Original Articles / *Özgün Araştırmalar*

Case Reports / *Olgu Sunumları*

Review / *Derleme*

Turkish Journal of Pediatric Disease / *Türkiye Çocuk Hastalıkları Dergisi*

Volume / *Cilt* 18 Number / *Sayı* 3 May / *Mayıs* 2024

ANKARA BİLKENT ŞEHİR HASTANESİ ÇOCUK HASTANESİ

Turkish J Pediatr Dis
Türkiye Çocuk Hast Derg

ISSN 1307-4490
E-ISSN 2148-3566

Turkish Journal of Pediatric Disease

Türkiye Çocuk Hastalıkları Dergisi

Vol/*Cilt* 18 • Number/*Sayı* 3 • May/*Mayıs* 2024

https://dergipark.org.tr/tr/pub/tchd

ISSN: 1307-4490 E-ISSN 2148-3566
Vol./*Cilt*. 18, No.3, **May**/*Mayıs*, 2024

Turkish Journal of Pediatic Disease
Türkiye Çocuk Hastalıkları Dergisi

Official Journal of Ankara Bilkent City Hospital, Children's Hospital
Ankara Bilkent Şehir Hastanesi, Çocuk Hastanesi Yayını

ANKARA BİLKENT ŞEHİR HASTANESİ
ÇOCUK HASTANESİ

Vol./*Cilt*. 18, No.3, **May**/*Mayıs*, 2024

Turkish Journal of Pediatic Disease
Türkiye Çocuk Hastalıkları Dergisi

ANKARA BİLKENT ŞEHİR HASTANESİ
ÇOCUK HASTANESİ

Owner on Behalf of the Ankara Bilkent City Hospital Children's Hospital *Ankara Bilkent Şehir Hastanesi Çocuk Hastanesi Adına Sahibi*	**Namık Yaşar ÖZBEK** Ankara Bilkent City Hospital Children's Hospital, Türkiye
Publishing Manager/*Yazı İşleri Müdürü*	**Metin YİĞİT** Ankara Bilkent City Hospital Children's Hospital, Türkiye
Head Office/*Yönetim Ofisi*	Ankara Bilkent City Hospital Children's Hospital, Türkiye Tel: +90 (312) 552 60 00 / 401506
Editor/*Editör*	**İbrahim İlker ÇETİN** Ankara Bilkent City Hospital Children's Hospital, Türkiye
Press Office/*Baskı Ofisi*	FABRİKA MATBAACILIK Tic. Sic. No: 393545, Mersis No: 0384 0359 0820 0013 İvedik O.S.B. Mah. 1372 Sk. No: 23 Yenimahalle / *Ankara*, Türkiye info@fabrikabaskida.com, www.fabrikabaskida.com Tel: +90 (312) 397 38 78 - Fax: +90 (312) 397 56 31
Publication Type/*Yayın Türü*	Common periodical / Yaygın süreli Published four issues per year: January, March, May, July, September, November *Yılda altı kez yayımlanır: Ocak, Mart, Mayıs, Temmuz, Eylül, Kasım*
Publishing Frequency/*Yayın Aralığı*	Bimonthly / *2 Ayda Bir*
Publication Language/*Yayın Dili*	English
This journal printed on acid-free paper *Dergimiz asitsiz kağıda basılmaktadır*	Printing Date / *Basım Tarihi* : 13.05.2024

Turkish Journal of Pediatric Disease has been a member of the DOI® system since March 2013.
Türkiye Çocuk Hastalıkları Dergisi Mart 2013 tarihinden itibaren DOI® sistemi üyesidir.

Turkish Journal of Pediatric Disease is indexed in Turkish Medical Index of TUBITAK/ULAKBIM, EBSCOhost Research Databases, CINAHL and Türkiye Citation Index.

Türkiye Çocuk Hastalıkları Dergisi TÜBİTAK/ULAKBİM Türk Tıp Dizini, EBSCOhost Research Databases, ve Türkiye Atıf Dizini tarafından indekslenmektedir.

The paper used to print this journal conforms to ISO 9706: 1994 standard (Requirements for Permanence).
The National Library of Medicine suggests that biomedical publications be printed on acid-free paper (alkaline paper).

Bu dergide kullanılan kağıt ISO 9706: 1994 standardına ("Requirements for Permanence") uygundur.
National Library of Medicine biyomedikal yayınlarda asitsiz kağıt (acid-free paper/alkalin kağıt) kullanılmasını önermektedir.

ENVIRONMENTAL INFORMATION / *ÇEVRE BİLGİSİ*

The company that manufactures the paper used in this journal has an ISO 14001 environmental management certificate. The company obtains all wood fiber in a sustainable manner. The forests and plantations of the company are certified. The water used in production is purified and used after recovery. Heavy metals or film are not used for the publication of this journal. The fluids used for developing the aluminum printing templates are purified. The templates are recycled. The inks used for printing do not contain toxic heavy metals.

22 PAP

This journal can be recycled. Please dispose of it in recycling containers.

Bu dergide kullanılan kağıdın üreticisi olan şirket ISO 14001 çevre yönetim sertifikasına sahiptir. Üretici şirket tüm odun elyafını sürdürülebilir şekilde temin etmektedir. Şirketin ormanları ve plantasyonları sertifikalıdır. Üretimde kullanılan su arıtılarak dönüşümlü kullanılmaktadır. Bu derginin basımında ağır metaller ve film kullanılmamaktadır. Alüminyum basım kalıplarının banyo edilmesinde kullanılan sıvılar arıtılmaktadır. Kalıplar geri dönüştürülmektedir. Basımda kullanılan mürekkepler zehirli ağır metaller içermemektedir.

Bu dergi geri dönüştürülebilir, imha etmek istediğinizde lütfen geri dönüşüm kutularına atınız.

AIMS and SCOPE / AMAÇ ve KAPSAM

The Turkish Journal of Pediatric Disease is an open access and a scientific publication journal of the Ankara Bilkent City Hospital, Children's Hospital. The journal is published in accordance with independent, unbiased, and double-blind peer review principles. The journal is published bimonthly (January, March, May, July, September, November).

In the Turkish Journal of Pediatric Disease original articles, reviews, case reports, editorials, short reports, book reviews, biographies and letters to the editor are also published in the journal. Besides if related with pediatrics, articles on aspects of pedatric surgery, dentistry, public health, genetics, pshyciatrics and nursery could be published. The publication language of Turkish Journal of Pediatric Disease is English.

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

Turkish Journal of Pediatric Disease is indexed in Turkish Medical Index of Tübitak / Ulakbim, EBSCOhost Research Databases and Türkiye Citation Index.

Journal is published on acid free paper.

Subscription

Turkish Journal of Pediatric Disease is delivered free of charge to library of Medical Faculties, Departments of Pediatrics in Universities and Research Hospitals and also to researchers involved in pediatrics. Full text of the artciles are available on http://dergipark.org.tr /tchd. For subscription please visit http://dergipark.org.tr /tchd.

Scientific and ethical responsibility

When submitting a manuscript to the Turkish Journal of Pediatric Disease, authors should accept to assign the copyright of their manuscript to the Turkish Journal of Pediatric Disease. If authors rejected for publication, the copyright of the manuscript will be assigned back to the authors. The Turkish Journal of Pediatric Disease requires each submission to be accompanied by a Copyright Transfer and Acknowledgement of Authorship Form (available for download at https://dergipark.org.tr/en/pub/tchd). When using previously published content including figures, tables, or any other material in both of the 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).

Türkiye Çocuk Hastalıkları Dergisi, Ankara Bilkent Şehir Hastanesi Çocuk Hastanesi'nin açık erişimli bilimsel yayındır. Dergi bağımsız, tarafsız ve çift-kör hakemlik ilkelerine uygun olarak yayınlanır. Dergi iki ayda bir yayınlanmaktadır (Ocak Mart, Mayıs, Temmuz, Eylül, Kasım).

Türkiye Çocuk Hastalıkları Dergisi'nde orijinal makale, derleme, olgu sunumu, editöryal, çalışma yöntemi, kısa rapor, kitap incelemeleri, biyografiler ve editöre mektup yayınlanmaktadır. Ayrıca pedatrik cerrahi, diş hekimliği, halk sağlığı, genetik, çocuk ve ergen psikiyatrisi ve hemşirelik konularında makaleler yayınlanabilir. Türkiye Çocuk Hastalıkları Dergisi'nin yayın dili İngilizcedir.

Türkiye Çocuk Hastalıkları Dergisi'ne gönderilen yazılar çift kör hakemlik sürecinden geçecektir. Her bir yazı tarafsız bir değerlendirme süreci sağlamak için alanda uzman en az iki harici, bağımsız hakem tarafından incelenecektir. Baş editör, tüm başvurular için karar alma sürecindeki nihai otoritedir.

Türkiye Çocuk Sağlığı ve Hastalıkları Dergisi Tübitak/Ulakbim Türk Tıp Dizini, EBSCOhost Research Databases ve Türkiye Atıf Dizini tarafından indekslenmektedir.

Dergimiz asitsiz kâğıda basılmaktadır.

Abonelik İşlemleri

Türkiye Çocuk Hastalıkları Dergisi Tıp Fakültesi olan Üniversite Kütüphanelerine, Çocuk Sağlığı ve Hastalıkları AD Başkanlıkları'na, Sağlık Bakanlığı'na bağlı Eğitim ve Araştırma Hastanelerinin Başhekimlikleri'ne ve konu ile ilgilenen bilim insanlarına ücretsiz olarak ulaştırılmaktadır. Yayınlanan makalelerin tam metinlerine https://dergipark.org.tr/tr/pub/tchd adresinden ücretsiz olarak ulaşılabilir. Abonelik işlemleri için lütfen https://dergipark.org.tr/tr/pub/tchd adresini ziyaret ediniz.

Yazarların Bilimsel ve Hukuki Sorumluluğu

Yazarlar Türkiye Çocuk Hastalıkları Dergisi'ne bir yazı gönderirken, yazıların telif haklarını Türkiye Çocuk Hastalıkları Dergisi'ne devretmiş olmayı kabul ederler. Yayınlanmamak üzere reddedilirse veya herhangi bir sebepten yazı geri çekilirse telif hakkı yazarlara geri verilir. Türk Türkiye Çocuk Hastalıkları Dergisi'ne ait Telif Hakkı Devri ve Yazarlık Formları (https://dergipark.org.tr/tr/pub/tchd adresinden indirilebilir). Şekiller, tablolar veya diğer basılı materyaller de dahil olmak üzere basılı ve elektronik formatta daha önce yayınlanmış içerik kullanılıyorsa yazarlar telif hakları sahiplerinden gerekli izinleri almalıdır. Bu konudaki hukuki, finansal ve cezai yükümlülükler yazarlara aittir.

INSTRUCTIONS FOR AUTHORS

The Turkish Journal of Pediatric Disease is an open access and a scientific publication journal that is published from the Ankara City Hospital, Children's Hospital. The journal is published in accordance with independent, unbiased, and double-blind peer review principles. The journal is published bimonthly (January, March, May, July, September, November)

In the Turkish Journal of Pediatric Disease original articles, reviews, case reports, editorials, short reports, book reviews, biographies and letters to the editor are also published in the journal. Besides if related with pediatrics, articles on aspects of pedatric surgery, dentistry, public health, genetics, pshyciatrics and nursery could be published. The publication language of Turkish Journal of Pediatric Disease is English.

The editorial and the publication processes of the journal are shaped in accordance with the guidelines of the World Association of Medical Editors (WAME), the Committee on Publication Ethics (COPE), the International Council of Medical Journal Editors (ICMJE), the Council of Science Editors (CSE), 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 (doaj.org/bestpractice).

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

Manuscripts submitted to the Turkish Journal of Pediatric Disease will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in the field, in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions. Articles accepted for publication in the Turkish Journal of Pediatrics are put in the order of publication, with at least 10 articles in each issue, taking into account the acceptance dates. If the articles sent to the reviewers for evaluation are assessed as a senior for publication by the reviewers, the section editor and the editor considering all aspects (originality, high scientific quality and citation potential), it receives publication priority in addition to the articles assigned for the next issue.

An approval of the research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for the experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts that are concerning experimental researchs on humans, a statement should be informed included that shows a written informed consent of the patients and the volunteers who were given a detailed explanation of the procedures that they may undergo. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information of the patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity carefully. For the photographs that may reveal the identities of the patients, releases signed by the patient or their legal representative should be enclosed.

A similarity report in terms of plagiarism will be requested in accordance with the agreement between DergiPark and intihal.net for all manuscript submissions. Authors will be informed during the submission process and the system will prepare a report during the file upload step and the result will be sent to the author via e-mail. The author will be able to complete the submission process at this stage. In order to submit a manuscript to the Turkish Journal of Pediatric Disease, the similarity rate should be maximum 20%.

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 the COPE guidelines.

Each individual 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 should be based on the following 4 criteria:

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

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

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

4. Agreement to be accountable of all aspects of the work in ensuring that questions related to the accuracy or the 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 had done, an author should be able to identify which co-authors are responsible for the specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

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

The Turkish Journal of Pediatric Disease requires corresponding authors to submit a signed and scanned version of the authorship contribution form (available for download through https://dergipark.org.tr/en/pub/tchd) 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 a further review. As a part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all of the responsibility for the authorship during the submission and review stages of the manuscript.

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

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

When submitting a manuscript to the Turkish Journal of Pediatric Disease, authors should accept to assign the copyright of their manuscript to the Turkish Journal of Pediatric Disease. If authors rejected for publication, the copyright of the manuscript will be assigned back to the authors. The Turkish Journal of Pediatric Disease requires each submission to be accompanied by a Copyright Transfer

and Acknowledgement of Authorship Form (available for download at https://dergipark.org.tr/en/pub/tchd). When using previously published content including figures, tables, or any other material in both of the 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).

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

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

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with the ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in May 2022 - http://www.icmje.org/recommendations).

CONSORT	Randomised controlled trials
STROBE	Observational epidemiological research
STARD	Diagnostic accuracy
PRISMA	Systematic reviews and meta-analysis
ARRIVE	Experimental animal studies
TREND	Non-randomized public behavior

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at

www.dergipark.org.tr/en/journal/2846/submission/step/manuscript/new. Manuscripts submitted via any other medium will not be evaluated.

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

Authors are required to submit the following:

Copyright Transfer and Acknowledgement of Authorship Form and

ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all of the contributing authors) during the initial submission. These forms are available for downloading at www.dergipark.org.tr/en/pub/tchd.

Manuscripts should be written using Microsoft Word™ (2010 and higher) software, in Times New Roman, 12 point size and double line spacing. There should be 2 cm margins on all sides on the pages. "System International" (SI) units should be used in manuscripts. Tables and graphics should be cited in the text. Abbreviations can be used provided that they are written openly at the first place they appear in the abstract and text, and the abbreviation is given in parentheses.

In the article, when giving the mean and percentile, 2 digits should be used after the decimal point (such as 231.69 or 231.70, instead of 231.7). In the representations other than integers, two digits should be written after the dot, and in the representation of statistical values (such as p, r, t, z values), three digits should be written after the dot. In the presentation of p values, instead of $p<0.05$ or $p>0.05$, the full p value should be given with three digits after the dot (eg p=0.029) with the test statistic. If this value is less than one thousandth, it should be displayed as $p<0.001$.

Preparation of the Manuscript Title page:

Title page should be submitted for all of the submissions and this page should include:

Title page of the manuscript should include the English title of the article. The title page should include the authors' names, degrees, ORCID number and the institutional/professional affiliations, a short title (max 50 character), abbreviations, financial disclosure statement, and the conflict of interest statement. For manuscripts sent by the authors in Türkiye, a title in Turkish is also required. If a manuscript includes authors from more than one institution, each author's name should be followed by a superscript number that corresponds to this/her institution, which is listed separately. Please provide a contact information for the corresponding author, including name, e-mail address, and telephone and fax numbers.

Important Notice: The title page should be submitted separately.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html). For manuscripts sent by the authors in Türkiye, key words in Turkish are also required.

MANUSCRIPT TYPES

Original Articles:

Word count: up to 3,500 (Introduction, Methods, Results, Discussion)

Title: maximum of 20 words

Structured abstract: up to 250 (Objective, Materials and Methods, Results and Conclusion)

Keywords: 3-6 word, listed in alphabetical order.

Figures and tables: are not limited, but must be justified thoroughly

References: It should be at least 20 and at most 40.

Original articles should include; English title, English structured abstract (structured as, English key words. If the article is in Turkish, Turkish title and English title, Turkish structured summary and English summary (structured as Purpose, Material and Method, Conclusion and Discussion), Turkish and English keywords are required.

for most readers, reading the abstract first, is critically important. Moreover, various electronic databases integrate only abstracts into their index, so important findings should be presented in the abstract.

The other sections of the manuscript should include Introduction, Materials and Methods, Results, Discussion, Acknowledgement (if required) and References. All sections of the manuscripts should start on a new page.

Review Articles:

Word count: up to 5000

Abstract: up to 500 (Objective, Materials and Methods, Results and Conclusion)

Keywords: 3-6 word, listed in alphabetical order.

Figures and tables: are not limited, but must be justified thoroughly

References: up to 80

Review articles are comprehensive analyses of the specific topics in medicine, which are written upon the invitation due to extensive experience and publications of authors on the review subjects. All invited review articles will also undergo peer review prior to the acceptance.

Review articles should include; English title, English abstract and English key words. For manuscripts sent by authors in Türkiye, a Turkish title, Turkish abstract and Turkish key words are also required.

Case Reports:

Word count: up to 2000

Abstract: up to 200

Keywords: 3-6 word, listed in alphabetical order.

Figures and tables: total 5

References: up to 15

There is a limited space for the case reports in the journal and reports on rare cases or conditions that constitute challenges in the diagnosis and the treatment, those offering new therapies or revealing knowledge that are not included in the literature, and interesting and educative case reports are being/ will be accepted for publication. The text should include Introduction, Case Presentation and Discussion.

Case reports should include; English title, English abstract and English key words. For manuscripts sent by authors in Türkiye, a Turkish title, Turkish abstract and Turkish key words are also required.

Letters to the Editor:

Word count: up to 1500

Figures and tables: total 3

References: up to 15

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

Letters to the Editor should include; English title. For the letter to the editor sent by authors in Türkiye, a Turkish title also required.

Study Protocols:

The Turkish Journal of Pediatric Disease welcomes study protocols to improve the transparency of research and inform the scholarly community about the trials that are being underway. Publication decision of study protocols will be by editorial decision. Study protocols for the pilot or feasibility studies are not generally taken into consideration.

Study protocol articles should follow the SPIRIT guidelines that provides a detailed account of the hypothesis, rationale, and methodology of the study. All study protocols must provide an Ethics Committee Approval. All protocols for the clinical trials require a trial registration number and the date of registration.

Tables

Tables should be included in the main document, presenting after the reference list, and they should be numbered consecutively in the order they are referred in the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by the footnotes (even if they were defined within the main text). Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text. The following symbols should be used for abbreviations in sequence: *, †, ‡, §, ||, ¶, **, ††, ‡‡.

Figures and Figure Legends

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

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

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses as in the following format: The skin prick tests were performed using a multi-prick test device (Quantitest, Panatrex Inc, Placentia, California, USA).

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

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

REFERENCES

While citing publications, the preference should be given to the latest, most up-to-date publications. Authors should avoid using references that are older than ten years. The limit for the old reference usage is 20% in the journal. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of the references. Reference numbers should be indicated at the end of the sentences in parentheses and references should be numbered consecutively in the order that they are mentioned in the text. Journal names should be abbreviated as listed in "Index Medicus" or in "ULAKBIM/Turkish Medical Index". References should be typed in consistence with the following examples. Native references should be used as much as possible.

If the reference is a journal;

Author(s)' surname and initial(s) of the first name (all authors if the number of authors are 6 or less, first 6 authors if the number of authors of an article is more than 6 followed by "ve ark." in Turkish references and "et al." in international references). Title of the article, title of the manuscript abbreviated according to Index Medicus

(http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi?db=nlmcatalog). Year;Volume:First and last page number.

Example: Benson M, Reinholdt J, Cardell LO. Allergen-reactive antibodies are found in nasal fluids from patients with birch polen-induced intermittent allergic rhinitis, but not in healthy controls. Allergy 2003;58:386-93.

If the reference is a journal supplement;

Author(s)' surname and initial(s) of the first name. Title of the article. Title of the manuscript abbreviated according to Index Medicus (http://www.ncbi.nlm.nih.gov/sites/entrez/query. fcgi?db =nlmcatalog). Year;Volume (Suppl. Supplement number): First and last page number.

Example: Queen F. Risk assessment of nickel carcinogenicity and occupational lung cancer. Envirol Health Perspect 1994;102 (Suppl. 1):S2755-S2782.

If the reference is a book;

Author(s)' surname and initial(s) of the first name. Title of the book. Edition number. City of publication; Publisher, Year of Publication.

Example: Ringsven MK, Bond N. Gerontology and leadership skills for nurses. 2nd ed. Albany, NY: Delmar Publishers, 1996.

If the reference is a book chapter;

Surname and initial(s) of the first name of the author(s) of the chapter. Title of the chapter. In: Surname and initial(s) of the first name(s) of

the editor(s) (ed) or (eds). Title of the book. Edition number. City of publication: Publisher, Year of publication: First and last page numbers of the chapter.

Example: Phillips SJ, Whistant JP. Hypertension and stroke. In: Laragh JH, Brenner BM (eds). Hypertension: Pathophysiology, Diagnosis and Management. 2nd ed. New York: Raven P, 1995:466-78.

If the reference is a conference paper presented in a meeting;

Author(s)' surname and initial(s) of the first name (all authors if the number of authors are 6 or less, first 6 authors if the number of authors of a conference paper is more than 6 followed by "et al.". Title of the conference paper, If applicable In: Surname and initial(s) of the first name(s) of the editor(s) (ed) or (eds). Title of the abstract book. Title of the meeting; Date; City of the meeting; Country. Publisher; Year: Page numbers.

Example: Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Reinhoff O (eds). MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. North-Holland; 1992: 1561-5.

If the reference is an online journal:

Author(s)' surname and initial(s) of the first name (all authors if the number of authors are 6 or less, first 6 authors if the number of authors of an article is more than 6 followed by "ve ark." in Turkish references and "et al." in international references).Title of the article, title of the manuscript abbreviated according to Index Medicus Year; Volume (Number). Available from:URL address. Accessed date:day.month. year.

Example: Arrami M, Garner H. A tale of two citations. Nature 2008;451(7177): 397-9. Available from: URL:www.nature.com/nature/journal/v451/n7177/full/451397a.html. Aaccessed 20 January 2008.

If the reference is a website:

Name of the web site. Access date. Available from: address of the web site.

Example: Centers for Disease Control and Prevention (CDC). Acsess date: 12 March 2013. Available from: http://www.cdc.gov/

If the reference is a thesis:

Author's surname and initial of the first name. Title of the thesis (thesis). City; Name of the university (if it is a university); Year.

Example: Özdemir O. Fibrillin-1 gene polymorhism and risk of mitral valve disorders. (Thesis). *Ankara*: Gazi University, 2006.

REVISIONS

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

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

CHANGE OF AUTHORSHIP AND WITHDRAWAL REQUEST

Change of Authoship

Any request to change the author list after submission, such as a change in the order of the authors or the deletion or the addition of author names, is subject to the Editorial Board's approval. To obtain this approval, please find and complete the change of authorship form on the Journal's website and send it to the Journal's office. This form should include the following information: The reason for the change of authorship signatures of all authors (including the new and/or removed author)

Please note, if you are adding or removing author/authors, a new copyright transfer form signed by all authors should also be sent to the editorial office after the Editorial Board approves the change of the authorship.

Withdrawal Policy

Turkish Journal of Pediatric Disease is committed to provide high quality articles and uphold the publication ethics to advance the intellectual agenda of science. We expect our authors to comply mbestly with the practice in publication ethics as well as in the quality of their articles.

Withdrawal of a manuscript will be permitted only for the most compelling and unavoidable reasons. For the withdrawal of a manuscript, authors need to submit an "Article withdrawal Form", signed by all of the authors mentioning the reason for withdrawaling to the Editorial Office. The form is available at the web page of the journal. Authors must not assume that their manuscript has been withdrawn until they have received appropriate notification to this effect from the editorial office.

In a case where a manuscript has taken more than six months' time for the review process, that this allows the author for withdrawing the manuscript.

YAZARLAR İÇİN BİLGİ

Türkiye Çocuk Hastalıkları Dergisi, Ankara Şehir Hastanesi Çocuk Hastanesi'nin açık erişimli bilimsel yayındır. Dergi bağımsız, tarafsız ve çift-kör hakemlik ilkelerine uygun olarak yayınlanır. Dergi iki ayda bir yayınlanmaktadır (Ocak Mart, Mayıs, Temmuz, Eylül, Kasım)

Türkiye Çocuk Hastalıkları Dergisi'nde orijinal makale, derleme, olgu sunumu, editöryal, çalışma yöntemi, kısa rapor, kitap incelemeleri, biyografiler ve editöre mektup yayınlanmaktadır. Ayrıca pedatrik cerrahi, diş hekimliği, halk sağlığı, genetik, çocuk ve ergen psikiyatrisi ve hemşirelik konularında makaleler yayınlanabilir. Türkiye Çocuk Hastalıkları Dergisi'nin yayın dili İngilizcedir.

Derginin yayın ve yayın süreçleri, Dünya Tıbbi Editörler Derneği (World Association of Medical Editors (WAME)), Yayın Etiği Komitesi (Committee on Publication Ethics (COPE)), Uluslararası Tıbbi Dergi Editörleri Konseyi (International Council of Medical Journal Editors (ICMJE)), Bilim Editörleri Konseyi (Council of Science Editors (CSE)), Avrupa Bilim Editörleri Birliği (EASE) ve Ulusal Bilgi Standartları Organizasyonu (National Information Standards Organization (NISO) (NISO)) kurallarına uygun olarak şekillendirilmiştir. Dergi, Bilimsel Yayıncılıkta Şeffaflık ve En İyi Uygulama İlkeleri'ne (Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice)) uygundur.

Yazıların yayına kabulü için en önemli kriterler özgünlük, yüksek bilimsel kalite ve atıf potansiyelidir. Değerlendirme için gönderilen yazılar daha önce elektronik veya basılı bir ortamda yayınlanmamış olmalıdır. Dergi, değerlendirilmek üzere başka bir dergiye gönderilen ve reddedilen yazılar hakkında bilgilendirilmelidir. Önceki inceleme raporlarının sunulması değerlendirme sürecini hızlandıracaktır. Kongre ve toplantılarda sunulan yazılarda yazının sunulduğu toplantının kongrenin adı, tarihi ve yeri de dahil olmak üzere ayrıntılı bilgi ile birlikte sunulmalıdır.

Türkiye Çocuk Hastalıkları Dergisi'ne gönderilen yazılar çift kör hakemlik sürecinden geçecektir. Her bir yazı tarafsız bir değerlendirme süreci sağlamak için alanda uzman en az iki harici, bağımsız hakem tarafından incelenecektir. Baş editör, tüm başvurular için karar alma sürecindeki nihai otoritedir. Türkiye Çocuk Hastalıkları Dergisinde yayınlanmak üzere kabul edilmiş makaleler kabul tarihleri dikkate alınarak her sayıda en az 10 makale olacak şekilde yayın sırasına alınır. Değerlendirilmek üzere hakemlere gönderilen makaleler tüm yönleri (özgünlük, yüksek bilimsel kalite ve atıf potansiyeli) dikkate alınarak hakemler, alan editörü ve editör tarafından öncelikli olarak yayınlanmaya aday bir makale olarak değerlendirilir ise bir sonraki sayıda o sayı için atanmış makalelere ek olarak yayınlanma önceliği alır.

Yazarlardan deneysel, klinik ve ilaç çalışmaları ve bazı vaka raporları için gerekirse, etik kurul raporları veya eşdeğer bir resmi belge istenecektir. İnsanlar üzerinde yapılan deneysel araştırmalarla ilgili yazılar için, hasta ve gönüllülerin yazılı bilgilendirilmiş olurlarının alınabileceği prosedürlerin ayrıntılı bir açıklamasının ardından elde edildiğini gösteren bir ifade eklenmelidir. Hayvanlar üzerinde yapılan çalışmalarda, hayvanların acı ve ıstıraplarını önlemek için alınan önlemler açıkça belirtilmelidir. Hasta onamı, etik komite adı ve etik komite onay numarası hakkında bilgi de makalenin Materyal-Metod bölümünde belirtilmelidir. Hastaların anonimliklerini dikkatlice korumak yazarların sorumluluğundadır. Hastaların kimliğini ortaya çıkarabilecek fotoğraflar için, hasta veya yasal temsilcisi tarafından imzalanan bültenler eklenmelidir.

Tüm makale başvurularında DergiPark ile intihal.net ile arasında yapılan işbirliği uyarınca intihal açısından benzerlik raporu istenecektir. Makale gönderim adımlarında yazarlar bilgilendirlecek ve dosya yükleme adımında sistem tarafından rapor hazırlanarak sonuç e-posta ile yazara bildirlecektir. Rapor açıklandığında yazar gönderim işlemini tamamlayabilecektir. Türkiye Çocuk Hastalıkları Dergisi'ne makale gönderebilmek için benzerlik oranı en fazla %20 olmalıdır.

İntihal, atıf manipülasyonu ve gerçek olmayan verilerden şüphelenilmesi veya araştırmaların kötüye kullanılması durumunda, yayın kurulu COPE yönergelerine uygun olarak hareket eder.

