethical Responsibilities of The Authors

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 submitted to another journal for evaluation but rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Individuals 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 be based on the following four criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of the data for the work;

2. Drafting the work or revising it critically for valuable intellectual content;

3. Final approval of the version to be published; AND

4. Agreement to be accountable for all aspects of the work and 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

other specific parts of the work. Besides, 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 on the title page of the manuscript.

When submitting a manuscript to The Journal, authors agree to assign the copyright of their manuscript to the Journal. If rejected for publication, the copyright of the manuscript will be assigned back to the authors. The Journal requires each submission to be accompanied by a Copyright and Ethical Form (available for download at Journal Website). When using previously published content, including figures, tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial, and criminal liabilities in this regard belong to the author(s).

Statements or opinions expressed in the manuscripts published in The Journal reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; thus, the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility regarding the published content rests with the authors.

To summarize, all authors submitting their works to the journal for publication as original articles attest that the submitted works represent their authors' contributions and have not been copied or plagiarized in whole or in part from other works. The authors acknowledge that they have disclosed all and any actual or potential conflicts of interest with their work or partial benefits associated with it.

Ethical Responsibilities of The Reviewer

Given the sensitive nature of peer review, there are ethical responsibilities of the review and review process that are critical to ensure their validity. It is important for reviewers to identify potential conflicts of interest with the authors and the topic of the manuscript. The Journal requires that reviewers disclose potential and relevant conflicts of interest related to employment, consultancies, stock ownership, receipt of honoraria, patents, a professional relationship with, or direct competition in the field of the authors. The reviewer has a responsibility to review the conflict-of-interest forms submitted by the authors, which specifically document any relevant financial relationships.

Given the role of reviewer as an expert in the topic area of the manuscript, a reviewer is requested to report any known situations of duplicate submissions (a manuscript that may be under concurrent review at another journal), fraudulent data, or plagiarism in a manuscript. If identified, these comments should be conveyed to the editor (in the confidential comments to the editor section) so they can be addressed as appropriate. Since peer review of a manuscript allows a reviewer to view data that is not yet in the public domain, strict confidentiality is critical to ensure scientific integrity; destruction of documents and files related to peer review is also mandatory once the review is complete.

The Committee on Publication Ethics (COPE) is a group comprised of editors of academic journals and others interested in publication ethics. Members of COPE were provided with the Ethical Guidelines for Peer Review, describing the basic principles to which reviewers should adhere. These guidelines provide a broad ethical context in which the peer review process should be performed.

COPE Ethical Guidelines for Peer Reviewers

Peer review in all its form plays an important role in ensuring the integrity of the scholarly record. The process depends to a large extent on trust and requires that everyone involved behaves responsibly and ethically. Peer reviewers play a central and critical part in the peer-review process, but too often come to the role without any guidance and may be unaware of their ethical obligations. The COPE Ethical Guidelines for Peer Reviewers set out the basic principles and standards to which all peer reviewers should adhere during the peer-review process. It is hoped they will provide helpful guidance to researchers, be a reference for journals and editors in guiding their reviewers, and act as an educational resource for institutions in training their students and researchers.

Basic principles to which peer reviewers should adhere

Peer reviewers should:

• only agree to review manuscripts for which they have the subject expertise required to carry out a proper assessment and which they can assess in a timely manner

• respect the confidentiality of peer review and not reveal any details of a manuscript or its review, during or after the peer-review process, beyond those that are released by the journal

• not use information obtained during the peer-review process for their own or any other person's or organization's advantage, or to disadvantage or discredit others

• declare all potential conflicting interests, seeking advice from the journal if they are unsure whether something constitutes a relevant interest

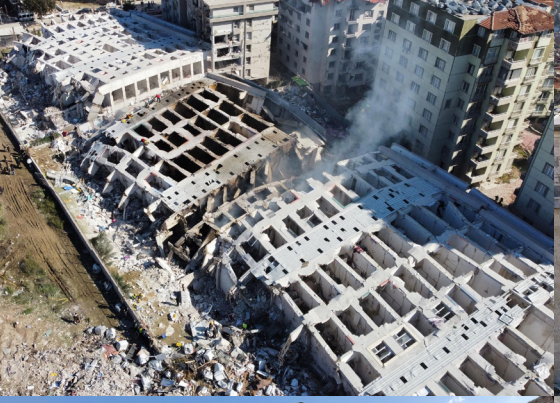
• not allow their reviews to be influenced by the origins of a manuscript, by the nationality, religious or political beliefs, gender or other characteristics of the authors, or by commercial considerations

• be objective and constructive in their reviews, refraining from being hostile or inflammatory and from making libelous or derogatory personal comments

• acknowledge that peer review is largely a reciprocal endeavor and undertake to carry out their fair share of reviewing and in a timely manner

• provide journals with personal and professional information that is accurate and a true representation of their expertise

• recognize that impersonation of another individual during the review process is considered serious misconduct









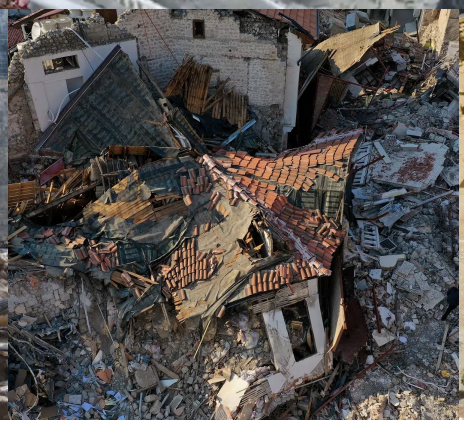








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INTERDISCIPLINARY MEDICAL JOURNAL
Interdiscip Med J 2023;14(48)

FROM THE EDITOR

On February 6, 2023, at 04:17, we awoke to a terrific earthquake at midnight on a cold and rainy night. Experts state that while the magnitude of the earthquake was 7.7, its destructive intensity was XI (11), and the energy released in this earthquake was more than the detonation of 300 atomic bombs. After those terrible moments that seemed like it would never end, we left our homes and tried to understand the magnitude of the earthquake in the rainy, cold and dark streets. It was as if the time had broken at one point, the clocks had stopped, there was no morning, the sun did not rise.

With the first light of day, we started to see the magnitude of the disaster with our own eyes. Then the sad news began to come one after another. Our lives have been filled with great pain, and we have been deeply shaken by the pain of losing our family members, relatives, loved ones, colleagues and students. Just 9 hours after the first earthquake, we were shaken again at 13:24, this time by a second big earthquake with a magnitude of 7.6. We watched many buildings become graves for the people living inside, leaving a huge cloud of dust behind in seconds. The scenery was unbearable, the size of the destruction was many times greater than expected. With the sad news we received, our hearts were filled with unnamable sadness on the one hand, and our pain was relieved a little with the health news of our friends. Many of us rushed to the hospital right after they buried their relatives, while others rushed to the hospital while their relatives were still under the collapsed buildings by burying their pain in their hearts.

A short time later, aid teams from all over Turkey and the world began to come to help. While our state's institutions and civil aid organizations mobilized all their means to meet the needs of our people, the rescue teams struggled for days with hope to save the right people under the rubble. Many tales of sacrifice and heroism were written during this period.

Our pain is still as fresh as yesterday. We commemorate all faculty members, research assistants, students and administrative staff of our university who lost their lives in the earthquake with mercy and respect. Their cherished memories will always live with us.

On the other hand, life still goes on for us as survivors. As the editorial board of the journal, our determination to reach the targets we have previously determined continues.

We had announced that, in order to increase its visibility in the international arena, the scientific quantity and quality of our journal, the number of international or multi-centered studies published, and to be recognized and become visible by researchers of the foreign institutions, editorial board of the Journal had decided to change the publication language to English and rename to Interdisciplinary Medical Journal.

So, what is interdisciplinary medicine? Interdisciplinary medicine can be defined as "an interdisciplinary approach that relies on health professionals from different disciplines, along with the patient, working collaboratively as a team. The most effective teams share responsibilities and promote role interdependence while respecting individual members' experience and autonomy. James I. Ausman in his article with the same title (*), examining the future of the medicine in the 21st century states that; medicine is going through a revolution, transitioning from the old systems of the industrial age (resident education, hospital systems) where everyone was treated as a mass, to the 21st century, where each person and patient is recognized as unique. Treatments also will have to be individualized for each patient even more so than now as each person's genetic code, when known, will allow physicians to design specific molecular treatments for that patient. This possibility is not achievable by an individual physician, but groups of physicians come closer to this goal by exchange of knowledge.

We should also add that such a major change in medical approach require more than the classical consultation medicine between specialties, an interdisciplinary approach to medicine in which team members interact more closely with each other. As an expression of this need, we see that the concept of interdisciplinary medicine is being mentioned more and more every day, and we update the focus and scope of our Journal accordingly.

In line with the new focus and scope of our journal, we have named it Interdisciplinary Medical Journal and we are happy to publish 10 articles in the April 2023 issue. Thank you to everyone who contributed to this process.

Best regards.

Dr. Uğur Koçak

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ORIGINAL ARTICLE

REVIEW

ORIGINAL RESEARCH
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The role of surgery in perianal disease developing in hematology malignancy patients

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Abstract

Objective: Perianal diseases may impair the comfort of life in immunosuppressive patients as well as cause life-threatening complications. In this study, we aimed to analyze the surgical management and results of perianal region diseases in hematological patients who were consulted by the hematology clinic and to present non-surgical treatment options in these patients.

Method: The files of the patients who were consulted by the Hematology Clinic of our hospital between January 2011 and January 2021 due to perianal disease were retrospectively reviewed. Patients with multiple data deficiencies were excluded from the study. Gender, age, hematological diagnosis, neutropenic status, number of consultations, surgical diagnosis, radiological imaging methods, treatment modalities and survival data of the patients were recorded.

Results: A total of 911 consultations were requested from 627 patients. The number of patients consulted with the diagnosis of perianal disease was 147. Ninety (61.2%) of the patients were male and 57 (38.7%) were female. Of the patients, 74 (50.3%) had acute myeloid leukemia, 29 (19.7%) lymphoma, 15 (10.2%) acute lymphoblastic leukemia, 12 (8.1%) myelodysplastic syndrome, 10 (6.8%) multiple myeloma, 7 (4.7%) of them were followed up with the diagnosis of chronic lymphocytic leukemia, 53 (36%) patients had additional systemic disease besides hematological disease. All patients were examined and prediagnosed. Magnetic resonance imaging was performed in 72 patients with suspected abscess and fistula. Surgical treatment methods were applied to 30 (20.4%) of the patients.

Conclusion: Perianal diseases frequently accompany hematological diseases, especially in neutropenic periods. With early examination, close follow-up and appropriate treatment, surgical necessity and the number of relapsed diseases can be minimized.

Keywords: Hematology, Neutropenia, Perianal Disease, Leukemia, Lymphoma

INTRODUCTION

Perianal disease and infections are the most feared complications in patients whose body defenses against microorganisms are weakened. Neutropenic patients are more prone to anorectal complications (1). Neutropenia is one of the most important risk factors for perianal sepsis. Anal mucosal damage and related lesions may develop in leukemic patients who develop neutropenia as a result of chemotherapy and radiotherapy. These neutropenic patients cannot show an adequate inflammatory response, and therefore even weaker virulent microorganisms can cause life-threatening perianal infections (2).

Perianal region infections are not rare in patients with hematological malignancies, and the rate is around 8-9% in acute leukemia patients (3,4). Disease severity ranges from self-resolving limited infection to life-threatening sepsis (5). Perianal infection may affect the quality of life with anal pain and discomfort, as well as cause mortality with a more severe course (6).

Surgical treatment is more effective in perianal abscesses and fistulas (5,7). However, since patients with leukemia are usually neutropenic during treatment, surgical intervention may cause sepsis and poor wound healing. Neutrophil counts influence treatment choice during anorectal infection.

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The optimal time for surgical treatment in patients with leukemia with perianal disease is still controversial (4). We aimed to analyze the surgical management and results of patients with hematological malignancies who were consulted to the general surgery clinic for perianal disease during hospitalization and present them to the literature.

METHOD

The files of the patients who were consulted to the General Surgery Clinic with the prediagnosis of perianal disease by the Hematology Clinic between January 2011 and January 2021 were reviewed retrospectively. We included 147 patients without missing data in the study. Patients under 18 years of age with multiple data deficiencies were excluded from the study. Pre-diagnosis was made by examining the patients at the bedside, radiological evaluations were made when necessary, and then their treatments were arranged and daily follow-ups were made. Patients' gender, age, hematological diagnosis, neutropenic status (<2000/mm3), number of consultations, surgical diagnoses, radiological imaging, treatment modalities, recurrence and mortality findings were recorded. The results were analyzed considering their neutropenic status and surgical or non-surgical treatment modalities.

Data were obtained from patient files and computer-based hospital operating system and analyzed using SPSS v16.0 (IBM) operating system. Frequency (n) and percentage were used in the evaluation of categorical variables as descriptive statistics, and median (minimum-maximum) values were used in the evaluation of numerical variables.

RESULTS

Of the 147 patients included in the study, 90 (61.2%) were male and 57 (38.7%) were female. The median age was 47.5 (17-82) (min-max) years. From the total patients, 74 (50.3%) had acute myeloid leukemia (AML), 29 (19.7%) had lymphoma, 15 (10.2%) had acute lymphoblastic leukemia (ALL), 12 (8.1%) had myelodysplastic syndrome (MDS), 10 (6.8%) had multiple myeloma (MM), 7 (4.7%) had chronic lymphoblastic leukemia (CLL). Ninety-six patients were in the neutropenic phase secondary to treatment. The number of patients with comorbid disease was 53 (36%). 128 (87%) of them were consulted once, and 19 (11.9%) more than once. Of the patients, fifty-eight (39.4%) abscesses, 33 (22.4%) fissures, 24 (16.3%) fistulas, 19 (12.9%) hemorrhoids, 5 (3.4%) edema, 6 (4%) normal examination, 2 (1.3%) necrotizing fasciitis surgery were diagnosed. Magnetic resonance imaging (MRI) was performed in 72 (48.9%) patients with suspected abscess and fistula. One hundred and seventeen (79.5%) patients were treated medically, while 30 (20.4%) patients underwent surgery. Twenty (13.6%) abscess drainage, 4 (2.7%) fistulotomy, 4 (2.7%) seton, 2 (1.3%) debridement were performed. There was no mortality. (Table 1).

DISCUSSION

Table 1. Distribution of findings according to patients

	n	%
Sex		
Male	90	61.2
Female	57	38.7
Age	47.5 (17-82 yıl)	
Hematological diagnosis		
Acute myeloid leukemia	74	50.3
Lymphoma	29	19.7
Acute lymphocytic leukemia	15	10.2
Myelodysplastic syndrome	12	8.1
Multiple myeloma	10	6.8
Chronic lymphocytic leukemia	7	4.7
Neutropenic patient	96	65.3
Comorbidity	53	36
Number of consultations		
Single	128	87
Multiple	19	12.9
Reason for consultation		
Pain	138	93.8
Discharge	4	2.7
Bleeding	3	2
Swelling	2	1.3
MRI	72	48.9
Surgical diagnosis		
Abscess	58	39.4
Anal fissure	33	22.4
Anal fistula	24	16.3
Hemorrhoids	19	12.9
Normal	6	4
Edema	5	3.4
Necrotizing fasciitis	2	1.3
Treatment		
Medical	117	79.5
Surgical	30	20.4
Abscess drainage	20	13.6
Fistulotomy	4	2.7
Seton	4	2.7
Debridement	2	1.3
Mortality	0	0

Anorectal diseases are more common in patients with hematological malignancies compared to other cancer patients (8,9). In some studies, it is mentioned that the incidence of anorectal disease is high in young male patients in relation to age (10). However, there are not many studies supporting this. Only one study reported a relationship between young age and septic disease, and septic complications were more common in younger patients (11). In this study, the proportion

of male patients (63.1%) was higher than female patients, and the mean age was 47 years.

The manifestation of perianal diseases differs according to the state of the body defense system in hematological patients (1). Neutrophils are a critical component of the innate immune system. Neutropenic patients cannot produce an adequate inflammatory response and are susceptible to infection by less virulent microorganisms. Therefore, there may be unusual infections (12). Qualitative deficits from underlying malignancy combined with periods of neutropenia from chemotherapeutic agents are the main risk factors for the development of bacterial and fungal infections in patients with acute leukemia (13). It has been shown that there is a consistent relationship between a lower absolute neutrophil count and an increased incidence of septic anorectal complications in patients with hematological diseases (11). Unlike patients with normal neutrophil counts, E. Coli and P. Aeruginosa are the most common bacteria in anorectal cultures in neutropenic patients. The coexistence of anorectal mucosal integrity, enteric colonization and neutropenia may be responsible for this condition (14). In the study, 63.1% of patients were neutropenic and perianal abscess was the most common anorectal disease. In this patient group, enteric bacteria such as E.Coli and P.Aeruginosa were predominant in the culture, consistent with the literature.

The clinical manifestation of anorectal infection is often masked by the absence of inflammatory cells. Therefore, it may be difficult to recognize signs and symptoms in neutropenic patients (15). Pain, swelling and constipation often accompany perianal disease and may cause systemic infection (16). 92.5% of our patients were consulted with complaints of pain in the perianal region.

Diagnosis of infection in neutropenic patients is difficult due to the absence of granulocytes to localize the perianal infection. The diagnosis is correct about 50% (17). Imaging is essential to determine the best treatment and disease management. The American Society of Colon and Rectal Surgeons recommend Computed Tomography (CT) and MRI imaging in selected patients (18). In the study, MRI imaging was used to support the differential diagnosis in patients with suspected abscess and fistula.

In a series of 92 patients with acute and chronic leukemia, the most common perianal disease was abscess (27%). This was followed by anal fissure (23%) and external hemorrhoids (19%) (1). In another study performed in 83 patients with hematological malignancies, anal fissure (36.1%) was the most common, followed by hemorrhoidal disease (26.5%), fistula (15.7%), and abscess (10.8%) (19). Abscess (39.4%) was the most common perianal disease in the study. We think that the immune status of the patients is effective in this.

There is no consensus in the literature on perianal disease management in hematological patients. While some studies advocate operative treatment, some studies have reported high mortality rates with operative treatment (4). Some concerns have been expressed about the development of septicemia and poor wound healing secondary to diagnostic or therapeutic procedures in patients with neutropenia (20). Treatment management should be determined according to the granulocyte count (21). Today, with the expansion of the pool of antibiotics against gram-negative and anaerobic bacteria, the need for surgery has decreased (11). Consistent with the literature, 117 (79.5%) patients were treated medically. Broad spectrum antibiotics (carbapenem, teicoplanin, ciprofloxacin) were used in the treatment. We believe that in addition to the antibiotic pool, the necessary examination and close examination follow-up are effective in the effectiveness of medical treatment.

In the literature, recurrence of perianal infectious disease is more common in neutropenic patients. Neutropenia and mucositis are common because patients with acute myeloid leukemia receive high-dose cytarabine-based chemotherapy. This may explain why patients with acute myeloid leukemia have a higher rate of recurrence of perianal infection than patients with acute lymphoblastic leukemia (16). In the study, in accordance with the literature, 46.2% of the patients were diagnosed with AML and 80% of the 15 patients who were consulted more than once for the same reason were neutropenic patients.

When septic complications develop in neutropenic patients, mortality rates range from 11-57% (19). There was no mortality in our patients. We believe that the reason for this is early diagnosis, effective treatment, strict patient follow-up and correct surgical indication.

The study includes various limitations such as not having a comparison group and not knowing the perianal disease history of the patients. Despite all these limitations, we believe that this clinical observation made in the homogeneous patient group will contribute to the literature.

CONCLUSION

Anorectal diseases are serious cause of morbidity that impairs the quality of life in hematology patients who receive chemotherapy and have active disease. Especially since perianal infectious diseases can cause life-threatening consequences, diagnosis and treatment are important. Due to the body defense system and wound healing problems in neutropenic patients, the surgical decision should be made with care. With early diagnosis and appropriate treatment plan, the need for surgery can be minimized and morbidity and mortality rates can be significantly reduced.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

For this study, permission was obtained from Başkent University Medical and Research Board with the letter dated 14.07.2021 and numbered E-94603339-604.01.02-47888, and the Helsinki Declaration criteria were taken into consideration.

Authorship Contributions

Concept: HY, Design: RG, MK, Supervising: MK, HY, Data collection and entry: SE, RG, Analysis and interpretation: SE, HY, Literature search: SE, MK, Writing: SE, Critical review: HY.

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Monocyte eosinophil ratio and red blood cell distribution width in the diagnosis of asthma

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Abstract

Objective: Bronchial asthma is often associated with chronic airway inflammation and airway hyperresponsiveness in which many cells and mediators are involved. Pulmonary function tests (PFTs) are used in the diagnosis of the disease. Yet PFTs are not available in every healthcare institution or some of the patients cannot cooperate with the procedure. The aim of this study was to determine whether mean platelet volume (MPV), platelet distribution width (PDW), monocyte lymphocyte ratio (MLR), monocyte eosinophil ratio (MER), and platelet lymphocyte ratio (PLR) can be used in the diagnosis of bronchial asthma.
Method: Two hundred and twelve patients who were diagnosed with bronchial asthma according to the Global Initiative for Asthma (GINA) criteria and 187 patients who were deemed not to have asthma were included in the study. Two patient groups were compared by examining the hemogram parameters at the time of diagnosis or exclusion of asthma.
Results: The levels of MPV, neutrophil-lymphocyte ratio (NLR), and PLR were significantly lower in the Asthma group ($p < 0.001$, 0.005, and 0.002 respectively) while the lymphocyte, eosinophil, monocyte, basophil, PDW, MLR, and MER levels were higher in the same group. On ROC analysis areas under the curve (AUC) for RDW, MER, and PDW were found as 0.81, 0.93, and 0.76 respectively.
Conclusion: In conclusion, higher lymphocyte, eosinophil, PDW, MLR, and MER whereas lower levels of MPV, NLR and PLR support the diagnosis of asthma.

Keywords: Asthma, Red Cell Distribution Width, Monocyte, Eosinophil

INTRODUCTION

Asthma is one of the most common chronic respiratory diseases that affects 1-20 % of the population in different countries and an estimated 300 million people around the World (1). The disease is often associated with chronic airway inflammation and airway hyperresponsiveness in which many cells and mediators are involved. It can be triggered by factors such as exercise and may improve with treatment or spontaneously. Asthma can be seen at any age, creates an obstacle in daily life, negatively affects the quality of life, and therefore becomes a global public health problem (2). Asthma; constitutes a significant social and economic burden and morbidity for patients, their families, and the health system (3). Asthma is a serious cause of labor loss and disability worldwide. Anamnesis, physical examination findings, and pulmonary function tests (PFTs) are used to diagnose the disease. Yet PFTs are not available in every healthcare institution, some patients cannot cooperate with the procedure, or due to their health conditions, PFTs cannot be performed. There is a need for objective tests without the need for the patient's effort to help doctors during the diagnosis. Hemogram parameters such as white blood cell (WBC) count, red blood cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), neutrophil-lymphocyte ratio (NLR), and platelet lymphocyte ratio (PLR) have been investigated as diagnostic parameters in some diseases (4, 5). In the COVID-19 pandemic, recent studies reported an association between high levels of PDW and COVID-19 mortality

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(6). All parameters mentioned above are studied by routine complete blood count tests that clinicians might overlook. This study aimed to determine whether MPV, PDW, monocyte lymphocyte ratio (MLR), monocyte eosinophil ratio (MER), and PLR can be used in diagnosing bronchial asthma.

METHOD

Files of patients admitted to Pulmonary Medicine Clinic in Bursa City Hospital/Bursa Turkey between January 2020, and October 2021 were investigated. Two hundred and twelve patients who presented with complaints such as shortness of breath and chest tightness underwent a complete blood count blood analysis and were newly diagnosed with asthma bronchiale according to the Global Initiative for Asthma (GINA) criteria as a result of the evaluation, and 187 patients who were deemed not to have asthma were included in the study. Pregnants, patients with chronic obstructive pulmonary disease (COPD), asthma attack, renal and cardiac failure, and respiratory insufficiency were excluded. All patients' demographic information and clinical data were recorded (Table 1). Two patient groups were compared by examining the hemogram parameters (Neutrophil, lymphocyte, monocyte, eosinophil, platelets, and PDW) at the time of diagnosis or exclusion of asthma. Leukocytes were measured using fluorescent flow cytometry; erythrocytes and platelets were measured using the impedance method. NLR, PLR, and monocyte lymphocyte ratio (MLR) values were calculated by dividing neutrophil, platelet, and monocyte levels to lymphocyte count. MER, neutrophil eosinophil ratio (NER), and lymphocyte eosinophil ratio (LER) were obtained by dividing monocyte, neutrophil, and lymphocyte levels to eosinophil, respectively. The ethics committee approval was obtained from Bursa City Hospital Clinical Research Ethics Committee (Ethics Committee Approval No:2021-22/4) in accordance with Helsinki Declaration. Statistical analyses were performed using SPSS 25.0 software. The normality of the sample data was evaluated with the Kolmogorov-Smirnow test, and the continuous variables were defined by the mean ± standard deviation, median (interquartile range 25-75 %), categorical variables were expressed as frequency and percent. A Student's t-test or a Mann-Whitney U test was used to compare the independent groups. The ROC analysis was performed for optimal cut-off values to predict asthma, and a p-value less than 0.05 was set as the statistical significance level.

RESULTS

The mean age was determined to be 29.5 (18-80) in the No-Asthma group and 30 (18-71) in the Asthma group (Table 1), with the Asthma group having a significantly greater median age ($p < 0.001$). The gender distribution in the No-Asthma group was 45.5% (85) women and 54.5% (102) men, while it was 59.9 % (127) women and 40.1% (85) men in the Asthma group.

Table 1. Demographic Data and Laboratory Findings of Patients with Asthma and Controls

Variable		No-Asthma		Asthma		p-value
		n=187		n=212		
		n	%	n	%	
Gender	Female	85	45.5	127	59.9	0.004
	Male	102	54.5	85	40.1	
Age (years)		29.5 (18-80)		30 (18-71)		0.001
WBC $10^3/\mu L$		7.1 (3.9-14)		7.3 (2.6-18.6)		0.09
Neutrophil $10^3/\mu L$		4.38 (1.02-13.6)		4.06 (1.06-13.83)		0.2
Lymphocyte $10^3/\mu L$		1.96 (0.49-3.85)		2.21 (0.6-5)		0.02
Eosinophil $10^3/\mu L$		0.17 (0.02-0.92)		0.22 (0.03-1.6)		0.05
Monocyte $10^3/\mu L$		0.4 (0.14-1.1)		0.51 (0.09-1.14)		0.001
Platelets $10^3/\mu L$		247 (132-454)		241 (130-556)		0.8
Basophil $10^3/\mu L$		0.04 (0.01-0.24)		0.05 (0.01-0.17)		0.001
RDW fL		12 (10.9-31.9)		13.9 (12-29)		0.05
MPV fL		8 (6.3-10.7)		7.8 (5.9-10)		0.001
PDW fL		16 (15-18)		16.7 (14.9-20.9)		0.001
NLR		2.06 (0.3-27.2)		1.8 (0.33-13)		0.005
PLR		122 (57-363)		107 (54-251)		0.002
MLR		0.18 (0.05-0.69)		0.22 (0.03-0.68)		0.004
MER		2.29 (0.33-16.5)		10 (1.3-161)		0.04
LER		11.9 (1.7-67)		10(1.36-161)		0.2
NER		24.5 (0.3-68)		18.6 (0.71-46)		0.04

RDW: Red blood cell distribution width, MPV: mean platelet volume, PDW, platelet distribution width, NLR: neutrophil-lymphocyte ratio, PLR: platelet lymphocyte ratio, MLR: monocyte lymphocyte ratio, MER: monocyte eosinophil ratio, LER: lymphocyte eosinophil ratio NER: neutrophil eosinophil ratio. Categorical variables were compared by the Chi-square test. Independent groups were compared by the Mann-Whitney U test. Bold values denote statistical significance at $p < 0.05$.