Yazar olarak listelenen her bireyin Uluslararası Tıp Dergisi Editörleri Komitesi (ICMJE - www.icmje.org) tarafından önerilen yazarlık kriterlerini karşılaması gerekir. ICMJE yazarlığın aşağıdaki 4 kritere dayanmasını önerir:

1. Çalışmanın tasarımı, verilerin elde edilmesi, analizi veya yorumlanması

2. Dergiye gönderilecek kopyanın hazırlanması veya bu kopyayının içeriğini bilimsel olarak etkileyecek ve ileriye götürecek şekilde katkı sağlanması

3. Yayınlanacak kopyanın son onayı.

4. Çalışmanın tüm bölümleri hakkında bilgi sahibi olma ve tüm bölümleri hakkında sorumluluğu alma

Bir yazar, yaptığı çalışmanın bölümlerinden sorumlu olmanın yanı sıra, çalışmanın diğer belirli bölümlerinden hangi ortak yazarların sorumlu olduğunu bilmeli ayrıca yazarlar, ortak yazarlarının katkılarının bütünlüğüne güvenmelidir.

Yazar olarak atananların tümü yazarlık için dört kriteri de karşılamalı ve dört kriteri karşılayanlar yazar olarak tanımlanmalıdır. Dört kriterin tümünü karşılamayanlara makalenin başlık sayfasında teşekkür edilmelidir.

Yazı gönderim aşamasında ilgili yazarların, yazarlık katkı formunun imzalı ve taranmış bir versiyonunu (https://dergipark.org.tr/en/pub/tchd adresinden indirilebilir) Türkiye Çocuk Hastalıkları Dergisi'ne göndermesini gerektirir. Yayın kurulu yazarlık şartarını karşılamayan bir kişinin yazar olarak eklendiğinden şüphe ederse yazı daha fazla incelenmeksizin reddedilecektir. Makalenin gönderilmesi aşamasında bir yazar makalenin gönderilmesi ve gözden geçirilmesi aşamalarında tüm sorumluluğu üstlenmeyi kabul ettiğini bildiren kısa bir açıklama göndermelidir.

Türkiye Çocuk Hastalıkları Dergisi'ne gönderilen bir çalışma için bireylerden veya kurumlardan alınan mali hibeler veya diğer destekler Yayın Kuruluna bildirilmelidir. Potansiyel bir çıkar çatışmasını bildirmek için, ICMJE Potansiyel Çıkar Çatışması Bildirim Formu, katkıda bulunan tüm yazarlar tarafından imzalanmalı ve gönderilmelidir. Editörlerin, yazarların veya hakemlerin çıkar çatışması olasılığı, derginin Yayın Kurulu tarafından COPE ve ICMJE yönergeleri kapsamında çözümlenecektir.

Derginin Yayın Kurulu, tüm itiraz durumlarını COPE kılavuzları kapsamında ele almaktadır. Bu gibi durumlarda, yazarların itirazları ile ilgili olarak yazı işleri bürosu ile doğrudan temasa geçmeleri gerekmektedir. Gerektiğinde, dergi içinde çözülemeyen olayları çözmek için bir kamu denetçisi atanabilir. Baş editör itiraz durumlarında karar alma sürecinde alınacak kararlarla ilgili nihai otoritedir.

Yazarlar Türkiye Çocuk Hastalıkları Dergisi'ne bir yazı gönderirken, yazıların telif haklarını Türkiye Çocuk Hastalıkları Dergisi'ne devretmiş olmayı kabul ederler. Yayınlanmamak üzere reddedilirse veya herhangi bir sebepten yazı geri çekilirse telif hakkı yazarlara geri verilir. Türk Türkiye Çocuk Hastalıkları Dergisi'ne ait Telif Hakkı Devri ve Yazarlık Formları (https://dergipark.org.tr/tr/pub/tchd adresinden indirilebilir). Şekiller, tablolar veya diğer basılı materyaller de dahil olmak üzere basılı ve elektronik formatta daha önce yayınlanmış içerik kullanılıyorsa yazarlar telif hakları sahiplerinden gerekli izinleri almalıdır. Bu konudaki hukuki, finansal ve cezai yükümlülükler yazarlara aittir.

Yazıların sonuçlarının rapor edilemesi sırasında genellikle istatistiksel analizler gereklidir. İstatistiksel analizler uluslararası istatistik raporlama standartlarına uygun olarak yapılmalıdır (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Tıp dergilerine katkıda bulunanlar için istatistiksel yönergeler. Br Med J 1983: 7; 1489-93). İstatistiksel analizler hakkında bilgi, Materyal ve Metot bölümünde ayrı bir alt başlık ile açıklanmalı ve bu süreçte kullanılan istatistiksel yazılımlar mutlaka belirtilmelidir.

Türkiye Çocuk Hastalıkları Dergisi'nde yayınlanan yazılarda belitilen ifade veya görüşler, editörlerin, yayın kurulunun veya yayıncının görüşlerini yansıtmaz; editörler, yayın kurulu ve yayıncı bu tür materyaller için herhangi bir sorumluluk veya yükümlülük kabul etmez. Yayınlanan içerikle ilgili nihai sorumluluk yazarlara aittir.

YAZININ HAZIRLANMASI

Yazılar, Tıbbi Çalışmalarda Bilimsel Çalışmanın Yürütülmesi, Raporlanması, Düzenlenmesi ve Yayınlanması için Uluslararası Tıbbi Dergi Editörleri Konseyi (International Council of Medical Journal Editors (ICMJE)) Önerileri'ne uygun olarak hazırlanmalıdır (Aralık 2019'da güncellenmiştir - http://www.icmje.org/icmje-recommendations). Bu liste aşağıda görülebilir.

CONSORT	Randominize kontrollü çalışma
STROBE	Gözlemsel epidemiyolojik çalışmalar
STARD	Tanı yöntemleri
PRISMA	Sistemetik derleme ve metaanaliz
ARRIVE	Deneysel hayvan çalışmaları
TREND	Randomize olmayan tutum ve davranış çalışmaları

Yazılar yalnızca derginin çevrimiçi (online) makale gönderme ve değerlendirme sistemi aracılığıyla gönderilebilir.

https://dergipark.org.tr/tr/journal/2846/submission/step/manuscript/new Başka herhangi bir araç aracılığıyla gönderilen yazılar değerlendirmeye alınmayacaktır.

Dergiye gönderilen yazılar öncelikle sekreterlik tarafından yazının

derginin kurallarına uygun olarak hazırlanıp hazırlanmadığı yönünden teknik bir değerlendirme sürecinden geçecektir. Derginin yazım kurallarına uymayan yazılar, düzeltme talepleriyle birlikte gönderen yazara iade edilecektir.

Yazarların yazıları hazırlarken ve sisteme yüklerken aşağıdaki konulara dikkat etmesi gerekmektedir:

Telif Hakkı Devri ve Yazarlık Formunun Kabulü ve ICMJE tyarafından önerilen Potansiyel Çıkar Çatışması Bildirim Formu İlk başvuru sırasında (katkıda bulunan tüm yazarlar tarafından doldurulmalıdır) sisteme yüklenmelidir. Bu formları www.dergipark.org.tr/tr/pub/tchd adresinden indirebilirsiniz.

Yazılar, Microsoft Word™ (2010 ve üstü) yazılım programı kullanılarak, Times New Roman karakterinde, 12 punto büyüklüğünde ve çift satır aralığı ile yazılmalıdır. Sayfalarda her yönden 2 cm boşluk bırakılmalıdır. Yazılarda "System International" (SI) birimleri kullanılmalıdır. Tablo ve grafiklere metin içinde atıf yapılmalıdır. Kısaltmalar öz ve metinde ilk geçtikleri yerde açık yazılıp, parantez içinde kısaltma verilmek kaydıyla kullanılabilirler.

Makale içinde, ortalama ve yüzdelik verilirken, ondalıklı hanelerin gösteriminde noktadan sonra 2 basamak kullanılması gerekmektedir (231.7 yerine; 231.69 veya 231.70 gibi). Tam sayı dışındaki gösterimlerde noktadan sonra iki hane, istatistiksel değerlerin gösteriminde ise (p, r, t, z değerleri gibi) noktadan sonra üç hane yazılması gerekir. p değerlerinin sunumunda $p<0.05$ veya $p>0.05$ yerine test istatistiği ile birlikte tam p değerinin noktadan sonra üç hane içerek şekilde verilmesi (ör: $p=0.029$) gerekmektedir. Bu değerin binde birden küçük olması durumunda $p<0.001$ şeklinde gösterim yapılmalıdır.

Kapak sayfasının hazırlanması:

Kapak sayfası tüm yazılarla birlikte gönderilmeli ve bu sayfa şunları içermelidir:

Yazının kapak sayfasında yazının İngilizce başlığı bulunmalıdır. Kapak sayfası yazarların adlarını, akademik ünvanlarının, ORCID numaralarını, kurumsal/mesleki bağlantılarını, yazının kısa başlığını (en fazla 50 karakter), kısaltmaları, finansal açıklama bildirimini ve çıkar çatışması bildirimini içermelidir. Yazı Türkiye'de bulunan bir merkez tarafından gönderilmişse yazılar için Türkçe bir başlık da gereklidir. Bir yazı birden fazla kurumdan yazar içeriyorsa, her yazarın adını, ayrı olarak listelenen kurumlarına karşılık gelen bir üst simge numarası izlemelidir. Tüm yazarlar için için isim soy isim, e-posta adresi, telefon ve faks numaraları dahili iletişim bilgileri verilmelidir. Ayrıca yazı ile ilgili olrak iletişim kurulacak sorumlu sorumlu yazarın kim olduğu belirtilmelidir.

Önemli Uyarı: Kapak sayfası ayrı bir belge olarak yüklenmelidir.

Anahtar kelimeler:
Özetin sonunda konu indeksleme için her gönderime en az üç en fazla altı anahtar kelime eklenmelidir. Anahtar kelimeler kısaltma olmadan tam olarak listelenmelidir. Anahtar kelimeler "National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html)" veritabanından seçilmelidir. Yazı Türkiye'de bulunan bir merkez tarafından gönderilmişse Türkçe anahtar kelimeler de gereklidir.

Yazı türleri:

Orijinal araştırma makalesi

Kelime sayısı: En çok 3500 kelime (Başlık, özet, anahtar kelimeler, kaynaklar, tablo ve figür yazıları hariç).

Ana metnin içereceği bölümler: Giriş, Yöntemler, Sonuçlar, Tartışma

Başlık: En çok 20 kelime

Yapısal özet: En çok 250 kelime. Bölümler: Amaç, Gereç ve Yöntem, Sonuçlar ve Tartışma

Anahtar kelimeler: En az 3 en fazla altı kelime, alfabetik olarak sıralanmıştır.

Şekiller ve tablolar: Sayı sınırı yok ancak tam olarak gerekçelendirilmeli ve açıklayıcı olmalıdır.

Referanslar: En az 20, en çok 40 olmalıdır.

Orijinal makaleler; İngilizce başlık, İngilizce yapılandırılmış özet (yapılandırılmış, İngilizce anahtar kelimeler. Yazı Türkiye'de bulunan bir merkez tarafından gönderilmişse Türkçe başlık, Türkçe yapılandırılmış özet (Amaç, Gereç ve Yöntem, Sonuç ve Tartışma olarak yapılandırılmıştır) ve Türkçe anahtar kelimeler de gereklidir.

Çoğu okuyucu ilk olarak başlık ve özeti okuduğu için bu bölümler kritik öneme sahiptir. Ayrıca, çeşitli elektronik veritabanları yazıların sadece özetlerini indeksledikleri için özette önemli bulgular sunulmalıdır.

Makalenin diğer bölümleri Giriş, Gereç ve Yöntemler, Sonuçlar, Tartışma, Teşekkür (gerekirse) ve Kaynaklar'dan oluşmalıdır. Makalelerin tüm bölümleri yeni bir sayfada başlamalıdır.

Derleme:

Kelime sayısı: En fazla 5000

Özet: En fazla 500 kelime

Anahtar kelimeler: En az üç en fazla altı kelime, alfabetik olarak sıralanmıştır.

Şekiller ve tablolar: Sayı sınır yok ancak tam olarak gerekçelendirilmeli ve açıklayıcı olmalıdır.

Referanslar: 80'e kadar

Derleme makaleleri, tıptaki belirli konuların kapsamlı olarak gözden geçirildiği, konunun tarihsel gelişimini, mevcut bilinenleri, araştırıma ihtiyacı olan alanları içeren yazılarır. Konu hakkında orijinal araştırmaları yazarlar tarafından yazılmalıdır. Tüm derleme yazıları kabulden önce diğer yazılara eşdeğer değerlendirme süreçlerine tabi tutulacaktır.

Derleme makaleleri şunları içermelidir; İngilizce başlık, İngilizce özet ve İngilizce anahtar kelimeler. Derleme Türkiye'de bulunan bir merkez tarafından gönderilmişse Türkçe başlık, Türkçe özet ve Türkçe anahtar kelimeler de gerekmektedir.

Olgu Sunumu:

Kelime Sayısı: En fazla 2000 kelime

Özet: En fazla 200 kelime

Anahtar Kelime: En az üç en fazla altı kelime

Tablo ve Şekil: Toplamda en fazla beş ile sınırlandırılmıştır.

Referans: En fazla 15

Dergiye sınırlı sayıda olgu sunumu kabul edilmektedir. Olgu sunumlarının tanı ve tedavide zorluk oluşturan, nadir, literatürde yer almayan yeni tedaviler sunan ilginç ve eğitici olguların seçilmesine dikkat edilmektedir. Olgu sunumu giriş, olgu sunumu ve tartışma içermelidir.

Olgu sunumları şunları içermelidir; İngilizce başlık, İngilizce özet ve İngilizce anahtar kelimeler. Türkiye'de bulunan bir merkez tarafından gönderilmişse Türkçe başlık, Türkçe özet ve Türkçe anahtar kelimeler de gereklidir.

Editöre mektup:

Kelime sayısı: En fazla 1500 kelime

Şekil ve tablolar: En fazla 3

References: En fazla 15

Editöre mektup daha önce yayınlanmış bir makalenin önemli

bölümlerini, gözden kaçan yönlerini veya eksik bölümlerini tartışır. Dergi kapsamında okurların dikkatini çekebilecek konularda, özellikle eğitici vakalarda yer alan yazılarda editöre mektup şeklinde de gönderilebilir. Okuyucular ayrıca yayınlanan yazılar hakkındaki yorumlarını editöre mektup şeklinde sunabilirler. Bir özet ve Anahtar Kelimeler dahil edilmemelidir. Tablo, şekil, görüntü içerebilir. Metin alt başlıkları içermemelidir. Yorum yapılan makaleye bu yazının içinde uygun şekilde atıfta bulunulmalıdır.

Editöre mektuplar; İngilizce başlık. Türkiye'de bulunan bir merkez tarafından gönderilmişse editör mektubu için Türkçe bir başlık da gerekmektedir.

Çalışma Metodları:

Türkiye Çocuk Hastalıkları Dergisi araştırmanın şeffaflığını artırmak ve devam etmekte olan araştırmalar hakkında ilgili kişileri bilgilendirmek için çalışma metodları yayınlamaktadır. Çalışma metodlarının yayın kararı editör tarafından verilmektedir. Pilot çalışmaların veya fizibilite çalışmalarının metodları genellikle yayınlanmamaktadır.

Çalışma metodları yazıları, çalışmanın hipotezi, gerekçesi ve metodolojisi hakkında ayrıntılı bir açıklama sunan SPIRIT yönergelerine uymalıdır. Tüm çalışmalar için etik kurul onayı alınmış olmalıdır. Klinik araştırmalar için tüm protokoller, araştırma kayıt numarasını ve kayıt tarihi verilmelidir.

Tablolar

Tablolar, referans listeden sonra ana belgeye dahil edilmelidir ana metin içine yerleştirilmemelidir. Ana metinde atıfta bulundukları sırayla numaralandırılmalıdır. Tabloların üzerine açıklayıcı bir başlık konulmalıdır. Tablolarda kullanılan kısaltmalar ana metinde tanımlansalar bile tabloların altında dipnotlarla tanımlanmalıdır. Tablolarda sunulan veriler, ana metinde sunulan verilerin tekrarı olmamalı, ancak ana metni desteklemelidir. Kısaltmalar için aşağıdaki semboller sırayla kullanılmalıdır: *, †, ‡, §, ||, ¶, **, ††, ‡‡.

Şekiller ve şekil alt yazıları

Şekiller, grafikler ve fotoğraflar, gönderim sistemi aracılığıyla ayrı dosyalar (TIFF veya JPEG formatında) olarak gönderilmelidir. Dosyalar bir Word belgesine veya ana metne yerleştirilmemlidir. Şekil alt birimleri olduğunda, alt birimler tek bir görüntü oluşturacak şekilde birleştirilmemeli, her alt birim, başvuru sistemi aracılığıyla ayrı ayrı yüklenmelidir. Resimlerin üzerine etiketleme (örneğin a,d,c,d gibi) yapılmamalıdır. Şekil altyazılarını desteklemek için görüntülerde kalın ve ince oklar, ok uçları, yıldızlar, yıldız işaretleri ve benzeri işaretler kullanılabilir. Görüntülerde bir bireyi veya kurumu gösterebilecek her türlü bilgi kör edilmelidir. Gönderilen her bir şeklin çözünürlüğü en az 300 DPI olmalıdır. Değerlendirme sürecinde gecikmeleri önlemek için, gönderilen tüm şekiller net ve büyük boyutlu olmalıdır (en küçük boyutlar: 100 × 100 mm). Şekil açıklamaları ana metnin sonunda metindeki sıraya göre ayrı ayrı listelenmelidir.

Makalede kullanılan tüm kısaltmalar ve akronimler, hem özet hem de ana metinde ilk kullanımda tanımlanmalıdır. Kısaltma, tanımın ardından parantez içinde verilmelidir.

Ana metinde bir ilaç, ürün, donanım veya yazılım programından bahsedildiğinde, ürünün adı, ürünün üreticisi ve şehri ve şirketin ülkesini (ABD'de ise eyalet dahil) içeren ürün bilgileri, parantez içinde aşağıdaki biçimde sağlanmalıdır: The skin prick tests were performed using a multi-prick test device (Quantitest, Panatrex Inc, Placentia, California, USA)

Tüm referanslar, tablolar ve şekiller ana metin içinde belirtilmeli ve ana metin içinde belirtildikleri sırayla numaralandırılmalıdır. Orijinal makalelerin kısıtlılıkları tartışma bölümü içinde sonuç paragrafından önce belirtilmelidir.

KAYNAKLAR

Yayınlara atıf yapılırken, en son ve en güncel yayınlar tercih edilmelidir. Yazarlar on yıldan eski referansları kullanmaktan kaçınmalıdır. Yazılarda 10 yıldan eski tarihli referans sayısının toplam referans sayısının %20'sini geçmemesine dikkat edilmelidir. Elektronik olarak yayınlanmış ancak cilt ve sayfa numarası verilmemiş yazılar atfedilirken DOI numarası verilmelidir. Yazarlar kaynakların doğruluğundan sorumludur. Referans numaraları metindeki cümlelerin sonunda parantez içinde metinde kullanıldıkları sıra ile numaralandırılmalıdır. Dergi adları "Index Medicus" veya "ULAKBIM/ Turkish Medical Index" de listelendiği gibi kısaltılmalıdır. Mümkün olduğunca yerel referanslar kullanılmalıdır. Kaynaklar aşağıdaki örneklere uygun olarak yazılmalıdır.

Kaynak dergi ise;

Yazar(lar)ın soyadı adının başharf(ler)i (6 ve daha az sayıda yazar için yazarların tümü, 6'nın üzerinde yazarı bulunan makaleler için ilk 6 yazar belirtilmeli, Türkçe kaynaklar için "ve ark.", yabancı kaynaklar için "et al." ibaresi) kullanılmalıdır. Makalenin başlığı. Derginin Index Medicus'a uygun kısaltılmış ismi

(http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi?db=nlmcatalog) Yıl;Cilt:İlk ve son sayfa numarası.

Örnek: Benson M, Reinholdt J, Cardell LO. Allergen-reactive antibodies are found in nasal fluids from patients with birch polen-induced intermittent allergic rhinitis, but not in healthy controls. Allergy 2003;58:386-93.

Kaynak dergi eki ise;

Yazar(lar)ın soyadı adının başharf(ler)i. Makalenin başlığı. Derginin Index Medicus'a uygun kısaltılmış ismi (http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi?db=nlmcatalog) Yıl;Cilt

(Suppl. Ek sayısı):İlk sayfa numarası-Son sayfa numarası.

Örnek: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; (102 Suppl 1):275–82.

Kaynak kitap ise;

Yazar(lar)ın soyadı, adının başharf(ler)i. Kitabın adı. Kaçıncı baskı olduğu. Basım yeri: Basımevi, Basım Yılı.

Örnek: Ringsven MK, Bond N. Gerontology and leadership skills for nurses. 2nd ed. Albany, NY: Delmar Publishers, 1996.

Kaynak kitaptan bölüm ise;

Bölüm yazar(lar)ının soyadı adının başharf(ler)i. Bölüm başlığı. In: Editör(ler)in soyadı, adının başharf(ler)i (ed) veya (eds). Kitabın adı. Kaçıncı baskı olduğu. Basım yeri: Yayınevi,

Baskı yılı:Bölümün ilk ve son sayfa numarası.

Örnek: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM (eds). Hypertension: Pathophysiology, Diagnosis, and Management. 2nd ed. New York: Raven P, 1995:466–78.

Kaynak toplantıda sunulan bildiri ise;

Yazar(lar)ın soyadı adının başharf(ler)i. (6 ve daha az sayıda yazar için yazarların tümü, 6'nın üzerinde yazarı bulunan bildiriler için ilk 6 yazar belirtilmeli, Türkçe kaynaklar için "ve ark.", yabancı kaynaklar için "et al." ibaresi kullanılmalıdır). Bildirinin başlığı. Varsa In: Editör(ler)in soyadı adının başharf(ler)i (ed) veya (eds). Kitabın adı. Toplantının adı; Tarihi; Toplantının yapıldığı şehrin adı, Toplantının yapıldığı ülkenin adı. Yayınevi; Yıl. Sayfa numaraları.

Örnek: Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet

P, Piemme TE, Reinhoff O (eds). MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. North-Holland; 1992. p. 1561-5.

Kaynak elektronik dergi ise;

Yazar(lar)ın soyadı adının başharf(ler)i. (6 ve daha az sayıda yazar için yazarların tümü, 6'nın üzerinde yazarı bulunan makaleler için ilk 6 yazar belirtilmeli, Türkçe kaynaklar için "ve ark.", yabancı kaynaklar için "et al." ibaresi kullanılmalıdır). Makalenin başlığı. Derginin Index Medicus'a uygun kısaltılmış ismi Yıl; Cilt (Sayı). Available from: URL adresi. Erişim tarihi: Gün.Ay.Yıl.

Örnek: Arrami M, Garner H. A tale of two citations. Nature 2008;451(7177): 397-9. Available from: URL:www.nature.com/nature/journal/v451/n7177/full/451397a.html. Aaccessed 20 January 2008.

Kaynak web sitesi ise:

Web sitesinin adı. Erişim tarihi. Available from: Web sitesinin adresi.

Örnek: Centers for Disease Control and Prevention (CDC). Erişim tarihi: 12 Mart 2013.

Available from: http://www.cdc.gov/

Kaynak tez ise:

Yazarın soyadı adının baş harfi. Tezin başlığı (tez). Tezin yapıldığı şehir adı: Üniversite adı (üniversite ise); Yılı.

Örnek: Özdemir O. Fibrillin-1 gen polimorfizmi ve mitral kapak hastalığı riski. (Tez). Ankara: Gazi Üniversitesi, 2006."

Düzeltme istenmesi aşaması:

Bir makalenin hakemler tarafından istenen değişiklikler yapılmış kopyası gönderilirken yazar, hakemler tarafından istenen her açıklama/düzeltmeye cevap vermekle yükümlüdür. Yazarlar hakemlerin düzeltme/açıklama isteklerini her isteğin ardından olacak şekilde madde madde açıklmalı, düzeltilmiş kopyaya yazılacak metin bu açıklamanın altına eklemelidir. Düzeltme yapılmış kopya dergiye ayrı bir kopya olarak yüklenmelidir. Düzeltilmiş yazılar düzeltme isteğinin gönderilmesinden itibaren 30 gün içinde gönderilmelidir. Yazının düzeltilmiş kopyası istenilen sürede gönderilmezse yazı sistemden ototmatik olarak düşürülecektir ve tekrar başvuru yapılması gerekecektir. Eğer yazarlar ek zaman talep ediyorlarsa bu taleplerini ilk 30 günlük süre sona ermeden önce dergiye iletmelidir.

Kabul edilen yazılar dilbilgisi ve noktalama işaretleri yönünden kontrol edilir. Kabul süreci ve düzenleme işlemleri tamamlandıktan sonra yazı son onay için yazara gönderilir ve yazar tarafından son defa onaylanması istenir. Bu işlem bittikten sonra yazı dergi web sayfasında cilt ve sayfa numarası verilmeden DOI verilerek yayınlanır.

Yazar Listesi/Sırası Değişimi

Yazı gönderildikten sonra yazar listesinin/sırasının değiştirilmesi (yazar adlarının silinmesi veya yeni yazar adı eklenmesi gibi) talepleri yayın kurulunun onayına tabidir. Bu talep yazar değişiklik formunun doldurulup dergiye yüklenmesi ile talep edilebilir. Bu form aşağıdakileri içerecek şekilde doldurulmalıdır: Talebin gerekçesi, yani yazar listesi, tüm yazarlar tarafından (yeni ve eski) imzalanan yeni bir telif hakkı transfer formu, yeni yazar tarafından imzalanmış çıkar çatışması formu.

Yazının geri çekilmesi talebi

Türkiye Çocuk Hastalıkları Dergisi yüksek kaliteli yazılar yayınlamayı ve yayın etiğini korumayı taahhüt etmektedir. Yazarlardan, yayın etiğinde ve yazıların kalitesinde tavsiye edilen kurallara uymaları beklenmektedir.

Yazının geri çekilme talebi olağanüstü durumlarda talep edilmelidir. Bir yazının geri çekilmesi için yazarların dergiye geri çekme nedenlerini belirten ve tüm yazarlar tarafından imzalanan bir "Makale geri çekme Formu" yüklemeleri gerekmektedir. Bu form derginin web sayfasından indirilebilir. Yazarlar dergiden bu konuda olumlu bir cevap alana kadar makalelerinin geri çekilme işleminin tamamlanmadığını bilmelidir.

Bir makalenin inceleme süreci altı aydan uzun bir zaman almış ve yazarlara karar bildirilmemişse yazının geri çekilme talebi olumlu karşılanır.

CONTENTS / İÇİNDEKİLER

Original Articles / *Özgün Araştırmalar*

Case Reports / *Olgu Sunumları*

Review / *Derleme*

Heart Diseases in Patients with Organic Acidemia

Organik Asidemi ile Takipli Hastalarda Kalp Hastalıkları

Sümeyra Zeynep ÖZBEY[1], Mehmet GÜNDÜZ[2], İbrahim İlker ÇETİN[3], Esra KILIÇ[4], Ahmet Cevdet CEYLAN[5], Özlem ÜNAL UZUN[6], Aynur KÜÇÜKÇONGAR YAVAŞ[1], Oya KIREKER KÖYLÜ[1], Burak YÜREK[1], Gülten Burcu CİVELEK ÜREY[1], Berrak BİLGİNER GÜRBÜZ[1], Çiğdem Seher KASAPKARA[1]

[1]Department of Pediatric Metabolism, Ankara Bilkent City Hospital, Ankara, Türkiye
[2]Dr.Sami Ulus Child Health and Diseases Training And Research Hospital, Ankara, Türkiye
[3]Department of Pediatric Cardiology, Ankara Bilkent City Hospital, Ankara, Türkiye
[4]Department of Pediatric Genetics, Ankara Bilkent City Hospital, Ankara, Türkiye
[5]Department of Medical Genetics, Ankara Bilkent City Hospital, Ankara, Türkiye
[6]Department of Pediatric Genetics, Faculty of Medicine, Kocaeli University, Kocaeli, Türkiye

ABSTRACT

Objective: Organic acidemias are intoxication-type inborn errors of the metabolism with multiple organ involvement. Patients with organic acidemia usually present in the neonatal or infantile period with high anion gap metabolic acidosis and hyperammonemia. The present study investigates the presence of congenital heart defects and secondary heart diseases in patients with organic acidemia.

Material and Methods: Included in the study were 31 patients of whom 14 were diagnosed with methylmalonic acidemia (MMA), 11 with propionic acidemia and six with isovaleric acidemia. The cardiac findings of all patients included in the study were evaluated.

Results: Of the sample, 63.64% were identified with accompanying congenital heart disease, with the most common diagnosis being propionic acidemia and the most common heart defects being atrial septal defects and mitral regurgitation.

Conclusion: The accumulation of toxic intermediate metabolites due to enzyme deficiency is thought to be the main mechanism behind the cardiac involvement noted in organic acidemias. In the presence of unexplained deterioration, the potential for organic acidemia to accompany congenital heart disease should be kept in mind, and so it is important to screen patients with organic acidemias by echocardiography.

Key Words: Echocardiography, Heart diseases, Organic acidemias

0009-0007-7970-1684 : ÖZBEY SZ
0000-0002-6005-5623 : GÜNDÜZ M
0000-0001-9480-8278 : ÇETİN İ
0000-0003-0522-1809 : KILIÇ E
0000-0003-4938-3420 : CEYLAN AC
0000-0001-7937-7721 : ÜNAL UZUN Ö
0000-0002-4766-300X : KÜÇÜKÇONGAR YAVAŞ A
0000-0001-6822-1713 : KIREKER KÖYLÜ O
0000-0002-1387-0502 : YÜREK B
0000-0001-7202-5897 : CİVELEK ÜREY GB
0000-0002-6197-0647 : GÜRBÜZ BİLGİNER B
0000-0002-3569-276X : KASAPKARA ÇS

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Ankara Bilkent City Hospital, Clinical Research Ethics Committee No. 2 (E2-22-2950/07.12.2022).

Contribution of the Authors / Yazarların katkısı*: *ÖZBEY SZ : Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in the writing of the whole or important parts of the study. ***GÜNDÜZ M:*** Planning methodology to reach the conclusions, Taking responsibility in logical interpretation and conclusion of the results. ***ÇETİN İ:*** Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments. ***KILIÇ E:*** Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments. ***CEYLAN AC:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***ÜNAL UZUN Ö:*** Taking responsibility in necessary literature review for the study. ***KÜÇÜKÇONGAR YAVAŞ A:*** Constructing the hypothesis or idea of research and/or article. ***KIREKER KÖYLÜ O:*** Taking responsibility in necessary literature review for the study. ***YÜREK B:*** Reviewing the article before submission scientifically besides spelling and grammar. ***CİVELEK ÜREY GB:*** Reviewing the article before submission scientifically besides spelling and grammar. ***GÜRBÜZ BİLGİNER B:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments. ***KASAPKARA ÇS:*** Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli* : Özbey SZ, Gündüz M, Çetin İİ, Kılıç E, Ceylan AC, Ünal Uzun Ö, et al. Heart Diseases in Patients with Organic Acidemia. Turkish J Pediatr Dis 2024;18:153-158.

Correspondence Address / Yazışma Adresi:
Sümeyra Zeynep ÖZBEY
Department of Pediatric Metabolism,
Ankara Bilkent City Hospital, Ankara, Türkiye
E-posta: s.zeynepozbey@hotmail.com

Received / Geliş tarihi : 08.06.2023
Accepted / Kabul tarihi : 25.12.2023
Online published : 05.02.2024
Elektronik yayın tarihi
DOI:10.12956/tchd.1311485

ÖZ

Amaç: Organik asidemiler, çoklu organ tutulumu ile seyreden ciddi kalıtsal metabolik hastalıklardır. Pek çok organik asidemi neonatal veya infantil dönemde artmış anyon açıklığı, metabolik asidoz ve hiperamonyemi ile birlikte bulunur. Bu çalışmada; organik asidemi ile takipli hastalarda konjenital kalp defektleri ile sekonder kalp hastalıklarının araştırılması amaçlandı.

Gereç ve Yöntemler: Çalışmaya 14'ü metilmalonik asidemi, 11'i propionik asidemi ve 6'sı izovalerik asidemi tanılı toplam 31 hasta dahil edildi. Bu olguların kardiak bulguları değerlendirildi.

Bulgular: En sık propionik asidemi tanısı ile takipli hastalarda %63.6 oranında eşlik eden konjenital kalp hastalığı saptandı. Bunlar arasında en sık görülen kalp anomalisi ise atriyal septal defekt ve mitral yetmezlikti.

Sonuç: Organik asidemilerde kardiyak tutulumun ana mekanizmasının enzim eksikliğine bağlı toksik ara metabolitlerin birikimi olduğu düşünülmektedir. Klinikte açıklanamayan kötüleşme varlığında konjenital kalp hastalıklarına organik asideminin eşlik edebileceği akılda tutulmalıdır. Bu nedenle organik asidemilerde ekokardiyografi ile tarama yapılması önemlidir.

Anahtar Sözcükler: Ekokardiyografi, Kalp hastalıkları, Organik asidemiler

INTRODUCTION

Organic acidemias (OA) are congenital metabolic diseases with potential multiple organ involvement that result from an accumulation of toxic metabolites. Propionic acidemia (PA), methylmalonic academia (MMA) and isovaleric acidemia (IVA) are most frequently encountered in clinical practice.

In PA, propionate and other metabolites accumulate due to a deficiency in propionyl CoA carboxylase. At the culmination of the reaction in which propionyl-CoA carboxylase catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA, succinyl-CoA enters the tricarboxylic acid (TCA) cycle, and any problems in this enzyme results in an accumulation of propionyl-CoA and the formation of pathological metabolites such as 2-methylcitrate, 3-hydroxypropionate, tiglylglycine and propionylglycine. Propionyl-CoA and 2-methylcitrate are thought to be the major endogenous toxins of PA, inhibiting the pyruvate dehydrogenase complex and multiple enzymatic steps in the TCA cycle. In addition to metabolic decompensation, long-term complexes include such pathologies as myopathy, pancreatitis and cardiomyopathy, as well as such potentially long-term signs as acute cardiac dysfunction and acquired long QT syndrome occurring during metabolic crisis (1,2).

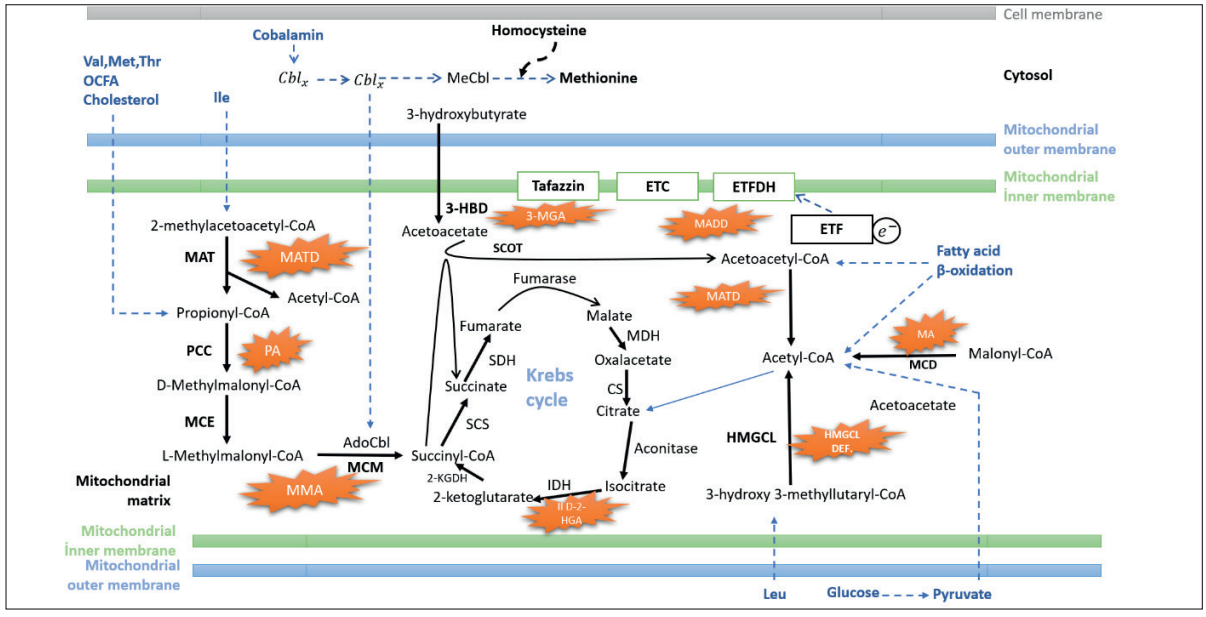

Figure 1: Schematic diagram of selected metabolic pathways in the heart that are affected and implicated in organic acidemias. AdoCbl: adenosylcobalamin; Cbl: cobalamin; 2-KGDH: 2-ketoglutarate dehydrogenase; CS: citrate synthase; ETC: electron transport chain; ETF: electron-transfer flavoprotein; ETFDH, electron-transfer flavoprotein dehydrogenase; II D-2-HGA, type II D-2-hydroxyglutaric aciduria; 3-HBD: 3-hydroxybutyrate dehydrogenase; HMGCL: 3-hydroxy-3-methylglutaryl-CoA lyase; IDH,: isocitrate dehydrogenase; Ile, isoleucine; Leu: Leucine; MA: malonic acidemia; MADD: multiple acyl-CoA dehydrogenase deficiency; MATD: mitochondrial acetoacetyl-CoA thiolase deficiency; MCD: malonyl-CoA decarboxylase; MCE: methylmalonyl-CoA epimerase (methylmalonyl-CoA racemase); MCM: methylmalonyl-CoA mutase; MDH: malate dehydrogenase; MeCbl: methylcobalamin; Met: methionine; 3-MGA: 3-methylglutaconic aciduria; MMA: methylmalonic acidemia; PA: propionic acidemia; PCC: propionyl-CoA carboxylase; SCOT: succinyl-CoA:3-ketoacid CoA transferase; SCS: succinyl-CoA synthetase (succinyl-CoA ligase); SDH: succinate dehydrogenase; Thr: threonine; Val: valine. Cblx refers to different oxidation states of the central cobalt ion in cobalamin. Blue dashed lines indicate the several metabolic steps.

Another potential autosomal recessive metabolism disorder is methylmalonic acidemia (MMA), which is caused by a deficiency in methylmalonyl-CoA mutase activity or the impaired transport and synthesis of its cofactor, cobalamin. MMA's clinical spectrum is broad, with phenotypes ranging from a relatively benign condition to fatal neonatal disease. Isovaleric acidemia (IVA) is an inborn error of leucine catabolism, caused by mutations in the isovaleryl-CoA dehydrogenase (IVD) gene and resulting in an accumulation of derivatives of isovaleryl-CoA, including isovaleryl (C5)-carnitine (3). It is rarer than other organic acidemias and the incidence of cardiac defects is less. Cardiac defects have been reported in several OA, among which cardiomyopathy and arrhythmia are the most common, although heart diseases may also be seen due to carnitine deficiency. The metabolic pathways of OAs are depicted schematically in Figure 1 (4). PA and MMA in particular can develop in the presence of cardiac dysfunction. Isovaleryl CoA rarely can be accompanied by cardiac pathologies (5). The association with congenital heart defect in the early period was remarkable in the patients in the present study who were diagnosed with organic acidemia, and so cardiac dysfunction should be kept in mind in such cases. The present study investigates the congenital heart diseases seen in patients with organic acidemia (6).

MATERIAL and METHODS

Included in the study were 31 patients with organic acidemias, all of whom were screened for congenital heart disease and possible cardiac pathologies by electrocardiogram (ECG) at a paper speed of 50 mm/sec. The echocardiographic parameters and ECG recordings were subsequently analyzed. The study was approved by Ankara Bilkent City Hospital, Clinical Research Ethics Committee No. 2 (E2-22-2950/07.12.2022).

Statistical Analyses

The study data were evaluated with IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics were presented as mean±standard deviation (mean±SD) and as minimum-maximum values, while distribution information was presented as numbers (n) and percentages (%).

RESULTS

The median age of the patients was 76±17 months (minimum 7 days; maximum 27 years). All of the patients had been diagnosed with organic acidemia during the neonatal or infantile periods. The clinical, cardiological and molecular features of the sample are presented in Table I. Of the total, 16 (52%) participants were male and 15 (48%) were female; and 14 (45%) had MMA, 11 (36%) had propionic acidemia and six (19%) had isovaleric acidemia. Accompanying congenital heart disease was identified in 63.6 % of the patients, the most common diagnosis of which was propionic acidemia, and among these, the most common heart defects were ASD and mitral valve insufficiency. Cardiac defects were detected in 50 percent of the patients with MMA, with the most encountered congenital heart defects being atrial septal defects, ventricular septal defects, aortic valve insufficiency, mitral valve insufficiency and left ventricular hypertrophy. Heart disease was rare in the patients followed up with isovaleric acidemia. ASD was detected in 25% of patients with isovaleric acidemia (Table II).

DISCUSSION

Then clinical presentation of organic acidemias can be very complicated, including the effects of the metabolites associated with the defect. The mechanism of cardiomyopathy is not clear, although it is thought to be caused by lactic acidosis and metabolic decompensation, while another known complication is arrhythmia.

The limitations of the study include the retrospective collection of data and the small number of patients in the sample. Studies reporting on the presence of cardiac defects in organic acidemia are rare (7). The accumulation of toxic intermediate metabolites due to enzyme deficiency is thought to be the main mechanism behind cardiac involvement in cases of organic acidemia. A recent study reported a reduction in reactive oxygen production, the presence of various respiratory chain deficiencies, and decreased detoxification in the tissues and fibroblast cultures taken from children with OA (8). Low levels of free carnitine as well as biotin deficiency have been suggested as potential risk factors for the development of cardiomyopathy in patients (9). Propionyl CoA has been reported to cause mitochondrial dysfunction and to impair adenosine triphosphate generation through oxidative phosphorylation, resulting in cardiotoxicity. Toxic metabolites such as propionate, propionyl-CoA and 2-methylcitrate cause cardiac pathologies. Previous studies have voiced a need for routine cardiac evaluations of patients with organic acidemias (10). Although cardiomyopathies are most seen in PA, they may also develop in MMA (11). Dilated cardiomyopathy is among the most frequent cardiac complications identified a long side PA, while there have been few studies to date reporting cases with isovaleric acidemia with congenital heart disease (12). To date, no specific marker of cardiomyopathy has been identified. While the major mechanism behind cardiac alterations in PA remains unclear, it is likely to be multifactorial. Metabolic decompensation and lactic acidosis are known to trigger cardiomyopathy, while other factors include myocarditis, carnitine deficiency and rhythm abnormalities. Cardiac diseases are complications of other known organic acidemias, although the causative pathophysiology has yet to be clarified. Identifying the molecular targets in the hearts of OA patients will provide a better understanding of the processes and may steer the development of new treatments in the future.

Table I: Clinical, laboratory and molecular genetic features of patients with organic acidemia

Patient	Diagnosis	Age	Sex	Genetic Analyses	Ecg	Age At Diagnosis	Ecocardiogrphy	Ecocardiographic Evaluation	Follow-Up Time
1	PA	5 Years	M	PCCB c.370C>G (p. Gln124Glu) Homozygous	Normal	2 Years	Secundum ASD	2 Years	3 Years
2	PA	6 Years	M	PCCB c.1369G>A (p. Gly457Ser) Homozygous	Normal	17 Days	Secundum ASD	4 Years	6 Years
3	MMA	5 Years	F	MMUT c.325C>T (p. Q109*) (p. Gln109Ter) Homozygous	Normal	2 Months	Normal	2 Years	5 Years
4	MMA	4.5 Years	M	MUT 0 : p. Leu674Phe c.2020c>T Homozygous	Normal	2 Years	VSD, PFO	2.5 Years	2.5 Years
5	MMA	7 Days	F	MMAB : c.571 C>T p. (Arg191Trp) Homozygous	Normal	3 Days	Hypoplastic left heart	3 Days	7 Days
6	PA	5 Years	M	PCCB c.1373C>T (p. Ala458Val) (p.A458v) Homozygous	Normal	9 Months	Normal	12 Months	4 Years
7	PA	6 Years	M	PCCB : c.1540C>t p.Arg 514*rs749908889 Homozygous	Normal	12 Months	Normal	2 Years	5 Years
8	MMA	9 Years	M	MUT : c.2020C>T p. Leu674Phe rs1164271240 : Homozygous	Normal	18 Months	VSD, ASD, MVI, TVI	5 Years	8 Years
9	MMA	2.5 Years	F	MMUT: c.1106G>A: Compound Heterozygous	Normal	1 Month	Normal	1 Month	2.5 Years
10	MMA	2 Years	F	MMAA: c.1104G>A p. Trp368Ter rs1131692023 Homozygous	Normal	32 Days	Secundum ASD	1 Month	2 Years
11	MMA	20 Years	F	MMADHC: c .211_212dupAG (p. Phe72fs*8) Homozygous	Long -QT	3 Years	Normal	18 Years	17 Years
12	MMA	3.5 Years	M		Normal	4 Months	Normal	2 Years	3 Years
13	PA	10 Years	F		Normal	2.5 Years	Normal	6 Years	10 Years
14	IVA	27 Years	F	IVD: c.158G>A/p. Arg53His and c.535A>G p. Met179Val Compound Heterozygous	Sinus Tachycardia	7 Years			20 Years
15	PA	11 Years	M		Normal	7 Years	Normal	7 Years	4 Years
16	IVA	4 Years	M		Normal	1 Years	Normal	1 Years	4 Years
17	IVA	5.5 Years	F	İVD: c.941C>T (p. Ala314Val) Homozygous	Normal	2 Years	Secundum ASD	4 Years	3 Years
18	IVA	19 Years	M		Normal	3 Years	LVH	17 Years	16 Years
19	MMA	5 Years	F	MUT: c.309_327del19; p. Arg103Ser Homozygous	Normal	4 Years			5 Years
20	IVA	4.5 Years	M		Normal	1 Month			4.5 Years
21	PA	2.5 Years	F	PCCA:c. I629delT (p.Q544Kfs*13) (p.Gln544LysfsTerl3) Homozygous	Normal	2.5 Months	MVI, LVH	6 Months	2.5 Years
22	MMA	3 Years	M	MUT (0): p. Val438serfsTer3(c.1311_1312insA) Homozygous	Normal	1 Years	Normal	1 Years	3 Years
23	MMA	3.5 Years	M			4 Days			3.5 Years
24	MMA	7 Years	M		Normal	2 Months	LVH, AF	4 Years	7 Years
25	MMA	28 Years	M	MMA: c.904A>T: Homozygous	Normal	12 Years	Normal	12 Years	28 Years
26	MMA	6 Years	F		Normal	1 Month	LVH, MVI, AF	3 Years	7 Years
27	IVA	7 Years	F	IVD: p.R398Q (c.1193G>A)/ p.E411K (c.1231G>A) Compound Heterozygous	Normal	2 Years	Normal	2 Years	5 Years
28	PA	4 Months	M	PCCA: c.2171T>A (p. L724H) (p. Leu724His) Homozygous	Normal	2 Months	BAV, AORT STENOZU(AS), AF, MVI, ASD	2 Months	4 Months

Patient	Diagnosis	Age	Sex	Genetic Analyses	Ecg	Age At Diagnosis	Ecocardiogrphy	Ecocardiographic Evaluation	Follow-Up Time
29	PA	13 Months	F	PCCB Homozygous	Sinus Tachycardia	1 Month	PDA, PFO, TVI	1 Years	1 Years
30	PA	3 Months	F	PCCB Homozygous	Normal	3 Months	PFO	3 Months	3 Months
31	PA	7 Months	F	PCCB: c.395_408delGTCTGTCAGGAGCA p. Ser132ThrfsTer24 Homozygous	Normal	1 Month	MVI, PFO, LVH	1 Month	7 Months

ASD: *Atrial septal defect,* ***AF:*** *Aortic failure,* ***BAV:*** *Bicuspid aortic valve,* ***F:*** *Female,* ***HLF:*** *Hypoplastic left heart,* ***IVA:*** *Isovaleric Acidemia,* ***LVH:*** *Left ventricle hypertrophy,* ***M:*** *Male,* ***MMA:*** *Methylmalonic Acidemia,* ***MVI:*** *Mitral valve insufficiency,* ***PA:*** *Propionic acidemia,* ***PDA:*** *Patent ductus arteriosus,* ***PFO:*** *Patent foramen ovale,* ***TVI:*** *Tricuspid valve insufficiency,* ***VSD:*** *Ventricular septal defect*

Table II: Distribution of organic acidemias according to echocardiography findings

Echo Findings	Methyl Malonic Acidemia (%)	Isovaleric Acidemia (%)	Propionic Acidemia (%)
NORMAL	50.00	75.00	36.36
ASD	16.67	25.00	27.27
AF	16.67	0.00	9.09
BAV	8.33	0.00	9.09
HLF	8.33	0.00	0.00
LVH	16.67	0.00	18.18
MVI	16.67	0.00	27.27
PDA	0.00	0.00	9.09
PFO	8.33	0.00	27.27
TVI	8.33	0.00	9.09
VSD	16.67	0.00	0.00
TOTAL	100.00	100.00	100.00

ASD: *Atrial septal defect,* ***AF:*** *Aortic failure,* ***BAV:*** *Bicuspid aortic valve,* ***HLF:*** *Hypoplastic left heart,* ***LVH:*** *Left ventricle hypertrophy,* ***MVI:*** *Mitral valve insufficiency,* ***PDA:*** *Patent ductus arteriosus,* ***PFO:*** *Patent foramen ovale,* ***TVI:*** *Tricuspid valve insufficiency,* ***VSD:*** *Ventricular septal defect*

The association between cardiomyopathies and organic acidemia is well known, as well as such inborn metabolism errors as mitochondrial disorders, fatty acid oxidation defects, carnitine transport defects and glycogen storage diseases (13). There have been few studies to date, however, reporting an association between cardiomyopathy and organic acidemia. In cases of PA, cardiac complications a leading factor in major morbidity and mortality. Besides cardiomyopathy, long QT syndrome is also an important ECG finding in PA (14,15). Cardiomyopathies appear mostly during childhood (mean age 7 years), while long QT syndrome emerges over time in patients with PA (16). Similar to the rates reported in previous studies, only one patient among the 31 cases reported in the present study was identified with long QT syndrome (Case 11).

In the present study, congenital heart defects were most associated with propionic acidemia and were most rarely seen in patients with isovaleric acidemia. In contrast to previous studies in literature, an important finding of the study was the detection of ASD at a rate of 25% in patients diagnosed with IVA, revealing the potential of cardiac defects to occur as a complication of organic acidemias, or congenital heart disease to accompany the pathology Such an association is rare, and so further studies are needed to clarify the relationship between heart defects and organic acidemia. Cardiac defects can cause sudden death or rapid clinical deterioration, and so clinicians should keep an eye out for cardiac pathologies in the presence of an unexpected sudden clinical deterioration or acute respiratory stress with organic acidemia, and cases of organic acidemia should thus undergo echocardiographic examinations. Further studies of organic acidemia are necessary with an increased number of patients in the sample.

REFERENCES

1. Wongkittichote P, Mew NA, Chapman KA. Propionyl-CoA carboxylase - A review. Mol Genet Metab 2017; 145–52.
2. Baumgartner MR, Hörster F, Dionisi-Vici C, Haliloglu G, Karall D, Chapman KA, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. Orphanet J Rare Dis 2014;9:130.
3. Kovacevic A, Garbade SF, Hoffmann GF, Gorenflo M, Kölker S, Staufner C. Cardiac phenotype in propionic acidemia - Results of an observational monocentric study. Mol Genet Metab 2020;130:41-8.
4. Richard E, Pérez B, Pérez-Cerdá C, Desviat LR. Understanding molecular mechanisms in propionic acidemia and investigated therapeutic strategies. Expert Opin Orphan Drugs 2015; 3:1427–38.
5. Fraser JL, Venditti CP. Methylmalonic and propionic acidemias: clinical management update. Curr Opin Pediatr 2016; 28:682– 93.
6. Park KC, Krywawych S, Richard E, Desviat LR, Swietach P. Cardiac Complications of Propionic and Other Inherited Organic Acidemias. Front Cardiovasc Med 2020; 7:617451.

7. Gilbert-Barness E. Review: metabolic cardiomyopathy and conduction system defects in children. Ann Clin Lab Sci 2004;34:15–34.
8. de Keyzer Y, Valayannopoulos V, Benoist JF, Batteux F, Lacaille F, Hubert L, et al. Multiple OXPHOS deficiency in the liver, kidney, heart, and skeletal muscle of patients with methylmalonic aciduria and propionic aciduria. Pediatr Res 2009; 66:91-5.
9. Mardach R, Verity MA, Cederbaum SD. Clinical, pathological, and biochemical studies in a patient with propionic acidemia and fatal cardiomyopathy. Mol Genet Metab 2005; 85:286– 90.
10. Lee TM, Addonizio LJ, Barshop BA, Chung WK. Unusual presentation of propionic acidaemia as isolated cardiomyopathy. J Inherit Metab Dis 2009;32 Suppl 1(01): S97-101.
11. Prada CE, Al Jasmi F, Kirk EP, Hopp M, Jones O, Leslie ND, et al. Cardiac disease in methylmalonic acidemia. J Pediatr 2011;159:862–4.
12. Romano S, Valayannopoulos V, Touati G, Jais JP, Rabier D, de Keyzer Y, et al. Cardiomyopathies in propionic aciduria are reversible after liver transplantation. J Pediatr 2010;156:128–34.
13. Nigel S, Tan MD, Ravi R, Bajaj MD, Chantal Morel MD, Sheldon M et al. Metabolic cardiomyopathy from propionic acidemia precipitating cardiac arrest in a 25-year-old man. CMAJ 2018; 23;190: E883-7.
14. Baumgartner D, Scholl-Burgi S, Sass JO, Sperl W, Schweigmann U, Stein JI, et al. Prolonged QTc intervals and decreased left ventricular contractility in patients with propionic acidemia. J Pediatr 2007; 150:192–7.
15. Pena L, Franks J, Chapman KA, Gropman A, Ah Mew N, Chakrapani A, et al. Natural history of propionic acidemia. Mol Genet Metab 2012;105:5-9.
16. Romano S, Valayannopoulos V, Touati G, Jais JP, Rabier D, de Keyzer Y, et al. Cardiomyopathies in propionic aciduria are reversible after liver transplantation. J Pediatr 2010;156:128-34.

Evaluation of Patients with Umbilical Hernia: 6 Years Experiences

Umblikal Hernili Hastaların Değerlendirilmesi: 6 Yıllık Deneyim

Elif Emel ERTEN[1], Derya ERDOĞAN[2]

[1]Department of Paediatric Surgery, Ankara Bilkent City Hospital, Ankara, Türkiye
[2]Department of Paediatric Surgery, Dr. Sami Ulus Obstetrics and Gynaecology Training and Research Hospital, Ankara, Türkiye

ABSTRACT

Objective: Umbilical hernia is a common anterior abdominal wall defect in childhood. Although the defect is present at birth, unlike other hernias of childhood, it may close spontaneously over time without the need for surgery. However, when these hernias do not close spontaneously, complications can develop that require emergency surgery. The aim of this study was to determine the incidence of spontaneous closure in patients diagnosed with umbilical hernia and the factors that influence this incidence, the complications that may develop during follow-up, the indications for surgery and the issues to consider when planning the follow-up of patients.

Material and Methods: Between January 2006 and December 2011, 1928 patients diagnosed with umbilical hernia and followed up and treated in our clinic were included in the study. In this retrospective cohort study the current size of umbilical hernia, comorbidities and demographic characteristics of the patients were analysed. Operative data, complications and postoperative follow-up of the operated patients were evaluated.

Results: The spontaneous closure rate of umbilical hernia was found to be 60%. The rate of spontaneous closure of umbilical hernia was higher in boys and the rate of operation was higher in girls. The risk of incarceration was higher in girls than in boys. The rate of spontaneous closure decreased with increasing defect size. It was found that comorbidities did not affect spontaneous closure of umbilical hernias. The rate of emergency surgery for incarceration was low (2%). Strangulation was noted in 1% of patients. All patients with incarceration were in Lassaletta group 2 (defect diameter 0.5-1.5 cm).

Conclusion: Conservative management is still the most accepted and safest method in the treatment of umbilical hernia. Incarceration and strangulation are very rare in umbilical hernias. Even if the umbilical hernia is complicated, surgical treatment is possible and peri- and post-operative complication rates are very low. Long-term morbidity and mortality due to incarceration have not been reported in the literature.

Key Words: Child, Complication, Spontaneous closure, Umbilical hernia

ÖZ

Amaç: Umblikal herni çocukluk çağında sıkça görülen karın ön duvarı defektidir. Defekt doğumda mevcut olmasına rağmen çocukluk çağının diğer hernilerinden farklı olarak ameliyat gerekmeden zamanla kendiliğinden kapanabilir. Ancak bu herniler spontan kapanmazsa acil ameliyat gerektiren komplikasyonlar gelişebilir. Bu çalışmada umblikal herni tanısı alan hastalarda spontan kapanma insidansının ve buna etki eden faktörlerin, takipte gelişebilecek komplikasyonların ve ameliyat endikasyonlarının belirlenmesi, hastaların takiplerini planlarken dikkat edilmesi gereken hususların ortaya konması amaçlanmıştır.

0000-0002-3666-295X : ERTEN EE
0000-0001-8753-7107 : ERDOĞAN D

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. This study was approved by the academic board of Dr. Sami Ulus Gynecology and Obstetrics Training and Research Hospital and was registered as 22/7 on 19/03/2009.

***Contribution of the Authors / Yazarların katkısı*: ERTEN EE:** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study. **ERDOĞAN D:** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli* : Erten EE and Erdoğan D. Evaluation of Patients with Umbilical Hernia: 6 Years Experiences. Turkish J Pediatr Dis 2024;18:159-165.

Correspondence Address / Yazışma Adresi:
E.Emel ERTEN
Department of Paediatric Surgery,
Ankara Bilkent City Hospital, Ankara, Türkiye
E-posta: elifemelerten@hotmail.com

Received / Geliş tarihi : 13.09.2023
Accepted / Kabul tarihi : 04.01.2024
Online published : 12.02.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1359548

Gereç ve Yöntemler: Kliniğimizde Ocak 2006-Aralık 2011 tarihleri arasında umblikal herni tanısı alan, takip ve tedavisi yapılan 1928 hasta çalışmaya dahil edildi. Retrospektif kohort olarak planlanan bu çalışmada hastaların mevcut umblikal herni boyutları, ek hastalıkları, demografik özellikleri incelendi. Ameliyat olan hastaların ameliyat verileri, komplikasyonları ve postoperatif takipleri değerlendirildi.

Bulgular: Hastaların umblikal hernilerinin spontan kapanma oranı %60 olarak bulundu. Erkeklerde umblikal herninin spontan kapanma oranları daha yüksek olduğu, kızlarda ise ameliyat olma oranının daha yüksek olduğu görüldü. Kızlarda inkarserasyon riski erkeklere göre yüksek bulundu. Defekt boyutu arttıkça spontan kapanma oranı azalmakta olduğu görüldü. Hastalarda izlenen ek hastalıkların umblikal herninin spontan kapanmasına etkisi olmadığı saptandı. İnkarserasyon nedeniyle acil operasyon oranının (%2) düşük olduğu gözlendi. Hastaların %1'inde strangülasyon saptandı. İnkarserasyon izlenen hastaların tamamı Lassaletta grup 2'de yer almaktaydı (defekt çapı 0.5-1.5 cm).

Sonuç: Umblikal herninin tedavisinde konsevatif izlem hala en kabul gören ve en güvenli yöntemdir. Umblikal hernilerde inkarserasyon ve strangülasyon oldukça nadir görülmektedir. Umblikal herni komplike hale gelmiş olsa dahi cerrahi tedavisi mümkündür ve peroperatif-postoperatif komplikasyon oranları oldukça düşüktür. İnkarserasyon nedeniyle uzun dönem morbidite ve mortalite literatürde saptanmamıştır.