The levels of MPV, NLR, PLR, and NER were significantly lower in the Asthma group ($p < 0.001$, 0.005, 0.002, and 0.04, respectively). In contrast, the red blood cell distribution width (RDW), lymphocyte, eosinophil, monocyte, basophil, PDW, MLR, and MER levels were higher in the same group. The Roc analysis with ROC curves was performed for optimal cut-off values to predict Bronchial Asthma (Figure 1). Youden Index was exploited to identify the optimal cut-off values. In addition, a p-value less than 0.05 was set as the statistical significance level. Areas under the curve for comparison of the No-Asthma group to the Asthma group were calculated. Areas under the curve (AUC) for eosinophil, RDW, MLR, MER, and PDW were found as 0.57, 0.81, 0.57, 0.93 and 0.76 respectively (Table 2).

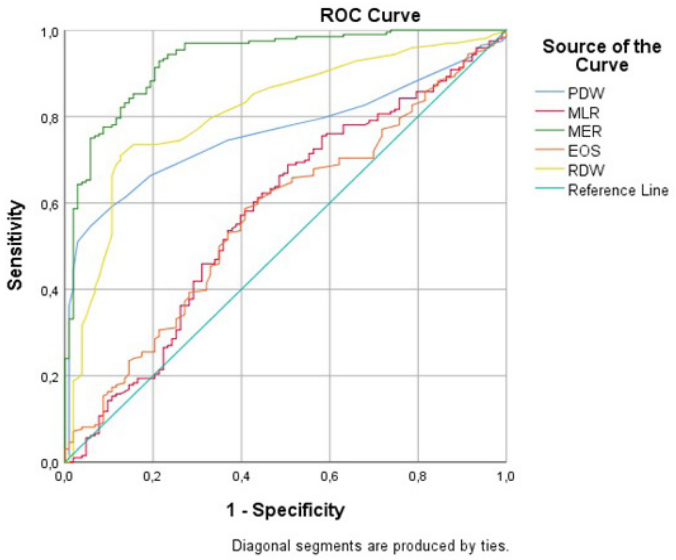

Figure 1. The ROC Curve of platelet distribution width (PDW), monocyte lymphocyte ratio (MLR), monocyte eosinophil ratio (MER), eosinophils (EOS), and red blood cell distribution width (RDW).

Table 2. ROC Analysis of patients with asthma and controls.

Variable	AUC (95%CI)	Cut-off	Sensitivity %	Specificity %	p
EOS	0.572	≥0.19	58	59	0.04
RDW	0.81	≥12.7	74	73	0.001
MLR	0.577	≥0.2	59	57	0.02
MER	0.930	≥4.83	84	83	0.001
PDW	0.76	≥16.1	70	72	0.001

EOS: Eosinophil, RDW: Red Blood Cell Distribution Width, MLR: monocyte lymphocyte ratio, MER: monocyte eosinophil ratio, PDW: platelet distribution width. Bold values denote statistical significance at the p <0.05

DISCUSSION

Lymphocytes play an essential role in the maintenance of immune system function. After a viral infection, changes in total lymphocyte numbers vary with different virus types. Asthma is a heterogeneous disease that may activate the immune system (7). In the present study Asthma group had significantly higher lymphocyte levels (p=0.02). Eosinophils are circulating and tissue-resident leukocytes that can potentially produce proinflammatory effects in some diseases. Eosinophils also have been shown to have various other functions like immunoregulation and antiviral activity. In severe eosinophilic asthma, high blood eosinophil levels have been associated with worse disease control and bad prognosis (8). The present study aimed to determine if eosinophil levels can help diagnose asthmatic subjects. In the present study, eosinophil levels of Asthmatic subjects were significantly higher.

Mean platelet volume is easily measured in the complete blood count analysis and reflects the functional and activity of platelets and their production rate from megakaryocytes. Some previous studies reported elevated levels of MPV in cerebrovascular diseases. (9), whereas other studies reported reduced MPV levels in rheumatoid arthritis (10). In the present study, MPV levels remained lower in the Asthma group. Similar to the present study, Sun et al. reported that patients with stable asthma had a lower MPV than controls. They included asthmatic subjects with exacerbation and found that asthmatic patients with exacerbations had lower MPV than stable asthmatic subjects (11). In light of these findings, investigating the mechanisms that cause the decrease in MPV values may be a method for treating and preventing the disease.

Red blood cell distribution width measures the variation of red blood cell volume. In a study, Gunbatar et al. reported a positive correlation between the apnea-hypopnea index of patients with obstructive sleep apnea syndrome and RDW (12). In this study, there were higher levels of RDW in patients with asthma compared to controls. High RDW values might be due to ongoing or recurrent inflammatory conditions. Previous studies reported low levels of PDW in COPD compared to healthy controls and smokers (13). In the present study, PDW levels were higher in the Asthma group. In a study, Ulucan et al. reported that major adverse cardiovascular events were more frequent in patients with a high PDW value (14). In infectious diseases, parameters such as C-reactive protein, erythrocyte sedimentation rate, and neutrophil levels are elevated, while some parameters, such as albumin, decrease. Today it's known that systemic inflammation observed in asthma has effects beyond the respiratory system (15). In light of this study and the studies mentioned above, systemic inflammation observed during asthma may be associated with a decrease in MPV. In contrast, an increase in RDW and PDW was observed in some other inflammatory conditions.

Recent studies suggest a preponderant role for monocyte-macrophage activation in the development of immunopathology and bad prognosis of COVID-19 patients (16, 17). In a study, macrophages were higher in asthmatic subjects' blood (18). In the present study, monocyte levels in the Asthma group were significantly higher. The present study hypothesized that the ratio of the values of these cells, which have an essential role in the inflammatory response, to the values of other inflammatory cells might guide the diagnosis of asthma. MLR and MER were both found to be higher in the asthmatic subjects whereas. In light of the obtained results and previous studies, higher levels of monocyte, MLR, and MER might indicate asthma. MER had an AUC of 93 (sensitivity 84 % and specificity 83 %).

In recent years, NLR and PLR have been in use by researchers in the diagnosis and prognosis of many inflammatory conditions. High levels of NLR were reported in patients with COVID-19 (19). In a study regarding patients with systemic lupus erythematosus, high NLR and PLR levels reflected inflammatory response and disease activity (20). In the present study, NLR, PLR, and NER were lower in the Asthma group. In a study, Yenigun et al. reported a high eosinophil-lymphocyte ratio and eosinophil counts in pediatric patients with allergic rhinitis (21). The present study aimed to investigate the usefulness of the LER in the diagnosis of asthma, yet no differences were found between groups.

Limitations of the Study

This study has limitations; besides its retrospective design, even though all parameters studied belong to the period before the treatment of asthma, information on unregistered diseases or therapies couldn't be obtained might have affected the parameters. The study results can guide the diagnosis stage but do not help the disease follow-up.

CONCLUSION

In conclusion, higher lymphocyte, eosinophil, monocyte, basophil, PDW, MLR, and MER, whereas lower levels of MPV, NLR, NER, and PLR support the diagnosis of asthma in patients who present to the clinic of the pulmonary disease with dyspnea in whom PFTs cannot be performed or who cannot cooperate with PFTs.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

The ethics committee approval was obtained from Bursa City Hospital Clinical Research Ethics Committee (Ethics Committee Approval No:2021-22/4) in accordance with Helsinki Declaration.

Authorship Contributions

Concept:İK, Design: İK, SD, Supervising: İK, Financing and equipment: İK, SD, Data collection and entry: İK, Analysis and interpretation İK, SD, Literature search: İK, SD, Writing: İK, SD.

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Evaluation of the Covid-19 vaccine literacy of the under-vaccinated community in a district in the Covid-19 pandemic: Pursaklar example

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Abstract

Objective: In this study, it was aimed to determine the reasons why individuals with incomplete Covid-19 vaccines in a district did not have their second dose of vaccine, even though they had their first dose of vaccine, and to evaluate their vaccine literacy.
Methods: The research is a cross-sectional study conducted between August and October 2021. It was identified and the population of the research was determined. 192 people participated in the study with the sample calculation. In the questionnaire used in the research, there are 29 questions and three sections: demographic characteristics of the participants, their knowledge and attitudes about the Covid-19 vaccine and other vaccines, and the Covid-19 vaccine literacy scale. The scale consists of 12 statements and two dimensions. Mann-Whitney U and Kruskal Wallis tests it was used as statistical methods in the study. Statistical significance value was accepted as $p<0.05$.
Results: Among the reasons why the participants did not receive the second dose of vaccines, the most common reason was stated as "I didn't have time/opportunity" with 51%. The Covid-19 vaccine literacy scale mean score of the participants was found to be 2.48 ± 0.53. A difference was found between education status and Covid-19 vaccine literacy, and it was determined that there was an increase in vaccine literacy level as the education level increased.
Conclusion: The mean score of the Covid-19 vaccine literacy scale was determined as 2.48 ± 0.54 for the under-vaccinated participants who had the first dose of Covid-19 vaccine, but did not receive the second dose. The Covid-19 vaccine literacy of the participants in the study is low. Increasing the vaccination literacy of individuals will make a positive contribution to their second dose vaccination.
Keywords: Covid-19, Missing Vaccine, Vaccine Literacy

INTRODUCTION

Vaccination is one of the greatest achievements of public health practice. As a result of immunization through vaccination, many epidemics have been prevented in the past and continue to be prevented today, and vaccination has been one of the most important weapons in building community immunity. Immunization programs are the best and most cost effective method for preventing and eliminating infectious diseases, reducing disease morbidity and mortality, and developing a healthy community (1). Vaccines are important for both individual immunity and social immunity, which occurs because the likelihood of unvaccinated individuals coming into contact with the pathogen decreases as the number of vaccinated individuals in the community increases (2).

Vaccination hesitancy today, as in the past, hinders the provision of community immunity to infectious diseases. The Covid-19 pandemic has brought a long standing debate on vaccine hesitancy back to the agenda. The reasons for vaccination hesitancy are not just a lack of information (3).

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Governments and health professionals play a critical role in shaping people's attitudes toward vaccines (4-6). Although it is extremely important that many entities (media, health organizations, educators, health professionals and educators, business or economic activities, general education institutions, public institutions, all academic fields, etc.) work together to combat vaccine hesitancy, society also has a major responsibility. However, for society to fulfill its responsibility, individuals must achieve a certain level of general education, followed by health literacy and vaccine literacy (7).

According to WHO, health literacy is defined as "the ability of individuals to acquire cognitive and social skills and abilities to use and understand information to maintain and improve health" (8). In addition to general health literacy, vaccine literacy should also be understood by society (9). Vaccine literacy is defined as the ability to have and use general health related information and services by processing and understanding them to make appropriate decisions about vaccines (10). In addition to providing information about vaccines, the role of vaccine literacy is to make vaccines more understandable by explaining them to individuals (3). Low vaccination literacy among people in the community leads to low vaccination rates in that community. There are studies that have investigated that vaccination is related to health literacy and vaccine literacy (11, 12). Therefore, proper communication about vaccination should be organized according to people's level of knowledge and vaccine literacy.

In this study, it was aimed to determine the reasons why individuals who were missing Covid-19 vaccine in a district did not receive the second dose of the vaccine even though they had received the first dose, and it was wanted to assess their vaccine literacy.

METHOD

In this study was a cross sectional study conducted between August 2021 and October 2021. From the Public Health Management System (PHMS) vaccination module, 6648 individuals it was identified who had received the first dose of Covid-19 vaccine by August 23, 2021, in the Pursaklar district of Ankara province but did not receive the second dose even though it was due. This number constitutes the population of the study. The sampling method used was systematic random sampling. Incomplete vaccination status was defined as a period of at least 4 weeks after the first vaccination, although the second vaccination was due. Pursaklar is a district in the north of Ankara. The population is 159676, of which 49.88% are male and 50.12% are female. 74% of the population has completed primary, middle or high school and 14% has attended university or higher education (13). In a literature study on Covid-19 vaccine literacy, the prevalence of limited vaccine literacy was found to be 11% (12). The study sample size was calculated using the online computer programme Openepi. The minimum sample size calculated with α= 0.05, d=5%, design effect 1 and 11% frequency % at a confidence interval of 95% was reported as 148. Considering that exceeding the calculated sample size increases the power of the study, the questionnaire was administered to 192 individuals who agreed to participate in the study.

The dependent variable of the study was the Covid-19 vaccine literacy scale score and its subdimensions of functional skills score and communicative/critical skills score. The independent variables were educational level, age, sex, employment status, marital status, presence of a chronic disease, history of Covid-19, self-assessment of own health, status of influenza vaccination in the last season, status of intention to get influenza vaccination in this season, questions about planning vaccination against other infectious diseases, and reasons for not receiving the second dose of Covid-19 vaccine.

The questionnaire, "Evaluation of Covid-19 vaccine literacy of the community with missing vaccines in a district during the Covid-19 pandemic," used as the data source in the study, was prepared by the researchers and administered to individuals by telephone. Those who could not be reached by telephone or who did not want to participate in the survey it was not surveyed and the next person on the list was passed on. The questionnaire included 29 questions and three sections: participant demographic characteristics, knowledge and attitudes about the Covid-19 vaccine and other vaccines, and a Covid-19 vaccine literacy scale. The question "Why did you not get the 2nd dose of Covid-19 vaccine?" was a closedended question, and options it was presented to participants by reading them.

Covid-19 Vaccine Literacy Scale

Biasio et al. adapted the scale developed by Ishikawa et al. to measure health literacy of individuals with chronic diseases as the Covid-19 vaccine literacy scale (12). The Turkish validity and reliability study of the Covid-19 vaccine literacy scale was conducted by Durmuş et al. in 2021 (14). In the scale, the functional dimension questions consist of 4 items and the communicative/critical dimension questions consist of 8 items, giving a total of 12 items and two dimensions. The scale is a 4-point Likert scale. The functional dimension items are rated 1=often, 2=sometimes, 3=rarely, 4=never, and the communicative/critical dimension statements are rated 4=often, 3=sometimes, 2=rarely, 1=never. No threshold or min-max value was determined for the scale used in this survey. A mean score close to 4 indicates a high level of vaccine literacy (14).

Functional vaccine literacy means that individuals have literacy skills to understand health information about vaccines and to use this information in their daily lives (15).

Communicative/critical immunization literacy focuses on areas such as the ability to solve problems more accurately and make decisions. Communicative health literacy means that individuals have cognitive and social skills, can adapt to current medical situations by using their health related knowledge, and benefit from various medical activities (15, 16). Critical health literacy refers to a more advanced cognitive level. It enables individuals to analyze acquired information in a critical dimension along with their social skills and use this information to gain control over the events or situations they experience (15, 16).

All subjects reached by telephone for the study were first given explanatory information about the study, and the informed consent form was read. Those who volunteered to participate in the study it was interviewed, and the questionnaire was used. The interview with one person took an average of 10-15 minutes.

The research data it was analysed using SPSS 23 statistical package program. Descriptive statistics it was presented as mean (±), standard deviation (min; max), median (IQR 25-75), frequency distribution, and percentage. Mann-Whitney U test was used for statistical analysis between two independent groups, and Kruskal-Wallis test was used for statistical analysis between more than two independent groups. Statistical significance value was accepted as $p<0.05$.

The ethics committee approval of the study was obtained from Ankara Yıldırım Beyazıt University Ethics Committee with the date 22.09.2021 and number 2021- 415.

RESULTS

Of the subjects who participated in the study, 55.2% were female and the mean age was 41.90±16.44 years. According to educational status, the highest number of individuals were primary school graduates with a rate of 38%. 73.9% of those over 40 years old had a primary school diploma or less. 43.2% of individuals were employed in an income generating job and 76% were married (Table 1).

25.5% of participants had a physician diagnosed chronic disease. 27.6% had been previously vaccinated with Covid-19. For the first vaccination dose, 79.7% of participants received the Biontech vaccine and 20.3% received the Sinovac vaccine (Table 2).

Table 3 lists the reasons why individuals who received the first dose of Covid-19 vaccine did not receive the second dose. Accordingly, it was found that 51% of participants did not receive the second dose of vaccine because they did not have time/opportunity and 17.2% did not get an appointment. The mean age of those who reported not having time/opportunity was 37.47±15.24 years, and 51.1% were employed (Table 3).

Table 1. Some demographic characteristics of the individuals participating in the research (n=192)

	n	(%)*
Age		
Mean± sd (min-max)	41.90 ± 16.44 (18-85)	
Median (25-75 %)	40 (28-53)	
Age group		
40 and below	100	52.1
41 and above	92	47.9
Sex		
Female	106	55.2
Male	86	44.8
Education		
Literate	15	7.8
Primary school graduate	73	38.0
Secondary school graduate	27	14.1
High school graduate	44	22.9
University graduate	33	17.2
Working status		
Working	83	43.2
Not working	109	56.8
Marital Status		
Married	146	76.0
Single	46	24.0
* Column Percentage		

Table 2. Some descriptive characteristics of the participants (n=192)

	n	(%)*
Chronic disease		
Yes	49	25.5
No	143	74.5
Previous Covid-19 status		
Yes	53	27.6
No	139	72.4
First dose of Covid-19 vaccine		
Biontech	153	79.7
Sinovac	39	20.3
* Column Percentage		

Table 3. The reasons why the participants who had the first dose of Covid-19 vaccine did not get the second dose of vaccine

	n	(%)*
Reasons for not getting the 2nd dose of vaccine **		
I didn't have the time/opportunity	88	46.0
I couldn't get an appointment	33	17.2
I experienced Covid-19-like symptoms with the first dose of vaccine. I am scared	24	12.5
I got Covid-19 before I got my 2nd dose of vaccine	10	5.2
I am affected by negative news in the media	4	2.1
I think a single dose of vaccine is enough	13	6.8
I regretted it after the first dose of vaccine	12	6.3
Conditions related to pregnancy/breastfeeding	4	2.1
Other	6	3.1
Age distribution of those who answered that I did not have time/opportunity (n=88)		
Mean± ssd	37.47± 15.24	
Median (min-max)	34.5 (18-85)	
Employment status of those who answered that I did not have time/opportunity		
Working	45	51.1
Not working	43	48.9

* Column Percentage
** More than one reason may be specified

Table 4. General health status of the participants and some attitudes and behaviors about vaccines

	n	(%)*
Flu vaccination status at last season (n=192)		
Yes	11	5.7
No	181	94.3
Age distribution of those who had the flu vaccine		
59 and below	4	5.7
60 and above	7	94.3
Considering getting the flu vaccine this season		
Considering getting vaccinated	97	26.6
Not considering	268	73.4
Planning to be vaccinated against other infectious diseases		
Considering getting vaccinated	35	18.2
Not considering	157	81.8
Self-evaluation of health		
Very good	50	26.0
Good	114	59.4
Middle	20	10.4
Bad	7	3.6
Too bad	1	0.5

*Column Percentage

Of those who participated in the study, 5.7% had received an influenza vaccination in the previous season, and 26.6% planned to receive influenza vaccination in this season. Of those who had received influenza vaccination, 63.6% were over 60 years of age, and 59.4% of participants rated their health as generally good (Table 4).

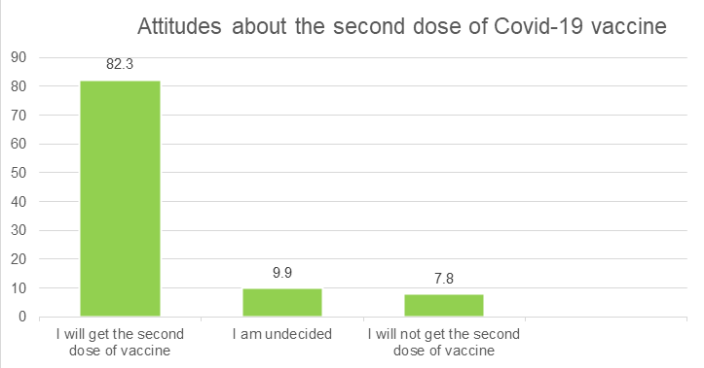

Figure 1. Attitudes of the individuals participating in the study about the second dose of Covid-19 vaccine

When study participants it was asked about their attitudes toward the second dose of Covid-19 vaccine, 82.3% of participants indicated that they would receive the second dose of vaccine, 7.8% indicated that they would not, and 9.9% indicated that they had not yet decided (Figure 1).

Table 5. Mean, standard deviation, minimum, maximum, median and 1st-3rd quartile (25-75 %) values for the Scale

	Mean±sd (min-max)	Median (25-75 %)
Functional Skills	2.71±0.91 (1-4)	2.5 (2-3.75)
Communicative/ Critical Skills	2.36±0.79 (1-4)	2.63 (1.78-2.88)
Covid-19 Vaccine Literacy	2.48±0.54 (1-3.67)	2.5 (2.08-2.83)

Participants' total and subscale scores on the Covid-19 Vaccine Literacy Scale were as follows: The mean score of the functional skills subscale was 2.71±0.91, the mean score of the communication/critical skills subscale was 2.36±0.79, and the mean score of the Covid-19 vaccine literacy scale was 2.48±0.54 (Table 5).

Table 6 shows the comparison of Covid-19 vaccine literacy scale and subdimensions scores according to some characteristics of the study participants. No difference was found in Covid-19 vaccine literacy scale score and its subdimensions by sex. While no difference was found between participants' educational levels for functional skills (p=0.186), Covid-19 scale scores for vaccination literacy and communication/critical thinking skills were higher in university graduates than in the other groups (p<0.001; p<0.001). While no significant difference was found between participants' communicative/critical skills scores by age group (p=0.263), functional skills and Covid-19 vaccine literacy scale scores were significantly higher among persons 40 years of age and younger than among persons 40 years of age and

older (p=0.006; p=0.010, respectively) (Table 6). Among those aged 40 and older, 73.9% had an primary school degree or less and 26.1% had a secondary school, high school, or university degree. Individuals over 40 years of age with at least secondary school, high school, or university education had a higher functional skills score (2.64±0.91), but this was not statistically significantly different from individuals with primary school education or less (p=0.528), while the communicative/critical skills score (2.69±0.64) and Covid-19 vaccine literacy scale score (2.67±0.43) were statistically significantly higher (p=0.010; p=0.002).

Table 6. Distribution of Covid-19 vaccine literacy scale and sub-dimension scores according to some demographic characteristics of the participants

N=192	Functional Skills		Communicative/Critical Skills		Covid-19 Vaccine Literacy	
	Mean ±sd	p	Mean ±sd	p	Mean ±sd	p
Education						
Primary school graduate and below	2.58±0.96	0.186[1]	2.07±0.79	<0.001[1]	2.24±0.51	<0.001[1]
Secondary school, high school graduate	2.75±0.90		2.46±0.70		2.58±0.48	
University graduate	2.79±0.75		2.85±0.57		2.89±0.38	
Age group						
40 and below	2.87±0.86	0.006[2]	2.43±0.77	0.263[2]	2.58±0.51	0.010[2]
41 and above	2.53±0.93		2.29±0.80		2.37±0.54	
Educational status of those over the age of 40						
Literate-Primary school graduate	2.50±0.94	0.528[2]	2.15±0.81	0.010[2]	2.27±0.55	0.002[2]
Secondary school, high school, university graduate	2.64±0.91		2.69±0.64		2.67±0.43	
Sex						
Female	2.75±0.89	0.445[2]	2.29±0.84	0.291[2]	2.44±0.56	0.369[2]
Male	2.65±0.93		2.46±0.70		2.52±0.50	
Working status						
Working	2.85±0.86	0.058[2]	2.49±0.74	0.092[2]	2.61±0.46	0.005[2]
Not working	2.60±0.93		2.27±0.80		2.38±0.56	
Marital Status						
Married	2.69±0.91	0.607[2]	2.25±0.80	0.002[2]	2.40±0.53	<0.001[2]
Single	2.77±0.89		2.70±0.64		2.72±0.46	

[1:] Kruskal Wallis test
[2:] Mann Withney U test

Table 7 shows the comparison of the results of the Covid-19 vaccine literacy scale and its subdimensions according to some characteristics of the study participants. There was no statistically significant difference in scores on the Covid-19 vaccine literacy and its subdimension communicative/critical skills depending on the presence of chronic disease and whether participants had previously had Covid-19, but there was a difference in functional skills (p<0.001; p=0.006). Accordingly, those who did not have chronic diseases and those who had not had Covid-19 before had higher functional skills scores. There was no difference in Covid-19 vaccine literacy and its subdimension communicative/critical skills score according to the ' status of participants considering influenza vaccination this season and planning to get vaccinated against other infectious diseases, while those who did not plan to get vaccinated against influenza and other infectious diseases this season had higher functional skills scores. This difference was statistically significant (p=0.029; p=0.034). In addition, although no statistically significant difference was found when comparing functional skills according to participants' self-assessed health status (p=0.078), those who rated their health status as very good-good had higher Covid-19 vaccine literacy and communicative/critical skills scale scores than those who rated their health status as moderate and very poor-bad. This difference was statistically significant (p<0.001; p=0.007) (Table 7).

Table 7. Comparison of Covid-19 vaccine literacy scale and subdimension scores according to some characteristics of the participants

	Functional Skills		Communicative/Critical Skills		Covid-19 Vaccine Literacy	
	Mean ±sd	p	Mean ±sd	p	Mean ±sd	p
Chronic disease						
Yes	2.30±0.90	<0.001[1]	2.40±0.80	0.457[1]	2.37±0.55	0.211[1]
No	2.85±0.87		2.35±0.782		2.52±0.53	
Previous Covid-19 status						
Yes	2.42±0.86	0.006[1]	2.44±0.79	0.447[1]	2.43±0.61	0.712[1]
No	2.81±0.91		2.34±0.78		2.49±0.51	
Self-evaluation of health						
Very good-good	2.76±0.88	0.078[2]	2.43±0.76	0.007[2]	2.54±0.51	<0.001[2]
Middle	2.43±1.05		2.05±0.82		2.18±0.52	
Bad-too bad	2.18±0.89		1.65±0.70		1.83±0.41	
Flu vaccination status at last season						
Yes	2.97±0.85	0.357[1]	2.09±0.82	0.278[1]	2.38±0.46	0.506[1]
No	2.69±0.91		2.38±0.78		2.48±0.54	
Considering getting the flu vaccine this season						
Considering	2.31±0.88	0.029[1]	2.48±0.69	0.275[1]	2.43±0.43	0.791[1]
Not considering	2.76±0.90		2.34±0.80		2.48±0.56	
Planning to be vaccinated against other infectious diseases						
Planning	2.40±0.81	0.034[1]	2.48±0.70	0.368[1]	2.45±0.53	0.910[1]
Not planning	2.77±0.92		2.34±0.80		2.48±0.54	

[1:] Mann Withney U testi , [2:] Kruskal Wallis testi

DISCUSSION

This study was conducted in Pursaklar district to determine the reasons why individuals who were missing Covid-19 vaccine did not receive their second vaccination dose even though they had received their first vaccination dose and to assess evaluate their vaccine literacy.