Anahtar Sözcükler: Çocuk, Komplikasyon, Spontan kapanma, Umblikal herni

INTRODUCTION

Umbilical hernia is a common anterior abdominal wall defect in childhood. Although the defect is present at birth, unlike other childhood hernias, it may close spontaneously over time without the need for surgery (1). However, complications requiring emergency surgery may develop in umbilical hernias that do not close spontaneously.

Umbilical and epigastric hernias constitute approximately 10% of all hernias (2). The estimated incidence of umbilical hernias is 15-32%, but the actual incidence is not known precisely as it mostly closes spontaneously after a while. It is accepted that approximately 10% of all umbilical hernias remain unclosed until adulthood (3).

Although umbilical hernias are usually seen in a silent clinic, they can also become quite complicated and may present with life-threatening conditions. Incarceration, strangulation, spontaneous rupture or perforation are serious complications that may be encountered in the course of umbilical hernia.

In this study, we aimed to determine the incidence of spontaneous closure in patients diagnosed with umbilical hernia and the factors affecting it, complications that may develop in follow-up, the indications for surgery, and the points to be considered while planning the follow-up of the patients.

MATERIAL and METHODS

This study was approved by the academic board of Dr. Sami Ulus Gynecology and Obstetrics Training and Research Hospital and was registered as 22/7 on 19/03/2009.

The study included children who were diagnosed with an umbilical hernia and received treatment at our clinic between January 2006 and December 2011. Conservative management was proposed for patients diagnosed with umbilical hernia who were aged under four. Families were educated about umbilical hernia and emergency situations were explained. Patients were scheduled for regular check-ups at intervals of 3-6 months. Surgery was advised for patients over four years old with umbilical hernia. Patients with incarceration or strangulation underwent surgery urgently.

In this retrospective cohort study, we examine the current sizes of umbilical hernias, comorbidities, and demographic characteristics of the patients. The patients were separated into three groups based on the Lassaletta classification, which is based on the sizes of the defects. Patients with umbilical defects smaller than 0.5 cm were categorised as group 1, whilst those between 0.5 cm and 1.5 cm were categorised as group 2, and those larger than 1.5 cm as group 3. The patients were additionally separated into 2 groups based on their clinical history. Those who completed follow-up were labelled as group A, and those who underwent surgery were classified as group B. The surgical data, complications, and postoperative follow-ups of those who received surgical intervention were assessed.

Statistical analysis was conducted using the SPSS 24.0 software package to determine the number of cases and ratios within the groups. Comparisons between ratios were made using Pearson's Chi-square test and Fisher's exact Chi-square test, while nonparametric comparisons of means for more than two groups were assessed using the Kruskal-Wallis Test. Pearson's chi-squared and Fisher's exact chi-squared tests were used to evaluate non-parametric data and when the number of parametric values was insufficient or the variances were not homogeneously distributed. A significance level of $p<0.050$ was deemed significant in the analyses.

RESULTS

Between January 2006 and December 2011, a total of 1928 patients were diagnosed with umbilical hernia in our clinic. Of these patients, 1185 did not return for follow-up after diagnosis and 743 were followed up with periodic checks. The mean age at diagnosis was 1.1 ± 0.9 years (min 1 week - max 16 years), the mean age at surgery was 5.0 ± 0.6 years (min 2 months

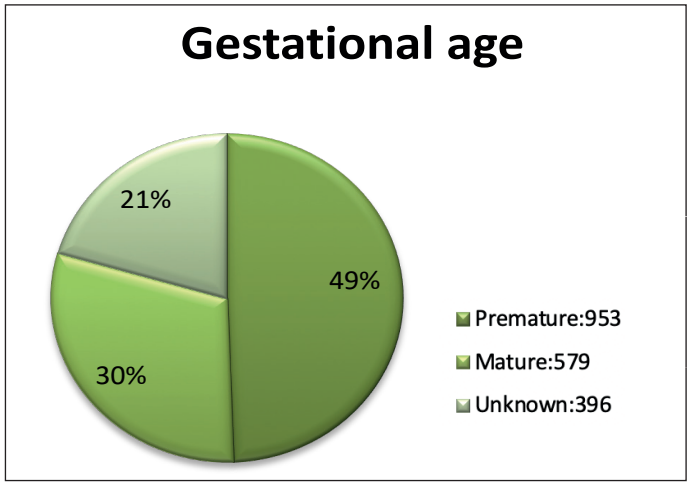

Figure 1: The Distribution of Gestational Age

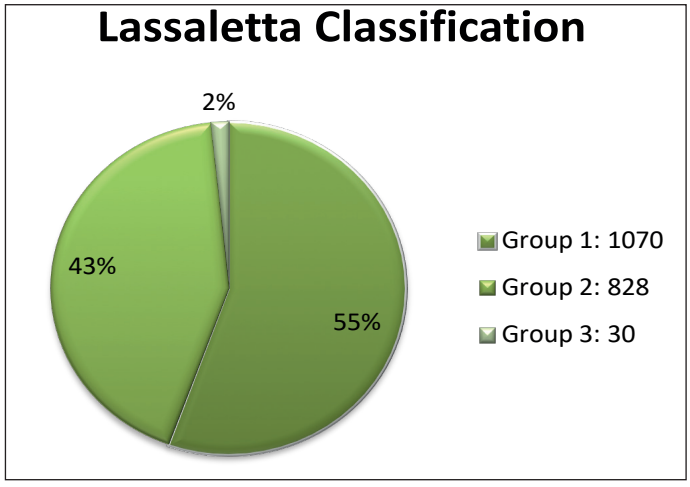

Figure 2: Classification of defect size at the time of diagnosis according to the Lassaletta Classification

- max 15 years). A total of 173 (8.9%) patients underwent surgery. The mean follow-up of all patients was 18.6 ± 7.3 months. Mean postoperative follow-up was 15.3 ± 7.4 months.

The sex, age group, gestational age and defect size of 1928 patients diagnosed with umbilical hernia were studied. In the study 1117 patients were male and 881 were female. The male/female ratio was 1.3 to 1 in patients with umbilical hernia. The gestational age of 396 patients was unknown. Almost half of the 1532 patients for whom gestational age data could be obtained had a history of preterm birth (25-36 weeks). The distribution is shown in Figure 1. 92% of patients were in the age group 0-4 years at the time of diagnosis.

The most common presenting symptom was umbilical swelling. 16% were diagnosed incidentally at our outpatient clinic for another reason. Other symptoms reported by families were itching and discolouration. When the size of the defect was classified, the patients were divided into 3 groups according to the Lassaletta classification. Their distribution is shown in Figure 2.

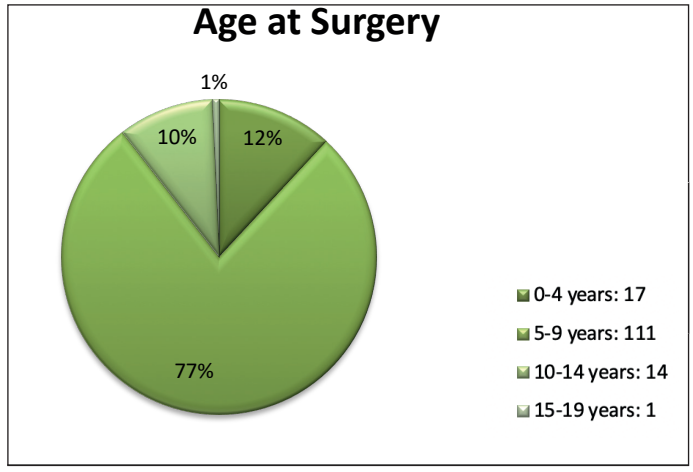

Figure 3: The distribution of age at surgery

While 91% of the patients were recommended for follow-up, 9% underwent surgery. Twenty-eight (16%) of the 173 patients who were operated on were younger than 4 years of age and were operated on after the age of 4 years. These patients were included in the operated group.

Additional disease was found in 21.7% of patients. Diseases related to the inguinal region were found in 109 (47%) patients with inguinal hernia, 101 (44%) with hydrocele and 19 (9%) with undescended testicles. Diseases of the cardiovascular system were found in 26 patients. Central nervous system disorders were observed in 27 patients and urogenital disorders in 25 patients. Congenital hypothyroidism was present in 13 patients and a history of chronic constipation in 67 patients.

When the relationship between defect size and clinical outcome was evaluated, 68% of patients in group 1 had spontaneous closure of the defect at follow-up, while this rate was 51% in group 2 and 42% in group 3 (Table I). As defect size increased, the rate of spontaneous closure decreased and this difference was statistically significant (p = 0.001). It was observed that the rate of surgery in group 3 was more than double that of the other two groups. This difference was statistically significant (p = 0.001). In group 3, 11 (58%) patients (4 girls/ 7 boys) whose umbilical defect did not close spontaneously had inguinal pathology in 2 (18%), Down syndrome in 1 (9%) and CNS pathology in 1 (9%).

The rate of spontaneous closure was higher in males than in females in patients followed for umbilical hernia. The rate of surgery was higher in girls than in boys (Table II). These differences were statistically significant (p=0.001).

When the relationship between co-morbidities and defect size was examined, a statistically significant relationship was found. (p=0.010). Additional diseases were found in 21.7% of the patients. Group 1 patients had the highest rate of comorbidities (25.1%). It was found that co-morbidities did not influence spontaneous closure of the umbilical hernia (p=0.214).

Table I: The relationship between defect size and clinical course

	Lassaletta Classification			
	Group 1	Group 2	Group 3	Total
Clinical outcome				
Remained	12 (3)	15 (5)	0	27 (5)
İncreased size	12 (3)	4 (1)	0	16 (2)
Spontaneous closured	284 (68)	154 (51)	8 (42)	446 (60)
Decreased size	10 (2)	70 (23)	1 (5)	81 (10)
Operated	99 (24)	64 (20)	10 (53)	173 (23)
Total	417	307	19	743(100)

p=0.001

Table II: The relationship between sex and clinical outcomes

	Boy	Girl	Total
Clinical outcome			
Remained	13 (3)	14 (5)	27
İncreased size	9 (2)	7 (3)	16
Spontaneous closured	304 (68)	142 (48)	446
Decreased size	43 (9)	38 (14)	81
Operated	83 (18)	90 (30)	173
Total	452	291	743

p= 0.001

743 patients were followed up for umbilical hernia and 173 (23%) were operated over time. The 743 patients who came to the controls and whose prognosis was known were compared with those who were followed up and operated on.

The patients were divided into 2 groups according to follow-up and surgery. Followed patients were classified as group A and operated patients as group B. While 65% of the followed patients were male, 52% of the operated patients were female. Female patients had a higher rate of surgery. In both groups of patients, more than 50% were patients with a history of preterm birth. The most common complaint on admission to hospital in both groups of patients was swelling of the umbilical region (Table III).

Considering the age distribution at the time of diagnosis, almost all patients aged >4 years underwent surgery, whereas clinical follow-up was preferred in patients aged <4 years. Defect sizes were classified according to the Lassaletta classification and the distribution of defect sizes was found to be similar in both groups of patients (Table III).

173 patients underwent surgery for umbilical hernia. The age distribution of patients who underwent surgery is shown in Figure 3. Five patients (3%) were operated for emergency indications due to strangulation or incarceration.

The inverted smile incision above the umbilicus was most commonly used to repair the patients' defects. The infraumbilical smiley incision was the other incision chosen. The supra-umbilical transverse incision was chosen to repair both defects, especially in cases of epigastric hernia. In patients who underwent intra-abdominal surgery for another reason, the defect was repaired intra-abdominally (Table IV).

Table III: The Comparison of Follow-up and Operated Patients Datas

	Followed up (Grup A)	Operated (Grup B)	Toplam
Sex			
Boy	369 (65)	83(48)	452
Girl	201 (35)	90 (52)	291
Gestational age			
Premature	312 (55)	90 (52)	402
Mature	153 (27)	38 (22)	191
Unknown	105 (18)	45 (26)	150
Symptoms			
Umbilical Swelling	453 (79.7)	136 (79)	589
Incidentally	115(20)	33 (19)	148
Other Umbilical Complaints	2 (0.3)	4 (2)	6
Age at diagnosis			
0-4 years	567 (99.5)	77 (44)	644
5-9 years	3 (0.5)	77 (44)	80
10-14 years	0	18 (11)	18
15-19 years	0	1 (1)	1
Lassaletta Classification			
Group 1	318 (55)	99 (60)	417
Group 2	243 (44)	64 (36)	307
Group 3	9 (1)	10 (4)	19
Total	570	173	743

Table IV: The Surgical Data

Incision types	
Infraumbilical smile incision	12 (7)
Inverted smile incision above umbilicus	145 (83)
Transvers or abdominal	16 (10)
Total	173

Table V: Postoperative complications (n=173)

Stick abscess	6 (3)
Recurrence	3 (1.5)
Pain	3 (1.5)
Total	12 (6)

While 20 of the patients who underwent umbilical hernia repair also had an epigastric hernia, 15 patients had an inguinal hernia, 5 patients had an undescended testicle, 2 patients had hypospadias, 2 patients had an umbilical polyp, 1 patient had an urachal remnant and 1 patient had an ovarian cyst. Simultaneous repairs were performed.

17 (10%) of these patients were younger than 4 years. Two (12%) patients younger than 4 years were operated for incarcerated umbilical hernia. In other patients younger than four years, simultaneous umbilical hernia repair was performed for epigastric hernia in 13 (76%) patients, for umbilical polyp in 1 (6%) patient and for inguinal hernia in 1 (6%) patient.

The postoperative complications were hernia abscess, recurrence and persistent pain and were observed at a rate of 6% (Table V). When the relationship between the size of the defect detected at surgery and the development of

postoperative complications was examined, no statistically significant difference was found (p=0.737).

Postoperative recurrence occurred in 3 patients (2 girls, 1 boy). All three patients had a history of prematurity. The age at surgery was 1-3-8 years. The size of the defect was 0.5-1-2 cm. While 2 of these patients underwent simultaneous epigastric hernia repair and 1 inguinal hernia repair, one of these patients had Down syndrome and the other had a history of chronic constipation. The other patient was found to have CNS pathology.

While co-morbidity was observed in 21.7% of all patients, the rate of co-morbidity was found to be 33.3% in 12 patients who developed complications (1 with Down syndrome, 1 with CNS disease, 2 with chronic constipation). Epigastric hernia repair was performed simultaneously in 3 (25%) of these patients. Although the rate of additional disease was higher in patients with postoperative complications compared to all patients, no statistically significant difference was found (p=0.528).

In our study there were 5 (2%) patients who underwent emergency surgery for strangulation and entrapment. The age of the patients was 1-11 years (mean 3.8 years). It was observed that only 2 (40%) patients were younger than 4 years. The defect diameter was 1 cm in 4 patients and 1.5 cm in 1 patient. It was noted that the defect diameters of the patients were in group 2 of the Lassaletta classification.

Five (2%) of the inguinal hernia cases we operated on were operated on for incarceration. Incarcerated omentum was present in 3 of these patients and strangulation was observed in 2 patients. In one of the 2 patients with strangulation, perforation of the ileum segment in the hernia sac was observed and the perforated area was primarily repaired. In the other, the ileal segment was irrigated with warm saline. No patient required bowel resection.

DISCUSSION

In general, hernias are a very important part of paediatric surgical practice. Approximately 75% of anterior abdominal wall defects occur in the inguinal region, 10% are incisional hernias, 10% are umbilical and epigastric hernias, approximately 4% are femoral hernias and the remainder are rare hernias (spigelian hernia, lumbar hernia, ventral hernia, etc.) (2) .

Umbilical hernia is a common defect of the anterior abdominal wall in childhood. Although the defect is present at birth, unlike other childhood hernias, it may close spontaneously over time without the need for surgery (1). However, if these hernias do not close spontaneously, complications may develop that require emergency surgery (1).

The incidence of umbilical hernias has been reported to be the same in boys and girls (4,5). Zendejas et al. (5) reported a male/female ratio of 1/1 in their study publishing their 53-year experience with umbilical hernia repair. In the study by Thomson et al. (6), this ratio was found to be 1/2,6 and was more common in girls. In our study, umbilical hernia was more common in males and the male/female ratio was 1.3/1. While this ratio was 1.4/1 in the group of patients recommended for follow-up, it was 0.9/1 in the group of patients recommended for surgery. It was observed that girls were more likely to be operated on for umbilical hernia than boys.

Most umbilical hernias present in childhood close by the age of 2 years, but the closure process may continue until the age of 5 years (2,4,7). A prospective study in Caucasian and African American populations showed spontaneous closure rates of 83-95% by 6 years of age (8). Another study reported that 50% of umbilical hernias that did not close by the age of 4-5 years could close by the age of 11 years (1). Because of the decreasing incidence of umbilical hernias with age and the tendency for spontaneous closure, it is widely accepted that patients should be followed clinically at regular intervals (1,2,4, 7-10). It is recognised that approximately 10% of all umbilical hernias remain unresolved into adulthood (2).

It was observed that umbilical hernias less than 0.5 cm in diameter tend to close spontaneously at around 2 years of age, and those with a defect diameter greater than 1 cm usually close by 4 years of age (11). Haller et al. (12) found that umbilical hernias did not close spontaneously in those with defects greater than 1.5 cm in diameter, in girls over 2 years of age, and in all children over 4 years of age, and recommended surgery in these patient groups. However, other authors in the literature have recommended that these patients should be followed up to the age of 5-7 years (8). In our study, patients under 4 years of age with umbilical hernia were followed up regularly. Those operated on before the age of 4 years were operated on urgently for incarcerated umbilical hernia or simultaneously for epigastric hernia, umbilical polyp, inguinal hernia.

Spontaneous closure of umbilical hernias can be predicted by looking at the diameter of the defect and the sharpness of the fascia at the edge of the defect. Defects larger than 1.5-2 cm are unlikely to close spontaneously. Papagrigoriadis et al found that the likelihood of spontaneous closure was low in those with an umbilical defect >2 cm (13). In our study the rate of spontaneous closure was 62%. This rate was 48% in female patients and 68% in male patients. A higher rate of spontaneous closure was observed in males. In our study it was observed that the umbilical defect closed spontaneously by the age of 4 years in 68% of patients in group 1, 51% of patients in group 2 and 42% of patients in group 3. Spontaneous closure was lower in patients with a defect diameter >1.5 cm and it was observed that the rate of spontaneous closure decreased with increasing defect size.

Complications of umbilical hernia are very rare and incarceration and strangulation are commonly reported in the literature. The

complication rate varies between 6-37% in different reports (1 3-19).

Chirdan et al. (16) repaired 52 umbilical hernias and incarceration was the indication for surgery in 44.2% of cases. It has been argued that incarceration in umbilical hernias is a rare complication, contrary to what has been reported in the literature (16). In our study there were 5 (2%) patients who underwent urgent surgery due to incarceration. Strangulation was found in only 2 patients (1%). It was noted that the rates of incarceration and strangulation were low when compared in the literature.

It is not clear why some umbilical hernias are incarcerated and others have an asymptomatic clinical course. Lassaletta et al. (20) found that a defect diameter of 0.5-1.5 cm doubled the risk of incarceration. Defects smaller than 0.5 cm are thought to be too small for the intestine to enter, and in defects larger than 1.5 cm, incarceration does not develop because the intestine entering the defect can easily exit (16,20). In our study there were 5 cases of incarcerated umbilical hernia. While the defect diameter in four of them was 1 cm, the defect diameter in one patient was 1.5 cm, and all of them were in Lassaletta group 2. In our study, incarceration was significantly higher in Lassaletta group 2, similar to the literature.

It has been argued that incarceration and strangulation are usually detected in infants younger than 6 months (17,21, 22). In our study, the median age of patients with incarcerated umbilical hernia was 4 years (min 1 year-max 11 years). This completely contradicts the reports in the literature that the risk of incarceration increases with younger age. Therefore, the opinion that age is a determining parameter in umbilical hernia incarceration is contradictory. The data from our study support that it is safe to wait for spontaneous closure of umbilical hernias until the age of 4 years.

Zendejas et al. (5) found that there was no association between incarceration and sex, whereas Lassaletta found incarceration mainly in male patients. In our study, 60% of the patients who underwent emergency surgery for incarceration were girls, and the incarceration rate was higher in girls.

The most common postoperative complications after umbilical hernia repair have been defined as wound infection, pain and recurrence, but they are very rare (16,19,22). In the Zendejas study, 96% of 489 children with umbilical hernia who underwent surgery had no postoperative complications. Recurrence was observed in 2% of patients, wound infection in 1% and haematoma in 1%. Repair technique, incision shape, hernia size, sex, prematurity and operative age were not associated with recurrence (5).

In our study, postoperative complications were found in 6% of the operated patients. The most common postoperative complication was rod abscess, which occurred in 6 patients (3%). Recurrence was observed in 3 patients (1.5%) and these patients underwent reoperation. Our complication rates were found to be consistent with the literature.

CONCLUSION

In our study we found that umbilical hernia was more frequent in boys, but the size of the defect was larger, the risk of incarceration was higher and the rate of spontaneous closure was lower in girls.

In conclusion, conservative management is still the most accepted and safest method of treating umbilical hernia in children under 4 years of age. In the absence of emergency complications in children under 4 years of age, there is no indication for surgery. In this condition, which tends to close spontaneously, the safest method is to explain the signs of incarceration to the family and to follow up regularly. Spontaneous closure is unlikely in umbilical hernias that persist beyond the age of four. Primary repair is recommended to prevent complications that may develop later in adulthood.

REFERENCES

1. Weber TR. Umbilical and Other Abdominal Wall Hernias. In: Ashcraft's Pediatric Surgery. 5 edn. Edited by Ashcraft KW, HolcombIII GW, Murphy JP: Elsevier 2005: 637-9.
2. Malangoni MA, Gagliardi RJ. Hernias. in: Sabiston Textbook of Surgery. edn. Edited by Towsend CM, Evers BM, Beauchamp RD, Mattox KL 2004:1199-218.
3. Başaklar AC. Göbek ve Karın Ön Duvarı Anomalileri. in: Bebek ve Çocukların Cerrahi ve Ürolojik Hastalıkları edn. Edited by Başaklar AC. Ankara: Palme 2006:1051-81.
4. Beres A, Christison-Lagay ER, Romao RL, Langer JC. Evaluation of Surgisis for patch repair of abdominal wall defects in children. J Pediatr Surg 2012;47:917-9.
5. Venclauskas L, Silanskaite J, Kiudelis M. Umbilical hernia: factors indicative of recurrence. Medicina (Kaunas) 2008;44:855-9.
6. Vohr BR, Rosenfield AG, Oh W. Umbilical hernia in the low-birth-weight infant (less than 1,500 gm). J Pediatr 1977;90:807-8.
7. Blumberg NA. Infantile umbilical hernia. Surg Gynecol Obstet 1980;150:187-92.
8. Morgan WW, White JJ, Stumbaugh S, Haller JA Jr. Prophylactic umbilical hernia repair in childhood to prevent adult incarceration. Surg Clin North Am 1970;50:839-45.
9. Zendejas B, Kuchena A, Onkendi EO, Lohse CM, Moir CR, Ishitani MB et al. Fifty-three-year experience with pediatric umbilical hernia repairs. J Pediatr Surg 2011;46:2151-6.
10. Scherer LR 3rd, Grosfeld JL. Inguinal hernia and umbilical anomalies. Pediatr Clin North Am 1993;40:1121-31.
11. Turnage RH, Li BD, McDonald JC. Abdominal Wall, Umbilicus, Periton and Omentum. in: Sabiston Textbook of Surgery. edn. Edited by Towsend CM, Evers BM, Beauchamp RD, Mattox KL 2004:1171-97.
12. Tank PW, Gest TR. The Abdomen. in: Lippincott Williams & Wilkins Atlas of Anatomy. 1 edn. Edited by Tank PW, Gest TR 2009:214-30.

13. Lassaletta L, Fonkalsrud EW, Tovar JA, Dudgeon D, Asch MJ. The management of umbilicial hernias in infancy and childhood. J Pediatr Surg 1975;10:405-9.
14. O'Donnell KA, Glick PL, Caty MG. Pediatric umbilical problems. Pediatr Clin North Am 1998; 45:791-9.
15. Papagrigoriadis S, Browse DJ, Howard ER. Incarceration of umbilical hernias in children: a rare but important complication. Pediatr Surg Int 1998;14:231-2.
16. Brown RA, Numanoglu A, Rode H. Complicated umbilical hernia in childhood. S Afr J Surg 2006;44:136-7.
17. Martis JJ, Rajeshwara KV, Shridhar MK, Janardhanan D, Sudarshan S. Strangulated Richter's Umbilical Hernia - A Case Report. Indian J Surg 2011;73:455-7.
18. Chirdan LB, Uba AF, Kidmas AT. Incarcerated umbilical hernia in children. Eur J Pediatr Surg 2006;16:45-48.
19. Fall I, Sanou A, Ngom G, Dieng M, Sankale AA, Ndoye M: Strangulated umbilical hernias in children. Pediatr Surg Int 2006; 22:233-5.
20. Komlatse AN, Komla G, Komla A, Azanledji BM, Abossisso SK, Hubert T. Meckel's diverticulum strangulated in an umbilical hernia. Afr J Paediatr Surg 2009;6:118-9.
21. Okada T, Yoshida H, Iwai J, Matsunaga T, Ohtsuka Y, Kouchi K,et al. Strangulated umbilical hernia in a child: report of a case. Surg Today 2001;31:546-9.
22. Sowula A, Groele H. (Treatment of incarcerated abdominal hernia). Wiad Lek 2003;56:40-4.

Turkish Journal of Pediatric Disease
Türkiye Çocuk Hastalıkları Dergisi

Psychosocial Functionality in Adolescents with Inborn Errors of Immunity During the COVID-19 Pandemic

COVİD-19 Salgını Sırasında Doğuştan Bağışıklık Yetersizliği Olan Ergenlerde Psikososyal İşlevsellik

Ayşe Gül GÜVEN[1], Saliha ESENBOĞA[2], Melis PEHLİVANTÜRK KIZILKAN[1], Cihan ASLAN[3], Ayşegül AKARSU[2], Erdem KARABULUT[4], Devrim AKDEMİR[3], Nuray KANBUR[1], Deniz ÇAĞDAŞ[2], İlhan TEZCAN[2], Orhan DERMAN[1]

[1]Division of Adolescent Medicine, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye
[2]Division of Immunology, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye
[3]Division of Child and Adolescent Psychiatry, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye
[4]Division of Biostatistics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye

ABSTRACT

Objective: We aimed to determine the impact of COVID-19 on the psychosocial functioning of adolescents with inborn errors of immunity (IEI).

Material and Methods: Thirty-six patients with IEI (18 mild, 18 severe) and 18 healthy controls aged between 10 and 17 were included in this study. Adolescents and their caregivers completed the Revised Child Anxiety and Depression Scale (RCADS) to measure symptoms of anxiety and depression and the Strengths and Difficulties Questionnaire (SDQ) to assess prosocial behaviors and emotional/behavioral problems. Additionally, the COVID-19 Phobia Scale (C19P-S) was answered by adolescents to assess the level of coronavirus phobia.

Results: According to SDQ, emotional scores of the adolescents in the control group are higher than the mild/severe patient group. Emotional scores of the severe patient group are higher than the mild group. The RCADS parent- and adolescent-reported depression scores and parent-reported social phobia scores of the control group were significantly higher than the mild group.There were no statistically significant differences between the groups according to COVID-19 Phobia Scale.

Conclusion: The healthy adolescents had higher psychiatric symptom scores, especially than the adolescents with mild IEI. We suggest that being exposed to health-related challenges even before the pandemics in adolescents with IEI helped them develop psychological resilience. Longitudinal and larger studies are needed to evaluate the long-term effects of the pandemic on the mental health of this vulnerable adolescent population.

Key Words: Adolescents, COVID-19 Pandemic, Primary immunodeficiency, Mental health

ÖZ

Amaç: Bu çalışma, doğuştan bağışıklık yetersizliği olan ergenlerin psikososyal işlevleri üzerinde COVİD-19'un etkisini belirlemeyi amaçlamıştır.

Gereç ve Yöntemler: Bu çalışmaya 36 doğuştan bağışıklık yetersizlik hastası (18 hafif, 18 şiddetli) ve yaşları 10-17 arasında 18 sağlıklı kontrol dahil edildi. Ergenler ve onların ebeveynleri, kaygı ve depresyon semptomlarını ölçmek için Gözden Geçirilmiş Çocuk Kaygısı ve Depresyon Ölçeği'ni (RCADS) ve sosyal davranışları ve duygusal/davranışsal sorunları

0000-0003-3129-4315 : GÜVEN AG
0000-0003-0562-9863 : ESENBOĞA S
0000-0002-0637-050X : PEHLIVANTÜRK KIZILKAN M
0000-0003-2116-4461 : ASLAN C
0000-0002-1544-3408 : AKARSU A
0000-0003-2116-4461 : KARABULUT E
0000-0003-2116-4461 : AKDEMIR D
0000-0001-5767-7926 : KANBUR N
0000-0003-2213-4627 : ÇAĞDAŞ D
0000-0001-7257-4554 : TEZCAN İ
0000-0003-4618-1110 : DERMAN O

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. This study was approved by the Ethics Committee of the Hacettepe University (Decision number: 2021/368) and the Turkish Ministry of Health.

Contribution of the Authors / Yazarların katkısı*:** All authors contributed to the study's conception and design. ***GÜVEN A, ESENBOĞA S, AKARSU A: collected the data. ***GÜVEN AG:*** wrote the first draft of the manuscript. ***KARABULUTE:*** Eperformed statistical analysis. ***PEHLİVANTÜRK KIZILKAN M, ASLAN C, KANBUR N, AKDEMİR D, ÇAĞDAŞ D, TEZCAN I, DERMAN O:*** commented on the document and improved the discussion. All authors read and approved the final manuscript.

How to cite / *Atıf yazım şekli* : Güven AG, Esenboğa S, Pehlivantürk Kızılkan M, Aslan C, Akarsu A, Karabulut E, et al. Psychosocial Functionality in Adolescents with Inborn Errors of Immunity During the COVID-19 Pandemic. Turkish J Pediatr Dis 2024;18:166-173.