When asked why they did not receive their second vaccine dose, the most common reason given was "I did not have time/opportunity" at 51%. Considering that 51% of respondents were employed and 61% were between 18 and 40 years old, this result is to be expected. According to the results of the survey conducted in October 2021 by Ipsos (Global Specialist in Marketing and Public Opinion Research), a global market research firm, the rate of nonvaccination in the 18- to 35 year-old age group was higher than the vaccination rate in this age group (17). An appointment system that can be adjusted to individual work schedules can change this result. On site vaccination practices and appointment free vaccination practices can improve the "I did not have time/opportunity" response. Vaccination points can be located in places that are easily accessible to people in public living spaces.

The majority of participants stated that they did not intend to get influenza vaccines or existing infectious disease vaccines other than Covid-19 vaccines. This indicates that these individuals with missing Covid-19 vaccines do not have the necessary care and dedication not only for the Covid-19 vaccine, but also for other vaccines. Only 5% of participants reported having been vaccinated against influenza in the last season. About 65% of those who received influenza vaccines were 60 years and older. Although the number of participants over 65 years old in the study was not very high (17 people), 29.4% of participants over 65 years old had received the flu vaccine in the last season. In another study conducted in Istanbul, the rate of influenza vaccination among those over 65 years of age was 26.5% (18). Experts strongly recommend seasonal influenza vaccination to reduce the burden of the two epidemics expected next winter (19, 20). Explaining the concepts of health literacy and vaccine literacy to people may positively influence their health related decisions and lead to an increase in vaccination rates. According to in this study, the mean score of participants in the functional skills dimension of the scale was 2.71±0.91, the mean score in the communicative/critical skills dimension was 2.36±0.79, and the mean score of the whole scale was 2.48±0.54. Similar to the study on vaccine literacy in Italy, the mean score of participants in the functional skills was higher than the mean score in the communicative/critical skills in this study (21). In the Turkish Validity and Reliability of the Covid-19 vaccine literacy scale, participants' mean score on functional skills (2.40±0.75) was lower than in this study, whereas mean scores on communicative/critical skills (2.60±0.69) and total scale (2.54±0.56) were higher than in this study (14). These differences could be due to the fact that the places where the studies it was conducted had different socioeconomic levels.

According to the results of the October 2021 survey by Ipsos (Global Specialist in Marketing and Public Opinion Research), a worldwide research firm, 85% of society has received at least one vaccination dose by the first week of October 2021, while the percentage of people who have never been vaccinated is 15%. According to the same study, the rate of those who have received a single dose of vaccination is 13% (17). In this study, 82% of the participants who had received the first dose of vaccine stated that they would also receive the second vaccine dose; whereas the others it was undecided or stated that they would not receive the second dose. The hesitations and reasons of those who it was undecided and those who stated that they would not get vaccinated can be listened to and their knowledge can be corrected with accurate information by healthcare professionals.

In both this study and the Turkish validity and reliability study of the Covid-19 vaccine literacy scale, no statistically significant difference was found in Covid-19 vaccine literacy, functional skills, and communicative/critical skills according to the gender of the participants (14). Covid-19 vaccine literacy scale and functional skills scores of individuals aged 40 years and younger were higher than those of individuals aged 40 years and older. Of note in the study, 73.9% of those over 40 years of age had primary school education or less. The higher Covid-19 vaccine literacy scale and functional skills score of individuals over age 40 may be due to this. A study of Covid-19 vaccine literacy in Japan found that Covid-19 vaccine literacy decreased as participants aged (22). Covid-19 risk increases with age, suggesting that vaccination literacy should be higher in older participants; however, this was not the case in either study. The study found a statistically significant difference between Covid-19 vaccine literacy and the communicative/critical skills subdimension according to the education level of the individuals. Accordingly, vaccine literacy was found to increase as the level of education increased. This was an expected result. In the Turkish validity and reliability study of the Covid-19 vaccine literacy scale, no significant difference was found in the Covid-19 vaccine literacy and communicative/critical skills dimension according to the educational level of the participants; however, a significant difference was found in the functional skills dimension. In the study on vaccine literacy in Italy, a strong association was found between vaccination, education level and vaccine literacy of participants (12). These three studies have shown us that the vaccine literacy scale score or the score of its subdimensions, in other words, the vaccine literacy level of individuals, has a positive relationship with educational status. This confirms the importance of improving health literacy and vaccine literacy skills through targeted interventions.

Appropriate and timely vaccination for the whole society is very important to survive the Covid-19 pandemic that the whole world has been struggling with for some time. In this context, it is important to increase the awareness of

the concept of vaccine literacy in the society, evaluate and improve the vaccine literacy of the society so that vaccines are accepted and used by the people in the society. There are very few studies on vaccination literacy in the literature. It is important to increase the number of studies in this area to prevent this and future infectious disease outbreaks.

Limitations of the Study

Since the survey of participants was conducted by telephone, some people over 65 years of age did not want to participate in the survey. Therefore, the number of participants over 65 years old is small. In addition, the survey was conducted in only one center, that is, only in Pursaklar district. The results can only be generalized for Pursaklar. This study can serve as a guide for other multicenter studies on vaccine literacy.

CONCLUSION

In this period from August to October 2021, when health experts emphasize the need for three doses of vaccination in the Covid-19 pandemic, there are still people in the Pursaklar district with a single dose of vaccination for various reasons. Low Covid-19 vaccine literacy is one of the most important reasons for incomplete vaccination. Identifying the vaccine literacy skills of the population and implementing the necessary measures to correct the inadequate vaccination competency can positively contribute to the reduction of incomplete vaccination rates.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Ethical permission was obtained from Ankara Yıldırım Beyazıt University Health Sciences Ethics Committee for this study with the letter dated 22.09.2021 and numbered 415, and the Helsinki Declaration criteria were taken into consideration.

Authorship Contributions

Concept: HD, MEG, Design: HD, FA, Supervising: MEG, HD, Data collection and entry: HD, FA, Analysis and interpretation: MEG, HD, Literature search: FA, HD, Writing: HD, FA Critical review: MEG.

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ORIGINAL RESEARCH
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Evaluation of serum adipocytokine and interleukin-18 levels in patients with epilepsy

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Abstract

Objective: Epilepsy is a neurological disease characterized by recurrent seizures. The underlying pathophysiological mechanisms in epilepsy are not fully known. Our aim is to investigate the relationship between serum adipocytokine and interleukin (IL)-18 levels in epilepsy patients receiving and not receiving antiepileptic therapy.

Method: Our study was established as three groups. I: Epilepsy patients receiving antiepileptic therapy (n=30), II: Newly diagnosed epilepsy patients (n=30) and III: Control group (n=30). Serum adipocytokine and IL-18 levels were measured by enzyme-linked immunoassorbent assay method.

Results: It was determined that serum adipocytokine and IL-18 levels were increased in epilepsy patients who received topiramate treatment and did not receive antiepileptic therapy compared to the control group. Serum glucose, total protein, cholesterol and albumin concentrations of patients who received antiepileptic treatment were decreased compared to the control group ($p<0.001$). It was found that serum adipocytokine and IL-18 concentration in epilepsy patients who received topiramate treatment decreased compared to patients who did not receive treatment, but it was not significant ($p>0.05$). It was found that the body mass index (BMI) ratio of epilepsy patients who received antiepileptic treatment decreased and was significant compared to the control group and the group that did not receive treatment ($p<0.01$).

Conclusion: In our study, it was shown that serum adipocytokine and IL-18 levels were increased in epilepsy patients who received and did not receive antiepileptic therapy. Findings from this study suggest that adipocytokine and IL-18 may be useful markers for the inflammatory process of epileptogenesis.

Keywords: Topiramate, Interleukin-18, Adipocytokine, Epilepsy

INTRODUCTION

Epilepsy, which affects millions of people around the world, is a neurological disease characterized by recurrent seizures. The underlying pathophysiological mechanisms in epilepsy have not been fully elucidated. Neurological injuries such as stroke, central nervous system (CNS) infections, inflammation, traumatic brain injury, cerebrovascular injuries play an important role in the pathophysiology of epilepsy (1-3). The process is activated when the blood-brain barrier function is impaired. In this process, it is thought that inflammatory mediators leaking into the CNS may cause neuroinflammation (4, 5).

Vaspin, isolated from white adipose tissue and visceral tissue, is a biomarker belonging to the adipokine family. Some studies have suggested that active mRNA expression of vaspin in human adipose tissue may be a compensatory mechanism for obesity and insulin resistance (6, 7).

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Visfatin is an adipokine with different functions and synthesized from different cells. Adipocytes, neutrophils, monocytes, lymphocytes, hepatocytes and pneumocytes are the main sources of visfatin. It has been stated that visfatin level is high in acute and chronic inflammatory diseases (8, 9).

Chemerin is a protein that is synthesized from the inactive form of prochemerin and is cleaved by serine proteases and activated via its C terminals (10). It has been stated that the chemerin molecule, which is involved in the maturation and differentiation of adipocytes, is associated with paracrine/autocrine signals (11).

Interleukin (IL)-18 is a pro-inflammatory cytokine, and IL-18, a molecule that belongs to the IL-1 family and was initially shown as an interferon gamma (IFN-γ) inducing factor, has been reported to be associated with a number of inflammatory and autoimmune diseases (12).

A limited number of studies were found related to epilepsy with and without topiramate treatment with the mentioned biomarkers (13). Hence the aim of the present study was to investigate the relationship between serum adipocytokine and IL-18 levels in epilepsy patients.

METHODS

This study was carried out in Dicle University Faculty of Medicine, Department of Neurology. The study was approved by the Dicle University Clinical Research Ethics Committee (ethic approval no:18/05/2018-176). The study was evaluated by two expert neurologists. Classification of epileptic seizures was carried out according to the epilepsy classification of the International League against Epilepsy, which was published in 2017 (14). Magnetic resonance imaging and electroencephalography (EEG) of the patients were taken. Patients with pathological EEG findings and patients with psychogenic seizures were not included in the study. Three groups were established: (I) The patient diagnosed with epilepsy (n=30): Patients who came one week after the seizure were included in the study. (II) Patients in this group included 30 epilepsy patients who received topiramate therapy as monotherapy for at least one year. (III) Healthy individuals who did not use any medication were selected as the control group (n=30). Conditions such as hypertension, acute and chronic infection, diabetes mellitus, autoimmune disease, fever, traumatic brain injury, psychiatric diseases, gastrointestinal disease, and obese individuals in all three groups were determined as the exclusion criteria of our study. An information form was signed by the volunteers in the patient and control groups in our study. It was conducted in accordance with the Declaration of Helsinki.

Biochemical Analyses

Blood samples were withdrawn from the epilepsy patient and from the control groups. Serum was obtained by centrifuging venous blood at 5000 rpm for 10 minutes. These serum samples were transferred to eppendorf tubes and kept at -80 degrees until the study day. Vaspin, visfatin, chemerin and IL-18 levels in the obtained serum were determined by enzyme-linked immunosorbent assay kits (Vaspin catalog no:YLB3664HU, the assay range for kit:1ng/mL→30ng/mL, sensitivity:0.48ng/mL, intra-interassay coefficients of variance <9%-<11%; visfatin catalog no:YLB3665HU, the assay rangefor kit: 1µg/L→20µg/L sensitivity:0.51µg/L, intra-interassay coefficients of variance: <9%-<11%, chemerin catalog no: YLB0782HU, the assay range for kit:2ng/L→40ng/L, sensitivity:0.96ng intra-interassay coefficients of variance <9%-<11%, IL-18:catalog no:YLB1955HU, the assay range for kit:5ng/L→100ng/L, sensitivity:2.35ng/L, intra-interassay coefficients of variance <9%-<11%, YL Biont, China) method. Serum triglyceride, albumin, globulin, total protein, cholesterol and glucose levels were studied by colorimetric method (Roche Modular Autoanalyzer; Roche, Tokyo, Japan).

Statistical Analysis

Statistical analyses were performed using SPSS 18.0 program (SPSS Inc., USA). The categorical variables were expressed as numbers and percentages. The conformity of the data to the normal distribution was checked with Kolmogorov-Smirnov and Shapiro-Wilk tests. 2-group student–T test for parameters with normal distribution. Three-group One Way ANOVA and Mann Whitney-U test were used to compare pairwise groups in parameters that were not normally distributed. Kruskal Wallis Test was used to compare more than two groups. Paired comparisons were made with Tukey HSD as a pot-hoc test in test by making bonferroni correction to understand which group caused the statistical difference. The cutoff values and corresponding sensitivity and specificity values for the prediction between the epilepsy group and the control group based on serum adipocytokine and IL-18 were estimated by receiver operating characteristic (ROC) curve analysis. Spearman correlation analysis was used to determine the relationship between the data. A result was accepted as statistically significant with a p-value < 0.05.

RESULTS

The mean age of the control group was 23.46±6.94 years, the mean age of the group that did not receive antiepileptic treatment was 27.83±13.20 years, and the mean age of the group that received topiramate therapy was 31.23±10.71 years, and there was no statistical difference between the three groups in terms of age. It was found that BMI ratio was lower in patients who received topiramate therapy compared to the control group and patients who did not receive treatment ($p<0.01$). There was no statistical difference between the genders in all three groups ($p>0.05$) (Table 1).

Table 1. Characteristics of control and patient groups

		Control group (n=30)		Epilepsy group not receiving antiepileptic therapy (n=30)		Epilepsy group receiving topiramate therapy (n=30)		p value
		n	%	n	%	n	%	
Gender	Female	16	53.3	16	53.3	15	50	
	Male	14	46.7	14	46.7	15	50	
Age (Year)		21.00(18.00-60.00) 23.46±6.94		20.50(18.00-60.00) 27.83±13.20		21.00(18.00-60.00) 31.23±10.71		0.093
BMI (kg/m²)		22.00 (14-36) 23.20±4.773		22.50(19-26) 22.23±2.063		20.50(18-24) * 20.90±1.517		0.020

Data are given as Mean±Standard deviation and median (minimum-maximum). p<0.001* The difference between the patient and control group is significant.
Abbreviations:BMI; Body mass index

Serum visfatin, vaspin, chemerin and IL-18 levels in patients who received and did not receive antiepileptic treatment were higher than the control group and were statistically significant. Serum total protein, albumin, glucose and cholesterol levels were found to be decreased and statistically significant compared to epilepsy patients who received and did not receive treatment (p<0.001). There was no statistically significant difference between the groups in terms of serum triglyceride, HDL and globulin values (p>0.05). (Table 2)

A positive correlation was observed between serum IL-18 levels and visfatin, chemerin in epilepsy patients receiving topiramate therapy (p<0.01). A positive correlation was observed between serum vaspin and visfatin and triglyceride levels (p<0.05). A positive correlation was observed between serum triglyceride level and BMI and visfatin (p<0.01). (Table 3)

Table 2. Comparison of epilepsy patients with and without antiepileptic treatment and the control group

Parameters	Control group (n=30)	Epilepsy group not receiving antiepileptic therapy (n=30)	Epilepsy group receiving topiramate therapy (n=30)	p value
Visfatin (ng/mL)	10.54 (4.75-14.64)	19.35 (12.88-68.95) *	15.10 (9.97-47.47) *	<0.001
IL-18 (µg/L)	12.23 (6.48-24.84)	16.08 (12.22-91.91) *	15.61 (11.11-44.58) *	0.007
Chemerin (ng/mL)	104.90 (73.86-165.40)	133.79 (95.46-958.67) *	122.93 (70.71-771.81) *	<0.001
Vaspin (ng/mL)	0.51 (0.16-0.96)	1.6703 (1.01-6.79) *	0.55 (0.23-1.32)*	<0.001
Glucose (mg/dL)	93.00 (59.00-104.00)	75.00 (55.00-95.00) *	74.00 (51.00-108.00) *	<0.001
Cholesterol (mg/dL)	149.00 (112.00-235.00)	162.00 (128.00-229.00)	144.00 (102.00-212.00) *	0.018
HDL (mg/dL)	45.55 (22.30-89.20)	49.10 (31.90-67.20)	47.80 (24.00-64.90)	0.992
Triglyceride (mg/dL)	95.00 (51.00-446.00)	117.00 (66.00-210.00)	119.00 (70.00-291.00)	0.394
Albumin (mg/dL)	5.18 (4.30-5.73)	5.03 (4.62-5.29) *	4.84 (4.42-5.22) *	<0.001
Globulin (mg/dL)	3.22 (2.77-3.76)	3.14 (2.64-3.43)	2.95 (2.75-4.06)	0.139
Total protein (mg/dL)	8.34 (7.20-9.24)	8.02 (7.62-8.61) *	7.8350 (7.17-8.67) *	0.001

Data are given as median (minimum-maximum). p<0.001* The difference between the patient and control group is significant.
Abbreviations: IL-18:Interleukin-18, HDL: High-density lipoprotein

Table 3. Correlation coefficients between parameters in the group receiving topiramate therapy

Parameters		BMI	Visfatin	IL-18	Chemerin	Vaspin
BMI		1				
Visfatin	r	0.323	1			
	p	0.082				
IL-18	r	-0.085	0.518**	1		
	p	0.656	0.003			
Chemerin	r	0.189	0.606**	0.728**	1	
	p	0,317	0.000	0.000		
Vaspin	r	0.311	0.444*	0.203	0.285	1
	p	0.094	0.014	0.283	0.127	
Triglyceride	r	0.413*	0.451*	0.223	0.218	0.043
	p	0.023	0.012	0.237	0.247	0.821

**p<0.01, *p<0.05 Abbreviations: IL-18: Interleukin-18, BMI: Body mass index

A negative correlation was found between serum IL-18 and visfatin and chemerin (p<0.01) serum vaspin levels and BMI in the epilepsy group who did not receive antiepileptic therapy (p<0.05). (Table 4)

Table 4. Correlation coefficients between parameters in the group not taking antiepileptic drugs

Parameters		BMI	Visfatin	IL-18	Chemerin	Vaspin
BMI		1				
Visfatin	r	-0.062	1			
	p	0.744				
IL-18	r	0.091	0.625**	1		
	p	0.632	0.000			
Chemerin	r	-0.078	0.458*	0.667**	1	
	p	0.681	0.011	0.000		
Vaspin	r	-0.424*	0.081	0.044	0.366*	1
	p	0.020	0.670	0.816	0.047	
Triglyceride	r	-0.274	0.162	-0.135	-0.280	-0.106
	p	0.143	0.393	0.478	0.134	0.578

**p<0.01, *p<0.05 Abbreviations: IL-18: Interleukin-18, BMI: Body mass index

In the ROC curve analysis performed between epilepsy patients and the control group, the cut-off value of the visfatin molecule was 14.24, sensitivity 81.7% and specificity 96.7%. Cut-off value of IL-18 molecule was determined as 24.21 sensitivity, 20% and specificity as 96.7%. The cut-off value of the chemerin molecule was determined as 163.68, sensitivity 35% and specificity 96.7%. The cut-off value of the vaspin molecule was 0.96, sensitivity of 58% and specificity of 96.7%. (Figure 1)

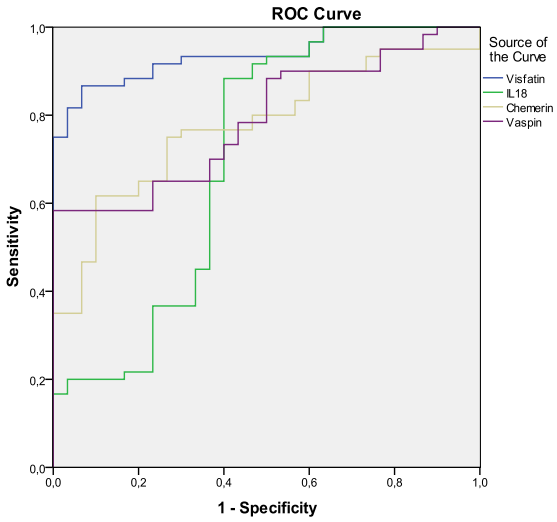

Figure 1. The ROC curve analysis of vaspin, visfatin, IL-18 and chemerin for prediction between the frequently control group between in patients with epilepsy. Abbreviations: IL-18: Interleukin-18, ROC, receiving operating characteristic.

DISCUSSION

Epilepsy is a disease characterized by recurrent seizures that cause behavioral, cognitive psychology and neurobiological disorders (15). The pathophysiology of epilepsy has not been fully explained. It has been observed that pro-inflammatory cytokines such as IL-1β, IL-2, and IL-6 increase after epileptic seizures (16).

In the study, it was determined that serum visfatin, vaspin, chemerin, and IL-18 serum levels increased in patients with epilepsy. It was observed that the levels of these biomarkers were decreased in patients receiving topiramate therapy, but not significantly. It was found that the BMI ratio of epilepsy patients who received topiramate therapy decreased compared to the control group and the group that did not receive antiepileptic treatment.

Studies in the literature on epilepsy patients and serum vaspin, visfatin, and chemerin levels were generally performed in pediatric patients. However, these studies are limited in number (13). This is the first clinical study in adult epilepsy patients.

IL-18, a classical pro-inflammatory cytokine, is associated with the IL-1 family released from the NLR family pyrin domain containing 3 (NLRP3) inflammasome. It has been reported that IL-18 is produced in the CNS and its receptors are expressed in neurons (17-19). Liu et al. (20) stated that IL-18, caspase 1, and NLRP3 inflammasome expression increased in their experimental epilepsy model. In this sense, Mochol et al. (21) suggested that there is an increase in serum IL-18, and IL-18BP receptor levels in patients receiving carbamazepine. In our study, it was found that there was a significant increase in serum IL-18 levels in patients with epilepsy. The elevation

of serum IL-18 level in epilepsy patients suggests that it may increase pro-inflammatory cytokine release in glial cells and impair the permeability of the blood-brain barrier. Mochol et al. found no correlation with serum IL-18 and BMI ratio in their study. They found that the serum IL-18 level was high in patients with a BMI ratio of less than 30 kg/m² and did not correlate with the BMI (21). In our study, no correlation was found between BMI and serum IL-18 levels. Hung et al. (22) suggested that high serum IL-18 level may be a risk marker for metabolic risk, but this is independent of obesity and insulin resistance. This study showed similar results with previous studies. In addition, there was a positive correlation between serum IL-18 level and serum chemerin, visfatin levels. These results indicate that adipose tissue may contribute to inflammation.

It has been stated that the concentration of chemerin rises in response to pro-inflammatory molecules to induce migration of macrophages, natural killer cells, and dendritic cells to the site of inflammation (23). The CNS has also been suggested that elevated chemerin levels in other tissues may serve as a biomarker of chronic inflammation (24). In our study, it was found that there was a significant increase in serum chemerin levels in patients with epilepsy. Elhady et al. (25) found significantly higher serum chemerin levels in idiopathic pediatric epilepsy patients receiving treatment, especially in those with uncontrolled seizures. In our study, it was shown that there was a positive correlation between serum chemerin levels and IL-18, visfatin and vaspin levels in patients with epilepsy. The high serum chemerin concentrations and its correlation with IL-18 indicate that angiogenesis is induced in endothelial cells and may induce inflammation by activating pro-inflammatory cytokines.

Studies with the visfatin molecule have generally focused on metabolic and immune diseases, and its inflammatory mediator role has been defined in some studies. Visfatin can induce the expression of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. NF-κB can also be increased by visfatin (26, 27). In our study, it was shown that serum visfatin levels were significantly increased in patients with epilepsy. It has been reported that the serum visfatin level is significantly increased in children treated with valproic acid (13). Sonmez et al. (28) stated that in pediatric patients receiving topiramate treatment, adiponectin levels increased in the 6th and 12th month, leptin levels decreased, and there was no statistical significance in visfatin levels. High serum visfatin concentrations in our study suggest that it may cause leukocyte infiltration by increasing adhesion molecules and pro-inflammatory cytokines, and may induce inflammation in microglial cells by increasing the release of cytokines in adipose tissue that functions as an endocrine organ. The positive correlation between serum visfatin and vaspin, chemerin and IL-18 concentrations in the epileptogenesis process in the correlation analyzes indicates that neuronal inflammation may contribute to the epilepsy process.

Vaspin has been shown to inhibit the expression of pro-inflammatory adipocytokines such as resistin, leptin and TNF-α in mesenteric and subdermal white adipose tissues (29). In our study, it was determined that there was a significant increase in serum vaspin levels in patients with epilepsy. Meral et al. (13) stated that serum vaspin concentration was increased in pediatric patients receiving valproic acid, but it was not significant. The high serum vaspin concentration suggests that it may increase free reactive oxygen radicals by increasing vascular adhesion molecules, and therefore, a molecular mechanism that can serve the metabolic pathway in the vascular inflammatory response of serum vaspin concentration will be used in physiopathology.

Uludag et al. (32) in their study on epilepsy patients receiving topiramate and valproic acid treatment, they reported that these values were higher in obese epilepsy patients, where serum leptin levels decreased significantly in non-obese patients. Generally, normal weight and overweight patients were selected in studies with adipocytokine biomarkers in epilepsy patients. Our patient group was selected from non-obese patients, so the fact that the biomarkers we studied were high in these patients makes our study valuable.

Topiramate, a new generation antiepileptic drug, has been reported to adversely affect body weight (30). The mechanism of topiramate-related weight loss has not been fully elucidated. It has been stated that topiramate treatment can reduce body weight by inhibiting the white adipose tissue deposits affected by the activity of lipoprotein lipase in brown adipose tissue. It has also been reported that topiramate stimulates the activation of lipoprotein lipase in skeletal muscles and therefore supports substrate oxidation (33). Li et al. (31) a study of 6.8-year-old children with epilepsy treated with topiramate showed a reduction in BMI. It has been stated that the increase in adiponectin biomarker levels in those receiving topiramate therapy may be an important factor in topiramate-related weight loss. In our study, it was shown that the BMI ratio decreased significantly in the group receiving topiramate therapy. In addition, a positive correlation was observed between serum triglyceride and visfatin concentration and BMI ratio in the group receiving topiramate therapy. It has been reported that glucose concentration decreased and glucose tolerance was impaired in the rat who received topiramate therapy (34). In our study, it was observed that the serum glucose concentration was significantly decreased in the treated and untreated group compared to the control group. Topiramate therapy has been reported to decrease serum triglycerides and cholesterol concentrations (35). In the study of Uludağ et al. (32) no

change was observed in serum triglyceride level. In our study, there was no significant decrease in serum cholesterol level and no significant difference in serum triglyceride and HDL concentrations in patients receiving topiramate therapy. In addition, it was determined that there was a significant decrease in serum cholesterol, total protein and albumin concentrations in the group receiving topiramate therapy. It is thought that the concentration of serum glucose, cholesterol, total protein and albumin in those receiving topiramate therapy may have suppressed the appetite center with the effect of topiramate, and thus their concentrations may have decreased. Ben-Menachem et al. (35) stated in their study that weight loss occurred and calorie intake decreased in the 3th month of topiramate treatment. In the same study, it was shown that leptin levels decreased as weight loss increased, and there was an improvement in glucose, cholesterol and insulin levels.