Correspondence Address / Yazışma Adresi:
Ayşe Gül GÜVEN
Division of Adolescent Medicine,
Department of Pediatrics, Faculty of Medicine,
Hacettepe University, Ankara, Türkiye
E-posta: aysegulguven1@gmail.com

Received / Geliş tarihi : 13.10.2023
Accepted / Kabul tarihi : 10.01.2024
Online published : 13.02.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1374480

değerlendirmek için Güçler Güçlükler Anketi'ni doldurdu. Ek olarak, ergenler tarafından koronavirüs fobisinin düzeyini değerlendirmek amacıyla COVİD-19 Fobi Ölçeği (C19P-S) yanıtlandı.

Bulgular: Güçler Güçlükler Anketi'ne göre kontrol grubundaki ergenlerin duygusal puanları hafif/ağır hasta grubuna göre daha yüksekti. Ağır hasta grubunun emosyonel puanları hafif gruba göre daha yüksekti. RCADS ebeveyn ve ergen skorlarına göre, kontrol grubunun depresyon puanları ve ebeveynlerin sosyal fobi puanları hafif gruba göre anlamlı derecede yüksekti.

Sonuç: Bu çalışma sonuçlarına göre sağlıklı ergenlerin psikiyatrik belirti skorları, özellikle hafif doğuştan bağışıklık yetersizliği olan ergenlere göre daha yüksektir. Doğuştan bağışıklık yetersizlikleri ergenlerin pandemiden önceki dönemde sağlıkla ilgili zorluklara maruz kalmalarının dayanıklılık geliştirmelerine yardımcı olduğunu düşünüyoruz. Pandeminin bu savunmasız ergen popülasyonunun ruh sağlığı üzerindeki uzun vadeli etkilerini değerlendirmek için uzun vadeli ve daha büyük çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Ergenler, COVİD-19, Primer immün yetmezlik, Ruh sağlığı

INTRODUCTION

The clinical course of COVID-19 infection in healthy children and adolescents has been reported to be milder than adults (1) and rarely progresses to acute respiratory distress syndrome, multiorgan system dysfunction and death (2). However, in the children and adolescents with inborn errors of immunity (IEI), the clinical course of COVID-19 differs from mild illness to death, which largely varies with the patient's age and the presence of immunosuppressive comorbidities (3). International studies show that more than 30% of patients with IEI have had mild COVID-19 infection and the 10% case-fatality rate was similar to the global data of the general population (4). On the other hand, in a Turkish study the COVID-19 infection mortality rate was reported as 7.6% among patients with IEI, which is eight times higher than the infection mortality rate (0.97%) of Turkey's general population (5). This wide range of mortality rates in different studies may be explained by the disease heterogeneity and concomitant comorbidities, as well as the differences in reporting systems in various countries.

Although adolescents' physical health was a less concern compared to other age groups, COVID-19-related mental health issues such as emotional reactions including concern, fear, and anxiety about being infected by the virus and depression, obsessive-compulsive symptoms, somatization, and behavioral issues have been found to be higher in young healthy individuals in most studies (6). However, there is limited data about the psychosocial effects of the pandemic on adolescents with chronic conditions (7). There are rare pre-pandemic studies about the behavioral, emotional and psychological well-being of adolescents with IEI and their parents. In these studies, it was reported that adolescents with IEI had significantly lower quality of life scores in adolescent and parent-rated questionnaires compared to healthy controls. In addition to this, higher rates of emotional, peer- relationship difficulties, anxiety and depressive scores were also reported in patients with IEI when compared to healthy controls (8,9). Psychosocial functionality in adolescents with IEI has not yet been the subject of extensive COVID-19 related psychological studies.

The management of a chronic illness during adolescence poses a significant burden not only for the adolescent but also for the parents, caregivers, and the health care providers (10). Recurrent and chronic infections, concomitant medical problems, regular hospital visits, the need for frequent hospitalization, and long-term treatment with immunoglobulin replacement therapy and immunomodulatory drugs add to the burdens imposed on patients with IEI and their families. Some studies reported that the parents of adolescents with chronic conditions experienced higher levels of anxiety and post-traumatic stress than controls during the pandemic (11,12).

We hypothesized that adolescents with IEI may have greater levels of behavioral, depressive, and anxiety symptoms than their healthy peers. The aim of this study was to investigate how COVID-19 affected the psychological, emotional, and behavioral well-being of adolescents with IEI.

MATERIAL and METHODS

Participants

This study was conducted by the divisions of Adolescent Medicine, Pediatric Immunology and Child and Adolescent Psychiatry of the Hacettepe University. This study took place between April and July 2021, during which pandemic curfews imposed on weekdays and weekends, school closures and intercity travel bans continued in Turkey. Between the ages 10 and 17 years, healthy adolescents who visited the Adolescent Medicine clinic formed the control group and adolescents with IEI who were followed up by the division of Pediatric Immunology formed the study group. The groups were age and gender-matched. The IEI group was further classified as mild and severe subgroups. Thirty-six patients with IEI (18 mild, 18 severe) and 18 healthy controls aged between 10 and 17 from a single children's hospital were included in this study.

All IEI patients were clinically diagnosed before the COVID-19 pandemic. The clinical diagnoses were made according to the European Society of Immunodeficiencies (ESID) guidelines (13). Since there is not any standardized form for the assessment of IEI severity, we modified a previously used scale to determine the IEI severity of the adolescent patients included. A severity score was calculated for each patient using the following: T-cell levels less than 500 cells/μl were assigned 8 points, for each immunoglobulin class impacted 1 point was assigned

(maximum of 4), receiving immunoglobulin replacement therapy was 4 points, and immunosuppressant/immunomodulatory therapy usage was 3 points. These points were tailed and the child was assigned to a severity group (mild 0-5, moderate >6). During the study period, no patients were diagnosed with COVID-19. None of the participants were vaccinated against COVID-19 at the time of the study since vaccination for that age group in the country had not yet started.

The exclusion criteria for IEI group were having a diagnosis of a psychiatric disorder and intellectual disability before pandemic. There were no patients with a previous psychiatric disorder or intellectual disability in the patient group. Having a previous chronic, systemic or psychiatric disorder and/or intellectual disability before pandemic period were also the exclusion criteria for the control group. All adolescents in the healthy control group were interviewed by HEEADSSS (home, eating, education, activities, drugs, sexuality, suicide, safety) psychosocial assessment which is a part of the standard care routine of the Adolescent Medicine clinic and offers a frame work for gathering detailed data about the young person's strengths and risky behaviors. At the end of this interview, adolescents who were determined not to have any psychiatric disease were included in the control group. Eight adolescents in the control group were excluded according to exclusion criteria. This study was approved by the Ethics Committee of the Hacettepe University (Decision number: 2021/368) and the Turkish Ministry of Health. Written informed consent were obtained from both adolescents and their parents.

Measures

Sociodemographic Information Form

This form was designed by the authors and included the parents' age and gender, the participants' age and gender, school grade, the number of children in the family, family structure (core, separated, large etc.), settlement (village, town, city), family income, parents' educational and employment status, family history of psychiatric disorders, and family history of COVID-19 infection.

Revised Child Anxiety and Depression Scale (RCADS)

The RCADS consists of 47 items developed to measure DSM-IV based symptoms of anxiety disorders and depression in children and adolescents. The subscales correspond to separation anxiety disorder (SAD) (7 items), social phobia (SP) (9 items), generalized anxiety disorder (GAD) (6 items), panic disorder (PD) (9 items), obsessions/compulsions (6 items) and major depressive disorder (MDD) (10 items). RCADS provides two summary scales: 1. Total anxiety and depression (total internalizing score), 2. Total anxiety and six subscales (14). In all instances, a higher score reflects a greater degree of symptom severity. Turkish reliability and validity study of the RCADS was provided by Gormez et al. (15) in children aged 8-17 years old. Inter-scale reliability is strong/excellent with a Cronbach's α of 0.95 and coefficients for the RCADS subscales ranging from 0.75 to 0.86 demonstrating good internal consistency. RCADS was administered face to face to both the participant and one of the parents.

Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a brief behavioural screening questionnaire designed to assess prosocial behaviors and emotional/behavioral problems of children. It contains five subscales, 25 questions (positive/negative). Subscales are inattention/hyperactivity, emotional problems, conduct problems, peer problems and prosocial behaviour. The scores related for inattention/hyperactivity, emotional problems, conduct problems, and peer problems can be summed to form a total difficulties score ranging from 0-40. The prosocial score is not included in the total score and is rated as positive behavior while others are rated as negative (16). High scores indicate greater clinical psychopathologies. The Turkish reliability and validity study was provided by Güvenir et al. (17) in children aged between 4-18. The Turkish SDQ was observed to be stable and reliable. SDQ was administered face to face to both the participant and one of the parents.

COVID-19 Phobia Scale (C19P-S)

C19P-S is a self-report five-point Likert-type scale which aims to assess the levels of coronavirus phobia developed by Arpacı et al. (18) in Turkey. The total score ranges from 20-100. It includes 20 items, 4 subscales (psychological factors, psychosomatic factors, economic factors, social factors). High scores indicate a greater phobia. C19P-S was administered face to face to the adolescents in both groups. The validity and reliability study of this scale was conducted between the ages of 12 and 92 (18).

Statistical Analyses

Statistical analyses were performed with the IBM SPSS Statistics for windows version 23. (IBM Corp., Armonk, NY) software. Frequencies and percentage for qualitative variables, mean ± standard deviation or median (min-max) for numerical variables were used as descriptive statistics. The Shapiro-Wilk test was used to determine if the numerical variables were normally distributed or not. Chi-square test was used to determine whether there was a difference in terms of qualitative variables among three groups. When numerical variables were normally distributed one-way ANOVA was used and pairwise comparisons were performed using the Tukey test; otherwise the Kruskal-Wallis test was used and pairwise comparisons were performed using Dunn's test. A statistically significant P value of 0.050 was used.

RESULTS

Demographic features of the participants are listed in Table I. There were no statistically significant differences between the

Table I: Demographic Features of the Groups

	Severe (n=18)	Mild (n=18)	Control (n=18)	F /X²	p
Participant age (mean±SD)	14.4 ± 2.1	14.0 ± 2.3	14.3 ± 1.57	0.176	0.839
Participant gender*					
Girl	6 (33.6)	6 (33.3)	6 (33.3)	0.000	1.000
Boy	12 (66.7)	ß€12 (66.7)	12 (66.7)		
Parent's gender*					
Mother	13 (72.2)	12 (66.7)	13 (72.2)	0.178	0.915
Father	5 (27.8)	6 (33.3	5 (27.8)		
Mother's age*					
<29	1(5.6)	1 (5.6)	0 (0.0)	7.422	0.217
30-39	4 (22.2)	8 (44.4)	5 (27.8)		
40-49	12 (66.7)	9 (50)	9 (50)		
≥50	1 (5.6)	0 (0.0)	4 (22.2)		
Father's age*					
<29	0 (0.0)	1 (5.6)	0 (0.0)	9.720	0.08
30-39	2 (11.1)	4 (22.2)	1 (5.6)		
40-49	14 (77.8)	11 (61.1)	9 (50.0)		
≥50	2 (11.1)	2 (11.1)	8 (44.4)		
Mother's grade at school*					
Illiterate	0 (0.0)	1 (5.6)	0 (0.0)	8.433	0.596
Literate	0 (0.0)	0 (0.0)	2 (11.1)		
Primary-middle school	7 (38.9)	5 (27.8)	6 (33.3)		
High school	7 (38.9)	10 (55.6)	6 (33.3)		
Graduate	4 (22.2)	2 (11.1)	3 (16.7)		
Postgraduate	0 (0.0)	0 (0.0)	1 (5.6)		
Father's grade at school*					
Illiterate	0 (0.0)	1 (5.6)	0 (0.0)	7.355	0.466
Literate	0 (0.0)	0 (0.0)	1 (5.6)		
Primary-middle school	9 (50)	8 (44.4)	4 (22.2)		
High school	5 (27.8)	5 (27.8)	9 (50.0)		
Graduate	4 (22.2)	4 (22.2)	4 (22.2)		
Postgraduate	0 (0.0)	0 (0.0)	0 (0.0)		
Mother's employment status*					
Not working	14 (77.8)	14 (77.8)	15 (83.3)	0.336	1.000
Working	4 (22.2)	4 (22.2)	3 (16.7)		
Father's employment status*					
Not working	3 (16.7)	2 (11.1)	4 (22.2)	0.857	0.898
Working	15 (83.3)	16 (88.9)	14 (77.8)		
Family Structure*					
Core	16 (88.9)	16 (88.9)	16 (88.9)	2.405	1.000
Large	1 (5.6)	2 (11.1)	2 (11.1)		
Other (divorced etc,)	1 (5.6)	0 (0.0)	0 (0.0)		
Living place*					
Village	0 (0)	0 (0)	0 (0.0)	3.060	0.349
Town	2 (11.1)	3 (16.7)	0 (0.0)		
City	16 (88.9)	15 (83.3)	18 (100)		
Family history of psychiatric disorders*					
No	15 (83.3)	14 (77.8)	14 (82.4)	0.316	1.000
Family work impact status from Covid*					
No	10 (55.6)	9 (50.0)	11 (61.1)	0.450	0.799
Family history of Covid*					
No	14 (77.8)	14 (77.8)	8 (44.4)	6.000	0.05

*****n (%)*

three groups according to the parents' and the participants' age and gender, school grade, the number of children in the family, family structure, settlement, family income, parent's educational and employment status, family history of psychiatric disorders, and family history of COVID-19 infection.

The median (min-max) values of the SDQ subgroup scores in the parent and adolescent groups are presented in Table III. The highest emotional symptom scores of adolescents were observed in the control group, the lowest were observed in the mild IEI group. Emotional scores of the control group

Table II: Diagnostic Subgroups of Adolescents with IEI

IEI Groups	n (%)	Severe /Mild (n)
Innate immune system defect	5 (14)	1/4
Immune deficiency with immune dysregulation	7 (20)	4/3
Primary antibody deficiency	4 (12)	3/1
Combined immune deficiency	10 (27)	10/0
Autoinflammatory disease	1 (3)	0/1
Other antibody deficiency	9 (24)	0/9

Table III: Parental and Adolescents' Scores of the SDQ

SDQ Subgroup	Severe (n=18) Median (min-max)	Mild (n=18) Median (min-max)	Control (n=18) Median (min-max)	Kruskal -Wallis H	p
Emotional Symptoms					
Parent	2.0 (0-6)	1.0 (0-6)	2.0 (0-6)	0.471	0.790
Adolescent	2.0 (0-7)[a]	1.0 (0-3)[a,b]	2.0 (0-10)[b]	8.300	0.016
Conduct Problems					
Parent	0.0 (0-3)	1.0 (0-5)	1.0 (0-4)	4.394	0.111
Adolescent	1.0 (0-5)	2.0 (0-5)	2.0 (1-6)	1.966	0.374
Inattention/ Hyperactivity					
Parent	3.0 (0-9)	3.0 (0-8)	4.0 (2-7)	3.004	0.223
Adolescent	3.0 (0-10)	3.0 (0-6)	3.5 (0-9)	0.704	0.798
Peer Problems					
Parent	4.0 (1-6)	2.0 (0-6)	3.5 (0-6)	2.895	0.235
Adolescent	3.0 (0-4)	3.0 (0-5)	3.0 (0-9)	0.451	0.796
Prosocial Behaviour					
Parent	8.0 (4-10)	7.5 (4-10)	7.0 (2-10)	1.830	0.400
Adolescent	8.0 (4-10)	8.0 (4-10)	8.0 (3-10)	0.933	0.627
Total Deviance/ Difficulties					
Parent	9.5 (2-18)	7.5 (2-23)	9.5 (6-19)	3.090	0.213
Adolescent	10.5(5-20)	8.5 (4-15)	11 (3-31)	3.722	0.155

SDQ: *Strength and Difficulties Questionnaire,* ***a, b:*** *There was a statistically significant difference between the groups indicated with the same letter (p<0.050).*

Table IV: Scores of the COVID-19 Phobia Scale in Adolescents

C19P-S Subgroup	Severe (n=18) Median (min-max)	Mild (n=18) Median (min-max)	Control (n=18) Median (min-max)	Kruskal -Wallis H	p
Psychological factors	20.5 (6-30)	14.0 (7-28)	14.0 (6-27)	0.867	0.648
Psycho-somatic factors	8.0 (5-20)	5.5 (5-17)	7.0 (5-15)	1.794	0.408
Social factors	13.0 (5-24)	9.0 (5-25)	11.5 (5-18)	2.639	0.267
Economic factors	7.0 (4-17)	6.5 (4-16)	6.0 (4-17)	1.872	0.392
Total score	35.5 (15-65)	27.5 (16-59)	29.0 (17-46)	1.606	0.448

were statistically higher than the severe and mild IEI groups (p <0.050). Also, the emotional scores of adolescents were statistically different between the severe-mild IEI and mild IEI-control groups (p=0.016). There were no statistically significant differences between the groups according to parent scores (p=0.790). The subgroup scores of C19P-S are presented in Table IV.

There were no statistically significant differences between the groups according to COVID-19 Phobia Scale. However, the depression and social phobia subgroup scores of parents in the control group were statistically higher than the mild IEI group in RCADS questionnaire (p=0.001 and p=0.031). The depression scores of adolescents in the control group were significantly higher than the mild group (p=0.006). Parental and adolescent scores in the severe group were higher than the mild group but lower than the control group, but the difference was not statistically significant (Table V). All adolescents with high scores were referred for further psychiatric evaluation.

DISCUSSIONS

In this study, emotional and behavioral problems and anxiety and depressive symptoms of adolescents with mild and severe

Table V: Parental and Adolescents' Scores of the RCADS

RCADS Subscores	Severe (n=18) Median (min-max)	Mild (n=18) Median (min-max)	Control (n=18) Median (min-max)	Kruskal -Wallis H	p
Separation anxiety					
Parent	2.5 (0-10)	2.0 (0-5)	2.0 (0-7)	2.048	0.359
Adolescent	2.0 (0-6)	1.5 (0-10)	1.0 (0-9)	0.234	0.890
Generalized anxiety					
Parent	4.9 (2-11)	4.4 (0-12)	5.0 (0-15)	1.019	0.601
Adolescent	6.0 (0-10)	6.0 (1-12)	7.0 (0-13)	0.137	0.934
Depression					
Parent	7.0 (2-15)	4.0 (1-15)[a]	9.0 (5-20)[a]	13.847	0.001
Adolescent	7.0 (0-21)	4.0 (1-19)[b]	10.5 (1-23)[b]	10.115	0.006
Panic disorder					
Parent	2.0 (0-14)	2.0 (0-6)	3.0 (0-9)	2.018	0.365
Adolescent	3.0 (0-10)	3.0 (0-15)	3.5 (0-18)	1.164	0.320
Social phobia					
Parent	5.5 (0-18)	5.2 (1-19)[c]	9.0 (0-19)[c]	6.976	0.031
Adolescent	7 (1-17)	5.8 (2-15)	9.0 (4-21)	4.148	0.126
Obsessions /compulsions					
Parent	3.5 (0-12)	2.0 (0-12)	2.5 (0-10)	0.365	0.833
Adolescent	5.0 (0-9)	4.0 (0-10)	5.0 (0-12)	2.815	0.245
Total anxiety					
Parent	20.0 (4-64)	16.0 (4-38)	27.5 (1-55)	3.396	0.183
Adolescent	23.0 (4-68)	21.3 (8-51)	28.5 (10-66)	2.252	0.324
Total anxiety & depression					
Parent	29.3 (7-76)	19.7 (5-45)	36.0 (9-75)	5.506	0.064
Adolescent	29.0 (4-59)	24.5 (10-70)	38.5 (11-86)	4.714	0.095

RCADS: *Revised Child Anxiety and Depression Scale,* [a, b, c]***:*** *There was a statistically significant difference between the groups indicated with the same letter (p<0.050).*

IEI were compared to healthy controls during the early stages of COVID-19 pandemic when isolation rules were strictly continued in Turkey. In contrast to our hypothesis, we found that healthy controls exhibited more emotional/depressive and social phobia symptoms than the adolescents with mild IEI. On the other hand, the severe IEI group had significantly higher emotional symptoms than the mild IEI group. The highest depressive symptoms were observed in the control group. The depressive symptoms of the severe IEI group were higher than the mild IEI group, but the only significant difference was between the control and the mild IEI group scores. In addition, coronaphobia levels and anxiety symptoms were not different between the three groups.

In contrast to our results demonstrating that the highest emotional symptoms were observed in the control group, P. Titman et al. (9) showed that children with primary antibody deficiency had significantly higher emotional scores of SDQ than the healthy controls. Their results were also consistent with studies designed in patients with different types of IEI and healthy children (19). Kuburovic, et al. (8) using different self and parent-rated assessments, found that children with IEI had significantly lower emotional functioning, higher anxiety and depressive symptoms than children with juvenil idiopathic arthritis (JIA) and healthy controls. In a different study designed by Ocakoğlu, et al. (20) the rate of psychopatology was found similar in IEI and JIA patients, being higher than the healthy control group. Piazza-Waggoner, et al. (21) also observed that as the severity of the disease increased, patients' psychosocial functioning deteriorated. However, all these studies showing that the psychosocial functioning of healthy children are higher than the IEI group, were conducted before the pandemic.

During the pandemic, the lockdowns, school closures, spending much more time at home, obligation to wear a mask, decreased social interaction, fear of getting infected had a negative impact on all adolescents. Some of these drastic changes occurred with the pandemic in the lives of healthy adolescents might have been already experienced before the pandemic at some level by the patients with IEI as a burden of their disease and related lifestyle. On the other hand, facing the risk of experiencing a serious health problem like the pandemic for the first time in the healthy control group may have increased their fear, anxiety and they may have failed to elicit a coping response. This might be the cause of our results contradicting the studies conducted prior to the pandemic. In addition, compared to the IEI group, higher family history of COVID-19 infection in the control group, although not statistically significant, may have affected their family functionality and this may have been reflected as emotional symptoms.

Another study from Turkey by Kılıç et al. (22) investigated the effects of COVID-19 pandemic on mental health of children with IEI found the depression scores significantly higher in patients with IEI than controls. However, they used only parent-reported scales and the study was performed at a different time period

during the pandemic with less limited social restrictions. A study evaluating the effect of pandemic on anxiety symptoms among Turkish adolescents with another chronic condition, cystic fibrosis (CF) found that COVID-19 had no effect on the anxiety of adolescents with CF (23). Similar to our results, healthy children had higher anxiety symptoms than patients with CF.

Although COVID-19 pandemic is expected to bring more stress for the immune compromised patients and their families, the lower emotional symptoms of mild IEI group than healthy control group in adolescents in our study may also be the result of both the patient's and parents' being exposed to the difficulties prior to the pandemic resulting from having a chronic condition, recurrent complicated medications and hospitalizations that leads to post-traumatic growth and resilience in the adolescents with mild IEI. Post-traumatic growth is defined as a significant positive change in an individual's life as an effect of exposure to a traumatic event which is a process that goes beyond the absence of symptoms or a return to baseline functioning following a trauma such as experiencing a serious pediatric illness (24,25). Eventually, these adolescents develop resilience as the ability to maintain healthy levels of functioning despite difficult experiences or returning to normal functioning when experiencing crisis and are more adaptive when responding (26,27). However, the adolescents with severe IEI having more difficulties in their disease management, poorer course of the disease with regard of disease activity and duration and more impaired quality of life may have caused higher emotional and depressive symptoms than the mild group.

In this study, adolescent-rated emotional symptoms scores of SDQ and parent-rated social phobia scores of RCADS were significantly different between three groups. Hence, adolescent and parent scores differed in terms of emotional symptoms and social phobia scores both in SDQ and RCADS scales, as with some other subscales. Similar to our study, parent-child agreement on reports of especially internalizing symptoms has been shown to be low in previous studies (29,30). Internalizing symptoms are generally better described by patients than parents (28).

This study has some limitations. First of all, it was conducted in a single center which limited reaching a larger sample of patients. To accurately analyze the effect of the pandemic, the data were collected before the COVID-19 restrictions were dropped in Turkey. Therefore, the limited number of participants is due to the restricted time between the onset of the study and the onset of the normalization period. Also, as this was a cross-sectional study, we could not analyze the premorbid characteristics and psychosocial functionality of the adolescents before the pandemic. We also could not conduct a formal interview with the IEI patients to identify psychosocial challenges because of the pandemic restrictions. However, participants in the IEI group had not been previously diagnosed with a psychiatric disorder. Despite these limitations, there are some strengths of this study. This is one of the first study evaluating the psychiatric effects of COVID-19 pandemic on adolescents with IEIs. The assessments being performed both for the parent and the adolescent in a limited time frame is another strength of the study.

In conclusion, healthy adolescents had more psychiatric symptoms than the adolescents with mild IEI during the pandemic, and the adolescents with IEIs did not have higher coronaphobia than their healthy peers. We suggest that being exposed to health-related challenges even before the pandemics in adolescents with IEI helped them to develop psychological resilience. However, the emotional and behavioral symptoms in adolescents with severe IEI were higher than the ones with mild IEI both in parental and adolescent reports. This finding is most probably because of the awareness of the increased burden of the disease, significant morbidity and poorer course of severe IEI. Although there is no significant difference between the COVID-19 Phobia Scale scores, family history of COVID was seen more frequently, close to a significant level, in the control group than in the disease group. This may be a limitation that affected the results. This study points out that routine psychosocial screening is essential for all adolescents especially in risky conditions that poses a threat to their mental health and wellbeing. Healthcare providers should pay attention to disease characteristics and personal strengths while evaluating for psychosocial stressors in adolescents with IEI.

REFERENCES

1. Ludvigsson JF. Systematic review of COVİD-19 in children show milder cases and better prognosis than adults. Acta Pediatr 2020; 109:1088-95.
2. PCM Williams, AR Howard-Jones, P Hsu, Palasanthiran P, Gray PE, McMullan BJ, et al. SARS-CoV-2 in children: spectrum of disease, transmission and immunopathological underpinnings. Pathology 2020; 52:801-8.
3. Williamson EJ, Walker AJ, Bhaskara K, Bacon S, Bates C, Morton CE, et al. OpenSAFELY: factors associated with COVID-19 death in 17 million patients. Nature 2020; 584:430-6.
4. Meyts I, Bucciol G, I Quinti I, Neven B, Fischer A, Seoane E, et al. Coronavirus disease 2019 in patients with inborn errors of immunity: An international study. J Allergy Clin Immunol 2021;147:520-31.
5. Esenboga S, Ocak M, Akarsu A, Bildik HN, Çağdaş D, Topeli A, et al. COVID-19 in Patients with Primary Immunodeficiency. Journal of Clin Immunol 2021;41:1515-22.
6. Burhamah W, Alkhayyat A, Oroszlanyova M, Kenane A, Almansouri A, Behbehani M, et al. The psychological burden of the COVID-19 pandemic and associated lockdown measures: Experience from 4000 participants. J. Affect Disord 2020; 277:977-85.
7. Lindoso L, Astley C, Bruni Queiroz L, Gualano B, Pereira RMR, Tannuri U, et al. Physical and mental health impacts during COVID-19 quarantine in adolescents with preexisting chronic immunocompromised conditions. J Pediatr 2022; 98: 350-61.
8. Kuburovic NB, Pasic S, Susic G, Stevanovic D, Kuburovic V, Zdravkovic S, et al. Health-related quality of life, anxiety, and

depressive symptoms in children with primary immunodeficiencies. Patient Prefer Adherence 2014; 8:323-30.

9. Titman P, Allwood Z, Gilmour C, Malcolmson C, Duran-Persson C, Cale C, et al. Quality of Life in Children with Primary Antibody Deficiency. J Clin Immunol 2014;34:844-52.
10. Suris JC, Michaud PA, Viner R. The adolescent with a chronic condition. Part I: developmental issues. Arch Dis Child 2004; 89: 938-42.
11. Yılmaz Topal Ö, Metin A, Çöp E, Şenses Dinç G, Şükran Üneri Ö. Anxiety among the parents of pediatric patients receiving IVID therapy during COVID-19 Pandemic. Turkish J Pediatr Dis 2021; 63: 801-10.
12. Akdağ B, Önder A, Gizli Çoban Ö, Kocacık Uygun DF, Sürer Adanır A, Erdem A, et al. Psychological State of Parents of Children with Primary Immunodeficiencies During the COVID-19 Pandemic. Pediatric Allergy Immunol Pulmonol 2022; 35:12-8.
13. Seidel MG, Kindle G, Gathman B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract 2019; 7: 1763-70.
14. Chorpita BF, Yim L, Moffitt C, Umemoto AL, Francis ES. Assessment of symptoms of DSM-IV anxiety and depression in children: a Revised Child Anxiety and Depression Scale. Behav Res Ther 2000; 38: 835–55.
15. Gormez V, Kılınçaslan A, Orengul AC, Ebesutani CE, Kaya İ, Ceri V, et al. Psychometric properties of the Turkish version of the Revised Child Anxiety and Depression Scale – Child Version in a clinical sample. Psychiatry Clin Psychopharmacol 2017; 27: 84-92.
16. Goodman R. The Strengths and Difficulties Questionnaire: A research note. J Child Psychol Psychiatry 1997;38:581-6.
17. Güvenir T, Özbek A, Baykara B, Arkar H, Şentürk B, İncekaş S. Psychometric Properties of the Turkish Version of The Strengths and Difficulties Questionnaire (SDQ) Turk J Child Adolesc Ment Health 2008;15: 65-74
18. Arpaci İ, Karatas Ķ, Baloğlu M. The development and initial tests for the psychometric properties of the COVID-19 Phobia Scale (C19P-S). Pers Individ Dif 2020; 164: 110108.
19. Cole TS, Jones L, Mc Grogan P, Pearce MS, Flood TJ, Cant AJ, et al. Emotional and behavioral difficulties in chronic granulomatosus disease. Arch Dis Child 2012; 97: 87.
20. Kayan Ocakoğlu B, Edeer Karaca N, Ocakoğlu FT, Erermis S. Psychological burden of pediatric primary immunodeficency. Pediatr Int 2018; 60: 911-7.
21. Piazza-Waggoner C, Adams CD, Cottrell L, Taylor BK, Wilson NW, Hogan MB. Child and caregiver psychosocial functioning in pediatric immunodeficiency disorders. Ann Allergy Asthma Immunol 2006; 96: 298-303.
22. Kılıç AO, Uzun N, Akın F, Akıncı MA, Yazar A, Bozkurt Alan H, et al. The Effects of COVİD-19 pandemic on Mental Health in Children with Primary Immunodeficiency. Pediatr Pract Res 2022; 10: 83-8.
23. Senkalfa BP, Sismanlar Eyuboglu T, Aslan AT, Ramaslı Gursoy T, Soysal AS, et al. Effect of the COVİD-19 pandemic on anxiety among children with cyctic fibrosis and their mothers. Pediatr Pulmonol 2020; 55: 2128-34.
24. Melissa Bernstein, Betty Pfefferbaum. Posttraumatic Growth as a Response to Natural Disasters in Children and Adolescents. Curr Pschiatry Rep 2018; 20:37.
25. Picoraro JA, Wormer WJ, Kazak AE, Feudtner C. Posttraumatic Growth İn Parents. J Palliat Med 2014;17:209-18.
26. Constanzo ES, Ryff C, Singer BH. Psychosocial adjustment among cancer survivors: Findings from a national survey of health and well-being. Health Psychol 2009;28: 147-56.
27. Rutter M. Implications of resilience concepts for scientific understanding. Ann N Y Acad Sci 2006; 1094: 1-12.
28. Kemper TS, Gerhardstein R, Repper KK, Kistner JA. Mother-Child Agreement on Reports of Internalizing Symptoms Among Children Referred for Evaluation of ADHD. J Psychopathol Behav Assess 2003; 25: 239-49.
29. Choudhury MS, Pimentel SS, Kendall PC. Childhood anxiety disorders: parent-child (dis)agreement using a structured interview for the DSM-IV. J Am Acad Child Adolesc Psychiatr 2003; 42: 957-63.
30. Sourander A, Hestela L, Helenius H. Parent-adolescent agreement on emotional and behavioral problems. Soc. Psychiatr Epidemol 1999; 34: 657-63.