The high specificity and probability ratios of serum visfatin, chemerin, vaspin and IL-18 values in ROC analyzes suggest that these biomarkers may be a reliable biomarker in the evaluation of epilepsy disease. It can be evaluated as a biomarker in the diagnosis of epilepsy with large-scale studies.

Limitations of this Study

The small number of patients in the study is among the main limitations of the study. Another limitation of our study is that the relationship between the duration of treatment and serum adipocytokine and IL-18 levels in patients receiving topiramate treatment was not evaluated.

CONCLUSION

İn this study, it was found that serum adipocytokine and IL-18 levels were increased in epilepsy patients who received and did not receive topiramate therapy, and BMI rate decreased in patients who received topiramate therapy. The fact that these cytokines were higher than the control group with the decrease in BMI in topiramate treatment may indicate that these cytokines play a role in the pathophysiology of the disease. Therefore, we think that neuronal inflammation is very important in the pathophysiology of epilepsy.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

This study was done in Mardin Artuklu University Graduate Education Institute and is the product of the thesis study registered with the number 698205 in the national thesis center.

Is Previously Presented?

Some part of this study was previously presented as oral presentation on 21st International Eastern Mediterranean Family Medicine Congress held in 12-15 May, 2022 Adana, entitled as Evaluation of serum adipocytokine and interleukin-18 levels in patients with epilepsy

Author Contributions

Concept: AD, DK, VJ, Design: AY, OA, MUÇ, AD, DK, Supervising: AD, DK, HA, Financing and equipment: AD, DK, HA, AY, VJ, OA, Data collection and entry: AY, OA, MUÇ, AD, DK, VJ, Analysis and interpretation: AD, VJ, OA, HA, DK, Literature search: DK, AY, VJ, AD, Writing: AD, OA, MUÇ, DK, Critical review: VJ, MUÇ, HA, AD, DK.

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ORIGINAL RESEARCH

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Another neglected symptom among the overweight young: an analysis of the self-reported anterior knee pain scores of the secondary school children

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Abstract

Objective: Knee pain is the most common joint complaint among the young and is linked with excessive weight by the World Health Organization. The study aimed to define the prevalence of anterior knee pain in secondary school children and search its' relations to weight and academic performance.
Method: The cross-sectional and descriptive study was conducted in Adana, between Sept 15 and Oct 15, 2020. Middle school students aged between 10 and 18 years were included. Anterior knee pain was assessed using the Anterior Knee Pain Scale short form (AKPS-SF), and the numerical rating scale (NRS) was used for pain intensity. Data were collected by questionnaires sent by e-mail.
Results: There were 709 replies eligible for analysis. The NRS mean score was 0.78±1.47 for 249 (35.12 %) participants who reported knee pain. The number of students with an AKPS-SF score of ≤40 in the study group was 46 (6.40%). AKPS-SF scores showed weak negative linear associations with weight and the school year (r=-0.346/p=0.019, and r=-0.292/p=0.049, respectively), and no correlations were found with academic performance.
Conclusion: The study has shown that more than a third of the students reported various levels of knee pain. The inverse correlation of AKPS-SF with weight and the weak link with the school year might attract the attention of healthcare givers to pay more attention to knee examination and help avoid future impairments in the knee joints of school age children.
Keywords: Knee Pain, Childhood Obesity, Academic Performance

INTRODUCTION

Anterior knee pain (AKP) is the most common joint complaint among the young. The incidence is between 6 and 33 % among school-age children (1–3). The causes include Osgood–Schlatter's disease, patellar tendinitis, patellofemoral instability, and growing pains (4,5). Studies indicate that knee pain was the most prevalent self-reported complaint compared to the other body sites, and the prevalence was slightly higher for girls (2). AKP was linked with excessive weight by the World Health Organization, signifying that any BMI outside the sample population's average levels was indicative of anterior knee pain syndrome (6). Childhood obesity is associated with many comorbidities and impaired quality of life (7). The reports show strong associations between obesity and joint pain and suggest that childhood obesity impairs musculoskeletal health (8,9). Obesity leads to knee impairment by the mechanical load, resulting in wear and tear and adiposity-related inflammation. The developing osteoarthritis may advance to the symptomatic stage, including pain and functional limitation, increasing the possibility to end with joint death (10). The number of studies searching for an association between knee pain and weight gain is increasing, with many resulting in strong links (11, 12). Based on the studies, it seems that childhood obesity and knee pain are two closely interrelated conditions.

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Moreover, the duration of knee pain might have been underestimated. Rathleff et al. showed that nearly half of the adolescents with knee pain continued to experience knee pain up to 5 years (13). The studies indicate that most children with knee pain miss a significant amount of class sessions and avoid engaging in routine exercises, including physical education (14,15). Thus, pointing to a link between knee pain and academic life.

The aims were to define the prevalence of anterior knee pain in secondary school children and evaluate anterior knee pain association with weight parameters and academic performance.

METHOD

The cross-sectional and descriptive study was conducted on 709 middle school students in Adana, between Sept 15 and Oct 15, 2020. The study participants were aged between 10 and 18 and were able to perform sports activities at school. Exclusion criteria were a history of trauma, disease, or disorder affecting or limiting the functions of the lower extremity in the past three months, being on medication that affects the lower extremity, and reported chronic knee pain. Chronic knee pain was defined as knee pain existing for more than three months (16). The age, gender, height, weight, academic performance score given by the participant's teacher, the Anterior Knee Pain Scale short form (AKPS-SF), the numerical rating scale (NRS), existing chronic diseases, a history of trauma of the lower extremity and medication data were collected. AKPS-SF was a modified, dichotomous 6-item of the original 13-item AKPS, a scale commonly used to investigate patellofemoral knee pain prevalence. The 6-item form was reported to have identical classification rates compared to the longer version and could screen at close accuracy and reliability and present highly similar reliability indices to the original (17). The Turkish version of the scale is reliable and valid (18). In the evaluation of the severity of the knee pain, the NRS was placed right after the AKPS-SF questions, consisting of a line of 10 cm including the first ten numbers, starting with a zero at the left end with a text next to zero stating "No pain", and ending with ten at the right end including a text "Excruciating pain" in Turkish. The NRS was a valid and reliable tool for screening pain in children of school-age (19).

The BMI percentiles were calculated by the Children's BMI Tool for Schools at the Microsoft Office 365 Excel software via the Group BMI Calculator V1.0 file downloaded from https://www.cdc.gov/healthyweight/xls/bmi_group_calculator_metric.xls. The participants were divided into four groups based on the BMI percentiles; underweight (<5th %), healthy weight (≥5th % and <85th %), overweight (≥85th % and <95th %, and obese (≥95th %).

The structured form consisting of sociodemographic, medical history, AKPS-SF, and NRS was e-mailed to the parents' e-mail addresses for completion by the student, including an informed consent letter and a broad explanation of how to complete the survey. There were 834 replies. After eliminating the uncompleted forms and ones with exclusion criteria, the remaining 709 forms were e-mailed to the participants' teachers for academic performance scoring.

Statistical Package for the Social Sciences (SPSS) 20.0 software was used in the data analysis. In descriptive analysis, mean, standard deviation, median, frequency, percentage, minimum, and maximum values were calculated. Group comparisons were made using the independent t-test and ANOVA. The Levene test was used to verify the homogeneity of the variance. Pearson Chi-square and Likelihood ratio tests were used to analyze the association between the categorical variables. The critical significance was set as 0.05.

Table 1. The descriptive analysis results of the 709 participants

	n	%	Min	Max	Mean
Total	709		10	18	13.55±2.25
Boys	231	32.6	10	18	13.15±2.14
Girls	478	67.4	10	18	13.75±2.27
Age			10	18	13.55±2.25
Height			110	188	157.22±11.62
Weight			23	108	50.75±13.71
BMI percentiles			1.20	99.3	56.43±29.93
Underweight	37	5.2			
Healthy Weight	514	72.5			
Overweight	103	14.5			
Obese	55	7.8			
AKPS-SF			25	50	47.48±3.69
NRS			0	8	0.78±1.47
Academic Performance					
A (100-85)	495	69.8			
B (84-70)	172	24.3			
C (69-54)	37	5.2			
D (<53)	6	0.7			

BMI: Body Mass Index, AKPS-SF: Anterior Knee Pain Scale Short Form, NRS: Numerical Rating Scale

RESULTS

There were 834 replies. After eliminating the 125 inadequate forms, the remaining 709 were e-mailed to the participants' teachers for academic performance scoring. In table 1, the baseline characteristics were presented.

Table 2. The distribution of the students according to AKPS-SF scores

	< 25	26-30	31-35	36-40	41-45	46-50
Total	1	3	8	34	93	570
Boys (n)	0	0	4	10	26	192
Girls (n)	1	3	4	24	67	378
Age (years)	16	15.33±1.53	14.63±2.97	13.65±2.29	13.87±2.18	13.46±2.24
Height (cm)	164	162.00±7.55	160.75±15.57	158.97±10.52	157.44±9.86	156.96±11.93
Weight (kg)	108	59.33±9.45	58.13±23.09	52.63±14.44	51.96±13.23	50.18±13.38
BMI percentiles	99.2	70.03±26.75	60.28±37.86	58.35±32.32	58.60±31.37	55.90±29.45
Underweight (n)	0	0	0	3	6	26
Healthy weight (n)	0	3	5	24	63	423
Overweight (n)	0	0	2	4	19	74
Obese (n)	1	0	1	3	5	47
AKPS-SF	25	30.00±0.00	33.25±1.16	38.38±1.44	43.89±1.31	48.95±1.38
NRS	1	6.00±1.73	5.63±2.92	3.00±1.92	2.28±1.53	0.31±0.78
Academic score						
A (100-85)	1	2	5	24	67	396
B (84-70)	0	0	2	7	19	143
C (69-54)	0	1	1	3	5	27
D (<53)	0	0	0	0	2	4

BMI: Body Mass Index, AKPS-SF: Anterior Knee Pain Scale Short Form, NRS: Numerical Rating Scale

The number of students with a perfect AKPS-SF score (AKPS-SF total score=50) was 346 (48.80 %). Four hundred sixty participants (64.88 %) reported no pain in the NRS. The number of participants reporting knee pain with an intensity varying between 1 and 8 was 249 (35.12 %). The high number of perfect and close to perfect scores required the need to determine a cut-off for AKPS-SF and select a relatively homogenous population to conduct a reliable and valid analysis of any potential links between the AKPS-SF scores, NRS, BMI, and academic performance. The cut-off value was calculated to be 40 in the ROC analysis. The number of students with an AKPS-SF score of ≤40 in the study group was 46. The summary of the initial data according to the AKPS-SF scores was presented in table 2.

The 46 reports with AKPS-SF scores ≤40 showed no significant associations of AKPS-SF, NRS, BMI percentiles, and the overall academic performance scores between the gender groups (p>0.05). There was no significance between overall academic score and AKPS-SF, NRS, and BMI percentiles (p>0.05).

However, the results showed a relation between weight groups and gender (p=0.049). Among boys, overweight ones were statistically high in number (p=0,047). On the contrary, girls with healthy weight outnumbered others (p=0,047) (Table 3). There was also a statistical significance between the weight groups and academic performance (p=0.029). The analysis to find the link with the subgroups showed that the B score group included more overweight students (p=0.045), and their obese peers were grouped in the C score rank (p=0.048).

Table 3. The variables according to the weight groups in students with AKPS-SF scores of ≤40

		Underweight	Healthy	Overweight	Obese	p
Boys	**n**	0	6	5	3	0.049
	%	0	20.7	55.6	60.0	
Girls	**n**	3	23	4	2	
	%	100	79.3	44.4	40.0	
AKPS-SF	**min-max**	38-40	30-40	30-40	25-40	0.342
	mean	39.00±1.00	36.90±2.89	36.22±3.73	34.60±6.15	
NRS	**min-max**	1-5	1-8	1-7	1-8	0.771
	mean	3.33±2.08	3.90±2.50	3.11±2.03	3.00±2.91	
Academic score						
A (100-85)	**n**	3	22	4	3	
	%	100	75.9	44.4	60.0	
B (84-70)	**n**	0	4	5	0	
	%	0	13.8	55.6	0	0.029
C (69-54)	**n**	0	3	0	2	
	%	0	10.3	0	40.0	
Total	**n**	3	29	9	5	
	%	100	100	100	100	

AKPS-SF: Anterior Knee Pain Scale Short Form, NRS: Numerical Rating Scale

Table 4. The association between the continuous variables

	Pearson Correlation	BMI percentile	AKPS-SF	NRS
Age	**r**	0.107	-0.216	0.286
	p	0.480	0.150	0.054
Height	**r**	0.258	-0.106	0.286
	p	0.083	0.484	0.054
Weight	**r**	0.685	-0.346	0.094
	p	<0.001	0.019	0.535
BMI percentile	**r**	1	-0.143	-0.088
	p		0.343	0.561
AKPS-SF	**r**	-0.143	1	-0.320
	p	0.343		0.030
School year	**r**	0.106	-0.292	0.324
	p	0.482	0.049	0.028

BMI: Body Mass Index, AKPS-SF: Anterior Knee Pain Scale Short Form, NRS: Numerical Rating Scale

Besides, AKPS-SF scores showed weak negative linear associations with weight and the school year (r=-0.346/p=0.019, and r=-0.292/p=0.049, respectively) in the Pearson correlation analysis (Table 4). The AKPS-SF and NRS values also showed weak negative linear association (r=-0.320/p=0.030). Also, there was a weak positive linear association between the NRS and the school year (r=0.324/p=0.028).

DISCUSSION

The number of participants reporting knee pain with an intensity varying between 1 and 8 was 249 (35.12 %). Unfortunately, we have failed to find a study researching knee pain prevalence among young Turkish students conducted outside the healthcare settings to compare our results. The prevalence rate was above the reports from other countries, indicating a range between 6 and 33 % (2,3). Female students' elevated participation rate (67.42 % in the initial group and 69.56 % in the AKPS-SF≤40 group) was eye-catching. The increased female participation rate might be due to the knee pain prevalence bias, which was higher in girls, similar to other research (20, 21). Another explanation might include child-parent communication. A study by David et al. conducted on gender issues in parental involvement presented similar ratios and indicated that girls were more willing to volunteer their parents in school issues (22).

There were no associations between the gender groups in terms of AKPS-SF, NRS, and BMI percentile values in the analysis of the AKPS-SF≤40 group. Various studies reported conflicting results on gender comparison, some with similar findings, and others indicating strong links between the female gender with AKPS-SF and NRS (23–25). Besides, a significant association with the AKPS and NRS might not have been found at all. In a study researching the associations among BMI, BMI z-scores, and relative body fat percentage in the development of AKP in an adolescent female athlete population, no relationship between BMI and AKP was found (26). Similarly, Selhorst et al. noted no meaningful change in NRS at the end of a six-week AKP syndrome treatment where the AKPS showed significance (27).

On the other hand, the Pearson correlation analyses of the subgroup demonstrated weak negative linear associations between the AKPS-SF scores and weight, and NRS and AKPS-SF scores. Associations of weight with knee pain and symptoms were already demonstrated in various studies, and a similar link strength between the pain and the scale was shown by Hott et al. in a reliability and validity report (28).

The school year showed a weak negative association with AKPS-SF and a parallel positive weak link with NRS. Although age was not associated with AKPS-SF and NRS, the former findings might be explained by the increased ability to express pain and define the physical findings after receiving a more comprehensive education.

The students' NRS scores in the underweight group were below 6, which might indicate less weight developing lower pain intensity. Yet again, the results lacked an association with the AKP-SF scores in the underweight group. Nevertheless, Kim et al. showed that patellofemoral stress during walking was increased in obese children (26), favoring the finding that children bearing more weight might express more pain.

The analysis showed no association between academic scores and AKPS-SF or NRS scores (p>0.05). Despite the lack of an association with academic scores in our study, studies link decreased knee pain with successful grades. In a study focusing on reducing knee pain in children with Joint Hypermobility Syndrome, Pacey et al. have shown significant pre-post improvements in the individual domains of self-esteem and mental health of the Childhood Health Assessment Questionnaire psychosocial summary scores (29).

Regarding the links between academic performance and BMI percentiles, it was worth mentioning that the numbers of overweight students in the B score group and the obese ones in the C score group were increased, parallel to other research (30,31).

A birth cohort study of 8579 individuals who were followed up throughout childhood and adulthood demonstrated a significant association of high BMI (lower than 30) with knee pain from as early as age 11 years (32). Therefore, for such patients, pieces of advice on a well-balanced physical activity, considering the metabolic benefits against the current or potential impairment of the knee joint, should be incorporated, avoiding weight-bearing activities.

Finally, the self-reports of children should be considered carefully. The findings of a meta-analysis searched for an association between self-report pain ratings of child and parent, child and nurse and parent and nurse dyads, indicated that both parents' and nurses' perceptions of a child's pain should only be considered an estimate of the pain experienced by the child but not the same as the child's self-report (33).

This research focusing on the prevalence of anterior knee pain in school children conducted outside a healthcare setting in Turkey is a first. This novel study has shown that the prevalence of self-reported knee pain was higher than in various reports from other countries.

Limitations of the Study

Using AKPS-SF and NRS for knee pain self-reports collected via e-mail might have decreased the data accuracy. The AKPS also had a limited correlation with pain (28). In addition, young children might select unreliable numbers on an NRS due to the lack of an understanding of the quantitative significance of those numbers (34). Although many other

studies used similar data collection procedures, the study results should be commentated cautiously in the absence of clinical examination.

CONCLUSION

The study has shown that more than a third of the students reported various levels of knee pain. Concerning the analysis of the pain scores; the lack of an association with academic performance, inverse correlation with weight and the weak link with the school year might attract the attention of healthcare givers to pay more attention to knee examination and help avoid future impairments in the knee joints of the school-age children.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

The study's ethical approval was given by the Adana City Training and Research Hospital Ethical Committee on Dec 4, 2019, with reference number 616. The Provincial Directorate of National Education of Adana's approval was obtained with reference number 98258552-604.01.01-E.6048517 on Mar 31, 2020. Informed consent was obtained from the parents or legal guardians.

Authorship Contributions

Concept: MCB, NAB, Design: MCB, NAB, Supervising: MCB, NAB, Financing and equipment: MCB, NAB, Data collection and entry: MCB, NAB, Analysis and interpretation: MCB, NAB, Literature search: NAB, Writing: MCB, Critical review: NAB.

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The effect of Covid-19 anxiety on prenatal distress and prenatal attachment in pregnant women

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Abstract

Objective: In this study, we aimed to investigate the effect of Coronavirus Disease 2019 (COVID-19) anxiety on pregnant women, whether it causes prenatal distress and may be changes on mother-infant attachment.
Method: The research population consists of pregnant women in all trimesters. Exclusion criteria from the study; anomaly risk in the fetus, abnormal examination findings, systemic chronic disease and drug use, presence of diagnosed psychiatric disease, consanguineous marriage between the pregnant woman and her spouse. 323 pregnant women who did not meet these exclusion criteria and agreed to participate in the study were included in this study. In the study data form: Income status, education level, age, gravidity, parity, presence and number of miscarriages, gestational week, smoking status, history of COVID-19 infection, if yes, in which week of pregnancy she had, history of COVID-19 infection in her close family, planned pregnancy, in vitro fertilization-intrauterine insemination (IVF-IUI) or a spontaneous pregnancy status were questionnaired. The patients included in the study were administered the Coronavirus Anxiety Scale (CAS), Revised Prenatal Distress Questionnaire (NuPDQ), Prenatal Attachment Scale (PAS).
Results: COVID-19 anxiety increases more if close relatives have coronavirus rather than participants themselves. We detected that high coronavirus anxiety also caused an increase in the sub-dimensions of prenatal distress. We also found that high COVID-19 anxiety negatively affects prenatal attachment.
Conclusion: Pregnant women should be given information and education about the possible effects of coronavirus on their pregnancy during their routine follow-up during the pandemic period. Otherwise, both psychologically and physically unhealthy generations await the whole world.
Keywords: Anxiety, Coronavirus Disease 2019, COVID-19, Pregnancy

INTRODUCTION

In December 2019, a new coronavirus isolated from the lower respiratory tract, called the new coronavirus, emerged in the city of Wuhan, Hubei province of China, and began to spread all over the world (1). In many countries, workplaces and schools were closed, people had to live in isolation from social life (2). Studies have shown that in addition to affecting physical health, the pandemic also has a profound psychological impact on society, leading to an increase in the number and severity of psychological diseases (3–5). It would not be wrong to think that pregnant women will also be more affected by this situation since there are studies proving that the symptoms of psychological diseases that occur during epidemic periods are more common in women (3–6). Anxiety, despair, suicidal ideation may develop in people due to both their own experiences and the news about the new coronavirus in the media. It has been determined that people with anxiety due to new coronavirus are more likely to not evaluate events correctly and develop reaction disorders. In this case, early detection of Coronavirus Disease 2019 (COVID-19) induced anxiety is important in order to protect personal health and improve health policies (4).

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Conditions such as prenatal anxiety and depression may cause lack of self-care, malnutrition and other psychological diseases in the mother, as well as adversely affect the pregnancy process. Newborns of mothers who have severe anxiety and psychiatric illness during pregnancy are more likely to develop psychological illnesses and cognitive disorders in later life (7–10). In addition, in women who had distress and severe psychiatric illness during pregnancy, this condition may cause miscarriage, premature birth, and low birth weight newborns (11–13).

Prenatal attachment is defined as the emotional attachment that has emotional, cognitive and behavioral consequences between the mother and her baby during pregnancy. The mother plays an important role in the attachment relationship (14,15). This bonding between the mother and the baby is important in terms of the formation of maternal identity in the mother, adaptation to pregnancy, and more conscious action during pregnancy (16,17). Attachment, which starts in the prenatal period and continues after birth, supports infant care positively, but also affects the child's psychological development and plays an important role in the child's future social, emotional and cognitive development (16–18). There are studies that found that mother-infant attachment is negatively affected in pregnant women with psychological problems accompanied by anxiety, stress and depressive symptoms (19,20).

For this reason, especially during the pandemic period we are in, it is very important to investigate all kinds of situations that may cause psychological stress in pregnant women and to take early precautions, in terms of world health and a healthier growth of new generations. In this study, our aim is to investigate the effect of COVID-19 anxiety on pregnant women, whether it causes distress in pregnant women and the effect of the situation on mother-infant attachment.

METHOD

This study is a descriptive cross-sectional study. The research population consists of pregnant women in all trimesters who applied to Sivas Cumhuriyet University Hospital Department of Obstetrics and Gynecology. Among the pregnant women who applied to the outpatient clinic between May and August 2021, 323 people who agreed to participate in the study were included. All pregnant women who did not meet the exclusion criteria and agreed to participate in the study were included. Exclusion criterias were; anomaly risk in the fetus, abnormal examination findings, systemic chronic disease and drug use, presence of diagnosed psychiatric disease, consanguineous marriage between the pregnant woman and her spouse. It was determined that approximately 2000 different pregnant women applied to the center in three months to calculate the sample size of the study. It was calculated that 320 participants should be reached with a 95% CI and a margin of error of 5%, according to the sample calculation of known population and unknown prevalence. The data collection form was applied to the participants by face-to-face interview method by one of the researchers (DK). Before the interview, the participants were informed about the research and informed consent was obtained.

Data Collection Tool

The data form contained a total of 70 questions. The first 15 questions were socio-demographic data, 5 questions were CAS, 17 questions were NuPDQ, 33 questions were PAS inventories (1,15,21,22).

The first 15 questions were about the descriptive features (e.g. age, occupation, education level, living place, income status, smoking status) of the pregnant women, their obstetric history (number of pregnancies, gestational week, previous pregnancy situations, planned pregnancy, in vitro fertilization-intrauterine insemination (IVF-IUI) or a spontaneous pregnancy status) and history of COVID-19 infection (if yes, in which week of pregnancy she had, in her close family). These questions were prepared by the researchers by scanning similar studies in the literature.

The patients included in the study were administered the Coronavirus Anxiety Scale (CAS), Revised Prenatal Distress Questionnaire (NuPDQ), Prenatal Attachment Scale (PAS) (1,15,21). The permission to use the CAS was obtained from Evren, the permission to use the PAS from Çevik Türkmen, and the permission to use the NuPDQ from Akın S were obtained via e-mail.

Coronavirus Anxiety Scale is a 5-item scale measuring anxiety caused by coronavirus. The scale was developed by Lee et al. in 2020 (23). The Turkish validity study of the scale was carried out by Evren et al (1). The Cronbach alpha coefficient of the scale, which was adapted into Turkish, was found to be 0.80. In this scale, there are questions to determine the ongoing coronavirus anxiety of the participants in the last two weeks. The scale is a 5-point Likert type scored from 0 to 4 (Not at all=0, Rare, less than a day or two=1, several days=2, more than seven days=3, nearly every day over the last two weeks=4). As the score obtained from the scale increases, coronavirus anxiety increases.

Revised Prenatal Distress Questionnaire was first created by Yali and Lobel as 12 items in 1999, and it was revised by Lobel et al. in 2008 and increased to 17 items (24). The Turkish validity and reliability study of the revised form was conducted by Yüksel et al. in 2011 (22). The Cronbach Alpha coefficient of the Turkish scale was calculated as 0.85. The scale consists of 17 items and 4 sub-dimensions. Sub dimensions; (1) physical and social changes due to pregnancy, concerns about the baby and childbirth, (2) concerns about the healthcare quality and health status, (3) concerns about

baby care and postpartum life, and (4) financial concerns. Scale items are scaled as "Not at all" (0), "Somewhat" (1) and "Very much" (2). There is no reverse-scored items in the scale. A minimum of 0 and a maximum of 34 points can be obtained from the scale. High scores obtained are interpreted as an increase in the level of distress perceived by pregnant women.

Prenatal Attachment Scale was developed by Çevik and Kurnaz in 2019 (15), but the first idea about the prenatal attachment scale was put forward by Muller (25). The Cronbach's alpha coefficient of the scale, which consists of three sub-dimensions and 33 items, ranges from 0.88 to 0.94. For each item, the total score is obtained by multiplying the "strongly agree" answer by three, the "partially agree" answer by two, and the "strongly disagree" answer by one point. There is no reverse-scored item in the scale. The scores of the curiosity and excitement factor vary between 13-39, the scores of the acceptance and enthusiasm factor vary between 9-27, and the scores of the hope factor vary between 11-33. A score between 33 to 99 can be obtained from the scale. High total scale scores are related to high prenatal attachment levels.