Turkish Journal of Pediatric Disease
Türkiye Çocuk Hastalıkları Dergisi

Is There a Relationship Between Urinary Tract Infections and Vitamin D and Cathelicidin Levels?: A Cross-Sectional Observational Study From the Pediatric Emergency Department

İdrar Yolu Enfeksiyonları ile D Vitamini ve Katelisidin Düzeyleri Arasında Bir İlişki Var mı?: Çocuk Acil Servisinden Kesitsel Gözlemsel Bir Çalışma

Alper ÇİÇEK[1], Pelin ELİBOL[1], Banu İŞBİLEN BAŞOK[2], Dilek ORBATU[3], Emel BERKSOY[1], Demet ALAYGUT[4], Oya BALTALI HIDIR[3]

[1]Department of Pediatric Emergency, University of Health Sciences, İzmir Tepecik Education and Research Hospital, İzmir, Türkiye
[2]Department of Medical Biochemistry, University of Health Sciences, İzmir Tepecik Education and Research Hospital, İzmir, Türkiye
[3]Department of Pediatric, University of Health Sciences, Izmir Tepecik Education and Research Hospital, İzmir, Türkiye
[4]Department of Pediatrics Division of Pediatric Nephrology, University of Health Sciences, Izmir Tepecik Education and Research Hospital, İzmir, Türkiye

ABSTRACT

Objective: Cathelicidin is a crucial antibacterial peptide that is produced in the urinary system and is induced by vitamin D. In order to distinguish between lower and upper urinary tract infections (UTIs), the association between cathelicidin levels and vitamin D levels was examined in this study.

Material and Methods: We analyzed complete blood count, biochemistry profile, C reactive protein (CRP), 25 hydroxyvitamin D, serum cathelicidin levels of pre-treatment children aged 0-18 years who were diagnosed with a UTI in the Pediatric Emergency Room.

Results: A total of 72 children (36 healthy and 36 patients) were included in the study. The mean age of the participants was 83.8±66.22 months, with 40 (56%) female and 32 (44%) male. Our patient group had higher white blood cell, neutrophil, and CRP levels than our control group (p=0.050). There was no significant difference in cathelicidin levels (5.7±3.7; 9.6±10.9; p=0.810) or vitamin D levels (23.3±9.5; 25.9±12.5; p=0.795) between patients with lower and upper UTI. We found a positive correlation between vitamin D and cathelicidin levels in the control group (r=0.346, p=0.030). There was no statistically significant difference in cathelicidin levels between patients with upper UTI and the control group (p=0.054).

Conclusion: Although there was no significant relationship between vitamin D and cathelicidin levels in children with urinary tract infections, a weak but positive correlation exists between vitamin D and cathelisidin in healthy children.

Key Words: Cathelicidin, Emergency, Pediatric, Urinary tract infection, Vitamin D

ÖZ

Amaç: Katelisidin, üriner sistemde üretilen ve D vitamini tarafından indüklenen önemli bir antibakteriyel peptittir. Bu çalışmada alt ve üst idrar yolu enfeksiyonlarını (İYE) ayırt etmek için katelisidin düzeyleri ile D vitamini düzeyleri arasındaki ilişki incelenmiştir.

0000-0001-5017-4525 : ÇİÇEK A
0000-0002-2195-7421 : ELİBOL P
0000-0002-1483-997X : İŞBİLEN BAŞOK B
0000-0002-5716-2938 : ORBATU D
0000-0002-6831-1353 : BERKSOY E
0000-0002-2164-4652 : ALAYGUT D
0000-0002-0062-9138 : BALTALI HIDIR O

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. This study was approved by the ethics committee of Izmir Tepecik Training and Research Hospital (15.06.2021-2021/06-52).

Contribution of the Authors / Yazarların katkısı*: *ÇİÇEK A: Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in the writing of the whole or important parts of the study. ***ELİBOL P:*** Taking responsibility in necessary literature review for the study. ***İŞBİLEN BAŞOK B:*** Reviewing the article before submission scientifically besides spelling and grammar. ***ORBATU D:*** Taking responsibility in necessary literature review for the study. ***ALAYGUT D:*** Constructing the hypothesis or idea of research and/or article, Organizing, supervising the course of progress and taking the responsibility of the research/study, Reviewing the article before submission scientifically besides spelling and grammar. ***BALTALI HIDIR O:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Taking responsibility in necessary literature review for the study, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli* : Çiçek A, Elibol P, İşbilen Başok B, Orbatu D, Berksoy E, Alaygut D, et al. Is There A Relationship Between UTI and Vitamin D and Cathelicidin Levels?: A Cross-Sectional Observational Study From the Pediatric Emergency Department. Turkish J Pediatr Dis 2024;18:174-180.

Correspondence Address / Yazışma Adresi:
Alper ÇİÇEK
Department of Pediatric Emergency,
University of Health Sciences,
İzmir Tepecik Education and Research Hospital, İzmir, Türkiye
E-posta: dr_alper_cicek@hotmail.com

Received / Geliş tarihi : 26.09.2023
Accepted / Kabul tarihi : 19.01.2024
Online published : 15.02.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1366493

Gereç ve Yöntemler: Çocuk Acil Servisinde idrar yolu enfeksiyonu tanısı alan 0-18 yaş arası tedavi öncesi çocukların tam kan sayımı, biyokimya profili, C reaktif protein (CRP), prokalsitonin, 25 hidroksivitamin D ve serum katelisidin düzeylerini analiz ettik.

Bulgular: Çalışmaya toplam 72 çocuk (36 sağlıklı ve 36 hasta) dahil edildi. Katılımcıların yaş ortalaması 83.8±66.22 ay olup, 40'ı (%) kadın, 32'si (%) erkektir. Hasta grubumuzun beyaz küre, nötrofil ve CRP düzeyleri kontrol grubumuza göre daha yüksekti (p=0.050). Alt ve üst İYE'li hastalar arasında katelisidin düzeyleri (5.7±3.7; 9.6±10.9; p=0.810) veya D vitamini düzeyleri (23.3±9.5; 25.9±12.5; p=0.795) açısından anlamlı fark yoktu. Kontrol grubunda D vitamini ile katelisidin düzeyleri arasında pozitif korelasyon bulduk (r=0.346; p=0.030). Üst İYE'li hastalar ile kontrol grubu arasında katelisidin düzeyleri açısından istatistiksel olarak anlamlı fark yoktu (p=0.054).

Sonuç: İdrar yolu enfeksiyonu olan çocuklarda D vitamini ile katelisidin düzeyleri arasında anlamlı bir ilişki bulunmazken, sağlıklı çocuklarda D vitamini ile katelisidin arasında zayıf fakat pozitif bir ilişki bulunmaktadır.

Anahtar Sözcükler: Katelisidin, Acil, Pediatri, Üriner sistem enfeksiyonu, Vitamin D

INTRODUCTION

Although based on observational research, it has been proposed that adequate vitamin D intake throughout childhood lowers levels of TNF-, C-reactive protein (CRP), and IL-6 and prevents the spread of inflammation. According to several epidemiological research, vitamin D insufficiency is linked to a variety of conditions outside those that affect the musculoskeletal system. Food allergies, hypertension, hyperglycemia, metabolic syndromes, and upper respiratory tract infections are all linked to decreased serum 25-hydroxyvitamin D3 (25(OH)D3) levels (1- 5). Recently, there was a correlation between vitamin D deficiency (serum 25(OH) D3 level 20 ng/mL) and recurrent urinary tract infections in children (6). Cathelicidin is a bactericidal agent that clears off intracellular mycobacteria and has a regulatory role in multiple different processes of autophagy activity. It helps positively to the fusion of mycobacterial phagosomes, autolysosomes, and autophagosomes (7,8). Also, it can stimulate chemokine and cytokine production with the help of various cell types. Its expression in neutrophils, monocytes, epithelial cells and macrophages is stimulated by 1,25 dihydroxyvitamin D (1, 25(OH)2D). Studies on urinary tract infections (UTI) have revealed that it is crucial for safeguarding the urinary tract (9,10). In addition, it is estimated that the defense system of the urinary tract may largely depend on some mediators, which are specifically soluble and epithelial cell-derived. It is presumed to be induced by bactericidal antimicrobial peptides such α, β-defensins, and cathelicidin (11,12). It is known that when exposed to *E. coli,* bladder and renal epithelial cells release cathelicidins, specifically LL-37 in humans (13,14). Cathelicidin and vitamin D levels in pediatric patients with upper or lower urinary tract infections were compared in this investigation before treatment. To find out the connection between the type and severity of urinary tract infections, they were compared with healthy controls.

MATERALS and METHODS

In this case control research, individuals with UTI who were hospitalized at the University of Health Sciences, İzmir Tepecik Hospital, Emergency Medicine Clinic between June and December 2021 were included. Prior to therapy, the values of the complete blood count, biochemistry profiles, C-reactive protein, procalcitonin, and 25(OH)D3 and serum cathelicidin levels were examined. Patients with immunological deficiencies, anatomical or functional abnormalities of the urinary system, or diabetes mellitus were excluded. All patients who participated in the study provided written and verbal consent. The patients' demographic data, UTI type (lower/upper), urine analysis outcomes, and results of blood tests were documented. Patients with at least one of the following symptoms of upper urinary tract infection (UTI) flank pain, costovertebral angle tenderness, fever, abdominal pain, the presence of pyuria/nitrite in the urine as well as growths of ≥50.000 CFU/ml in the catheter culture and ≥100.000 CFU/ml in the midstream urine were considered to have an upper UTI. In infants and young children, UTI usually presents with nonspecific symptoms and signs (e.g. fever, irritability, vomiting, diarrhea, poor feeding). Fever may be the sole manifestation of UTI in infants and children <2 years of age. Urine nitrite and bacteriuria positivity and significant growth in urine culture were accepted as upper UTI even if the infants had fever condition and nonspecific symptoms. Patients with at least one of the following symptoms, as well as pyuria (≥5 leukocytes per high magnification in urine microscopy), nitrite, and ≥50.000 CFU/ml in catheter culture, ≥100.000 CFU/ml in midstream pee a growth of CFU /ml were classified as having a lower UTI. Serum 25 (OH) D3 levels were classified as follows: normal ≥ 30ng/ml, deficient 20-30 ng/mL, and severely deficient < 12ng/mL (14). Age and gender-matched healthy children whose blood samples were taken during routine control were included in the control group (Group 2). Plain blood collection tubes (BCTs) (BD Vacutainer® SST II Advance Tube, 5mL, 13x100 mm, USA) were used to collect venous blood samples. Within an hour of blood collection, serum samples were isolated from cellular debris by centrifugation for 10 minutes at 1.500 g. Before further investigation, serum samples were divided into smaller amounts and kept at 80 °C.

Serum cathelicidin concentrations were determined by a commercial "Human LL37(Anti-bacterial protein LL-37)" kit employing quantitative sandwich enzyme immunoassay technique (Elabscience, Houston, TX, USA)(Catalog No: E-EL-H2438). In accordance with the manufacturer's instructions, the analysis was completed. The kit's CVs (coefficients of variation) were less than 10% both within and

between assays. The test's limit of detection (LOD) was 0.94 ng/mL.

Serum 25-OH-Vitamin D levels were measured by an immunoassay analyzer (Advia Centaur XP; Siemens Healthineers, Siemens Healthcare GmbH, Germany) according to the manufacturers' instructions.

This study was approved by the ethics committee of Izmir Tepecik Training and Research Hospital (15.06.2021-2021/06-52). Every method carried out during the study complied with the Declaration of Helsinki's guiding principles as well as the institutional and national research committee's ethical requirements.

Statistical Analysis

Using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA), a statistical software product, we assessed the data. For descriptive statistics, the frequency (n), percentage (%), mean and standard deviation were all given. The Shapiro Wilk test of normality and Q-Q graphs were used to assess the normal distribution of the numerical data. Using Levene's test, we looked at the homogeneity of variances. Independent samples T test was used to compare the means of two independent groups consisting of normally distributed continuous data. Differences between two groups which were not normally distributed were examined with the Mann-Whitney U Test. For categorical data, Pearson's chi-square test and Fisher's exact test were used. Correlations were assessed using the Spearman's correlation test. A p value less than 0.05 was considered statistically significant.

RESULTS

A total of 72 children were included in the study. Thirty-six of these children (n=22 girls (61.1%), n=14 boys (38.9%)) with a mean age of 50.4±53.8 months were in the patient group diagnosed with UTI, and 36 of them (n=18 girls (50%), n=18 boys (50%)) with a mean age of 117.2±60.8 months were in the healthy group. Age and gender did not significantly difference between the patient and control groups (p=0.343) (Table I).

Fever was experienced by 29 patients (80.6%) and stomach pain by 16 patients (44.4%) as the most frequent UTI symptoms.

Table I: Demographic data, patient symptoms and types of UTI in the study group

Demographic data of study subjects	UTI group n: 36	Control group n: 36	p
Age (mean±SD) (months)	50.40±53.84	117.27±60.82	0.343
Sex (girl/boy)	22/14	18/18	0.920
Type of UTI n(%)			
Lower UTI	7 (19.4)	7 (19.4)	
Upper UTI	29 (80.6)	29 (80.6)	

UTI: *Urinary tract infections*

The presence of an unpleasant odor, suprapubic pain, dysuria, frequent urination, and flank pain were other symptoms (Table II).

The most frequent etiological agent identified in 25 patients (69.4%) with substantial growth in urine culture was *Escherichia coli* (18/69.2%). We found that patients with significant growth in the urine culture had significantly higher white blood cell counts, urinary nitrite positivity, and only abdominal pain symptoms at admission when compared to patients without growth (p<0.050) (Table II).

Comparing the UTI group to the healthy group, WBC, neutrophil count, and C reactive protein (CRP) levels were higher in the UTI group (p<0.050) (Table III).

Cathelicidin and vitamin D levels did not differ significantly between lower and upper UTI (p=0.810 and p=0.795, respectively) (Table IV). Cathelicidin levels (9.6±10.9) in patients with upper UTI did not differ significantly from the control group (5.2±5.7) (p=0.054) . There was a positive correlation between cathelicidin and vitamin D levels in the control group (r=0.346; p=0.030) (Figure 1).

DISCUSSION

UTI is a frequent and dangerous bacterial illness identified by pediatricians (15). The infection can spread to the lower urinary tract (cystitis) or the upper urinary tract (pyelonephritis). Unfortunately, based on clinical signs and symptoms in infants and young children, it can be challenging to distinguish pyelonephritis from cystitis (16). Uncircumcised boys have a 10 to 12-fold increased risk of developing a UTI during the first six months of life. Above the age of one, girls are more prone to develop an UTI than the boys (13,16). Similar to the literature, there are a lot of girls in our study. The most typical sign in the first two years of life is unexplained fever (13,14). Non-specific symptoms include irritability, malnutrition, recurrent abdominal pain, vomiting, anorexia, and growth retardation (17). After the second year of life, UTI symptoms and indicators become increasingly obvious. Signs and symptoms of pyelonephritis include chills, fever, malaise, vomiting, costovertebral angle tenderness, flank pain, and back pain, lower urinary tract signs and symptoms include abdominal or suprapubic pain or tenderness, dysuria, cloudy urine, foul-smelling urine, increased urinary frequency, daytime wetting, new-onset nocturnal enuresis (13,17,18). In our study, we recorded the symptoms of the patients at admission: abdominal pain, foul-smelling urine odor, and fever were the most common symptoms in the literature. Most UTIs occur in the lower urinary tract, and only a tiny percentage of them progress to pyelonephritis (19). In contrast to the literature, our investigation revealed that pyelonephritis was more prevalent. The distinction might exist because our hospital is the only pediatric tertiary care facility in the area. As a result, patients with conditions like pyelonephritis

Table II: Comparison of patients with and without growth in urine culture

Variables	Culture Growth Yes	Culture Growth No	Total	p
Parameters, mean (SD)				
WBC(×10³/mm) (n=34)	13.42±4.25	9.44±3.55	12.25±4.40	0.023*
CRP (mg/L) (n=35)	53.86±71.92	29.80±54.19	46.98±67.46	0.324*
D vitamin (n=36)	25.90±13.10	24.53±9.15	25.48±11.91	0.932*
Cathelicidin (n=36)	8.79±10.11	9.16±10.47	8.90±10.07	0.904*
Neutrophil/lymphocyte ratio (n=34)	3.61±4.17	3.62±3.22	3.61±3.87	0.558*
Urine test(n=36)				
pH	5.82±0.73	5.68±0.78	5.77±0.74	0.383*
Density	1021.04±9.98	1023.91±10.10	1021.92±9.96	0.419*
Pyuria				
Yes	23 (69.7)	10 (30.3)	33 (91.6)	
No	2 (66.7)	1 (33.3)	3 (8.3)	1.000‡
Nitrite				
Yes	14 (93.3)	1 (6.7)	15 (41.6)	
No	11 (52.4)	10 (47.6)	21 (58.3)	0.011‡
Proteinuria				
Yes	19 (67.9)	9 (32.1)	28 (77.7)	
No	6 (75)	2 (25)	8 (22.2)	1.000‡
Symptoms (n=36)				
Fever				
Yes	22 (75.9)	7 (24.1)	29 (80.5)	0.167‡
No	3 (42.9)	4 (57.1)	7 (19.4)	
Dysuria				
Yes	2 (50)	2 (50)	4 (11.1)	0.570‡
No	23 (71.9)	9 (28.1)	32 (88.8)	
Flank pain				
Yes	1 (50)	1 (50)	2 (5.5)	0.524‡
No	24 (70.6)	10 (29.4)	34 (94.4)	
Frequent urination				
Yes	1 (50)	1 (50)	2 (5.5)	0.524‡
No	24 (70.6)	10 (29.4)	34 (94.4)	
Urgency				
Yes	0	0		-
No	25 (69.4)	11 (30.6)	36 (100)	
Abdominal pain				
Yes	8 (50)	8 (50)	16 (44.4)	0.034‡
No	17 (85)	3 (15)	20 (55.5)	
Foul-smell odor				
Yes	9 (90)	1 (10)	10 (27.7)	0.127‡
No	16 (61.5)	10 (38.5)	26 (72.2)	
Suprapubic pain				
Yes	4 (66.7)	2 (33.3)	6 (16.6)	1.000‡
No	21 (70)	9 (30)	30 (83.3)	

***:** *Mann-Whitney U test,* **‡:** *Chi-square test, Fisher's Exact test,* ***CRP:** C-reactive protein,* ***WBC:** White blood cell*

who need more in-depth follow-ups are sent to our facility. Another factor is that patients with low sociocultural levels who applied to our facility might not have been accepted because they were unable to express their concerns or symptoms clearly.

In patients with suspected UTIs, we measured the erythrocyte sedimentation rate (ESR), CRP, or procalcitonin level (PCT). Acute pyelonephritis is indicated by neutrophils, high serum ESR, high serum CRP, and elevated white blood cells in the urine sediment. These tests, however, have a limited level of specificity and are unable to distinguish between acute pyelonephritis and lower urinary tract infection (20,21). Sensitivity varies from 81 to 93% and specificity from 37 to 76% in a meta-analysis of studies examining the reliability of PCT, CRP, and ESR levels in predicting dimercaptosuccinic acid-confirmed pyelonephritis in children with culture-confirmed UTIs. Although CRP 20 mg/L (2 mg/dL) and PCT>0.5 ng/mL (0.5 mcg/L) seem to be useful in eliminating and confirming pyelonephritis, respectively, the studies do not guarantee the outcomes (22). As expected, the UTI group in this study had higher levels of WBC and CRP. Only CRP values were found to be greater in the upper UTI group in the comparison between lower and higher UTIs, which is an remarkable finding.

Table III: Comparison of laboratory findings between UTI and control group

Variables	UTI (Group 1)	Control (Group 2)	Total	p*
CBC parameters, mean (SD)				
WBC(×10³/mm)	12.25±4.41	8.80±3.31	10.50±4.23	<0.001
Hemoglobin	11.19±1.71	12.13±1.21	11.66±1.54	0.010
Platelet count (×10³/µl)	330.50±122.05	287.06±79.42	308.46±104.21	0.086
Neutrophil count (×10³/uL)	7.22±4.57	5.18±2.93	6.18±3.93	0.033
Lymphocyte count (×10³/uL)	3.69±2.49	2.77±1.44	3.22±2.07	0.068
Neutrophil/lymphocyteratio	3.61±3.87	2.57±2.53	3.09±3.28	0.194
Other parameters, mean (SD)				
BUN (U/L)	21.20±9.18	23.08±5.94	22.13±7.75	0.313
Creatinin (U/L)	0.48±0.16	0.60±0.15	0.54±0.16	0.003
AST (ng/L)	34.60±22.18	26.60±10.63	30.60±17.73	0.059
ALT (µg/L)	19.82±21.72	16.85±9.09	18.34±16.59	0.458
CRP (mg/L)	46.98±67.46	1.75±2.11	25.03±53.20	<0.001
D vitamin	25.48±11.91	26.82±9.71	26.15±10.81	0.603
Cathelicidin	8.90±10.07	5.20±5.77	7.05±8.36	0.061

***:** *Student t test,* ***ALT:** *Alanine aminotransferase,* ***AST:** *Aspartate aminotransferase,* ***BUN:** *Blood urea nitrogen,* ***CBC:** *Complete blood count,* ***CRP:** *C-reactive protein,* ***UTI:** *Urinary tract infections ,* ***WBC:** *White blood cell*

Table IV: Comparison of laboratory findings between Lower UTI and Upper UTI group

Variables	Lower UTI group	Upper UTI group	Total	p*
CBC parameters, mean (SD)				
WBC(×10³/mm)	9.30±4.17	12.75±4.31	12.25±4.41	0.061
Hemoglobin	12.52±2.37	10.95±1.50	11.19±1.71	0.098
Platelet count (×10³/µl)	243.80±88.63	345.44±121.90	330.50±122.05	0.061
Neutrophil count (×10³/uL)	4.52±3.84	7.68±4.58	7.22±4.57	0.138
Lymphocyte count (×10³/uL)	3.30±1.71	3.76±2.62	3.69±2.49	0.789
Neutrophil/lymphocyte ratio	1.92±1.73	3.90±4.08	3.61±3.87	0.319
Other parameters, mean (SD)				
BUN (U/L)	20.50±15.1	21.34±7.81	21.20±9.18	0.335
Creatinin (U/L)	0.42±0.11	0.49±0.16	0.485±0.16	0.251
AST (ng/L)	37.50±17.10	34.00±23.30	34.60±22.18	0.614
ALT (µg/L)	16.33±9.50	20.55±23.53	19.82±21.72	0.568
CRP (mg/L)	2.33±3.38	56.22±70.77	46.98±67.46	0.015
D vitamin	23.38±9.52	25.99±12.51	25.48±11.91	0.795
Cathelicidin	5.73±3.77	9.67±10.98	8.90±10.07	0.810

***:** *Many Whitney U,* ***ALT:** *Alanine aminotransferase,* ***AST:** *Aspartate aminotransferase,* ***BUN:** *Blood urea nitrogen,* ***CBC:** *Complete blood count,* ***CRP:** *C-reactive protein,* ***UTI:** *Urinary tract infections ,* ***WBC:** *White blood cell*

For UTI diagnosis, urine culture is still the gold standard (23). The most prevalent germ responsible for 80–90% of UTIs in children is *Escherichia coli* (13,18). Our culture results showed a large proportion of *Escherichia coli* growth, similar to the literature.

There was no growth in urine culture in some of the cases who presented with high fever and did not have a specific fever focus but were accompanied by upper urinary tract infection symptoms. However, pyuria and bacteriuria were present in the urine.

Culture negativities were attributed to the fact that some of the cases came with antibiotic treatment and some of them did not have laboratory-induced reproduction.

Cathelicidin is a protein that is produced by neutrophils, bone marrow cells, and epithelial cells and is encoded by the antimicrobial protein (CAMP) gene. It is antibacterial and effective against viruses and bacteria. It is a chemical molecule for attracting defense cells by interacting with fMLP (N-formyl-methionyl peptides) receptors (13, 24). Human cationic antimicrobial protein 18 (hCAP18) is one of the members of the cathelicidin family which is found in humans. LL-37, on the other hand, is an alpha-helical peptide produced by splitting the C-terminal end of the hCAP18 protein by serine proteases and proteinase 3 (25). Hacihamdioglu et al. (26) analyzed the relationship between cathelicidin and vitamin D in UTI in their study; they found no significant difference between the study and healthy groups in terms of UTI and cathelicidin levels. They connected elevated cathelicidin levels during UTIs to insufficient vitamin D levels. They discovered a link between vitamin D and cathelicidin levels in both people with urinary tract infections and healthy people. A similar study found a positive association between vitamin D levels and cathelicidin levels (27). In our study, we compared children with UTI and healthy controls. There was

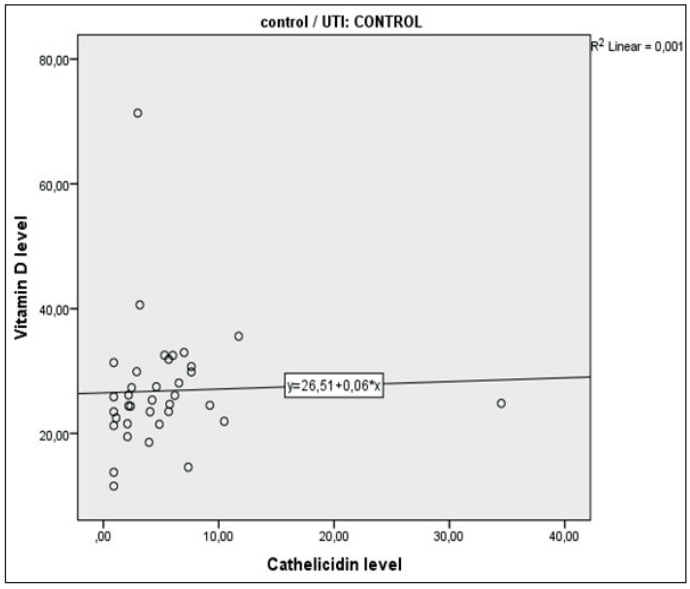

Figure 1: Cathelicidin - vitamin D correlation graphic in control group

no significant difference between vitamin D-cathelicidin levels in UTIs. Likewise, we found no discernible variation in vitamin D and cathelicidin levels between patients with upper and lower UTIs. There were no differences in cathelicidine and vitamin D levels between upper UTI patients and the control group. We hypothesized that it might be because there weren't enough patients with upper UTI. However, in the healthy group, we discovered a significant association between vitamin D and cathelicidin levels, which is consistent with previous research.

Numerous research have been done on the role of vitamin D and cathelicidin as biomarkers in individuals with asthma, cystic fibrosis, *Staphylococcus aureus*, *Clostridium difficile*, and UTIs (28,29). A study found no correlation between blood hCAP18/LL-37 levels and pulmonary conditions, the *Mycobacterium avium* complex, or serum vitamin D levels (30). Also, there was no correlation between vitamin D levels in the bronchoalveolar lavage fluids of children with cystic fibrosis and the production of LL-37, the only human antimicrobial peptide of the cathelicidin family (31). But another study concluded that serum cathelicidin correlated with vitamin D levels, and they were associated with a reduced frequency of UTIs in younger children (27). In addition, another study utilizing a model of acute infection with non-typeable *Haemophilus influenza* shown that infection and lung inflammation cleared more quickly in vitamin D-deficient animals due to elevation of cathelicidin-related antimicrobial peptide (32). These findings suggest that during infection, in vivo cathelicidin synthesis is controlled by vitamin D-dependent and independent mechanisms. It depends on the bacterial species, cell types, and immune status of the host. In our investigation, serum cathelicidin levels were greater in the UTI group than in the control group, although this difference did not reach statistical significance. This might be because there aren't enough patients in the upper UTI group and because their cathelicidin levels are higher. We wanted to increase the number of patients. But due to the pandemic and the expiry date of cathelicidine kits approaching, we had to study the collected patient samples. Our study's limitations include an insufficient sample size of UTI and control group patients with low vitamin levels and an absence of information about pre-disease vitamin D and cathelicidin levels in the UTI group.