Statistical Analysis

The collected data were analyzed by using the SPSS program (Statistical Package for Social Sciences) for the Windows Version 25 package. Normality analysis of numerical data was performed using Shapiro-Wilk test. Firstly, descriptive statistical analysis of the data was carried out. Next, frequencies for categorical data and measures of central distribution (Mean ± Standard Deviation) for numerical data were calculated. Whether the means of normally distributed numerical data differed significantly between two independent groups and more than two independent groups was analyzed using the independent samples t-test or the one-way ANOVA test. The chi-square test was used to compare categorical data. Pearson Correlation test was conducted to analyze the relationship between scale and sub-dimension scores and different numerical data. Binary logistic regression analysis was used to investigate the factors affecting prenatal attachment and prenatal distress. The scores of the prenatal attachment and prenatal distress scales were outcome variables and were analyzed in two categories (Above-mean/Mean and below-mean scores). Wald chi-square test was used to determine the significance of the variables in the model. Continuous variables in the Prenatal Distress Model; age, gravidity, gestational week, coronavirus anxiety scale score. Categorical variables in this model are presence of miscarriages (Compared to absence), smoking (Compared to non-smoker), university and above education level (Compared to high school and below), planned pregnancy (Compared to unplanned pregnancy), natural conception (Compared to conception with the help of assisted reproductive technologies), history of COVID-19 infection in pregnancy (Compared to not) and history of COVID-19 infection in her close family (Compared to not). Continuous variables in the Prenatal Attachment Model are age, gravidity, gestational week, coronavirus anxiety scale score and prenatal distress scale score. Categorical variables in this model are presence of miscarriages (Compared to absence), university and above education level (Compared to high school and below), planned pregnancy (Compared to unplanned pregnancy), natural conception (Compared to conception with the help of assisted reproductive technologies) and history of COVID-19 infection in pregnancy (Compared to not). A p-value of less than 0.05 was considered statistical significance, with a 95% CI.

RESULTS

323 pregnant women volunteered to participate in the study. The descriptive features of the pregnant women are shown in Table 1.

Table 1. The descriptive features of the pregnant women

Age †	28.1±5.4 (18-43)	
Gravidity †	2.3±1.3 (1-8)	
Parity †	0.9±1.0 (0-5)	
Living †	0.9±1.0 (0-5)	
Presence of miscarriages *	90 (27.9)	
Number of miscarriages †	1.4±0.7 (1-4)	
Smoking status *	**n**	**%**
Still smoking	22	6.8
Cessation during pregnancy	3	0.9
Never smoked	298	92.3
Income level *	**n**	**%**
Low	33	10.2
Middle	242	74.9
High	48	14.9
Education level *	**n**	**%**
High school and below	220	68.1
University and above	103	31.9

† Continuous variables expressed as Mean ± Standard deviation (minimum - maximum).
* Categorical variables were expressed as frequency (percentage) values.

The mean week of gestation of the participants was 27.4±10.2 (min=5- max=41). Woman's 6.2% (n=20) had COVID-19 infection during pregnancy. The mean week of gestation, when they had COVID-19 infection, was 15.7±8.3 (min:5-max:28). This pregnancy of 85.1% (n=275) woman was planned. Participants' became pregnant with natural conception (94.1%; n=304), ovulation induction (3.4%; n=11), and assisted reproductive techniques (2.5%; n=8).

The CAS mean score of the pregnant women was 1.1±1.9 (min=0-max=11). There was no significant difference between the status of having COVID-19 infection and the mean CAS

score (p=0.298). The mean CAS score of pregnant women who had a close relative with COVID-19 infection (1.8±2.6) was found to be significantly higher than the others (0.9±1.6) (p=0.007). There was no significant difference between continuing smoking during pregnancy, the type of pregnancy (natural/help of assisted reproductive technologies), whether the pregnancy was planned or not, and the CAS score (respectively; p=0.057, p=0.959, p=0.128).

The mean scores of NuPDQ, PAS and their sub-dimensions are given in Table 2.

Table 2. The mean scores of NuPDQ, PAS and their sub-dimensions

	Mean ± SD	Min – Max
Revised Prenatal Distress Questionnaire	27.9 ± 5.7	17 – 47
Physical and social changes due to pregnancy, concerns about the baby and childbirth	16.2 ± 3.6	9 – 25
Concerns about the healthcare quality and health status	4.3 ± 1.2	3 – 9
Concerns about baby care and postpartum life	4.2 ± 1.4	3 – 13
Financial concerns	3.1 ± 1.2	2 – 12
Prenatal Attachment Scale	90.6 ± 10.8	33 – 129
Curiosity and excitement	34.5 ± 4.5	13 – 39
Acceptance and enthusiasm	25.0 ± 3.4	9 – 27
Hope	31.0 ± 4.1	11 – 63

Low-level significant correlations were found between coronavirus anxiety and NuPDQ and its sub-dimensions (p<0.05). The data for this analysis are shown in Table 3.

Table 3. The relationship between coronavirus anxiety and prenatal distress

n=323		NuPDQ	Factor 1	Factor 2	Factor 3	Factor 4
Coronavirus Anxiety Scale	r	0.172**	0.108	0.177**	0.135*	0.138*
	p	0.002	0.053	0.001	0.015	0.013

** The correlation is significant at the 0.01 level.
* The correlation is significant at the 0.05 level.
NuPDQ. Revised Prenatal Distress Questionnaire
Factor 1. Physical and social changes due to pregnancy, concerns about the baby and childbirth
Factor 2. Concerns about the healthcare quality and health status
Factor 3. Concerns about baby care and postpartum life
Factor 4. Financial concerns

Low-level significant correlations were found between coronavirus anxiety and PAS and its sub-dimensions (p<0.05). The data for this analysis are shown in Table 4.

Table 4. The relationship between coronavirus anxiety and prenatal attachment

n=323		PAS	Curiosity and excitement	Acceptance and enthusiasm	Hope
Coronavirus Anxiety Scale	r	-0.144**	-0.127*	-0.131*	-0.131*
	p	0.009	0.022	0.019	0.018

** The correlation is significant at the 0.01 level.
* The correlation is significant at the 0.05 level.
PAS. Prenatal Attachment Scale

They were divided into two groups as 28 points and below (n=182; 56.3%) and above 28 points (n=141; 43.7%) according to the mean score (27.9±5.7) of NuPDQ. Increasing coronavirus anxiety had a positive effect by 0.2 times (p<0.001) on high prenatal distress score. While having a coronavirus infection during pregnancy had a negative effect 1.2 times (p=0.049) and increased maternal age had a negative effect 0.05 times (p=0.031); increasing coronavirus anxiety had a positive effect by 0.2 times (p<0.001) on high prenatal distress scores. Binary logistic regression analysis results are shown in Table 5. The sensitivity of the binary logistic regression model was 81.3%, and the specificity was 41.1%. The Nagelkerke R2 of the model was 0.108. The Omnibus significance value of the model is p=0.004.

Table 5. Analysis of factors affecting high scores on the prenatal distress scale with Binary Logistic Regression Model (Compared with below-average score)

						95% CI	
	Coefficient β)	SE (β)	W	p	OR	Lower	Upper
Age	-0.059	0.028	4.659	0.031	0.942	0.893	0.995
Gravidity	0.127	0.142	0.799	0.371	1.135	0.860	1.498
Gestational week	0.007	0.012	0.374	0.541	1.007	0.984	1.031
Presence of miscarriages (compared to absence)	0.411	0.348	1.400	0.237	1.509	0.763	2.982
Smoking (compared to non-smoker)	0.178	0.475	0.141	0.707	1.195	0.471	3.032
University and above education level (compared to high school and below)	-0.062	0.267	0.055	0.815	0.939	0.557	1.585
Planned pregnancy (compared to unplanned pregnancy)	-0.072	0.354	0.041	0.840	1.074	0.537	2.149
Natural conception (compared to conception with the help of assisted reproductive technologies)	-0.251	0.518	0.234	0.629	0.778	0.282	2.149
History of coronavirus infection in pregnancy (compared to not)	-1.210	0.614	3.887	0.049	3.353	1.007	11.162
History of coronavirus infection in her close family (compared to not)	-0.010	0.297	0.001	0.973	1.010	0.564	1.807
Coronavirus anxiety scale score	0.227	0.068	11.144	0.001	1.255	1.098	1.434

n = 323, Nagelkerke R^2 = 0,108, SE, standard error; W, Wald chi-square; OR, odds ratio; CI, confidence interval

They were divided into two groups as 91 points and below (n=119; 36.8%) and above 91 points (n=204; 63.2%) according to the mean score (90.6 ± 10.8) of PAS. While an increase in gravidity had a negative effect of 0.3 times (p=0.039), and the increase in the coronavirus anxiety had a negative effect of 0.2 times (p=0.002); having planned pregnancy had a positive effect of 1.1 times (p=0.002) on high prenatal attachment.

Binary logistic regression analysis results are shown in Table 6. The sensitivity and specificity of the binary logistic regression model were found to be 33.6% and 89.2%. The Nagelkerke R2 of the model was 0.141. The Omnibus significance value of the model is p<0.001.

Table 6. Analysis of factors influencing high prenatal attachment with Binary Logistic Regression Model (compared with below-average scores)

						95% CI	
	Coefficient (β)	SE (β)	W	p	OR	Lower	Upper
Age	0.031	0.028	1.163	0.281	1.031	0.975	1.090
Gravidity	-0.308	0.149	4.253	0.039	0.735	0.548	0.985
Gestational week	-0.009	0.013	0.553	0.457	0.991	0.967	1.015
Presence of miscarriages (compared to absence)	0.383	0.363	1.114	0.291	1.467	0.720	2.987
University and above education level (compared to high school and below)	-0.050	0.280	0.031	0.859	1.051	0.607	1.818
Planned pregnancy (compared to unplanned pregnancy)	1.061	0.348	9.290	0.002	0.346	0.175	0.685
Natural conception (compared to conception with the help of assisted reproductive technologies)	-0.507	0.614	0.683	0.409	0.602	0.181	2.005
History of coronavirus infection in pregnancy (compared to not)	0.260	0.508	0.263	0.608	0.771	0.285	2.084
Coronavirus anxiety scale score	-0.207	0.067	9.667	0.002	0.813	0.714	0.926
Revised prenatal distress questionnaire score	-0.038	0.022	3.052	0.081	0.963	0.922	1.005

n = 323, Nagelkerke R^2 = 0.141, SE, standard error; W, Wald chi-square; OR, odds ratio; CI, confidence interval

DISCUSSION

The COVID-19 infection has not only caused physical illness but also had serious social and psychological effects on communities. It seems impossible not to be affected by the psychological and social destruction of the pandemic in a period such as pregnancy, when the person is more sensitive both mentally and physically. In our study, we determined the anxiety caused by the COVID-19 in pregnant women of all gestational weeks and examined the effect of this situation on the occurrence of distress in pregnancy and mother-baby attachment.

Among the participants, the coronavirus anxiety scale score of the pregnant women who had a close relative who caught the COVID-19 infection was higher than the pregnant women who caught the COVID-19 infection and uncaught. The rapid spread of COVID-19 infection, exposure to false information from the media and the immediate environment cause more fear and anxiety in people (3,23). In a study conducted by Vally and Alowais in 2021 (26), it was shown that the diagnosis of COVID-19 to a loved one or relative increases coronavirus anxiety more than individuals who were not exposed to these experiences. There are also studies showing that when social support decreases, psychological distress increases during pregnancy (17). In the literature, it has been shown that the uniqueness of the disease, its unexpected global impact, the uncertainty of physical outcomes (for both mothers and babies), the risk of transmission, and social contact restrictions are effective on this anxiety in pregnant women (27). This effect can be seen more in societies like Turkey where communication with family and relatives is important. During the pandemic period, when social isolation and restriction increased, pregnant women were able to communicate only with their close relatives. Learning that their close relatives also got the COVID-19 infection may have led to a narrowing of their social circle and an increase in the fear of infecting their baby and themselves.

Pregnant women are at high risk for distress. Especially not knowing what kind of consequences having a COVID-19 infection during pregnancy will cause for the baby and herself, and the lack of sufficient information in the literature on this subject, increases the fear of catching COVID-19 even more. This situation causes more anxiety and anxiety in pregnant women (28,29). In our study, prenatal distress was less in those who had a history of COVID-19 during pregnancy compared to those who did not. We also observed that prenatal distress increased significantly in pregnant women with high coronavirus anxiety. Among the sub-dimensions of prenatal distress, 'Concerns about health care quality and health status','Concerns about baby's care and postpartum life' and 'Financial concerns' increased significantly. The distress score of pregnant women who had COVID-19 infection during pregnancy was lower. We think that this is due to the disappearance of the fear that her baby will be harzzmed and the uncertainty of the effects of COVID-19 infection. However, there are many studies stating that the transmission of COVID-19 infection from mother to baby during pregnancy cannot be proven (30,31). As Nwafor (32) et al.said in their study, curfews taken, removal of elective patient care in hospitals, dismissal of many pregnant women and their husbands may cause concerns about transportation to health care providers, pregnancy follow-up, postpartum baby care and increased costs in pregnant women. There are studies showing that women who have anxiety during pregnancy have miscarriage, low birth weight in their babies, growth restriction and preterm delivery (11,13). Therefore, we would like to underline the necessity of providing information and psychological support to pregnant women during the COVID-19 pandemic.

In addition, experiencing anxiety during pregnancy may affect the fetus in the intrauterine period and lead to negativities in the psychosocial development of the baby in the future. Anxiety, feelings of depression, and lack of social support also hinder the development of prenatal attachment. Prenatal attachment can be defined as the feelings, perceptions and behaviors of the parents about the fetus (17). When the stress caused by coronavirus turns into anxiety during pregnancy, the mother cannot spare enough time to establish a healthy bond with her baby (33). If an adequate prenatal attachment is not provided, neonatal care may be adversely affected, and as the newborn grows up, it may lag behind its peers

in terms of physical, mental, emotional, social and language development (18). Many studies have found that women with low-quality fetal attachment have significantly higher levels of anxiety and depression during their pregnancy (17,34,35). There are studies showing that the lack of social support also negatively affects prenatal attachment (17). Therefore, it would not be wrong to think that the isolation measures taken due to the coronavirus pandemic may affect mother-baby bonding in pregnant women. In our study, we found that prenatal attachment of pregnant women with high coronavirus anxiety was significantly lower. In the only similar study in the literature, Craig et al. found that prenatal attachment in Italian pregnant women was negatively affected in those with anxiety and depression, and it was positively related to the risk perception of COVID-19. For this reason, they also underlined that prenatal attachment may be negatively affected when the risk perception of COVID-19 becomes psychological distress (33). In our study, the effect of coronavirus anxiety on prenatal attachment was investigated. Our research is the first known study in the literature that looks at the subject from this perspective.

In our study, 85.1% of pregnant women had this pregnancy voluntarily and planned. We found that while voluntary pregnancy had a positive effect on prenatal attachment, increasing the number of pregnancies had a negative effect on it. This situation is compatible with the literature. In a large-scale study conducted by Damato et al. (36), they found that psychiatric problems of the mother affected attachment negatively, but voluntary pregnancy had a positive effect Malm et al. (16) reported that younger women expecting their first baby had more prenatal attachment than multiparous women (36,16). They attributed this to the fact that as the number of pregnancies decreased, they had more free time to focus on their relationships with the baby (16). Multiparous pregnant women may not be able to spare enough time for their babies in the womb because they have experienced situations such as the development and movement of their babies before and they need to spend time in the care of previous children. At this point, it is necessary to underline the importance of social support.

As a result of our research, we found that COVID-19 anxiety increases more if close relatives have coronavirus rather than participants themselves. High coronavirus anxiety also caused an increase in the sub-dimensions of prenatal distress, ' Concerns about the quality of health care and health status', ' Concerns about the care of the baby and postpartum life' and ' Financial concerns '. We also found that high COVID-19 anxiety negatively affects prenatal attachment.

CONCLUSION

We think that the results found are due to the lack of information about the effect of coronavirus on pregnancy and to focus only on the physical results. It is obvious that psychiatric disorders that increase during pandemic periods can be seen more in a sensitive period such as pregnancy. Pregnant women should be given information and education about the possible effects of coronavirus on their pregnancy during their routine follow-up during the pandemic period. Screening tests should be performed to determine pregnant women with high COVID-19 anxiety in advance. Necessary psychological support should be started before prenatal distress develops and prenatal attachment is negatively affected in pregnant women with high COVID-19 anxiety. Otherwise, both psychologically and physically unhealthy generations await the whole world.

Limitations of the Study

The most important limitation of our research is that it was carried out in a single center and in a tertiary hospital. Multicenter and large-participant studies are needed. The results of our research evaluate the effects of coronavirus on pregnant women both before and after delivery from different perspectives. In this context, it is expected to shed light on the special psychological support policies to be developed for pregnant women during the pandemic period.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

Ethical permission was obtained from the Sivas Cumhuriyet University, Medical Faculty noninvasive clinical Ethics Committee for this study with date 2021 and number 04/27 and Helsinki Declaration rules were followed to conduct this study. An application has been made to the Turkish Republic Ministry of Health COVID-19 Scientific Research Evaluation Commission.

Authorship Contributions

Concept: DK, EA, SK Design: DK, SK, Supervising: DK, EA, SK, Financing and equipment: DK, Data collection and entry: DK, EA, SK, Analysis and interpretation: EA, SK, Literature search: DK, Writing: DK, Critical review: DK, EA, SK.

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ORIGINAL RESEARCH
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Evaluation of notifiable infectious diseases between 2018 and 2021 in Van province: A descriptive study

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Abstract

Objective: In this study, it was aimed to examine the infectious diseases reported in Van province between 2018 and 2021 in terms of various characteristics.
Method: It is a descriptive study that was conducted between January 1 and January 31, 2022. Necessary permissions and an ethics committee were obtained for the study. Infectious diseases reported in Van province between January 1, 2018 and September 30, 2021 were examined using the Infectious Diseases Surveillance and Early Warning System (IZCI) of the Ministry of Health. It was examined in terms of various variables. The study's data were analysed with the Jamovi 2.2 statistical program. Descriptive data were given as numbers and percentages. The Pearson Chi-square test was used for comparisons between categorical variables.
Results: There were 16778 notifications between 01.01.2018-30.09.2021. Of the people with the reported disease, 59.5% were men and 40.5% were women. The mean age was 23.3±19.0 (min: 0-max: 99). The 3 most frequently reported diseases/conditions were 32.51% (n=5455) Brucellosis, 31.07% (n=5212) Rabies-risk contact, and 12.70% (n=2131) Rotavirus. There were 5555 (33.1%) disease reports in 2018, 4769 (28.4%) in 2019, 3411 (20.3%) in 2020, and 3043 (18.1%) in the first nine months of 2021. The incidence of Brucellosis, Rabies Risky Contact, Echinococcosis, and Anthrax diseases was found to be higher in rural districts than in urban districts (for each $p<0.001$).
Conclusion: The most common infectious diseases reported in the city where the study was conducted were Brucellosis, Rabies Risky Contact, and Rotavirus disease, respectively. The fact that the main livelihood of Van province is animal husbandry may explain the prevalence of zoonotic diseases.
Keywords: Notification, Infectious Disease, Surveillance

INTRODUCTION

Surveillance is the continuous and systematic examination of all aspects of the emergence and spread of a disease or health condition in accordance with effective control of the condition (1,2). Monitoring of infectious diseases is essential to detect outbreaks that require public health response and control measures. Therefore, effective and reliable surveillance and notification systems are vital for monitoring public health trends and the early detection of outbreaks (3). Notifiable infectious diseases (NID) determined by law and regulation are among the basic components of surveillance (4).

With the Public Health Law published in 1930 in Turkey, the notification of some infectious diseases became a legal obligation (5,6). Through the data obtained through the notification of communicable diseases, health policies are determined in order to reduce the morbidity and mortality of these diseases, and new regulations are made when necessary by creating control programmes for diseases. With the Communique on the Notification System of Infectious Diseases published in 2004, 51 diseases collected in four groups as "Group A", "Group B", "Group C" and "Group D" were updated to 73 diseases and conditions with the circular published in 2015 (7,8).

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Notification systems included traditional methods using mail, telephone, fax, or e-mail. With the development of electronic software systems in recent years, the availability of data such as patient file records, laboratory test records, and laboratory reports in electronic form has facilitated instant notification and follow-up for public health services at both the regional and national level (3,9). In Turkey, NID data collected through software systems are monitored, and necessary interventions are made by authorised persons using the Infectious Diseases Surveillance and Early Warning System (IZCI) of the Ministry of Health.

The types, numbers, and distribution of infectious diseases seen in a particular region according to certain characteristics are among the most important health indicators of that region. At the same time, these data are critical for the planning of infectious disease prevention studies and the direction of health care (5).

In this study, it was aimed to examine the data of notifiable infectious diseases and conditions in Van province between 2018 and 2021 in terms of various characteristics.

METHODS

This is a descriptive study that was conducted between January 1 and January 31, 2022. Necessary permissions for the study were obtained from the Van Provincial Health Directorate (Letter dated 24.11.2021 and numbered E-73040253-129-1100), and ethics committee approval was obtained from the Clinical Research Ethics Committee of SBU Van Training and Research Hospital (Date of 29.12.2021 and decision no. 2021/23). Infectious diseases reported in Van province between January 1, 2018 and September 30, 2021 were examined using the IZCI system of the Ministry of Health. The sample was not determined because the population of the study consisted of the disease records reported within the specified date range. Reports of SARS-CoV-2 infection (COVID-19) were excluded from the study.

Van is a province located in the Eastern Anatolia Region of Turkey, with a population of 1149342 (45.6% rural district population, 54.4% urban district population) according to the data of the Turkish Statistical Institute (TUIK) for the year 2020, and the main source of livelihood is animal husbandry (10). 42.8% of the population is 19 years old or younger, 52.8% is between 20 and 64 years old, and 4.4% is 65 years old or older. In the study, the districts affiliated with the metropolitan municipality were taken as the central district (Edremit, İpekyolu and Tuşba) and the other districts as rural districts (Bahçesaray, Başkale, Çaldıran, Çatak, Erciş, Gevaş, Gürpınar, Muradiye, Özalp and Saray).

Statistical Analysis

The study's data were analysed with the Jamovi version 2.2 statistical program. Descriptive data were given as numbers and percentages. The Pearson Chi-square test was used for comparisons between categorical variables. Cases with $p \leq 0.05$ were accepted for statistical significance.

RESULTS

There were 16778 notifications between 01.01.2018-30.09.2021. Of the people with the reported disease, 59.5% (n=9984) were male and 40.5% (n=6794) were female. The mean age was 23.3±19.0 (min: 0-max: 99). When the notifiable infectious disease groups were examined, there were 13130 (78.3%) notifications from group A, 1007 (6.0%) notifications from group C, and 2641 (15.7%) from group D, while there were no notifications from group B diseases. When the diagnostic status was evaluated, 71.6% (n=12019) were definite, 17.9% (n=2995) probable, and 10.5% (n=1764) suspected. There were 5555 (33.1%) disease reports in 2018, 4769 (28.4%) in 2019, 3411 (20.3%) in 2020, and 3043 (18.1%) in the first nine months of 2021. When analysed according to seasons, 4410 (26.3%) diseases were reported in spring, 4489 (26.8%) in summer, 3450 (20.6%) in autumn, and 4429 (26.4%) in winter. There were 8877 (52.9%) notifications from rural districts and 7901 (47.1%) notifications from central districts (Table 1) (Figure 1) (Figure 2).

Table 1. Distribution of reported diseases grouped according to some characteristics

	Number (n)	Percent (%)
Gender		
Male	9984	59.5
Female	6794	40.5
Age Group		
0-9 years	4965	29.6
10-19 years	3943	23.5
20-29 years	2359	14.1
30-39 years	1926	11.5
40-49 years	1570	9.4
50-59 years	1160	6.9
60-69 years	572	3.4
70-79 years	229	1.3
80 years and older	54	0.3
Notification Group		
A	13130	78.3
C	1007	6.0
D	2641	15.7
Diagnostic status		
Definite	12019	71.6
Possible	2995	17.9
Suspicious	1764	10.5
Notification Year		
2018	5555	33.1
2019	4769	28.4
2020	3411	20.3
2021	3043	18.2
Season		
Spring	4410	26.3
Summer	4489	26.8
Autumn	3450	20.5
Winter	4429	26.4
District		
Central District	7901	47.1
Rural District	8877	52.9

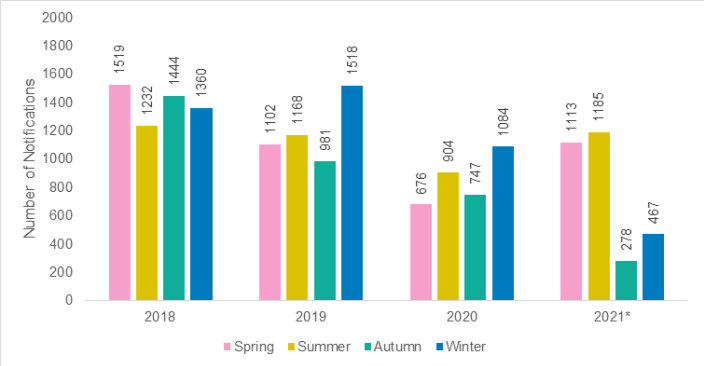

Figure 1. Distribution of notifications by years and seasons

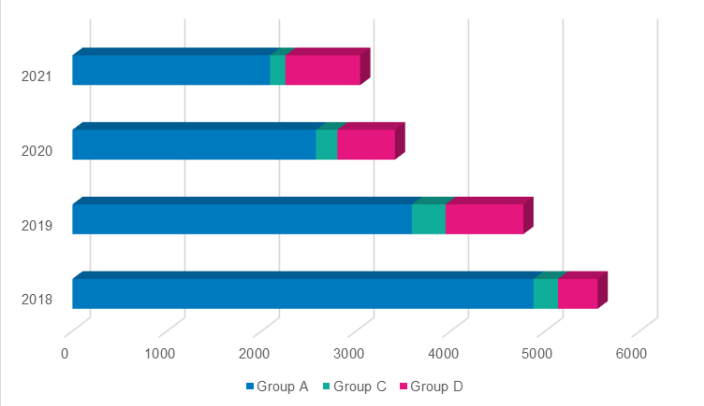

Figure 2. Group distribution of notifications by years

Table 2. Distribution of infectious diseases reported between 2018 and 2021

Reported Disease	2018 (n)	2019 (n)	2020 (n)	2021* (n)	Total (n)	Percent (%)
Brucellosis	2134	1357	1104	860	5455	32.51
Rabies Risky Contact	1513	1471	1164	1064	5212	31.07
Rotavirus	327	552	512	740	2131	12.70
Varicella	927	515	146	28	1616	9.63
Echinococcosis	164	212	165	143	684	4.08
Giardia Intestinalis	59	123	73	46	301	1.79
Hepatitis B	50	80	56	47	233	1.39
Tuberculosis	86	54	24	30	194	1.16
Entamobea Histolytica	26	112	14	2	154	0.92
Anthrax	58	21	23	24	126	0.75
Toxoplasmosis	41	35	13	9	98	0.58
Influenza	16	32	41	0	89	0.53
Crimean Congo Hemorrhagic Fever	11	65	6	6	88	0.52
Syphilis	27	33	12	10	82	0.49
Hepatitis C	11	24	21	17	73	0.44
Measles	17	16	14	3	50	0.30
Mumps	27	12	6	1	46	0.27
Salmonella	6	25	6	0	37	0.22
Tularemia	16	3	2	0	21	0.13
Other	39	27	9	13	88	0.52
Total	5555	4769	3411	3043	16778	100

*It covers the period from January 1 to September 30, 2021.