CONCLUSION

In our study, there was no relationship between vitamin D and cathelicidine in children with UTI. But in healthy children, there is a weak but positive correlation between vitamin D and cathelicidin levels. Further research using a bigger population may clarify the role of vitamin D and cathelicidin in UTIs. Additionally we think that our study will lead to other studies to determine the relationship between infections and vitamin D-cathelicidin.

Main Points

1. Cathelicidin and vitamin D levels have a positive correlation.

2. Future large-scale research may provide insight into whether cathelicidin can be used as a biomarker to support clinical findings, particularly in the differentiation of upper and lower UTIs.

REFERENCES

1. Mailhot G, White JH. Vitamin D and Immunity in Infants and Children. Nutrients 2020;12:1233.
2. Al-Khalidi B, Kimball SM, Rotondi MA, Ardern CI. Standardized serum 25-hydroxyvitamin D concentrations are inversely associated with cardiometabolic disease in U.S. adults: a cross-sectional analysis of NHANES, 2001-2010. Nutr J 2017;16:16.
3. Science M, Maguire JL, Russell ML, Smieja M, Walter SD, Loeb M. Low serum 25-hydroxyvitamin D level and risk of upper respiratory tract infection in children and adolescents. Clin Infect Dis 2013;57:392-7.
4. Riverin BD, Maguire JL, Li P. Vitamin D Supplementation for Childhood Asthma: A Systematic Review and Meta-Analysis. PLoS One 2015;10:e0136841.
5. Liu X, Arguelles L, Zhou Y, Wang G, Chen Q, Tsai HJ, et al. Longitudinal trajectory of vitamin D status from birth to early childhood in the development of food sensitization. Pediatr Res 2013;74:321-6.
6. Tekin M, Konca C, Celik V, Almis H, Kahramaner Z, Erdemir A, et al. The Association between Vitamin D Levels and Urinary Tract Infection in Children. Horm Res Paediatr 2015;83:198-203.
7. Yuk JM, Shin DM, Lee HM, CS Yang, HS Jin, KK Kim, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6:231-43.
8. Li A, Zhang W, Zhang H, Yi B. Vitamin D/vitamin D receptor, autophagy and inflammation relevant diseases. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2017;42:979-85.

9. White JH. Emerging Roles of Vitamin D-Induced Antimicrobial Peptides in Antiviral Innate Immunity. Nutrients 2022;14:284.
10. Avila EE. Functions of Antimicrobial Peptides in Vertebrates. Curr Protein Pept Sci 2017;18:1098-119.
11. Weichhart T, Haidinger M, Hörl WH, Säemann MD. Current concepts of molecular defence mechanisms operative during urinary tract infection. Eur J Clin Invest 2008;38 Suppl 2:29-38.
12. Ching C, Schwartz L, Spencer JD, Becknell B. Innate immunity and urinary tract infection. Pediatr Nephrol 2020;35:1183-92.
13. Chromek M, Slamová Z, Bergman P, Kovács L, Podracká L, Ehrén I, et al. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nat Med 2006;12:636-41.
14. Kai-Larsen Y, Lüthje P, Chromek M, Peters V, Wang X, Holm A, et al. Uropathogenic Escherichia coli modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. PLoS Pathog 2010;6:e1001010.
15. Asadi Karam MR, Habibi M, Bouzari S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli. Mol Immunol 2019;108:56-67.
16. Balighian E, Burke M. Urinary Tract Infections in Children. Pediatr Rev 2018;39:3-12.
17. Leung AKC, Wong AHC, Leung AAM, Hon KL. Urinary Tract Infection in Children. Recent Pat Inflamm Allergy Drug Discov 2019;13:2-18.
18. Korbel L, Howell M, Spencer JD. The clinical diagnosis and management of urinary tract infections in children and adolescents. Paediatr Int Child Health 2017;37:273-9.
19. Morello W, La Scola C, Alberici I, Montini G. Acute pyelonephritis in children. Pediatr Nephrol 2016;31:1253-65.
20. Tullus K, Shaikh N. Urinary tract infections in children. Lancet 2020;395:1659-68.
21. Zhang H, Yang J, Lin L, Huo B, Dai H, He Y. Diagnostic value of serum procalcitonin for acute pyelonephritis in infants and children with urinary tract infections: an updated meta-analysis. World J Urol 2016;34:431-41.
22. Shaikh KJ, Osio VA, Leeflang MM, Shaikh N. Procalcitonin, C-reactive protein, and erythrocyte sedimentation rate for the diagnosis of acute pyelonephritis in children. Cochrane Database Syst Rev 2020;9:CD009185.
23. Schmidt B, Copp HL. Work-up of Pediatric Urinary Tract Infection. Urol Clin North Am 2015;42:519-26.
24. Spencer JD, Schwaderer AL, Becknell B, Watson J, Hains DS. The innate immune response during urinary tract infection and pyelonephritis. Pediatr Nephrol 2014;29:1139-49.
25. Danka ES, Hunstad DA. Cathelicidin augments epithelial receptivity and pathogenesis in experimental Escherichia coli cystitis. J Infect Dis 2015;211:1164-73.
26. Övünç Hacıhamdioğlu D, Altun D, Hacıhamdioğlu B, Çekmez F, Aydemir G, Kul M, et al. The Association between Serum 25-Hydroxy Vitamin D Level and Urine Cathelicidin in Children with a Urinary Tract Infection. J Clin Res Pediatr Endocrinol 2016;8:325-9.
27. Georgieva V, Kamolvit W, Herthelius M, Lüthje P, Brauner A, Chromek M. Association between vitamin D, antimicrobial peptides and urinary tract infection in infants and young children. Acta Paediatr 2019;108:551-6.
28. Kafle S, Basnet AK, Karki K, Thapa Magar M, Shrestha S, Yadav RS. Association of Vitamin D Deficiency With Pulmonary Tuberculosis: A Systematic Review and Meta-Analysis. Cureus 2021;13:e17883.
29. Dao CN, Patel P, Overton ET, Rhame F, Pals SL, Johnson C, et al. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. Clin Infect Dis 2011;52:396-405.
30. Fujita K, Ito Y, Oguma T, Mio T, Niimi A, Hirai T. Association between Mycobacterium avium complex lung disease and serum vitamin D status, antimicrobial peptide levels, and bone mineral density. Medicine (Baltimore) 2018;97(38):e12463.
31. Thursfield RM, Naderi K, Leaver N, Rosenthal M, Alton EWFW, Bush A, et al. Children with cystic fibrosis demonstrate no respiratory immunological, infective or physiological, consequences of vitamin D deficiency. J Cyst Fibros 2018;17:657-65.
32. Serré J, Mathyssen C, Ajime TT, Korf H, Maes K, Heulens N, et al. Airway infection with Nontypeable Haemophilus influenzae is more rapidly eradicated in vitamin D deficient mice. J Steroid Biochem Mol Biol 2019;187:42-51.

Evaluation of Clinical Features of Pediatric Patients with Wheat Sensitivity

Buğday Duyarlılığı Saptanan Çocuk Hastaların Klinik Özelliklerinin Değerlendirilmesi

Gökhan YÖRÜSÜN[1], Funda AYTEKİN GÜVENİR[1], Ragıp DERE[1], Zeynep ŞENGÜL EMEKSİZ[1], Emine DİBEK MISIRLIOĞLU[2]

[1]Department of Pediatric Allergy and Immunology, Ankara Bilkent City Hospital, Ankara, Türkiye
[2]Department of Pediatric Allergy and Immunology, University of Health Sciences, Ankara Bilkent City Hospital, Ankara, Türkiye

ABSTRACT

Objective: This study aimed to determine the frequency of true wheat allergy among pediatric patients with wheat sensitivity detected by skin prick test (SPT) in our center and to evaluate the clinical features and prognosis of the patients.

Material and Methods: This study was conducted with 63 patients who were found to have wheat sensitivity on skin prick test (SPT) between January 2017 and May 2023 in the Pediatric Allergy and Immunology Clinic of our hospital. Demographic and clinical characteristics, oral provocation tests (OPT) and prognosis of these patients were analyzed.

Results: In 9432 food SPTs, wheat positivity was detected in 63 patients and the wheat sensitivity rate was found to be 0.6%. Sixty-one point nine percent of these patients were girls. In patients with wheat atopy, 55.5% were infants between 0-6 months of age. Out of 63 patients, six (9.5%) presented with a history suggestive of IgE-mediated reaction, while 57 (90.5%) presented with a history suggestive of atopic dermatitis. Among these patients with a history of atopic dermatitis, 34 (59.6%) were found to have cow's milk and egg atopy in addition to wheat sensitivity. Eczema exacerbation was observed in three patients on OPT performed after 2-4 weeks of short term elimination. These patients were able to consume wheat without any reaction after 6-12 months of elimination diet.

Conclusion: Wheat sensitivity not confirmed by oral provocation tests leads to unnecessary elimination of wheat, an essential nutrient. This shows the importance of OPT in patients with wheat atopy.

Key Words: Sensitivity, Oral provocation test, Wheat

ÖZ

Amaç: Bu çalışma ile merkezimizde deri prick testi (DPT) ile buğday atopisi saptanan çocuk hastalar içinde gerçek buğday alerjisi sıklığının belirlenmesi, hastaların klinik özelliklerinin ve prognozlarının değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma hastanemiz Çocuk Alerji ve İmmünoloji Kliniği'nde Ocak 2017 ile Mayıs 2023 tarihleri arasında deri prick test (DPT)' lerinde buğday atopisi saptanan 63 hasta ile yapılmıştır. Bu hastaların demografik ve klinik özellikleri, oral provokasyon testleri (OPT) ve prognozları incelenmiştir.

Bulgular: Yapılan 9432 besin DPT'nde 63 hastada buğday pozitifliği saptandı ve buğday atopi oranı %0.6 olarak bulundu. Bu hastaların %61.9'u kızdı. Buğday atopisi saptanan hastaların %55.5'i 0-6 ay arasındaki süt çocuklarından oluşmaktaydı. Altmış üç hastandan altı (%9.5)'i IgE aracılıklı reaksiyon düşündüren öykü ile, 57 (%90.5)'i ise atopik

0000-0002-0558-7192 : YÖRÜSÜN G
0000-0002-2703-8055 : AYTEKİN GÜVENİR F
0000-0003-2218-5854 : DERE R
0000-0001-7648-0352 : ŞENGÜL EMEKSİZ Z
0000-0002-3241-2005 : DİBEK MISIRLIOĞLU E

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. Ethics Approval was granted by Ankara City Hospital Clinical Trials (according to decision number E2-23-50360).

Contribution of the Authors / Yazarların katkısı*: *YÖRÜSÜN G: Planing methodology to reach the conclusion, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Providing personnel, environment, tools that are vital for the study. ***AYTEKİN GÜVENİR F:*** Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Providing personnel, environment, tools that are vital for the study. ***DERE R:*** Taking responsibilty logical interpretation and conclusion of the result, Biological marerials, taking responsibility of the referred patients. ***ŞENGÜL EMEKSİZ Z:*** Constructing the hypothesis or idea of rescharch and article, Reviewing the article before submission scientifically besides spelling and grammer. ***DİBEK MISIRLIOĞLU E:*** Organizing, supervising the course of progress and taking the responsibility of the study, Reviewing the article before submission scientifically besides spelling and grammer

How to cite / *Atıf yazım şekli* : Yörüsün G, Aytekin Güvenir F, Dere R, Şengül Emeksiz Z and Dibek Mısırlıoğlu. Evaluation of Clinical Features of Pediatric Patients with Wheat Sensitivity. Turkish J Pediatr Dis 2024;18:181-185.

Correspondence Address / Yazışma Adresi:
Gökhan YÖRÜSÜN
Department of Pediatric Allergy and Immunology,
Ankara Bilkent City Hospital, Ankara, Turkey
E-posta: gokhan_yrsn33@hotmail.com

Received / Geliş tarihi : 26.01.2024
Accepted / Kabul tarihi : 01.03.2024
Online published : 03.04.2024
Elektronik yayın tarihi
DOI:10.12956/tchd.1425845

dermatit düşündüren öykü ile başvurmuştu. Atopik dermatit öyküsü olan bu hastalardan 34 (%59.6)'sında buğday atopisine ek olarak inek sütü ve yumurta atopisi de saptandı. İki ile dört haftalık kısa eliminasyondan sonra yapılan OPT' de üç hastada egzama alevlenmesi görüldü. Bu hastalar 6-12 aylık eliminasyon diyeti sonrasında buğdayı sorunsuz bir şekilde tüketebildi.

Sonuç: Oral provokasyon testleri ile doğrulanmayan buğday atopisi, temel besin maddesi olan buğdayın gereksiz eliminasyonuna neden olmaktadır. Bu durum buğday atopisi olan hastalarda OPT'nin önemini göstermektedir.

Anahtar Sözcükler: Duyarlılık, Oral provokasyon testi, Buğday

INTRODUCTION

Food allergies are an important public health problem that is gradually increasing and adversely affecting the life quality of patients and their parents. Food allergy prevalence is thought to be as high as 10% in developed countries (1,2). Cow's milk, eggs, wheat, soy, peanuts, and fruits are responsible for more than 80% of food-related hypersensitivity reactions (3).

Wheat is the most commonly consumed cereal since it can grow in various climates and is a relatively cheap staple food. Even though the sensitivity rates are higher, the real wheat allergy rate verified by the oral provocation test (OPT) is between 0.2% and 0.5%. Clinical findings of wheat-related hypersensitivity reactions vary depending on the routes of allergen exposure and underlying immunologic mechanisms (4).

Following wheat consumption, it is possible to observe various reactions such as urticaria, angioedema, bronchial obstruction, nausea, stomachache and anaphylaxis characterized by classical IgE-mediated early-type reaction findings. Children commonly develop tolerance to these reactions during their school years, just like milk and egg allergies.

In adolescents, food-dependent exercise-induced anaphylaxis occurs in combination with food intake and physical exercise as well as nonsteroidal anti-inflammatory drugs or alcohol. And also wheat allergy may present with occupational asthma (known as baker's asthma) and rhinitis or contact urticaria-like clinical conditions in these age group (5).

In these study, it was aimed to determine the real wheat allergy rate among pediatric patients diagnosed with wheat sensitivity and to evaluate their clinical characteristics and prognosis.

MATERIALS and METHODS

The research was carried out at the Pediatric Allergy and Immunology Clinic of Ankara Bilkent City Hospital. Ethics approval was granted by Ankara Bilkent City Hospital Clinical Trials (27.09.2023/E2-23-50360). The study was conducted in accordance with the principles of the Declaration of Helsinki.

SPTs performed between January 2017 and May 2023 were examined retrospectively. Patients who were found to have wheat sensitivity and whose full medical records were accessible were included in the study.

Data such as demographic, presenting complaints of the patients, the duration between wheat consumption and symptoms, clinical characteristics of the reaction, and accompanying allergic diseases were obtained from the medical records of the patients. Wheat-specific IgE values, existing food atopy, and the SPT results and tolerance statuses were recorded. Wheat-specific IgE below 0.35 kU/L was categorised as negative, between 0.35-100 kU/L as high and >100 kU/L as very high. The patients were categorized according to their ages 0-6 months, 6-12 months, 12-24 months, and >24 months to analyze a detailed evaluation of their diet and developmental characteristics.

Skin prick test: This test is applied to flexors on the back or forearm using commercial extracts (Lofarma®, Milan, 1945) and the prick method. As a negative control, 0.9% sodium chloride is used, while histamine hydrochloride serves as the positive control. The results are evaluated 15-20 minutes after application. A pitting of three millimetres or more accompanied by a circle of erythema around the test area is considered a positive result.

In the event of a suspected food-related reaction, the SPT is performed with a food panel including wheat (milk, eggs, wheat, peanuts, fish, and soy). When there is no related clinical history, the positive result of a wheat SPT is accepted as 'sensitivity'. In the case of a compatible clinical history in the SPT positivity, a diagnostic OPT is applied, and the patient is diagnosed with 'wheat allergy' or the allergy is excluded.

Oral provocation test: OPT was performed at baseline to confirm the diagnosis of wheat allergy or during follow-up to assess tolerance. OPTs were performed as open OPTs after obtaining written consent from the patient or parent, under the supervision of experienced personnel and taking every precaution for a possible anaphylaxis intervention. All patients were examined in detail before starting OPT. Vital signs and physical examination findings of the patients were recorded. Oral wheat provocation tests were performed according to the Turkish National Allergy and Clinical Immunology Society Food Loading Tests: According to the 2019 Guidelines of the National Allergy and Clinical Immunology Society of Türkiye and it was performed using pasta equivalent to 10 grams of wheat protein without any other product in wheat. The oral provocation test was started with 0.01 grams of wheat protein and terminated when the equivalent of 10 grams of wheat protein was reached (6). Patients were kept under observation for at least two hours after the last dose was given, and in case of a reaction, until the symptoms completely regressed. If objective findings were present during OPT, the test was considered positive, the test was terminated and the reaction was treated as required. If

negative, food was added to the diet. At this stage, clinical follow-up of the patient was continued in terms of late reactions.

Statistical analyses: Statistical analysis was performed using SPSS version 22.0 (SPSS Inc. Chicago, IL, USA). Numbers and percentages were reported for discrete variables. Continuous variables were expressed as mean, minimum and maximum for data with a normal distribution and as median and interquartile range (IQR, 25th–75th percentile values) for non-normally distributed data. A value of $p<0.050$ was considered statistically significant.

RESULTS

Wheat sensitivity was detected in 63 of 9432 patients who underwent food SPT, including wheat, during the study period and the wheat sensitivity rate was 0.66%. Of the patients, 61.9% were girls. The average age at admission was 16 months. The median age was 6 months (4 months to 8.5 months, IQR; 5 months). When considering the most common age group for initial presentation, 55.5% were infants aged between 0 and 6 months. Only six patients (9.5%, n= 63) were over 24 months old at the time of initial assessment.

When the patients are evaluated according to their complaints, six patients (9.6%, n= 63) at the age of 5-132 months had a skin rash after wheat consumption, suggesting an IgE-mediated reaction, and 57 (90.4%, n= 63) patients presented due to skin lesions, which suggested atopic dermatitis (AD).The age and gender characteristics of patients with wheat sensitivity are summarized in Table I.

One of the patients (a 5-month-old boy) with the complaint of a skin rash had a high wheat-specific IgE value of 62.8 kU/L, while the other five patients had a low wheat-specific IgE value (under 0.35 kU/L) at admission. All patients underwent a diagnostic OPT at admission, and none of them showed acute reactions. Therefore, wheat was added into their diet without any issues and so wheat allergy was excluded. Additional food sensitivity was not detected by SPT in any of these patients.

Table I: Demographic and clinical characteristics of patients with wheat sensitivity (n= 63)

Parameter	
Age at wheat atopy, months (median-IQR)	16 (6.5)
Sex*	
Male	24 (38.1)
Female	39 (61.9)
Age groups*	
0-6 months	35 (55.5)
6-12 months	19 (30.1)
12-24 months	3 (4.8)
>24 months	6 (9.6)
Application complaint*	
IgE-mediated reaction	6 (9.6)
Male	3 (4.8)
Female	3 (4.8)
AD	57 (90.4)
Male	21 (33.3)
Female	36 (57.1)

***** *n(%)*

Table II: Laboratory findings according to age groups.

Age group / n (%)	Total IgE* (IU/mL)	Eosinophil Number* ($\times 10^7$/L)	Eosinophil Percentage (%)
0-6 months / 35 (55.5)	155.6	903	7.5
6-12 months / 19 (30.1)	614	703.3	5.2
12-24 months/ 3 (4.8)	209.9	343	3.3
>24 months/ 6 (9.6)	492.8	350	3.1
Total (n= 63)	315.2	769.4	6.4

***** *mean*

Table III: Wheat specific IgE status according to clinical features (n= 63)

Parameter	n (%)
Wheat specific IgE	63 (100)
Low	34(54)
High	29 (46)
Very High	0 (0)
IgE-mediated reaction	6 (9.6)
Low	3 (4.8)
High	3 (4.8)
Very High	0 (0)
AD	57 (90.4)
Low	31(49.2)
High	26 (41.2)
Very High	0 (0)

Most of the patients (n= 34/57, 59.60%) who had skin lesions, which suggested AD, consisted of 0-6 months old infants. Fifty-two of these patients (91.2%) had additional food sensitivity. Fifteen of the patients (26.3%) had accompanying egg atopy, three (5.20%) had milk atopy, and 34 (59.60%) had both milk and egg atopy. The patients' atopy pattern is summarized in Figure 1.

The mean total IgE level of the patients was 315.20 IU/mL (1.5-525) and eosinophil number was 769.4 × 10^7/L (60-1100) at admission. The data are summarized in Table II.

Table III summarizes the distribution of wheat sp IgE levels in 57 patients admitted with atopic dermatitis. Of these patients, 29 (50.9%) had low levels below 0.35 kU/L, while 28 (49.1%) had high levels between 0.35-100 kU/L. No patient had a wheat sp IgE value above 100 kU/L.

It was observed that the patient, who presented with atopic dermatitis clinic, and/or his mother when he was breastfeeding, was recommended short-term wheat elimination for 2-3 weeks, and then wheat was added to his diet in the form of a food appropriate for his age. While 54 (94.7%, n= 57) patients consumed wheat without any eczema exacerbations, in the

Table IV: Characteristics of patients with wheat allergy confirmed by Oral Food Challenge (OFC)

Admission	Wheat SPT at admission (mm)	Wheat-specific IgE at admission (kU/L)	Elimination diet duration (months)	Before OFC Wheat sp IgE (kU/L) at the end of elimination
3 m/M	3 mm	23.90	12	0.43
4 m/F	5 mm	1.90	9	<0.35
6 m/M	4 mm	19.80	6	<0.35

m: *Months,* ***F:*** *Female,* ***M:*** *Male*

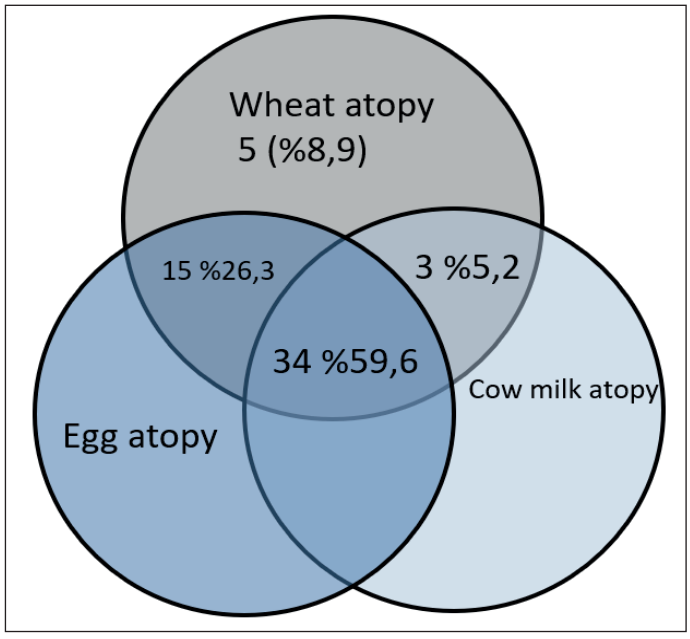

Figure 1: Association of cow's milk and egg atopy in atopic dermatitis patients with wheat atopy.

other three (5.3%, n= 57) , who had multi-food atopy and wheat specific IgE increase, eczema exacerbation was observed. The clinical characteristics of these patients are summarized in Table IV.

DISCUSSION

There are many studies in the literature evaluating the characteristics of food allergies, especially milk and egg allergies, in childhood; however, the data on wheat allergy are rather limited. The aim of our study was to evaluate the clinical and prognostic characteristics of patients diagnosed with wheat atopy and wheat allergy.

Wheat is regarded as a staple food around the world. In our country, wheat is included in diets starting from a very young age in various forms, primarily as bread. However, wheat allergy is not a widely known phenomenon. Although the real wheat allergy prevalence verified by OPT is not known clearly, it is estimated to be less than 0.5% in the general population (7). The data obtained from positive SPTs indicate that up to 3% of the general American pediatric population is sensitive to wheat; however, the allergy rate is estimated to be between 0.2% and 1% (7). In another study, in which 256 children patients were evaluated, while the sensitivity to wheat was 9.4% by SPT, the real wheat allergy verified by OPT was 0.4% (8). It was stated that wheat allergy is the third most common food allergy after milk and egg in countries such as Germany, Japan, and Finland and that its prevalence varies according to age and geographic region and is thought to be 1% (0.4%-4%) (9). In the studies of Unsal et al. (10) where they evaluated 613 pediatric patients with food atopy, wheat sensitivity was detected in 37 (6%) patients, and in wheat OFCs, real wheat allergy was diagnosed in 2.6% of children under 2 years of age and in 2.8% of those between 2-18 years of age. In our study, both the wheat atopy rate (0.6%) and the confirmed wheat allergy rate (0.03%) were found to be much lower than literature data.

Wheat is usually introduced into the diet of infants between 4 and 6 months of life, but sensitivity can develop much earlier through breast milk or extra-intestinal exposures such as skin and rhinoconjunctival (11). The fact that sensitivity was detected in the infantile period between 0-6 months in the majority of patients (55.5%) in our study supports this data.

When the complaints of our patients were evaluated, no acute reactions were observed in those who underwent a diagnostic OPT following their referral to the center due to a skin rash occurring shortly after wheat consumption, suggesting an early IgE-mediated reaction. Wheat was added into the diet of these patients and wheat allergy was excluded. In our study, no patients describing severe IgE-mediated reactions to wheat. Considering the literature, in a study in which the clinical characteristics of 100 children experiencing IgE-mediated reactions due to wheat consumption were evaluated, the researchers stated that while only the skin and mucosa were affected in 49 patients, 51 patients had anaphylaxis. SPT size and wheat-specific IgE were found to be a significant predictor for anaphylaxis. Although there are studies that show how wheat-specific IgE predicts reaction severity, it must be noted that there might be cases of anaphylaxis development despite low specific IgE values (12,13).

In another study where OPT results were evaluated in 108 children with an average age of 1.5 years due to suspicion of wheat allergy, the test was found to be negative in approximately half of the patients and wheat could be added to the diet (4). While this procedure is difficult to apply to pediatric patients, that study showed the significance of OPT application during the diagnostic process in an environment equipped with opportunities for a possible anaphylaxis intervention.

In previous studies, the most common application symptom associated with wheat consumption was moderate severity AD (5). A similar picture was also seen in our study, with a large portion of our patients (90.4%) presenting to our center with the same history. In such food allergies, observing recovery in lesions by short-term elimination and, later on, added the food into the diet are diagnostic in cases of re-exacerbation. Following this diagnostic provocation applied in our patients, wheat was associated with exacerbation of eczematous rash in only three (5.20%). Wheat was added into the diet of the other children without any reactions.

AD is known to be the most powerful and best-known risk factor for developing food allergies. This is explained by the dual allergen exposure hypothesis, which suggests that allergic sensitivity to food might originate from cutaneous exposure, and the disturbed skin barrier in atopic dermatitis results in increased permeability for food allergies (14,15). Examination of the literature reveals that wheat atopy is the third most common atopy after milk and eggs in patients with AD (8,16). In our study, 15 (26.3%) patients had egg atopy, 3 (5.2%) patients had milk atopy, 34 (59.6%) patients had both wheat and egg atopy, and three patients with OFC and eczema exacerbation had both milk and egg atopy, indicating that wheat atopy should be considered in cases of AD not responding to milk and egg diet.

There is very limited literature data on the prognosis and natural course of wheat allergy. Keet et al. (17), who evaluated 103 patients with wheat-related IgE-mediated clinical history diagnosed with SPT-positive results and showed tolerance by OPT, found the mean tolerance age to be 79 months. They highlighted that wheat specific IgE is the most significant prognosis indicator, and tolerance might be delayed up to adolescent age in those with values over >50 kU/L; however, those with delayed tolerance were a minority. Additionally, there some patients developed tolerance earlier despite having high specific IgE values. However, it is difficult to determine when children have their peak specific IgE values. Compared to other food allergies, specific IgE is less helpful in predicting clinical reactivity. At this stage, since the related molecular mechanisms are not entirely understood, there are no treatments for wheat allergy except for oral immunotherapy, and avoiding food with wheat is the best option. The patients should be periodically tested by OPT, and their tolerance status must be evaluated (17, 18).

In conclusion, skin test positivity that cannot be verified by OPT can cause unreal food allergy stigma, the patients to unnecessarily eliminate foods, and nutritional deficiency that might result from removing a staple food from their diet, such as wheat. This indicates the significance of diagnostic OPTs. Additionally, SPT size and specific IgE cut-off values, which can predict severe reactions to wheat, are not as clear-cut as for milk and eggs. This requires the provocation tests to be performed by experienced healthcare staff in an environment equipped with emergency intervention facilities.