The five most frequently reported diseases/conditions were 32.5% (n=5455) Brucellosis, 31.1% (n=5212) Rabies risky contact, 12.7% (n=2131) Rotavirus, 9.6% (n=1616) Chickenpox, 4.1% (n=684) was Echinococcosis (Table 2). The four-year incidences (per 10000 people) of the most frequently reported diseases/conditions by rural and urban regions are as follows: Brucellosis 66.9, Rabies risk contact 65.7, Rotavirus 4.9, Chickenpox 11.9, Echinococcosis 7.6 in the rural area, while Brucellosis 31.1, Rabies risk contact 65.7, Rotavirus 29.9, Chickenpox 15.8, Echinococcosis 4.5 in the urban area.

The incidence of Brucellosis ($p<0.001$), Rabies Risky Contact ($p<0.001$), Echinococcosis ($p<0.001$) and Anthrax ($p<0.001$) diseases/conditions was higher in rural districts than in central districts. Diseases seen more frequently in central districts than in rural districts are: Rotavirus ($p<0.001$), Chickenpox ($p<0.001$), Giardia Intestinalis ($p<0.001$), Hepatitis B ($p=0.008$), Entamobea Histolytica ($p<0.001$), Toxoplasmosis ($p<0.001$), Influenza ($p=0.041$), Crimean-Congo Hemorrhagic Fever ($p=0.001$), Syphilis ($p=0.001$) and Salmonella ($p=0.015$) (Table 3).

Table 3. Distribution of reported diseases by central and rural districts

Reported Disease	Rural Districts		Central Districts		Statistical Analysis	
	Number (n)	Percent (%)	Number (n)	Percent (%)	X^2	p
Brucellosis	3510	64.34	1945	35.66	775.877	<0.001
Rabies Risky Contact	3446	66.12	1766	33.88	887.955	<0.001
Rotavirus	257	12.06	1874	87.94	968.460	<0.001
Varicella	627	38.80	989	61.20	30.217	<0.001
Echinococcosis	402	58.77	282	41.23	47.828	<0.001
Giardia Intestinalis	103	34.22	198	65.78	15.735	<0.001
Hepatitis B	86	36.91	147	63.09	7.103	0.008
Tuberculosis	100	51.55	94	48.45	2.761	0.097
Entamobea Histolytica	26	16.88	128	83.12	51.221	<0.001
Anthrax	93	73.81	33	26.19	40.408	<0.001
Toxoplasmosis	25	25.51	73	74.49	15.954	<0.001
Influenza	31	34.83	58	65.17	4.165	0.041
Crimean Congo Hemorrhagic Fever	25	28.41	63	71.59	10.491	0.001
Syphilis	22	26.83	60	73.17	10.910	0.001
Hepatitis C	29	39.73	44	60.27	1.017	0.313
Measles	19	38.00	31	62.00	0.879	0.348
Mumps	28	60.87	18	39.13	3.727	0.054
Salmonella	9	24.32	28	75.68	5.924	0.015
Tularemia	8	38.10	13	61.90	0.223	0.637
Other	31	35.23	57	64.77	3.821	0.051
Total	8877	52.91	7901	47.09	366.081	<0.001

X^2: Pearson Chi-square Test result

DISCUSSION

Infectious diseases are still an important cause of mortality and morbidity in underdeveloped and developing countries. Knowing the regional distribution of infectious diseases will facilitate field studies and preventive interventions on this subject. The literature on the reporting of infectious diseases in Turkey mostly focuses on a limited number of diseases, and there are not many studies that evaluate all diseases together. Aside from the studies conducted in İzmir in 2012 and Erzurum in 2008, no current studies were found at the regional level (5, 11).

When the reports were evaluated according to gender and age, it was found that NID was reported more frequently in males (59.5%) and in individuals under the age of 20 (53.1%). It has also been reported in other studies that NID is more common in males (5,11,12). Given Turkey's social structure, particularly in the Eastern Anatolia Region, it is expected that men will be more likely than women to be in a risky environment in terms of infectious diseases. When the age distribution is examined, the fact that a significant portion (53.1%) of the communicable disease notifications are seen in the under-20 age group can be explained by the fact that 42.8% of the population is under the age of 20 when the population characteristics of Van are taken into account.

When NID groups are examined, group A and group B diseases include diseases to be reported from all health institutions and organisations serving throughout the country; group C diseases are reported only from inpatient treatment institutions; and group D diseases include agents detected by laboratories with diagnostic capacity (8). Although group A (n=13130) was the most reported group in this study, it is seen that group A disease reports decreased from year to year. On the other hand, there was an increase in the notifications of group C and group D diseases in 2019 and 2021 compared to the previous years. The decrease in notifications in 2020 may be due to the COVID-19 pandemic. There were no reports from group B. Similar to this study, in a study conducted at a university hospital in Izmir, it was reported that group A diseases decreased by years, while group D reports increased by years (11).

In this study, when the notifications are analysed by years, it is observed that the notifications are decreasing gradually. In a study covering the years 2005–2008, it was reported that the number of notifications increased by year, whereas in another study covering the years 1997–2000, it decreased compared to the year (5,11). Since there is a long time between this study and other studies, it is thought that an accurate comparison by year cannot be made due to the differences in both the reporting system and scope of NID in this process. In addition, the significant decrease in notifications in 2020 and 2021 in the study may be due to the COVID-19 pandemic. In a study conducted in China, it was reported that the incidence of infectious diseases decreased during the restrictions applied due to the COVID-19 pandemic and increased again when the restrictions were lifted (13). In a study conducted in Australia, it was shown that during the COVID-19 pandemic, vaccine-preventable diseases decreased, while there was an increase in some sexually transmitted diseases and vector-borne diseases (14). The fact that the spread of infectious diseases has decreased due to the restrictions applied during the COVID-19 process and that the health personnel workforce has been shifted to pandemic services may explain the decrease in notifications in this study. In addition, since this study does not have data for the last three months of 2021, there may be a relative low for this year.

The most frequently reported diseases/conditions in this study were Brucellosis, Rabies Risky Contact, and Rotavirus disease, respectively. In a study conducted in Erzurum, the most frequently reported diseases were presented as Streptococcal angina, Measles, and Amoebic dysentery, respectively (5). Rabies Risky Contact, Tuberculosis, and Salmonellosis were reported as the most common diseases or conditions in a study of notifications in an Izmir university hospital (11). Again, in a study conducted in a paediatric hospital in İzmir, the most frequently reported diseases were chickenpox, pertussis, and influenza (12). Considering the regional characteristics, it can be assumed that animal husbandry is the main source of livelihood and a significant part of the population lives in the countryside, which explains the high number of Brucella and Rabies Risky Contact notifications in Van.

In this study, notifications in central and rural districts were compared both according to incidence and number of notifications. The distribution according to the number of notifications is presented in Table 3, since the total populations of the central and rural districts are close to each other and the differences between the districts are similar according to the incidence and the number of notifications. Brucellosis, Rabies Risky Contact, Echinococcosis and Anthrax notification rates were higher in rural districts than in central districts. It can be said that these diseases are expected to be seen in rural districts that deal with livestock and are more open to contact with animal/animal products. Reports of Rotavirus, Chickenpox, Giardiasis, Hepatitis B, Amoebic dysentery, Toxoplasmosis, Influenza Crimean Congo Hemorrhagic Fever, Syphilis and Salmonella were higher in central districts compared to rural districts. Human-to-human transmission is possible for many of these diseases. Risk factors include being in public living areas, living in unsuitable housing, being unable to access clean water, insufficient waste management, and consuming foods that have not been stored and processed under proper conditions. Therefore, it is not surprising that it is reported more in urban areas than in rural areas (15,16).

The lack of adequate studies evaluating notifiable infectious diseases in Turkey and the Eastern Anatolia Region, where the study was conducted, constitutes a limitation for conducting a comparative current situation assessment both at the regional level and across the country. At the same time, due to this situation, this study is considered one of the rare ones in which NID is evaluated comprehensively.

CONCLUSION

In the province of Van, where the study was conducted, the most frequently reported infectious diseases/conditions are Brucellosis, Rabies Risky Contact, and Rotavirus disease, respectively. The prevalence of diseases such as Brucellosis, Echinococcosis and Anthrax can be explained by the fact that animal husbandry is the main source of livelihood in rural areas and people consume animal products under inappropriate conditions. Since studies examining the epidemiology of communicable diseases will guide the interventions to be made, it is very important for our region to increase the number of similar studies. In line with the data obtained within the scope of this study, it is recommended to plan studies to increase the effectiveness of primary prevention studies for common infectious diseases.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Presentation

A part of this study was presented as an oral presentation at the 5th International 23rd National Public Health Congress, held online on December 13–18, 2021, with the title "Evaluation of infectious disease notifications in a province between 2018 and 2021".

Ethical Declaration

The Clinical Research Ethics Committee of Health Sciences University, Van Training and Research Hospital granted ethical approval with the date 12.29.2021 and the number 2021/23 to conduct this study, and the rules of the Helsinki Declaration were followed.

Authorship Contributions

Concept: ÖFT, MÇT, Design: ÖFT, MÇT, Data collection and entry: MÇT, Analysis and interpretation: ÖFT, Literature search: ÖFT, MÇT, Writing: ÖFT, MÇT, Critical review: ÖFT, MÇT

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Prognosis and risk factors of chronicity in childhood idiopathic thrombocytopenic purpura: a single-center experience

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Abstract

Objective: In previous studies, chronicity risk factors for idiopathic thrombocytopenic purpura (ITP) are unclear. This study aimed to evaluate the outcome of children with ITP and determine the chronicity risk factors.
Methods: This study retrospectively examined the demographics, laboratories, outcome, and chronicity risk factors among sixty children with ITP and obtained the data from the computer system. We analyzed demographics, treatment, and laboratory risk factors for chronic ITP by IBM SPSS and used binary logistic regression analysis.
Results: Of 60 children with ITP, 32 (53.3%) had acute, 25 (41.7%) had chronic, and 3 (5%) had persistent ITP. Demographics, laboratories (age <4 years, thrombocyte count at diagnosis, serum LDH, neutrophil count, mean platelet volume, status and grade of bleeding, infection in the last month) were unrelated to chronic ITP. As a new finding, loss of treatment response rates predicts chronicity in both univariate OR [2.56 (1.25 – 5.25)](p=0.01) and multivariate analysis OR [3.873 (1.488–10.08](p=0.006). Among second-line therapies, eltrombopag (n=6) achieved a durable response of thrombocyte count for more than 50.000/mm³ in five. However, two required the cessation of treatment two due to renal failure. Of two splenectomized patients, one could not achieve remission.
Conclusion: Lower platelet counts (<20.000/mm³), younger age, male gender, and initial treatment regimens (IVIG, steroid, IVIG plus steroid) did not influence chronicity in our study. We suggest that loss of response rates predicts chronicity as a new factor.
Keywords: Idiopathic Thrombocytopenic Purpura, Chronic, Children

INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disease with increased thrombocyte destruction and decreased production. The condition resolves spontaneously (1).

First-line treatment includes steroids (high-dose dexamethasone or prednisone), IV immunoglobulin (IVIG), or both for selected cases. The second-line therapies are thrombopoietin reseptör agonists (eltrombopag, romiplostim), rituximab, and splenectomy. Immunosuppressive agents (e.g., azathioprine, cyclosporine, mycophenolate mofetil, etc.) are rarely used (1).

Risk factors for chronic ITP are age, thrombocyte level, insidious symptoms, no history of vaccination or infection, or chronic diseases at diagnosis. As a chronicity risk factor, age >4 years (2,3,4), thrombocyte count <20.000/mm³ at diagnosis and male sex are defined in other studies (5,6,7). However, male sex predominance is frequent at younger ages. Finally, the thrombocyte count at diagnosis was not associated with chronicity in ITP (8). Low white blood cell count, which differs according to age, was related to chronicity in ITP. However, it was not considered an independent variable. IVIG treatment was found to lower the risk of chronic ITP. However, IVIG is associated with lower thrombocyte counts and symptomatic illness at

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diagnosis (9). A randomized control trial found that IVIG was not an independent risk factor for chronic ITP (10).

In this retrospective study, we aim to report the outcomes of children with ITP. In addition, chronicity risk factors (age, neutrophil level, sex, the severity of bleeding, treatment with IVIG, steroid, or both, and rate of loss of response to treatment) were also investigated.

METHOD

In this study, the University computer database is retrospectively examined, and the data of children with primary ITP is recorded. This data included age at diagnosis, sex, infection in the last month, type and severity of bleeding, treatments and their cycles, thrombocyte levels at diagnosis, and the sixth month, first, and second year of diagnosis. In addition, each patient's disease course was evaluated. The subjects were diagnosed from 2010 to 2021 in our University Pediatrics Clinic. We followed all for more than or equal to one year. The exclusion criteria were inadequate follow-up (<1 year), having secondary ITP like collagen tissue disorders and malignancies.

The disease course lasted <3 months is defined as acute, 3-12 months as subacute, and >12 months as chronic. Chronic ITP has a prevalence of 10%-20%. Response to treatment was having a thrombocyte count ≥ 30.000/mm³ or two-fold the baseline level without bleeding. Complete loss of response to treatment is defined as having a thrombocyte count <100.000/mm³ or bleeding. Partial loss of response treatment is having a thrombocyte count of <30.000/mm³ or less than the basal thrombocyte count or bleeding. Recovery was defined as having a thrombocyte count over 150.000/mm³ for at least one month without any treatment. Corticosteroid dependency was the continuation of steroid treatment to avoid bleeding and maintain a platelet count above 30.000/mm³. Corticosteroid resistance is defined as having thrombocyte levels below 30.000 or bleeding or below the two hold of the beginning thrombocyte level despite adequate corticosteroid treatment. First-line therapies were IVIG, corticosteroid, IVIG plus corticosteroid. Second-line treatment included eltrombopag, mycophenolate mofetil, and splenectomy (11).

Statistical Analysis

This study analyzed demographics, treatment, and laboratory risk factors for chronic ITP by IBM SPSS and used binary logistic regression analysis. Categorical variables are represented as absolute numbers and percentages. Mean and standard deviation represented typically distributed quantitative variables. Not usually, allocating variables were expressed as medians and ranges. For example, a $p<0.050$ was reported as significant. The local ethics committee approved the study on 09.05.2022 with the approval number 2022/85. Informed consent was obtained.

RESULTS

Of sixty children diagnosed between 2010-2021 with primary ITP in our university pediatrics clinic, 32 (53.3%) had acute, 25 (41.7%) had chronic, and 3 (5%) had persistent ITP. Most of them, 38 (63.3%), were male. Skin manifestations included children with petechia, purpura, and ecchymosis 31 (52.5%). In forty-three (86%), the severity of bleeding was mild (Table 1).

Table 1. Demographics of children with *ITP

Variables	mean	SD
Age year)	7.1	4.74
	n	**(%)**
Sex		
Male	38	63.3
Female	22	36.7
Disease course		
Acute	32	53.3
Chronic	25	41.7
Subacute	3	5
Bleeding type		
none	9	15.3
Skin	31	52.5
Epistaxis	10	16.9
All	7	11.9
Mucosal	2	3.4
Bleeding severity		
Mild	43	86
Moderate	5	10
Severe	2	4
Treatment		
¶ IVIG alone	18	30
Corticosteroid alone	19	31.6
IVIG plus corticosteroid	19	31.6
Corticosteroid dependency	11	19
Corticosteroid resistance	3	5
Eltrombopag	6	10
╬MMF	2	3.3
Splenectomy	3	5
Remission		
Not in remission	14	24.1
In remission	44	75.9

Abbreviations: *ITP (immune thrombocytopenia), ¶IVIG (intravenous immunoglobin), ╬MMF (mycophenolate mofetil)

Table 2. Thrombocyte levels during the follow-up and first-line treatments

	Median	[minimum – maximum]
Thrombocyte count at 6th month (/mm³)	155000	[200 - 603000]
Thrombocyte count in the first year (/mm³)	213000	[5000 - 389000]
Thrombocyte count in the second year (/mm³)	201000	[4000 - 459000]
The last thrombocyte count (/mm³)	232000	[18390 - 543000]
IVIG cycles	1	[1 - 33]
IVIG cycles in the first year	1	[1 - 9]
Steroid cycles	1	[1 - 10]
IVIG plus steroid cycles	1	[1 - 6]

Abbreviations: ¶IVIG (intravenous immunoglobin)

First-line treatment included IVIG alone in 18 (30%), corticosteroid alone in 19 (31.6%), and IVIG plus corticosteroid in 19 (31.6%). Eleven (19%) had corticosteroid dependency. Three (5.2%) had corticosteroid resistance. In addition, mycophenolate mofetil was administered in two (3.4%), resulting in an inadequate response (Table 1). As a second-line treatment, eltrombopag (n=6), (10%) achieved platelet count response >50.000/mm³ in 5 (8.6%). However, in two cases, treatment resulted in a cessation of eltrombopag due to adverse reactions. These adverse events were renal failure (n=1), renal failure, elevated transaminases, vomiting, and diarrhea (n=1)]. Three (2 chronic, one acute) patients had undergone splenectomy. Of three (5%) splenectomized cases, one recovered. However, one had persistent thrombocytopenia, and the other's prognosis was unknown. Six (24%) of 25 chronic patients received eltrombopag; two (8%) had undergone splenectomy, and two (8%) took mycophenolate mofetil. Additionally, acute and persistent cases all recovered. Ten chronic issues achieved remission, and thirteen chronic patients had persistent thrombocytopenia. The current status of two children with chronic ITP is unknown.

Thrombocyte counts were recorded in the sixth month, first, and second year of diagnosis. The median IVIG cycle was 1 [1-33] (Table 2).

Table 3. Demographics, clinic status, laboratories, and chronicity

	Chronicity				Univariate			Multivariate		
	Non-chronic (n=35)		Chronic (n=25)				p	OR	(%95 CI)	p
Age at diagnosis (year)	**n**	**%**	**n**	**%**	**OR**	**(%95 CI)**				
≥4	24	55.8	19	44.2	**Reference**					
<4	11	64.7	6	35.3	0.689	(0.215– 2.204)	0.53	0.214	(0.027– 1.703)	0.145
Infection in the last month	n	%	n	%						
Absent	26	59.1	18	40.9	**Reference**					
Present	9	60	6	40	0.963	(0.29– 3.18)	0.95	0.692	(0.117– 4.093)	0.684
Bleeding	n	%	n	%						
Absent	4	44.4	5	55.6						
Present	30	60	20	40	0.533	(0.127– 2.232)	0.39	0.104	(0.01– 1.115)	0.061
Severity of bleeding	**n**	**%**	**n**	**%**						
Mild	24	55.8	19	44.2	---		---	---		---
Moderate	4	80	1	20	---		---	---		---
Severe	2	100	0	0	---		---	---		---
Thrombocyte count at diagnosis (/mm³)	**n**	**%**	**n**	**%**						
≥20000	9	60	6	40	**Reference**					
<20000	24	58.5	17	41.5	1.062	(0.318– 3.547)	0.92	1.5	(0.181– 12.418)	0.707
Neutrophil count at diagnosis (/mm³)	**n**	**%**	**n**	**%**						
≥1000	23	56.1	18	43.9						
<1000	1	100	0	0						
Laktat dehidrogenase at diagnosis (U/L)	**mean**	**SD**	**mean**	**SD**	0.995	(0.987– 1.004)	0.32			
	326.3	87	289.9	102.3						
Mean Platelet volume at diagnosis (fl)	**n**	**%**	**n**	**%**						
<11	11	50	11	50	---		---	---		---
≥11	4	100	0	0	---		---	---		---

OR (%95 CI): Odds ratio (%95), SD: Standard deviation

Among 60 patients with ITP, the chronicity risk factors were investigated. Age ≥ 4 years since diagnosis, presence of infection, bleeding and its severity, thrombocyte count ≥20.000/mm³, neutrophil count <1000/ mm³, mean platelet volume (>11 fL) diagnosis did not increase the risk for chronic ITP (Table 3). Treatment cycles also did not affect the chronicity. However, loss of response rates is strongly associated with chronic ITP and can be a good predictor of chronicity. Loss of response rates increased chronicity 2.56 times (p=0.010) and 3.87 times (p=0.005), respectively (Table 4).

Table 4. Treatment and chronicity

					Univariate			Multivariate		
IVIG treatment	**Non-chronic**		**Chronic**		**OR**	**(%95 CI)**	**p**	**OR**	**(%95 CI)**	**p**
	n	**%**	**n**	**%**						
Absent	15	65.2	8	34.8	**Reference**					
Present	20	54.1	17	45.9	1.594	(0.54-4.67)	0.395	5.223	(0.58- 46.9)	0.140
Steroid cycles										
	n	**%**	**n**	**%**						
Absent	14	66.7	7	33.3	**Reference**					
Present	21	55.3	17	44.7	1.619	(0.53-4.91)	0.395	2.871	(0.38- 21.56)	0.305
IVIG plus steroid										
	n	**%**	**n**	**%**						
Absent	26	63.4	15	36.6	**Reference**					
Present	9	47.4	10	52.6	1.926	(0.6–5.8)	0.244	0.158	(0.015-1.721)	0.130
	Mean	**SD**	**Mean**	**SD**						
Loss of response (n)	0.2	0.58	3.72	4.51	2.563	(1.25-5.25)	0.010	3.873	(1.49- 10.1)	0.006

OR (%95 CI): Odds ratio (%95), SD: Standard deviation

DISCUSSION

Children with ITP achieve remission spontaneously; only 20% develop chronic disease (1). However, our study reports a 40 % chronicity rate, higher than expected. The higher rates of our center may be associated with the fact that our center is nearly in the middle of Istanbul and Ankara; the chronic patients of Istanbul visit our center temporarily on holidays when they probably visit their relatives. Predictors for chronic ITP were investigated in several studies. Unfortunately, not many studies examined the variables of chronic ITP. Therefore, there are inconsistent results on this topic. Bennet et al. reported that at diagnosis younger age (<1 year, 1-6 years), the severity of bleeding and the treatment decreased the risk of chronicity for ITP. Sex and thrombocyte levels <20.000/mm³ did not affect chronicity in ITP (2). On the other hand, Jaime-Pérez et al. carried out a study that showed that children of older ages (≥ six years) male sex, infection history, and leukocyte level <6.25x10⁹/L were at higher risk for chronic ITP (3).

In another study, leukocyte levels above 6250/mm³ decreased, and thrombocyte levels above 20.000/mm³ increased the risk of chronic ITP. In addition, older age (>4 years) increased the risk of chronic ITP in another study(4). However, this study found no relationship between chronicity in ITP and older age (>4 years). They also reported age that ≥ five years is a risk factor. Treatment with IVIG (Intravenous Immunoglobulin) did not affect the chronicity rate in that study (4).

In previous studies and meta-analyses, lower thrombocyte counts have been associated with chronic ITP (4,5,6,7). However, Italian AIEOP studies found no relationship with platelet count (8). Opposing the previous studies, except for Italian AIEOP studies, we found that thrombocyte levels ≤20.000/mm³ didn't decrease the risk of chronic ITP.

The male gender decreased the risk of chronic ITP in various studies. Finally, this relation was related to the fact that the male gender is predominant in younger patients (4, 7). Our study supports the results that gender does not influence the chronicity of ITP.

Deel et al. and Ahmed et al. suggested low leukocyte counts predicted chronic ITP. However, leucocyte count that differs according to age is not an independent variable (13, 14). Therefore, we didn't find infection status in the last month, bleeding level, or neutrophil count predictive for chronic ITP.

IVIG treatment decreased chronic ITP rates in a few previous studies due to the lower platelet counts and an acute presentation (9,15). However, Heitink-Polle et al. concluded that immunoglobulin treatment is not associated with chronicity (10). Additionally, we investigated steroid and IVIG plus steroid effects, and none of the initial therapies was related to chronicity. In logistic regression analysis, loss of response rates significantly predicted this study's chronicity (2.56-fold and 3.87-fold).

Second-line treatments for chronic and persistent ITP include thrombopoietin receptor agonists, rituximab, and splenectomy. Additionally, mycophenolate mofetil and 6-mercaptopurine are other second-line drugs (16). Unfortunately, two of the six patients in our study needed to stop the treatment due to renal failure despite the eltrombopag's efficacy. In addition, one of the splenectomized patients couldn't achieve remission. Splenectomy in idiopathic thrombocytopenic purpura treatment doesn't always lead to a cure for the disease.

Limitations of the Study

The limitations of our study are that more patients should be followed to reach more objective results, and the local factors (being a small city near big cities) did not let to increase the number of patients. Also, due to the rarity of pediatric hematology-oncology specialists, more than one hospital doctor followed the patient. In addition, data is heterogenous

due to different doctors' treatment approaches. Finally, this study is retrospective. Therefore, randomized control trials will show more accurate results.

CONCLUSION

Lower platelet counts (<20.000/mm³), younger age, male gender, and initial treatment regimens (IVIG, steroid, IVIG plus steroid) did not influence chronicity in our study. Therefore, different studies have different results in the literature, depending on the independent variables (gender, leukocyte counts, treatment regimens) (4-15). We suggest that loss of response rates predicts chronicity as a new factor.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Ethical permission was obtained from the Duzce University Medical Faculty Clinical / Human Research Ethics Committee for this study with the date 09.05.2022 and number 2022/85, and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: HMÇ, K.K., Design: HMÇ, K.K., Supervising: HMÇ, K.K., Financing, and equipment: HMÇ, KK, Data collection and entry: HMÇ, Analysis, and interpretation: HMÇ, K.K., Literature search: HMÇ, Writing: HMÇ, Critical review: KK

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ORIGINAL RESEARCH
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Genetic and clinical characteristics of Turkish children with Maturity Onset Diabetes of the Young Type 2 (MODY2): A single center experience

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Abstract

Objective: The aim of the study was to investigate the clinical and molecular genetic characteristics of children with maturity-onset diabetes of the youth-glucokinase (MODY-GCK, MODY type 2).
Method: Medical files of 21 patients with suspected MODY-GCK were reviewed retrospectively. The file records of the clinical findings, laboratory results and the suspected clinical diagnoses of MODY were based on (1) asymptomatic fasting hyperglycemia (glucose ≥100mg/dl, HbA1c < 7.5% (at least twice measurement) 2) parents with a history of diabetes without complications or mild fasting hyperglycemia (100-144mg/dL).
Results: The mean age at diagnosis was 11.5±4.3 years (min-max, 1.9-17.2). The mean (SD) fasting blood glucose level was 119.1 (9.8) mg/dL. The mean (SD) fasting C-peptide level was 1.3 (0.7) ng/mL, the mean (SD) insulin level was 5.9 (2.3) IU/ml, and the mean (SD) HbA1c level at diagnosis was 6.2 (0.5) %. Among 12 variants detected in the GCK gene, 8 were missense mutation, 2 were non-sense mutation, 1 of them was splice site and 1 of them was frameshift mutation. Eight of them (p. Val227Met, p. Ser282Ala, p.Val183Met, p.Met239Thr, p.Arg304Gln, p.Thr229Met, p.Gly163Asp, p.Cys130Ter) have been previously reported in the literature and 4 variants (c.582+4delA, p.Glu436Ter, p.His106ThrfsTer11, p.Asp133Gly) were novel.
Conclusion: We found similar phenotype characteristic of children with GCK-MODY among the children with different variants. The most common mutation type was missense and followed by nonsense, splice site and frameshift mutations. Detection of the molecular defect in patients with MODY is vital for the implementation of appropriate treatment approaches.
Keywords: Children; Maturity Onset Diabetes of Youth; Youth-Glucokinase; Next Generation Sequencing

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a rare, insulin independent, autosomal dominantly inherited type of diabetes typically diagnosed before 25 years of age and that results from heterozygous mutations in various transcription factors acting in the development and maturation of pancreatic β-cells (1). MODY is reported to be the most common form of monogenic diabetes and it is estimated to account for about 1–5% of all cases of diabetes (2-4). MODY2 (also referred as Glucokinase (GCK)-MODY), which is caused by heterogenous inactivating mutations in the glucokinase (GCK) gene, is one of the most common types of MODY (5). GCK mutations (MODY2) have been reported to be the most common cause of MODY in Germany, Spain, France, Poland, Austria, the Czech Republic, Italy, and Greece (6).

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GCK catalyzes the rate limiting reaction of the glucose metabolism and therefore, it is the key enzyme in regulation of insulin release in pancreatic β-cells (7). The GCK gene is expressed in liver and beta cells (8). The GCK gene is located at chromosome 7p15.3–p15.1. Until now, missense, nonsense, frameshift, splice site, promoter mutations and deletions have been reported in GCK gene (7). While homozygous or compound heterozygous mutations in the GCK gene lead to permanent neonatal diabetes mellitus, heterozygote-inactivating mutations of the GCK gene cause mild, subclinical, non-progressive hyperglycemia, which is generally present at birth (8, 9). Typically, fasting glucose in MODY2 patients is between 100 and 144 mg/dL, and hemoglobin A1c (HbA1c) is between 5.6-7.3%. Patients with MODY2 are usually asymptomatic and most of them are diagnosed by random blood glucose measurements.

In this study, we aimed to present the clinical features of the patients with suspected MODY2 diagnosis and to identify genetic variations in GCK genes.

METHOD

Subjects

A total of 21 Turkish children and adolescents suspected MODY2 between 2019 and 2022 in Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital were enrolled. The file records of the clinical findings, laboratory results and the suspected clinical diagnoses of MODY were based on (1) asymptomatic fasting hyperglycemia (glucose ≥100mg/dl, HbA1c < 7.5%) (at least twice measurement), 2) parents with a history of diabetes without complications or mild fasting hyperglycemia (100-144mg/dL). The cases with insulin resistance, stress hyperglycemia, and those with diseases or using medication that may cause hyperglycemia were excluded from the study.

Informed consents were obtained from all patients' parents. This study was approved by the Ethics Committee of Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, (approval number: 640, date: 12.09.2021) and conducted after obtaining written informed consent from the patients or their guardians. Helsinki Declaration rules were followed to conduct this study.

DNA extraction and Next-generation sequencing

Peripheral blood samples were collected from these patients and the affected family members if required. Genomic DNA was extracted from leukocytes using the MagPurix kit (Zinexts Life Science Corp., New Taipei City 235, Taiwan), according to manufacturer's instructions. For the molecular genetic evaluation, NEXTflex® MODY1 Amplicon Kit (Bioo Scientific Corp., Perkin Elmer, USA) was used for all coding regions and exon-intron boundaries (±10 bases) of GCK (ENST00000345378) gene. Initially, DNA was amplified. Subsequently, ligation and indexing procedures were performed according to manufacturer's instructions. Sequencing reactions was performed with MiniSeq® NGS system (Illumina Inc., San Diego, CA, USA). FASTQ sequencing files were collected and transferred to "SEQ" variant analysis software (Genomize, Istanbul, Turkey). The Integrative Genome Viewer (IGV) (http://software.broadinstitute.org/software/igv/) was used for visualizing the status of each read alignment.

Pathogenicity Assessment

In an appropriate reference population, the pathogenic variant should have a frequency of much less than 1%. Therefore, we excluded all the common variants (minor allele frequency, MAF > 1%) reported in the following public databases: 1000 Genomes Project (http://www.1000genomes.org/) and dbSNP database. Possible variants that were not presented in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), or genetic studies in the published literature were considered as novels and included in the further analysis. Novel variants were evaluated according to 2015 publication of standards and guidelines for the clinical interpretation of sequence variants by the American College of Medical Genetics and Genomics (ACMG). The pathogenicity of the identified sequence variants was reported using an automatic variant classifier that evaluated the submitted variant according to the American College of Medical Genetics (ACMG) guidelines, classifying it as one of 'pathogenic', 'likely pathogenic', 'likely benign', 'benign' or 'uncertain significance'. (10). We also used the search engine Varsome (https://varsome.com/), which has information from 30 external databases, to investigate the pathogenicity of the novel variant. For confirmation and to detect the status of the specific familial variant in relatives, Sanger sequencing was also performed.

Statistical Analysis

Analyses were performed using the Statistical Package for the Social Sciences 18.0 (SPSS). Whether the quantitative variables were suitable for normal distribution or not was tested with the single-sample Kolmogorov Smirnov test. Mann-Whitney U test was used to compare data that was not normally distributed, and the chi-square test was used to compare categorical data between groups. Descriptive statistics for the data were given as median (minimum-maximum) for non-normally distributed parameters and mean ± SDS for normally distributed parameters. A p value of <0.05 was considered significant.

RESULTS

A total of 21 children suspected with the diagnosis of MODY were included in study. Among 21 patients, 12 variants were identified in 12 patients (12/21 patients; 57.14%). Eight out of 12 children (66.6%) were boys and the remaining (4 children,

33.3%) were girls. Body mass index SDS mean (SD) was found to be 0.2 (0.8). The mean age at diagnosis was 11.5±4.3 years (min-max, 1.9-17.2). The mean (SD) fasting blood glucose level was 119.1 (9.8) mg/dL. The mean (SD) fasting C-peptide level was 1.3 (0.7) ng/mL, the mean (SD) insulin level was 5.9 (2.3) IU/ml, and the mean (SD) HbA1c level at diagnosis was

6.2 (0.5) %. Clinically, all of the patients with GCK mutation had random hyperglycemia, none of them have glycosuria, polydipsia and polyuria, ketonemia/ketonuria, ketoacidosis, or dyslipidemia at the time of diagnosis. Five of the 12 patients had consanguinity. Clinical data on each of the 12 patients with mutations in the *GCK* gene are shown in Table 1.

Among 12 variants detected in *GCK* gene, 8 were missense mutation, 2 were non-sense mutation, 1 of them was splice site and 1 of them was frameshift mutation. Eight of 12 variants were previously reported while 4 of the rest were novel according to in ClinVar. Seven of the 8 previously reported variants were missense mutation and 1 of them was nonsense mutation, while among the novel variants there were 1 frame shift, 1 nonsense, 1 splice site and 1 missense mutations. An overview of these mutations was shown in Table 2.

Table1. Clinical and genetic characteristics of the patients with GCK-MODY related gene variations

Patient number	Age/ Gender	Gestational week	DKA	Weight (kg)/ Weight SDS	Height (cm)/ Height SDS	BMI/BMI SDS	Fasting Glucose (mg/ dl)	Fasting İnsulin (IU/ml)	C-peptide (ng/Ml)	HbA1c	Ketonemia /ketonuria	Consanguinity
P1	15.97/M	37	No	61/-0.52	164/-1.41	22.68/0.28	118	10.1	2.1	6.41	-	Yes
P2	4.51/F	39	No	16/-0.58	104/-0.45	14.7/-0.46	124	2.2	0.31	6.23	-	No
P3	7.44/M	38	No	44.3/3.34	133.8/1.94	24.7/2.95	124	10.2	2.7	7.03	-	Yes
P4	16.01/F	36	No	63.5/0.9	158.6/-0.6	25.2/1.4	129	6.5	1.9	6.1	-	Yes
P5	10.8/M	39	No	43/0.7	152.1/1.4	18.8/0.28	136	5.1	1.8	6.6	-	Yes
P6	13.36/M	38	No	39/-1.42	157.6/-0.35	15.7/-1.74	139	4.3	0.9	6.6	-	Yes
P7	11.44/F	37	No	54/1.29	157/1.49	21.9/1.09	108	2.2	1.1	6.15	-	No
P8	9.11/M	34	No	42.3/1.41	142.2/0.86	20.9/1.20	108	4.4	1.6	6.5	-	No
P9	10.85/M	38	No	34/-0.42	132/-1.7	19.51/0.5	124	7.2	2.2	7.01	-	No
P10	14.62/F	39	No	59/0.63	153/-1.4	25.2/1.46	121	11.6	3.8	6.03	-	No
P11	11.01/M	36	No	58.8/1.95	152.6/1.35	25.25/1.78	124	11.7	2.56	6.57	-	No
P12	1.9/M	38	No	12.2/-0.2	85.3/-0.54	16.7/0.22	114	6.93	0.36	6.93	-	No

P: Patient, DKA: Diabetic ketoacidosis, SDS: Standard deviation score, NA: not available, M: male, F: female, kg: kilogram

Table 2. Glucokinase (GCK) gene mutations in 12 patients and their family members

Patient Number	Zygosity	cDNA	Protein	Type of mutation	Inheritance	Reported/Novel	Pathogenicity (ACGM 2015)	SIFT	Mutation Taster
P1	HT	c.679G>A	p. Val227Met	Missense	From the mother	Reported	Pathogenic	Damaging	Disease causing
P2	HT	c.582+4delA	-	Splice site	NA	Novel	Likely Pathogenic	NA	Disease causing
P3	HT	c.844T>G	p. Ser282Ala	Missense	From the mother	Reported	Pathogenic	Damaging	Disease causing
P4	HT	c.1306G>T	p. Glu436Ter	Nonsense	From both of the parents	Novel	Likely Pathogenic	NA	Disease causing
P5	HT	c.316delC	p. His106ThrfsTer11	Frameshift	From the father	Novel	Likely Pathogenic	NA	Disease causing
P6	HT	c.547G>A	p. Val183Met	Missense	De novo	Reported	Pathogenic	Damaging	Disease causing
P7	HT	c.398A>G	p. Asp133Gly	Missense	From the mother	Novel	Likely Pathogenic	Damaging	Disease causing
P8	HT	c.716T>C	p. Met239Thr	Missense	From the father	Reported	Pathogenic	Tolerated	Disease causing
P9	HT	c.911G>A	p. Arg304Gln	Missense	NA	Reported	Pathogenic	Damaging	Disease causing
P10	HT	c.686C>T	p. Thr229Met	Missense	NA	Reported	Pathogenic	Damaging	Disease causing
P11	HT	c.488G>A	p. Gly163Asp	Missense	NA	Reported	Pathogenic	Damaging	Disease causing
P12	HT	c.390C>A	p. Cys130Ter	Nonsense	NA	Reported	Pathogenic	NA	Disease causing

HT: heterozygous, NA: not available

DISCUSSION

In this study, we aimed to investigate the clinical and molecular spectrum of patients with *GCK*-MODY in pediatric population. GCK mutation is a common cause of incidental hyperglycemia and individuals with *GCK*-MODY are generally first diagnosed during routine investigations or from blood glucose measurements performed to investigate another complaint. The ratio of *GCK*-MODY has been reported as 40–50% among children with asymptomatic or coincidental hyperglycemia (6, 11, 12). Our study was conducted in patients who had randomly detected hyperglycemia. We found that the fasting blood glucose levels between 106-139 mg/dl and HbA1C levels between 6.1-7.03 ng/ml at the first admission and these values were consistent with the literature. GCK mutations have been reported as account for 8%-56% of MODY, and it also reported as the most common cause of MODY in European countries (13, 14, 15). The prevalence of GCK mutations in Turkish population has been reported by Ağladıoğlu et al. (16), Anik et al. (17) and Aykut et al. (18) as 64%, 25% and 25.42 %, respectively. In our study, the frequency of the patients with *GCK* gene mutations was also 57.14%. In none of our patients with GCK-MODY pharmacological treatment including oral anti-diabetic medicines and insulin regimen was recommended and in all of them diet alone is sufficient to achieve metabolic control.

To the best of our knowledge, to date, more than 900 mutations of the GCK gene have been documented (19). According to different studies conducted in our country, different mutations were detected. Ağladıoğlu et al. (16) identified seven novel and five known GCK mutations among 18 patients. Anık et. al (17) detected mutation in GCK gene in 8 of 42 patients and 3 of these mutations were novel. Aykut et al. (18) identified 45 different mutations in the GCK gene, 20 of which were novel. In this study, we reported 12 (57,14%) different likely pathogenic/pathogenic variants. Eight of them (p.Val227Met, p.Ser282Ala, p.Val183Met, p.Met239Thr, p.Arg304Gln, p.Thr229Met, p.Gly163Asp, p.Cys130Ter) have been previously reported in the literature and 4 variants (c.582+4delA, p.Glu436Ter, p.His106ThrfsTer11, p.Asp133Gly) were novel. While the most frequently reported mutation among GCK-MODY mutations are missense mutation, followed by nonsense, frameshift or splice site mutations; pancreatic islet promoter mutations and partial or complete gene deletions are the rare mutations that cause GCK-MODY (20, 21). In a study conducted in our country, among 45 GCK mutations, 32 were missense mutations, 5 were nonsense mutations, 6 small deletions/insertions resulting in frameshifts and 2 splice site mutation (18). Another study conducted in our country, Bolu et al (22) detected sixteen different variants in the GCK gene of the 40 cases; 33 were missense mutations, six were deletions, and one was a nonsense mutation. Consistent with the literature, in our study the most common mutation type was missense and followed by nonsense, splice site and frameshift mutations.

However, in recent studies the mutations observed in individuals with GCK-MODY have showed that the phenotype may significantly differ in patients with GCK-MODY depending on the type of the mutation, it is mostly known that the phenotypic characteristics of GCK-MODY patients are very similar with the unaffected alleles compensate for the mutations (6, 23, 24). In our study, we found similar phenotype characteristic of children with GCK-MODY among the children with different variants.

Patients with GCK-MODY have hyperglycemia due to the altered physiological set point of glucose homeostasis and they have no risk of developing long-term complications of diabetes, and they can only be treated with diet and do not require medication. Genetic confirmation of GCK-MODY will not only prevent patients from being mistakenly diagnosed with type 1 or type 2 diabetes but will also prevent unnecessary oral antidiabetic medication and insulin therapy. Children with hyperglycemia should underestimated for GCK-MODY. Clinical findings can be similar different variants.

Limitations of the Study

Our study has some limitations. The small number of patients is major limitation of the study. Second, in vitro functional studies are essential to prove the disease-causing effects of novel variants that were interpreted to be as pathogenic / likely pathogenic by various bioinformatics tools. Third, genetic analysis of all the parents of the patients with positive variant could not be performed. Finally, large copy number or deep intronic variants could not be analyzed in the current study.

CONCLUSION

In conclusion, we identified *GCK* gene variants in 12 of 21 (57.14%) patients with asymptomatic hyperglycemia and family history of diabetes. Four of these mutations were novel. We found similar phenotype characteristic of children with GCK-MODY among the children with different variants. The most common mutation type was missense and followed by nonsense, splice site and frameshift mutations. Detection of the molecular defect in patients with MODY is vital for the implementation of appropriate treatment approaches.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Ethical permission was obtained from the University of Health Sciences Turkey, Dr. BehçetUz Child Disease and Pediatric Surgery Training and Research Hospital Clinical / Human Research Ethics Committee for this study with date 12.09.2021 and number 640(2021/19-10) and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: ÖN, BÖ, Design: ÖN, SG, Supervising: ÖN, BÖ, TK, Financing and equipment: ÖN, TK, Data collection and entry: TK, ÖN, Analysis and interpretation: SG, FH, Literature search: FH, TK, Writing: ÖN, SG, Critical review: BÖ.

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REVIEW
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Breast cancer and the molecular mechanism of estrogen signaling

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Abstract

Cancer is a complex pathology that occurs due to the uncontrolled proliferation and growth of cells in any organ or tissue of the body. Breast cancer is the most frequently diagnosed cancer among women worldwide and is the second leading cause of cancer-related deaths. Breast cancer is a pathology that exhibits heterogeneity in which genetic and environmental risk factors play a role. Although many treatment approaches have been developed for breast cancer today, the frequency of the number of patients diagnosed with breast cancer and lost their lives due to this reason is increasing in the world. The most significant limitation to the success of the treatment approaches developing drug resistance in breast cancer cells, and the disease relapses after a certain period and exhibits a more aggressive profile. Therefore, understanding the molecular biology of breast cancer is essential for developing potent therapeutic approaches. It is known that the development of breast cancer is related to changes in direct and indirect signaling mechanisms mediated by estrogen and estrogen receptor. These signaling mechanisms exhibit highly complex interaction patterns. This review summarizes the pathology of breast cancer, estrogenic compounds, estrogen receptors, genomic and non-genomic molecular signaling mechanisms mediated by estrogen and estrogen receptor.

Keywords: Breast Cancer, Estrogen, Estrogen Receptor

INTRODUCTION

Breast Cancer

Cancer is a commonly observed pathology due to the uncontrolled proliferation of cells in any organ or tissue of the body. Today, breast cancer is the most commonly diagnosed cancer type in women worldwide and is the second leading cause of cancer-related deaths (1). Breast cancer has a wide variation in gene expression level and is a complex disease with genetic and clinical heterogeneity. (1). This heterogeneity determines the progression of cancer, treatment success and survival rates of patients (2). Breast cancer refers to a malignant tumor originating from cells in the breast tissue. Breast tissue has 15 to 20 sections called lobes, which are organized into much smaller sections called lobules. Lobes and lobules are connected by thin tubes called ducts (3). While breast cancer is limited in the channel system (ducts) that carries milk during its initial development, tumoral cells are infused into the connective tissue by advancing over the basement membrane, depending on the progression in the carcinogenesis. Breast cancer can spread to different parts of the body through the inclusion of cancerous cells in the blood or lymphatic system. This process is referred to as metastasis. (4). Although breast cancers can originate from different parts of the breast tissue, they often develop over the inner wall of the lobules in the breast tissue. Theoretically, breast cancer tissue with a mass of one gram is estimated to develop on average in eight years (4).

According to GLOBOCAN 2023 data, it is estimated that 1,958,310 new cancer cases will be diagnosed, and 609,820 people will die from cancer in the United States. Also, according to these data, the most commonly diagnosed cancer type is breast

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cancer in females for the estimated new cancer cases, which will account for 31% of cancer cases.(6%), breast (11.6%), and colon (10.2%) cancers, respectively (5). Furthermore, according to 2015 data from the Turkish Republic Ministry of Health, breast cancer ranks first among the top 10 cancer types observed in women in Turkey. According to these data, the incidence of breast cancer is 43.8 per hundred thousand (6). Additionally, the International Agency for Research on Cancer reported that the incidence of breast cancer in women increased by 20% and breast cancer-related deaths by 14% compared to previous estimates (5). Today, it is determined that one out of every four women diagnosed with cancer has breast cancer. Considering the general age distribution of breast cancer in Turkey, it is seen that 44.5% of the women diagnosed with breast cancer are between the ages of 50-69, and 40.4% are between the ages of 25-49.(6).

Breast cancer cells are a type of cancer that can often be observed on X-ray examinations or felt as a lump during physical examination. Although it can be seen primarily in women, it can also be seen in men at low frequency. Early diagnosis is essential for determining whether the lumps felt in the breast are benign or malignant breast tumors and for the success of the treatment to be applied. Although non-cancerous breast tumors are mostly foci of abnormally growing cells, they do not spread beyond the breast tissue and are not life-threatening (7,8). However, it is known that some benign and stable breast lumps can increase the risk of developing breast cancer (9). Therefore, it is crucial for early diagnosis, especially for female individuals, to participate in regular screening programs in proportion to their age (10). The most apparent findings related to breast cancer, except the mass development in the breast tissue, are, swellings and collapse in the breast or armpit, discharge, cupping, pain, enlargement, focal asymmetry, ulceration, inflammation findings, orange peel appearance and deformity in the breast (9,11).

Stages of Breast Cancer

Knowing the stage of breast cancer is essential to understanding how quickly cancer cells can grow and spread. Therefore, cancerous tissues taken from patients are staged according to specific parameters by laboratory studies (12). The stage depends on how similar the cancer cells are to normal cells. Breast cancer is classified from stage 0 (zero) to stage 4 (four). Accordingly, stage-0 is not literally defined as breast cancer. At stage-1, the tumor size is less than 2 cm, and the tumor does not spread to another site. In stage-3, the tumor size can be greater than 5 cm or less than 5 cm and it can also be highly adherent in the axillary glands, attached to the chest muscle wall, or spread to lymph nodes in the neck. In stage-4, a large spread of breast cancer to other tissues and organs is observed (13).

The Importance of Inherited and Acquired DNA Mutations in the Development of Breast Cancer

Alterations in DNA sequence or mutations can cause normal breast cells to turn into cancerous. Some alterations in DNA can be inherited from generation to generation, and these can significantly increase the risk of breast cancer. Although other risk factors related to lifestyle and environmental factors may increase the rate of breast cancer development, how some of these risk factors contribute to the cancerization of normal cells is still not fully understood (14).

Some mutations in cells can be an important risk factor for developing various types of cancer. It is seen that a significant part of the mutations observed in DNA is acquired later. Mutations in some genes that are critical for cells lead to uncontrolled division of cells (14). In particular, alterations in tumor suppressor and proto-oncogene genes often result in cancer development. Tumor suppressor genes are associated with cell division control, repairing of DNA errors or programmed cell death. When these genes lose functionality, cells uncontrolled divide and initiate the cancerous (15). The most well-known genes responsible for susceptibility to breast cancer are breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2). Studies have shown a strong relationship between the germline mutations of BRCA1 and BRCA2 and the development of breast cancer (16). In addition, it has been reported that BRCA1 and BRCA2 mutations increase the risk of ovarian, uterine tubes, and peritoneal cancers, while BRCA2 mutations increase the risk of breast cancer, pancreatic cancer, and melanoma in men (17). These groups are being studied under Hereditary Breast and Ovarian Cancer (HBOC) (18). Both genes are inherited in an autosomal dominant manner and constitute a well-defined example of tumor suppressor genes (18). Genes with carcinogenic properties are called oncogenes and these group genes contribute to cell development, controlling the division cycles and normal proliferation of cells. When a proto-oncogenic gene is mutated or has multiple copies, it can become a carcinogenic gene that can remain continuously active. In this case, cells can divide uncontrollably, leading to cancer (19,20). Some effective proto-oncogenes in breast cancers are Ras, human epidermal growth factor receptor 2 (HER2), and c-Myc genes (21,22).

Types of Breast Cancer

Today, many different types of breast cancer have been described. Among the most common types of breast cancer are ductal carcinoma in situ (DCIS) and invasive carcinoma. Some breast tumors, such as phyllodes and angiosarcomas, are less common. Receptor expression levels are used in molecular typing of breast cancer. Following the biopsy, breast cancer cells are analyzed to determine the expression profiles of proteins called estrogen receptor (ER), progesterone

receptor (PR) and HER2. A detailed set of methodological approaches, such as advanced molecular analyzes and extensive histological staining, are used to define the stage of tumor cells. Also, the tumor stage and specific protein expression levels help to decide treatment options (23).

In Situ and Invasive Breast Cancers:

The type of breast cancer can provide information about whether cancer has spread. If cancer originates from the mammary gland is called lobular carcinoma; otherwise, it is named ductal carcinoma. These two groups are divided into two subgroups depending on whether the cancer is still inside or outside the mammary gland or milk duct. Cancer inside the mammary gland is called lobular carcinoma in situ (LCIS) and the outside mammary gland is called invasive lobular carcinoma. The terms invasive and infiltrative mean that cancer cells have spread beyond the ducts of the mammary gland (24).

Ductal Carcinoma in Situ (DCIS):

DCIS; Intraductal carcinoma is a non-invasive or pre-invasive type of breast cancer. One in five newly diagnosed breast cancers determines to be DCIS (25). It is also referred to as intraductal carcinoma or stage 0 breast cancer. DCIS means that the duct cells have transformed into cancer cells but have not spread to the breast tissue near the ducts. In addition, since there is no spread to the breast tissue in DCIS, metastasis is not observed (26).

Invasive Breast Cancer:

Breast cancers that have spread to the surrounding breast tissue are known as invasive breast cancer. It constitutes the majority of diagnosed breast cancers. Invasive breast cancer divided into subclasses. The two most well-known classes are invasive ductal carcinoma and invasive lobular carcinoma. Invasive ductal carcinoma accounts for approximately 70-80% of all breast cancers. Inflammatory breast cancer and triple-negative breast cancer are also types of invasive breast cancer (27).

Invasive (Infiltrative) Ductal Carcinoma (IDC):

IDC is the most common type of breast cancer. About 8 out of 10 invasive breast cancers are diagnosed as invasive (or infiltrative) ductal carcinoma. IDC begins in the cells surrounding the milk duct in the breast and grows from there through the duct wall to nearby breast tissues. In this case, it can metastasize to other body parts through the lymphatic system and blood circulation (28).

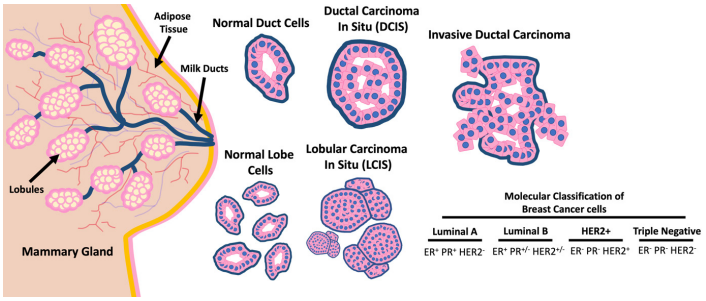

Figure 1: Schematic representation of types of breast cancers.

Invasive Lobular Carcinoma (ILC):

1 in 10 invasive breast cancers is diagnosed with ILC. ILC can metastasize to other body parts, such as IDC, starting in the milk-producing glands (lobules). Compared with other types of invasive carcinoma, ILC could be present in both breasts in 1 out of 5 women (29).

Less Common Types of Invasive Breast Cancer:

Less common invasive breast cancers are also known as special types of invasive breast cancers. Some special types of breast cancers are classified into invasive carcinoma subtypes. These types of breast carcinomas are less common than other types of breast cancer, and each usually accounts for less than 5% of all types of breast cancer (23).

Triple Negative Breast Cancers (TNBC):

Unlike other types of invasive breast cancer, TNBC spreads much faster and has limited treatment options. It also has a worse prognosis compared to other breast cancer types. TNBC is classified as a type of breast cancer in which cancer cells do not have ER or PR. Also, it doesn't express three receptor proteins, including ER, PR, and HER2. TNBC is an aggressive invasive breast cancer that accounts for 10-15% of all diagnosed breast cancers. Also, the treatment options are more limited than in other breast cancer. It has a low treatment success and high recurrence rates. Moreover, it has several subtypes, including Luminal A (ER^+, PR^+, $HER2^-$, Ki-67^{low}), Luminal B (ER^+, PR^{low} or $^-$, $HER2^-$ or $^+$, Ki-67^{high}), $HER2^+$ (ER^-, PR^-, $HER2^{high/overexpressed}$) and Basal-like ($ER^-$, PR^-, $HER2^+$). While various approaches can be offered with hormonal intervention for treating breast cancer subtypes that are positive for one or more receptors (ER, PR, or HER2), hormone-focused therapies cannot be utilized in TNBC (30). Therefore, new treatment approaches for TNBC are under intense research.

Estrogenic Compounds

Estrogens are classified into two main groups as natural and synthetic estrogenic compounds (31). Natural estrogens are classified into three subgroups ovarian steroids, mycoestrogens, and phytoestrogens. PPhytoestrogens have weaker biochemical activity than estrogens produced in the endogenously synthesized estrogens. They are abundantly

found in foods such as soybeans, garlic, parsley, cereals, carrots, potatoes, cherries, apples, and coffee, which are frequently consumed in the daily diet. In addition, phytoestrogens are classified into two groups phenolics (lignans, stilbenes, flavonoids, isoflavonoids) and non-phenolics (terpenoids, saponins). Synthetic estrogens are divided into two groups: birth control pills, estrogen drugs such as diethylstilbestrol, cimetidine, and environmental estrogens, known as lxenoestrogens, which are long-term and persistent organic pollutants and difficult to recycle in nature (32).

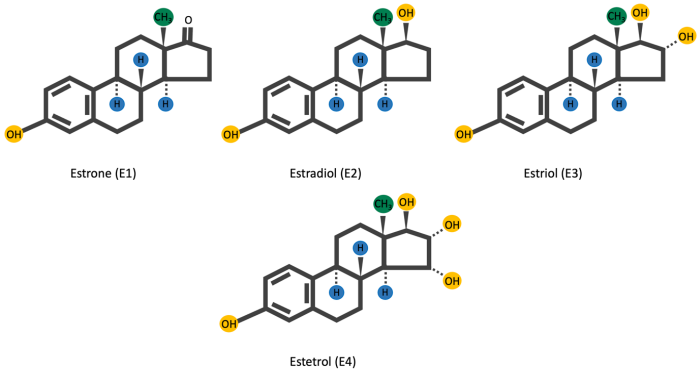

Figure 2: Natural estrogenic compounds. Representation of the chemical structures of Estrone (E1), Estradiol (E2), Estriol (E3) and Estetrol (E4) compounds.

E1 (Estrone), E2 (17β-Estradiol), E3 (Estriol), and E4 (Estetrol), which are especially important for female physiology, are among the natural steroid estrogens (Figure 2). In addition to the adrenal gland, E1, produced in the ovaries, placenta, testicles, and adipose tissue, is the primary estrogen in men, and women who have gone through menopause. E1 is secreted less than other forms of estrogen and is weakly effective (33). E2, dominant in the reproductive period, is secreted from the ovarian follicles and is produced in the ovaries in premenopausal women and the testicles in men. E2, the most potent and abundant estrogen in premenopausal women, is 12 times more active than E1 and 80 times more active than E3 (34). E2 levels in postmenopausal women are usually below 20 pg/ml. During the ovulation cycle, serum concentrations of E2 range from 30 pg/ml in the early follicular phase, 150-350 pg/ml in the preovulatory stage, and 100-210 pg/ml in the luteal phase. During pregnancy, E2 levels increase 100 times (35). E3 is produced in the placenta in women, while it is in the adrenal glands in men. In non-pregnant women, circulating E3 serum concentrations are very low (approximately 10 pg/ml) as E3 is rapidly cleared from circulation. During pregnancy, the level of E3 reaches from 12 ng/ml to 210 ng/ml (35,36). E4 is an estrogenic steroid molecule synthesized only by the fetal liver during pregnancy in women and reaches the maternal circulation via the placenta. It is only synthesized during pregnancy, while other forms of estrogen can be continuously observed in circulation (37).

Breast Cancer Associated Receptors

Breast cancer is a complex pathology that exhibits a high level of heterogeneity and has different genetic alterations. After the 2000s, breast cancers have been begun to be categorized according to their gene expression profiles and histopathological typing. It is classified into four subtypes, mainly Luminal A, Luminal B, HER2 overexpression, and triple negative, also known as basal-like. ER and PR are positive in Luminal A and Luminal B subtypes. There are also differences between subtypes regarding clinical behavior, survival rates, and response to treatment. The evaluation of ER, PR, and HER2 expression levels is used as prognostic and predictive factors. Tumor cells are classified as hormone receptor-positive or negative, depending on their receptor expression profile. Knowing the expression status of the hormone receptor is an essential factor in selecting the treatment strategy to be applied (38). In the follow-up of this section, basic information about the ER protein, which is known to be closely associated with breast cancer, and signaling pathways controlled by the ER will be shared.

Estrogen Receptor

ER is a steroid/nuclear receptor superfamily member. It is a specialized transcription factor stimulated by estrogens and regulates a series of complex signaling processes within the cell (39). Physiologically, ERs are involved in women's menstrual cycle, during pregnancy and lactation, as well as the functioning of the cardiovascular system, nervous system, musculoskeletal and immune system, and the regulation of the responses of these systems to stimuli (40,41).

The ER protein is encoded by the estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) genes located on separate chromosomes in the human genome. These genes mediate the formation of two isoforms, ER alpha (ERα) and ER beta (ERβ) proteins. The ESR1 gene is located at position 6q24-27, and the ESR2 gene is at 14q22-24 (42). ERα and ERβ are involved in the regulation of many complex physiological processes in humans. The full-length ERα isoform consists of a polypeptide chain of 595 amino acids and has a molecular weight of approximately 67kDa. The shorter isoforms of ERα, which have 36kDa and 46kDa molecular weights, are synthesized through alternative splicing or start codon. Some of these short isoforms lack the activation function-1 (AF-1) region located in the N-terminal domain (NTD) required for transcriptional activation. Although these isoforms are not incapable of transcriptional activation, they can heterodimerize with full-length ERα and block transcriptional activity (43). ERβ is 530 amino acids long and has a molecular weight of 59kDa (Figure 3). Both receptor forms regulate

the transcriptional program of target genes by interacting with specialized regulatory DNA sequences in the nucleus. The main difference observed between full-length ERβ and shorter ERβ isoforms is in the C-terminal ligand binding domain (LBD). Therefore, ERβ isoforms lacking transcriptional activity are capable of suppressing ERα signaling by dimerizing with ERα (44). In addition, ERα and ERβ receptor forms have subtypes with different functionality. Some of these types are ERα36 and ERα46, which lack the AF-1 region, and ERβ2/cx and ERβ5, which lack the F region and show differences in the length of the LBD (45).

ERα and ERβ have different expression patterns in tissues. ERα is mainly expressed in the mammary gland, uterus, thecal cells of the ovary, bone, and male reproductive organs such as the testis and epididymis, liver, and adipose tissue (46). ERβ is expressed in prostate epithelium, bladder, ovarian granulosa cells, colon tissue, immune system cells, and adipose tissues. Differences are observed in the expression foci of both subtypes of ER, it is known that both ERs are expressed in the tissues of the cardiovascular and nervous systems. Although the effects of ERs on the mammary gland and uterus are more widely known, they have several critical roles, including maintaining the cardiovascular system, homeostasis of skeletal muscle, and regulating metabolic flow (47). Besides, ERβ actively regulates the central nervous system and immune system responses. In contrast, it has an antagonistic biological effect against cell hyperproliferation supported by ERα in tissues such as the breast and uterus. Also, ERα has active roles in regulation of metabolism and maintaining skeletal system homeostasis as well as on the mammary gland and uterus (39).

Like other nuclear hormone receptors, ERs have structurally specialized functional domains and segments organized within these domains to perform individual functions. These domains are NTD, DNA binding domain (DBD), and LBD. Moreover, the AF-1 and activation function-2 (AF-2) regions located in NTD and LBD, respectively, are responsible for regulating the transcriptional activity of the ER. The AF-1 region is hormone-independent, while the AF-2 region functions in the presence of hormones. ER protein is divided into 5 main structural divisions: A/B domain, C domain, D domain, E domain, and F domain. Basically, the difference between the two forms of receptors is that ERβ has a shorter NTD than ERα. The A/B region is represented by a zinc finger-containing NTD domain that participates in the transactivation of gene transcription and mediates binding to the target sequences (48).

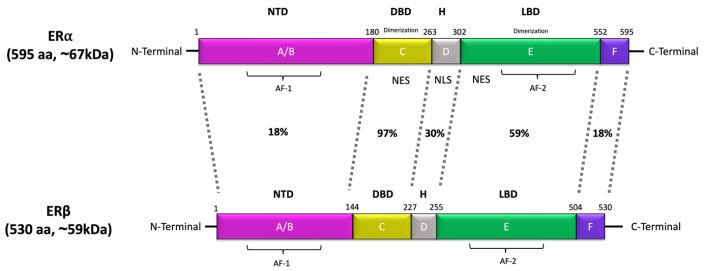

Figure 3: The protein organization of ERα and ERα. Representation of protein structure, functional domains and locations, homology ratios and protein structures of ERα and ERα.

Region C corresponds to the DBD of the ER, which helps dimerize and interact with specialized sequences in the chromatin structure. The DBD domain interacts with the 5′-AGGTCAnnnTGACCT-3′ estrogen response element (ERE), usually a palindromic hexanucleotide consensus motif on DNA. The DBD domain of ERα and ERβ isoforms may share the same DNA response elements (49). Further studies have determined that EREs also regulate the participation of coregulators in the ER transcriptional module (50). Furthermore, these elements share a high degree of sequence similarity, while it is known that the intrinsic sequence composition of EREs can regulate the receptor's binding affinity to DNA (51). The P-box, located in the ER's protein structure, interacts effectively with EREs in the interaction of ERs with nucleic acids. The D-box mediates the formation of an interface for dimerization. Moreover, D domain connects the C and E domains, is located between the DBD and LBD, and forms a flexible hinge region that allows interaction with molecular chaperone proteins. In addition, this region contains the signal sequence required for nuclear localization (52,53).

LBD is located in the E domain of the ER protein and consists of 12 helix structures. LBD functions as a ligand binding and dimerization interface. Also, it contains a hormone-binding pocket. When the LBDs of ERα and ERβ are compared in terms of structural similarity, it is seen that the homology between the LBD domains of both proteins does not exceed 55%. Although the homology ratio between the LBDs of the nuclear receptor superfamily members is low, there is a remarkable similarity in their 3-D structural organization. This structural feature is likely the product of adaptive plasticity gained in evolution to make the interaction of each receptor protein with the corresponding steroid hormone specific. The LBD of ERα contains the hormone-binding domain, the interface that mediates homo- and heterodimerization, and interaction surfaces on which the interactions of activators and inhibitors are coordinated. The C-terminal F domain downstream of LBD regulates the ligand-specific gene expression. Besides, it has been reported to be effective in receptor dimerization (52,54). In addition, the E/F region contains binding sites for the interaction of accessory proteins and cofactors (55).

Depending on the composition of various post-translational modifications, the activity and interactive protein dynamics of ERα and ERβ change. Numerous modifications for ERα and ERβ have been reported in the literature. These modifications include phosphorylation, methylation, acetylation, SUMOylation, ubiquitination, acetylation, palmitoylation, and glycosylation (45). Phosphorylation of ERα at positions Y52, S102, S104/106, and S118 through kinase proteins can increase or decrease its transactivation ability. For example, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and E2 stimulation-mediated mitogen-activated protein kinase (MAPK) induced S118 phosphorylation of ER result in the transcriptional activation. In contrast, reactive oxygen species (ROS)-mediated MAPK stimulation may cause a decrement in receptor expression levels. Phosphorylation at the S80 position for ERβ has been reported to promote ERβ degradation. The ubiquitination at positions K302 and K303 has been shown to target ERα for proteasomal degradation (45). Post-translational modifications of different isoforms and subtypes of ERs and tight interaction dynamics with their co-regulatory proteins mediate ER-mediated tissue-specific responses. Therefore, the mechanism of ER-mediated signaling is discussed under several sub-headings in the following sections.

ER-mediated Signaling

Nuclear Estrogen Receptors

Genomic Signaling: Estrogens directly pass the plasma membrane and fulfill the specific biological functions through inducing the ERα and ERβ receptors. Depending on the targeted cellular effect and molecular response in estrogen signaling, the estrogen response has been divided into genomic and non-genomic signaling. Genomic signaling encompasses a series of processes, including translocation of the complex formed after the estrogen-ER interaction to the cell nucleus and direct interactions with chromatin-mediated by specific DNA sequences known as ERE. Non-genomic signaling refers to the control of estrogen signaling through other intracellular signaling networks (56). Directly maintained genomic signaling is also known as the classical estrogen signaling pathway. After binding of estrogen to ERα or ERβ in the cytoplasm of the cell, a series of conformational changes occur that induce dimerization of the receptor (57). Subsequently, the activated receptor complex translocates into the cell nucleus. It regulates gene expression processes by selectively binding to ERE sequences in the chromatin structure, 3'-untranslated regions of target genes, or areas adjacent to the promoter region (58).

Non-genomic signaling: ERs can regulate the expression of some genes through interactions with other co-regulatory proteins without directly interacting with DNA. Recent studies suggest that 35% of genes regulated by estrogens do not have a putative ERE sequence in their promoter region (44,56). Regulation of estrogen-mediated expression of these genes is controlled through protein-protein interactions that ERs maintain with protein groups that interact with other transcription factors and response elements. In this, estrogens can activate or suppress the expression of target genes. In addition, non-genomic steroid signaling responses tend to be rapid and sensitive (59,60). Non-genomic signaling includes a series of events, including mobilization of seconary messengers, membrane receptors such as insulin-like growth factor-1 receptor (IGF-1R), epidermal growth factor receptor (EGFR), and interaction with Src, phosphoinositide 3-kinases (PI3K), and protein kinase B (PKB) (61).

One of the most important mediators in the non-genomic signaling process is specificity protein 1 (Sp-1). The presence of ER enhances the interaction of Sp-1 with its targets. Today, many genes related to this mechanism have been characterized. A few of these targets are PR-B, signal transducer and activator of transcription 5 (STAT5), low-density lipoprotein receptor (LDLR), GATA Binding Protein 1 (GATA1), and retinoic acid receptor-1 alpha (RAR-1α) (55, 62, 63). In addition, ERα is capable of interacting with other transcriptional modulators, such as activating transcription factor-2 (ATF-2), c-jun, and activating transcription factor-1/cAMP-responsive element (CRE)-binding protein (ATF-1/CREB) (62). Also, ERα regulates the expression levels of target genes containing activator protein-1 (AP-1) transcription factor interaction domains through protein-protein interactions (64). AP-1 is a transcription factor that regulates essential cellular processes such as cell differentiation, proliferation, and apoptosis (55). The best-known examples of target genes controlled by AP-1 are IGF-1, collagenase, IGF-1R, ovalbumin, and cyclin D1 (56,65). It is known that the two main isomers of ER regulate the expression level of target genes with different patterns. E2, a potent estrogenic molecule, regulates AP-1-dependent transcription through ERα, while ERβ can inhibit this mechanism (66). A well-understood example of this mechanism is the regulation of cyclin D1. Estrogen-stimulated ERβ suppresses cyclin D1 expression, while ERα has an opposite effect on cyclin D1 expression in the presence of both receptors (56,67). This regulation model provides a good example of the mechanisms involved in controlling tissue-specific estrogen-mediated gene expression processes based on the tissue expression profiles of ER isoforms and other transcription factors. Also, other steroid hormones and their receptors regulate gene expression processes by similar mechanisms. Therefore, elucidating the dynamics of receptor isoforms and their interactions with other coregulator proteins is essential in understanding the processes related to hormone-dependent cancers, including breast cancer.

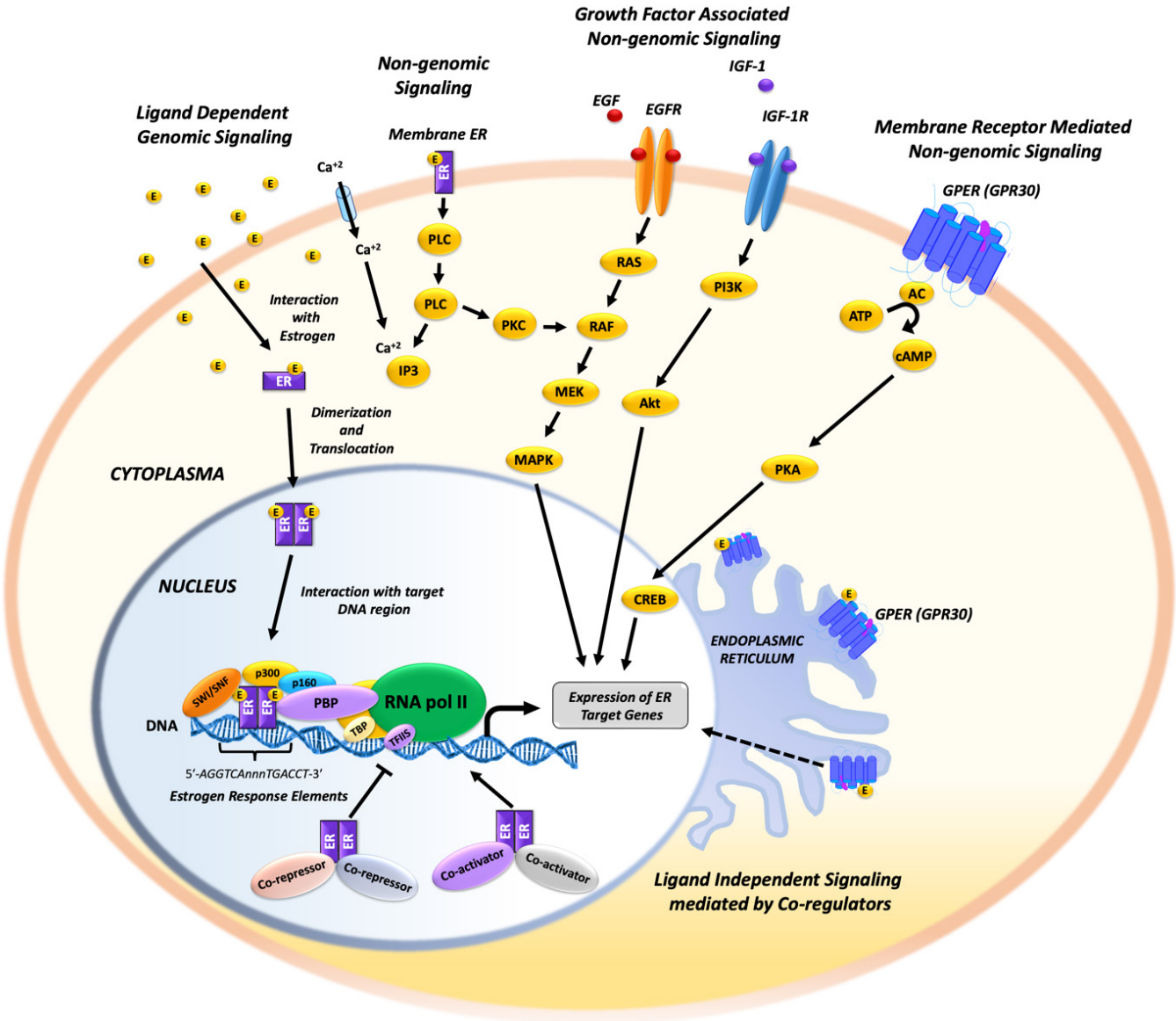

Figure 4: ER-related signaling mechanisms. Demonstration of ER-mediated ligand-dependent genomic signaling, non-genomic signaling, growth factor-related non-genomic signaling and membrane-mediated non-genomic signaling mechanisms in breast cancer.

Non-genomic Signaling Mediated by Membrane Receptors

The activity of the non-genomic signaling mediated by estrogen stimulation is driven by a series of signaling cascades mediated by intracellular levels of secondary messengers such as cyclic adenosine monophosphate (cAMP) and activation of protein kinases (68). The activities of target proteins can be highly coordinated through post-translational modifications such as the phosphorylation of proteins. By this way, many cellular signaling mechanisms, including ERα and ERβ, can be highly coordinated. ERα and ERβ are good molecular targets for kinase proteins. G Protein-Coupled Estrogen Receptor 1 (GPER1), a membrane-bound ER, and some variants of ERα and ERβ have been associated with non-genomic estrogen signaling (69,70). ERα, Erβ, and GPER can function synergistically or antagonistically at the cellular level. These cross-interactions mediate the formation of complex physiological responses to various stimuli (71). Therefore, to improve our understanding of the biology of breast cancer, all signaling cascades regulated by estrogen, ER-mediated direct and cross-interactions-coordinated mechanisms need to be further characterized.

GPER was identified in the late 1990s and is a protein belonging to the G protein-coupled receptor (GPCR) family that possesses seven membrane-spanning domains and is a protein belonging to the G protein-coupled receptor (GPCR) family that possesses seven membrane-spanning domains GPER was identified in the late 1990s. GPER, also known as GPR30, interacts with a heterotrimeric G protein and mediates the regulation of multiple intracellular signal transductions. It is also involved in rapidly activating extracellular signal-regulated protein kinase 1/2 (ERK1/2)

mediated by E2. The ability of GPER to activate adenylate cyclase has been implicated as a mechanism involved in the activation and/or inhibition of ERK1/2. Moreover, GPER activates the PI3K/Akt pathway and responds to E2 by EGFR transactivation. Furthermore, GPER can control the various signals such as c-fos and cyclin D1, connective tissue growth factor, fatty acid synthetase, and vascular endothelial growth function at the gene expression level. These different GPER-mediated signals regulate various physiological processes such as cell proliferation, metabolism, migration, and secretion (73). ERα and ERβ can interact with G proteins, tyrosine kinases, membrane receptors such as IGF-1R and EGFR, and signaling pathway molecules such as Ras, Src, and PI3 kinases, and HER2. These interactions can precisely regulate the expression levels of target genes with MAPK and Akt signal transduction pathways, which are associated with various cellular responses (74).

Ligand Independent ER Signaling

Ligand-independent ER signaling is tightly regulated with regulatory molecules required for phosphorylation, such as kinase cascade components, including protein kinase A (PKA), Protein kinase C (PKC) and MAPK, inflammatory cytokines, cell adhesion molecules, cell cycle regulators, and peptide growth factors; EGF, insulin, IGF-1 and transforming growth factor beta (TGF-β) (75). However, the details of signal transduction mechanisms involving ER proteins in the absence of estrogenic compounds and other receptor agonists are still poorly understood (44). Post-translational modifications regulate the special biochemical functions of numerous proteins. In particular, the phosphorylation of serine, threonine, and tyrosine residues, mediated by various kinase enzymes, plays a vital role in regulating many biochemical functions. Momentary alteration of phosphorylation patterns has an essential role in coordinating specific responses in ligand-independent ER signaling. Furthermore, it has been suggested that estrogen receptor-mediated intracellular signaling is highly regulated through specific modification motifs in the ER protein structure of ERs (44).

The transcriptional activity of the ER-mediated signaling mechanism is regulated by groups of proteins expressed as coregulators. While interaction with coactivators increases the transcriptional activity of ERs, corepressors cause transcriptional repression. Coregulators play a crucial role in many step of the signal transduction process, such as rearranging chromatin structure, transcriptional initiation, RNA chain elongation, mRNA processing, and translation (76). One of the first identified coregulators for ERα is steroid receptor coactivator-1 (SRC-1) (77). Although a limited number of coregulators have been characterized for ERβ, numerous coregulatory proteins have been identified that regulate the activity of ERα today (78). Some of these regulators for ERα are ATP-dependent chromatin remodeling systems such as SRC/p160, histone acetyltransferase, CREB-binding protein (CBP)/p300, SWItch/Sucrose Non-Fermentable (SWI/SNF), and E3 ubiquitin ligase enzymes (78,79). Protein dynamics regulated by diverse protein complexes, including coregulatory proteins, have critical importance in terms of precise control of expression levels of target genes and the formation of tissue-specific responses (79).

Coregulator proteins have specific structural motifs and selectively maintain interactions through these motifs (78). In particular, LxxLL motifs mediate these interactions. The interactions between corepressors and free ER proteins competitively take place with coactivators (79). On the other hand, intracellular levels of coregulators and post-translational modifications such as phosphorylation, methylation, or ubiquitination control the ER-mediated gene expression processes by modulating coregulator dynamics. In this way, ER-mediated signal transduction is indirectly fine-regulated by highly coordinated protein interactions (76,80).

All this information summarized in this review regarding estrogenic signaling in breast cancer cells reveals the complexity of breast cancer biology. The cross-protein interactions, altered chromatin dynamics, and dynamic processes of alternative post-translational modifications occurring in the absence and presence of estrogenic stimulation is the most compelling obstacle to a complete understanding of breast cancer biology. This situation constitutes the biggest obstacle in front of new treatment strategies to be developed. Therefore, there is a great need for extensive further analysis and ongoing studies on discovering new regulation models.

CONCLUSION

Breast cancer biology constitutes an area on which intensive studies continue, especially the signaling mechanisms regulated by breast cancer-related receptors are tried to be understood. In this review, the basic information about breast cancer and the mechanisms related to estrogen and ER-mediated signaling, which are thought to play crucial roles in breast cancer, are summarized at a basic level. To achieve effective treatment success in breast cancer, there is a need for multidisciplinary approaches to clarify the unknowns of breast cancer-related signaling mechanisms and their possible interactions with other cellular signaling networks.

Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Since this study is a review article, ethics committee approval is not required, and the Helsinki Declaration rules were followed to conduct this study.

Author Contribution

Concept: YE, Design: YE, Supervision: YE, Literature search: YE, HKD, Writing: YE, HKD, Critical review: YE, HKD.

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