REFERENCES

1. Peters RL, Krawiec M, Koplin JJ, Santos AF. Update on food allergy. Pediatr Allergy Immunol 2021;32:647-57.
2. Savage J, Johns CB. Food allergy: epidemiology and natural history. Immunol Allergy Clin North Am 2015;35:45-59.
3. Inomata N. Wheat allergy. Curr Opin Allergy Clin Immunol 2009;9:238-43.
4. Ricci G, Andreozzi L, Cipriani F, Giannetti A, Gallucci M, Caffarelli C. Wheat allergy in children: a comprehensive update. Medicina 2019;55:400.
5. Elli L, Branchi F, Tomba C, Villalta D, Norsa L, Ferretti F, et al. Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity. World J Gastroenterol 2015;21:7110-9.
6. Immunology TNSoAaC. Buğday Yükleme Testleri. In: Saçkesen C, editor. Allergy. Türkiye: Buluş tasarım ve yayıncılık 2019;114-5.
7. Cianferoni A. Wheat allergy: diagnosis and management. J Asthma Allergy 2016:13-25.
8. El-Sayed ZA, Sakr H, Shousha GAH. Sensitivitys to wheat and strawberry: are they a tangible threat to atopic Egyptian. Am J Clin Exp Immunol 2022;11:84.-91
9. Czaja-Bulsa G, Bulsa M. What do we know now about IgE-mediated wheat allergy in children? Nutrients 2017;9:35.
10. Ünsal H, Ocak M, Akarsu A, Şahiner ÜM, Soyer Ö, Şekerel BE. Oral food challenge in IgE mediated food allergy in eastern Mediterranean children. Allergol Immunopathol (Madr) 2021;49:185-92.
11. Fukutomi Y, Taniguchi M, Nakamura H, Akiyama K. Epidemiological link between wheat allergy and exposure to hydrolyzed wheat protein in facial soap. Allergy 2014;6:1405-11.
12. Rutrakool N, Piboonpocanun S, Wangthan U, Srisuwatchari W, Thongngarm T, Jirapongsananuruk O, et al. Children with wheat anaphylaxis and with low wheat specific IgE have a different IgE immunoblot pattern than those with high wheat specific IgE. Asian Pac J Allergy Immunol 2023:2. doi: 10.12932/AP-140622-1387.
13. Srisuwatchari W, Vichyanond P, Jirapongsananuruk O, Visitsunthorn N, Pacharn P. Characterization of children with IgE-mediated wheat allergy and risk factors that predict wheat anaphylaxis. Asian Pac J Allergy Immunol 2022;40:263-8.
14. Samady W, Warren C, Kohli S, Jain R, Bilaver L, Mancini AJ, et al. The prevalence of atopic dermatitis in children with food allergy. Ann Allergy Asthma Immunol 2019;122:656-7. e1.
15. Singh AM, Anvari S, Hauk P, Lio P, Nanda A, Sidbury R, et al. Atopic dermatitis and food allergy: best practices and knowledge gaps—a work group report from the AAAAI Allergic Skin Diseases Committee and Leadership Institute Project. J Allergy Clin Immunol Pract 2022;10:697-706.
16. Mavroudi A, Karagiannidou A, Xinias I, Cassimos D, Karantaglis N, Farmaki E, et al. Assessment of IgE-mediated food allergies in children with atopic dermatitis. Allergol Immunopathol 2017;45:77-81.
17. Keet CA, Matsui EC, Dhillon G, Lenehan P, Paterakis M, Wood RA. The natural history of wheat allergy. Ann Allergy Asthma Immunol 2009;102:410-5.
18. Quirce S, Boyano-Martínez T, Díaz-Perales A. Clinical presentation, allergens, and management of wheat allergy. Expert Rev Clin Immunol 2016;12:563-72.

Assessing Iron Deficiency Anemia in Obese Adolescents and Identifying Contributing Factors

Obez Ergenlerde Demir Eksikliği Anemisinin Değerlendirilmesi, Rol Oynayan Faktörlerin Belirlenmesi

Şeyma KILINÇ[1], Alkım ÖDEN AKMAN[2], Tülin Revide ŞAYLI[2]

[1]Department of Developmental Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[2]Department of Adolescent Medicine, Ankara Bilkent City Hospital, Ankara, Türkiye
[3]Pediatrics (Retired), Ankara, Türkiye

ABSTRACT

Objective: Obesity and iron deficiency, which are public health problems that maintain their prevalence and for which the adolescent population is particularly at risk, may have important clinical consequences. This study aimed to assess the iron parameters and blood vitamin B12 levels in obese adolescents and identify the contributing variables to the development of anemia.

Material and Methods: The present study involved a retrospective evaluation of 260 children (130 obese-130 control) who were admitted to the Ankara Children's Haematology Oncology Training and Research Hospital, Pediatric Outpatient Clinics, between March 2013 and May 2015. Children aged 12 to 18 years without acute or chronic illnesses and body mass index (BMI) above the 95th percentile for age and gender were required for inclusion in the study group. Data from patient files were used to collect information on physical examination findings, sociodemographic characteristics, daily dietary status, and level of physical activity. All patients had evaluations for CRP, iron parameters, vitamin B12, and complete blood count.

Results: The study revealed that the obese group had significantly higher serum ferritin levels (p=0.002) and lower serum iron and vitamin B12 levels (p=0.036 and 0.047, respectively) as compared to the control group. In the obese population, elevated BMI has been demonstrated to be correlated with elevated CRP and ferritin levels.

Conclusion: Obesity's chronic inflammation state may lead inflammatory pathophysiological pathways to activate resulting in iron deficiency and other nutritional deficiencies. Obesity should be followed up as a chronic disease and monitored in terms of nutritional deficiencies, especially in adolescents who have not completed their growth.

Key Words: Adolescence, Iron deficiency, Obesity

ÖZ

Amaç: Ergen nüfusun özellikle risk altında olduğu, yaygınlığını koruyan halk sağlığı sorunlarından obezite ve demir eksikliğinin önemli klinik sonuçları olabilmektedir. Bu çalışmada obez ergenlerde demir parametrelerinin ve serum vitamin B12 düzeylerinin değerlendirilmesi ve anemi gelişiminde rol oynayan faktörlerin belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmada T.C. Sağlık Bakanlığı Ankara Çocuk Sağlığı ve Hastalıkları Hematoloji Onkoloji Eğitim ve Araştırma Hastanesi Çocuk Polikliniklerine Mart 2013-Mayıs 2015 tarihleri arasında başvuran 260 çocuk (130 obez, 130 kontrol) geriye dönük olarak değerlendirilmiştir. Akut veya kronik hastalığı olmayan ve vücut kitle indeksi (VKİ)

0000-0001-9788-062X : KILINÇ Ş
0000-0001-8080-7127 : ÖDEN AKMAN A
0000-0002-9537-7390 : ŞAYLI TR

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study received Ethics Committee approval from Ankara Child Health and Diseases Hematology Oncology Hospital (2017-096/ 03.07.2017).

Contribution of the Authors / Yazarların katkısı: KILINÇ Ş: Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study. ***ÖDEN AKMAN A:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***ŞAYLI TR:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli*: Kılınç Ş, Öden Akman A and Şaylı TR. Assessing Iron Deficiency Anemia in Obese Adolescents and Identifying Contributing Factors. Turkish J Pediatr Dis 2024;18:186-191.

Correspondence Address / Yazışma Adresi:
Şeyma KILINÇ
Department of Developmental Pediatrics,
Hacettepe University Faculty of Medicine, Ankara, Türkiye
E-posta: drseymakilinc@gmail.com

Received / Geliş tarihi : 08.01.2024
Accepted / Kabul tarihi : 06.03.2024
Online published : 02.05.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1416473

yaş ve cinsiyete göre 95. persentilin üzerinde olan 12-18 yaş arası çocuklar çalışma grubuna dahil edilmiştir. Fiziksel muayene bulguları, sosyodemografik özellikler, günlük beslenme durumu ve fiziksel aktivite düzeyi hakkında bilgi toplamak için hasta dosyalarından elde edilen veriler kullanılmıştır. Tüm hastalarda CRP, demir parametreleri, B12 vitamini ve tam kan sayımı değerlendirmeleri yapılmıştır.

Bulgular: Serum demir ve vitamin B12 düzeyleri, obez grupta kontrol gruba oranla düşük (sırasıyla p = 0.036, p = 0.047), serum ferritin düzeyi ise obez grupta kontrol gruba kıyasla istatistiksel olarak anlamlı derecede yüksek saptanmıştır (p=0.002). Obez grupta yüksek CRP düzeylerinin, artmış VKI ile ilişkili olduğu gösterilmiştir.

Sonuç: Obezitenin kronik enflamasyon durumu, enflamatuar patofizyolojik yolakların aktive olmasına neden olarak demir eksikliği ve diğer beslenme yetersizliklerine yol açabilir. Obezite kronik bir hastalık olarak takip edilmeli ve özellikle büyümesini tamamlamamış ergenlerde beslenme yetersizlikleri açısından izlenmelidir.

Anahtar Sözcükler: Ergenlik dönemi, Demir eksikliği, Obezite

INTRODUCTION

Over 650 million people and 340 million children suffer from obesity, an energy metabolism condition that can cause both physical and psychological issues (1, 2). Different population segments have varying prevalence rates. Compared to children aged 2–5, this rate is higher in children aged 6–11 and 12–19 (3). According to the World Health Organization (WHO), obesity presents a serious risk to public health and is classified as a chronic illness.

Children and adolescents with obesity appear to have a paradoxical malnutrition in terms of their nutritional condition. Micronutrient deficits are prevalent even with high dietary consumption rates (4). Apart from increasing the risk of diabetes mellitus, cancer, and cardiovascular disease, obesity has also been connected to iron deficiency anemia (IDA), which is another issue related to general public health (2, 5). According to the WHO, globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. Iron deficiency (ID) with or without anemia may lead to a broad spectrum of signs and symptoms. Even if iron deficiency in adolescents is not severe enough to cause anemia, it can nevertheless induce cognitive and physical problems. Adolescence is considered a period of increased risk of ID due to accelerated growth, rise of blood volume, menstrual blood loss in girls, greater muscle mass in boys, and unbalanced diets (6).

Growing evidence supports the existence of an association between obesity and ID. This link was observed among children, adolescents, and adults. The low-grade systemic inflammation that is present in obese people is the primary mechanism that connects obesity and ID (7). Interleukin-6 and serum hepcidin levels were considerably higher in overweight and obese people than in people of normal weight. Pro-inflammatory cytokines like interleukin-6 enhance the liver's hepcidin production. According to a recent study, even with iron-rich diets, overweight and obese women with central adiposity had greater blood hepcidin, lower iron levels, and reduced iron absorption (8). Even so, some research has determined the higher risk of vitamin and mineral deficiencies in malnourished children with obesity, overweight, and metabolic syndrome. Therefore, it was thought that inadequate dietary composition, brief, frequently restricted diets, or higher requirements could be the cause of the drop in serum vitamin B12 levels (9). A study revealed that among Mexican children aged 8 to 15, the relationship between serum concentrations of vitamin B12 and Body Mass Index (BMI) was inverse (10).

This study aims to assess the iron parameters and blood vitamin B12 levels in obese adolescents and identify the contributing variables to the development of anemia.

MATERIAL and METHODS

The present study involved a retrospective evaluation of 260 children (130 obese and 130 control) who were admitted to the Ankara Children's Haematology Oncology Training and Research Hospital, Pediatric Outpatient Clinics of the Ministry of Health, between March 2013 and May 2015. Patients with BMIs above the age and gender-specific 95th percentile, between the ages of 12 and 18 years, without known acute or chronic illnesses, and admitted to our hospital due to obesity were included. The control group consisted of individuals with a BMI between the 5 and 95 percentile and no acute or chronic illnesses.

The results of the physical examination, body weight, height, BMI, waist circumference, family history, socioeconomic factors, physical activity levels, daily diet, and menarche status in girls were all documented along with the evaluation of the patient files. The BMI percentile values developed by Bundak and colleagues (11) were applied to evaluate obesity. The metabolic equivalent task score (MET) was used to calculate the levels of physical activity (12). The daily intake of iron was categorized as being below or above 10 mg based on the quantity and composition of nutrients (13). The three socioeconomic levels are those with a wage below the minimum (1st level), those with a wage greater than twice the minimum (3rd level), and those in between (2nd level).

All patients had their levels of ferritin, vitamin B12, serum iron (SI), total iron binding capacity (TIBC), C-reactive protein (CRP), and complete blood count assessed. The obese group also

Table I: Grouping patients based on hematological characteristics

	Hb	MCV	SI	Ferritin
Iron deficiency	N	N	↓	↓
Iron deficiency anemia	↓	↓	↓	↓

had evaluations of liver function tests, triglycerides, cholesterol, fasting blood glucose, and fasting insulin levels. For girls and boys, respectively, the lower limit of hemoglobin (Hb) values was determined to be 12 g/dl and 13 g/dl. Red cell distribution width (RDW) values in the range of 11.5%–14.5% were regarded as normal, and the lower limit of the mean corpuscular volume (MCV) value was accepted as 78 fL (14). As indicated in Table I, patients were categorized based on hematological characteristics.

The statistical analysis was carried out using the SPSS for Windows 22.0 (IBM Corp., Armonk, NY, USA) package. The descriptive statistics were displayed as the number of cases (n) and percentage (%) for categorical variables, as the mean ± standard deviation (SD) and median/interquartile range (IQR) for continuous and discrete numerical variables. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. For normally distributed values, the Student's t-test demonstrated the significance of the group difference; for non-normally distributed and ordinal variables, the Mann-Whitney U test was used. The chi-square test was used for comparisons of categorical data. Ferritin, BMI, and CRP levels have been linked using the Spearman correlation test. The significance threshold was set at 0.05, and a significant relationship between the groups was acknowledged when p<0.050.

RESULTS

The control group had an average age of 14.68 ± 1.52 years, whereas the obese group had an average age of 14.07 ± 1.65 years. There was no statistically significant difference in the gender and mean age distributions between the groups (p=0.606 and p=0.266, respectively). Regarding the menarche status, duration of menarche, hypermenorrhea in females, birth weight, and breastfeeding in all children, there was no difference between the control and obese groups. Monthly income level 1 was lower in the obese group (3.8%) compared to the control group (13.1%), while monthly income level 3 was significantly higher in the obese group (46.9%) compared to the control group (34.6%). The groups' sociodemographic information is shown in Table II.

The control and obese groups did not differ statistically significantly in terms of daily iron intake or level of physical activity. The prevalence of diabetes, cardiovascular disease,

Table II: Demographic data for each group

	Control group*	Obese group*	p
Birth weight			
< 2500 g	8 (6.3)	11 (8.5)	0.508
≥ 2500 g	118 (93.7)	118 (91.5)	
Breastfeeding			
< 1 year	59 (46.8)	63 (48.8)	0.748
≥ 1 year	67 (53.2)	66 (51.2)	
Menarche status			
(-)	11 (12.9)	12 (14.8)	0.727
(+)	74 (87.1)	69 (85.2)	
Menarche duration			
< 1 year	7 (9.5)	12 (17.4)	0.163
≥ 1 year	67 (90.5)	57 (82.6)	
Hypermenorrhea			
(-)	64 (86.5)	59 (85.5)	0.866
(+)	10 (13.5)	10 (14.5)	
Socioeconomic status			
1st level	17 (13.1)	5 (3.8)	0.011
2nd level	68 (52.3)	64 (49.2)	
3rd level	45 (34.6)	61 (46.9)	

**n (%), Chi-square Test. p<0.050 is significant.*

Table III: Hb, RBC, MCV, RDW, SI, TIBC, ferritin and vitamin B12 levels

	Control group	Obese group	p
Hb (g/dL)*	13.79 ± 1.33	13.72 ± 1.18	0.616
RBC (10^6/µL)*	4.89 ± 0.43	4.97 ± 0.41	0.137
MCV (fL)*	82.73 ± 6.18	81.79 ± 5.20	0.187
TIBC (ng/mL)*	367.12 ± 65.77	365.71 ± 64.03	0.862
RDW (%)†	13.8 /13.1-14.95	13.9 /13.3-14.62	0.765
SI (µg/dL)†	67 /46.5-105.5	65.5 /43-86	0.036
Ferritin(ng/mL)†	17.6 /8.1-30-95	24.7 /14.95-36	0.002
Vit B12 (pg/mL)†	202 /157.25-254	186.5 /136.75-245.75	0.047

**:mean ±SD(standard deviation), †: median / IQR (Interquartil Range). The Student's t-Test, Mann Whitney U Test. p<0.050 is significant.*

and obesity in the family was shown to be greater in the obese group (p<0.001, p=0.033, and p<0.001, respectively). The groups did not differ in terms of how much milk, eggs, cheese, red meat, vegetables, or oranges they consumed daily when asked about their nutritional condition (p = 0.620, p = 0.119, p = 0.281, p = 0.123, p = 0.483, and p = 0.500, respectively).

The study revealed that the obese group had significantly higher serum ferritin levels (p = 0.002) and lower SI and vitamin B12 levels (p = 0.036 and 0.047, respectively) as compared to the control group (Table III). Compared to the control group (n = 58), there were fewer patients in the obese group (n = 32) with low blood ferritin levels (p = 0.001). Regarding IDA (low Hb, MCV, SD, and ferritin combined), there was no statistical difference between the groups (p = 0.349).

Patients with daily iron intake >10 mg had statistically substantially higher levels of Hb and ferritin than those with daily

Table IV: The relationship between daily iron intake and Hb, RBC, MCV, RDW, TS, TIBC, vitamin B12, SI, and ferritin levels in the groups

Daily iron intake	Control group	Obese group	p*	p†
Hb (g/dL)‡				
<10 mg	13.46±1.23	13.60±1.24	0.001	0.176
>10 mg	14.23±1.35	13.89±1.10		
RBC (10^6/µL)‡				
<10 mg	4.84±0.43	4.93±0.42	0.076	0.106
>10 mg	4.98±0.45	5.05±0.42		
MCV(fL)‡				
<10 mg	81.88±6.98	81.85±5.10	0.074	0.913
>10 mg	83.84±4.86	81.75±5.40		
TIBC (ng/mL)‡				
<10 mg	373.43±67.39	366.05±61.26	0.222	0.944
>10 mg	359.14±63.37	365.25±68.11		
RDW (%)§				
<10 mg	13.95 /13.12-15.45	13.95 /13.2-14.5	0.073	0.803
>10 mg	13.6 /13-14.35	13.85 /13.4-14.95		
Vit B12 (pg/mL)§				
<10 mg	197 /144-254	180.5 /129.75-230.5	0.698	0.280
>10 mg	203 /175-254	190.5 /144.5-277		
SI(µg/dL)§				
<10 mg	65 /40.25-99	65 /42.75-79.75	0.094	0.478
>10 mg	73/ 54.5-115.5	67.5 /44.5-91.25		
Ferritin (ng/mL)§				
<10 mg	14.15 /6.52-29.27	26 /13.82-37.25	0.015	0.959
>10 mg	23.1/13.45-31.9	24.55 /16-35.3		

***:** *Control group,* **†:** *Obese group,* **‡:** *mean ±SD (Standard Deviation),* **§:** *median / IQR (Interquartil Range). The Student's t-Test, Mann Whitney U Test. p<0.050 is significant*.

Table V: Hb, RBC, MCV, RDW, TS, TIBC, vitamin B12, SI, and ferritin levels with BMI in all patients

	BMI (kg/m²)	
	p	r
Hb (g/dL)	0.625	0.031
RBC (10^6/µL)	0.017	0.148
MCV (fL)	0.207	-0.079
RDW (%)	0.683	0.026
TS (%)	0.078	-0.110
TIBC (ng/mL)	0.587	-0.034
Vit B12 (pg/mL)	0.073	-0.112
SI (µg/dL)	0.207	-0.079
Ferritin (ng/mL)	0.001	0.200

Spearman Test. p<0.05 is significant.

iron intake <10 mg in the control group (p = 0.001; p = 0.015) (Table IV).

Table V illustrates a positive correlation between BMI and the levels of ferritin (p=0.001; r=0.200) and RBC (p=0.017; r=0.148). Figure 1 shows for every unit increase of 1 kg/m² in BMI, the ferritin levels increased by 0.562 ng/mL.

In the obese group, Table VI presents the correlation between elevated CRP levels and elevated BMI (p = 0.024; r = 0.199). In the obese group, RDW levels rose in parallel with rising CRP levels (p = 0.039; r = 0.181).

Table VI: The correlation between ferritin levels, vitamin B12, SI, BMI, Hb, RBC, MCV, RDW, TS, TIBC and CRP in the obese group

	CRP (mg/dL)	
	p	r
BMI (kg/m²)	0.024	0.199
Hb (g/dL)	0.153	0.126
RBC (10^6/µL)	0.845	0.017
MCV (fL)	0.112	-0.140
RDW (%)	0.039	0.181
TS (%)	0.141	-0.130
TIBC (ng/mL)	0.255	-0.101
Vit B12 (pg/mL)	0.451	0.067
SI (µg/dL)	0.055	-0.169
Ferritin (ng/mL)	0.077	0.156

Spearman Test. p<0.050 is significant.

DISCUSSION

Clinical outcomes can be significant for obesity and ID, two public health issues that continue to be prevalent and for which adolescents are especially vulnerable (15). Obesity prevalence is currently 16%–31%, and it has dramatically increased in recent years (16). The inverse association between iron and obesity has been described in both adults and children, but it is not currently sufficiently clarified. Many studies demonstrated

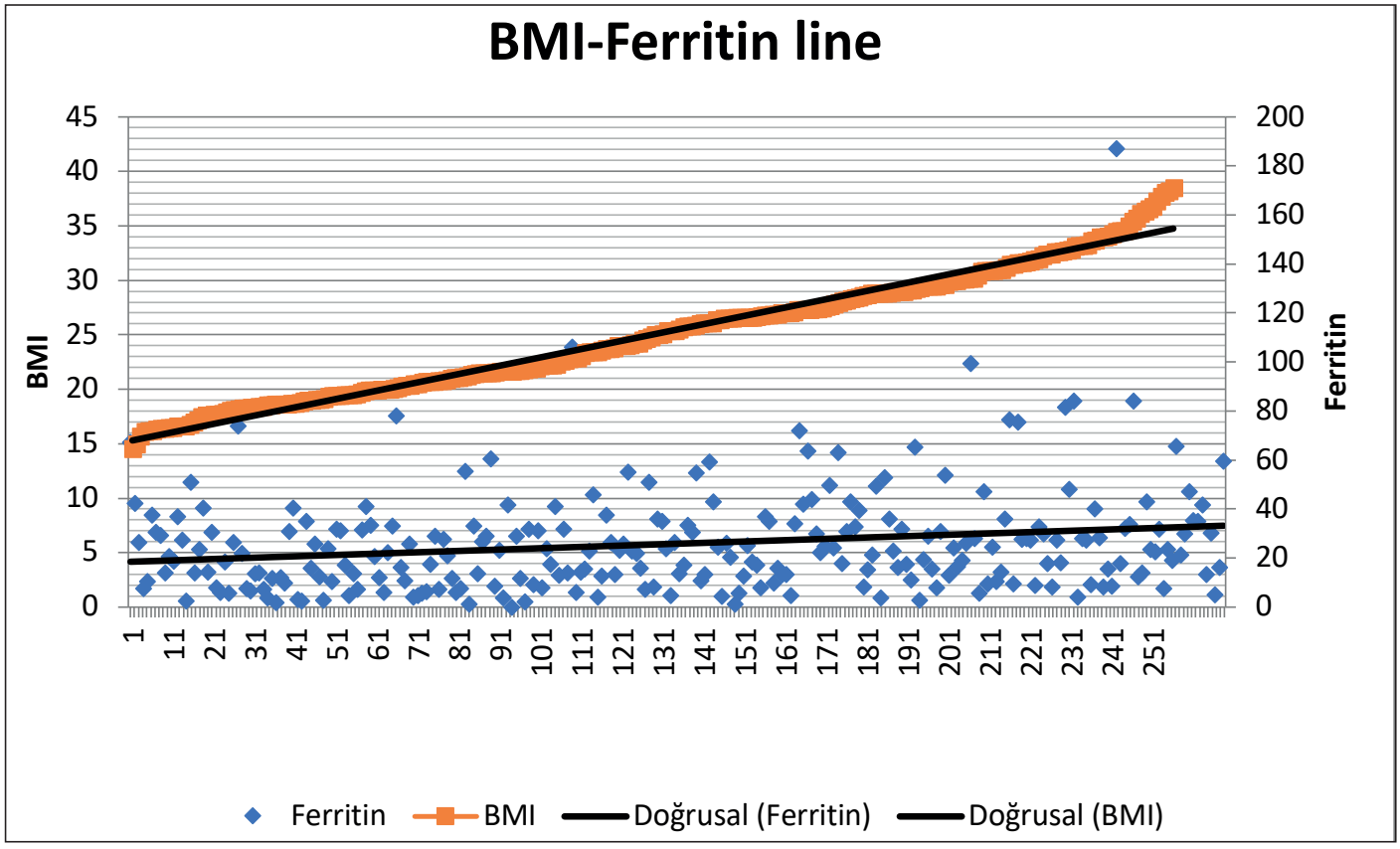

Figure 1: BMI-Ferritin

an increased risk of ID in line with the BMI. This relationship could be explained by: 1) an increase in iron requirements, due to the greater blood volume required by the increased body weight, provoking a real ID; 2) a decrease in the bioavailability of iron due to its sequestration in the reticuloendothelial system (assuming that obesity is a chronic inflammatory state, which would imply functional ID); and 3) both situations. Current evidence identifies hepcidin, a hormonal peptide secreted by the liver and the adipocytes, as a major factor in the alteration of iron metabolism that is observed in subjects with obesity. The proinflammatory cytokines that are characteristic of obesity would induce an increase in the production of hepcidin, thus reducing the absorption of iron in the intestine and its release from the macrophages (causing functional ID from decreased iron delivery for erythropoiesis), and an increase in the synthesis of ferritin in the reticuloendothelial cells (15). Other factors that could account for iron insufficiency in obese children include a diet rich in calories, a diet low in iron, a sedentary lifestyle that reduces myoglobin dissolution, and an increased need for iron because of larger red blood cells (17).

There is evidence that ID, whose risk increases in adolescence, impacts neurocognitive outcomes. Therefore, it may be essential to work to reduce health disparities among populations that are at risk during this critical period (18). A study revealed that the 12–16 age group was more likely to suffer from iron insufficiency and IDA (19).

According to a Chilean study, children from low socioeconomic properties were far more likely than those from high socioeconomic families to be overweight or obese (20). A study performed in our country revealed the high-income group had greater rates of obesity (21). Similarly, we found the obese group had a higher income level in our study. As reported in the literature, our study's findings indicated that the obese group also had higher incidences of diabetes, cardiovascular disease, and obesity in their families (22).

Obesity has been reported to be associated with ID frequently (19). Serum iron levels in the obese group in our study were lower than those in the control group, but there was no difference in IDA. The inadequate daily intake of iron in the majority of children in both groups and the lack of variation in consumption between the groups disproved the theory that the low serum iron level in the obese group was caused by low daily iron intake. Hb and ferritin levels were found to be statistically significantly higher in the control group with daily iron intake >10 mg compared to those with daily iron intake <10 mg, highlighting the significance of nutrition in healthy children, even though no correlation was found between daily iron intake and Hb and ferritin levels in the obese group.

Ferritin is an acute-phase protein that is upregulated during infections, inflammatory states, and malignant diseases. It is suggested that serum ferritin level is elevated in response to inflammation in obesity, even in persons with ID (17). Ferritin

levels were found to be higher in the obese group in our study than in the control group (p=0.002). Ferritin level and BMI were found to be significantly positively correlated. The ferritin levels increased by 0.56 ng/mL for every 1 kg/m² increase in BMI, according to the results of a linear regression analysis. The fact that the rate of anemia in our study's obese patients was the same as that of the control group suggests that the patients' high ferritin and low SI levels could be more consistent with chronic inflammation than with chronic infection anemia. In our research, we found that elevated BMI in obese patients was correlated with elevated CRP levels. However, the interpretation of chronic inflammation anemia is limited because the inflammatory indices hs-CRP, TNF-α, IL-6, and hepcidin could not be assessed.

The principal mechanism that links obesity and iron deficiency is low-grade systemic inflammation. Obesity's chronic inflammation state may lead inflammatory pathophysiological pathways to activate resulting in iron deficiency and other nutritional deficiencies. Obesity should be followed up as a chronic disease and monitored in terms of nutritional deficiencies, especially in adolescents who have not completed their growth.

REFERENCES

1. Tsoi MF, Li HL, Feng Q, Cheung CL, Cheung TT, Cheung BM. Prevalence of childhood obesity in the United States in 1999–2018: A 20-year analysis. Obes Facts 2022;15: 560-9.
2. Wang T, Gao Q, Yao Y, Luo G, Lv T, Xu G,et al. The causal relationship between obesity and iron deficiency anemia: a two-sample Mendelian randomization study. Front Public Health 2023; 11: 1188246.
3. Ogden CL, Fryar CD, Hales CM, Carroll MD, Aoki Y, Freedman DS. Differences in obesity prevalence by demographics and urbanization in US children and adolescents, 2013-2016. Jama 2018; 319: 2410-8.
4. Calcaterra V, Verduci E, Milanta C, Agostinelli M, Todisco CF, Bona F, et al. Micronutrient Deficiency in Children and Adolescents with Obesity—A Narrative Review. Children (Basel) 2023; 10: 695.
5. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. Lancet 2021; 397: 233-48.
6. Ibáñez-Alcalde MM, Vázquez-López MÁ, López-Ruzafa E, Lendínez-Molinos FJ, Bonillo-Perales A, Parrón-Carreño T. Prevalence of iron deficiency and related factors in Spanish adolescents. Eur J Pediatr 2020; 179: 1587-95.
7. Alshwaiyat NM, Ahmad A, Wan Hassan WMR, Al-Jamal HAN. Association between obesity and iron deficiency. Exp Ther Med 2021;22: 1-7.
8. Stoffel NU, El-Mallah C, Herter-Aeberli I, Bissani N, Wehbe N, Obeid O, et al. The effect of central obesity on inflammation, hepcidin, and iron metabolism in young women. Int J Obes 2020; 44: 1291-300.
9. Kozioł-Kozakowska A, Kozłowska M, Jagielski P. Assessment of diet quality, nutrient intake, and dietary behaviors in obese children compared to healthy children. Pediatr Endocrinol Rev 2020; 26: 27-38.
10. Gunanti IR, Marks GC, Al-Mamun A, Long KZ. Low serum vitamin B-12 and folate concentrations and low thiamin and riboflavin intakes are inversely associated with greater adiposity in Mexican American children. J Nutr 2014; 144: 2027-33.
11. Bundak R, Furman A, Gunoz H, Darendeliler F, Bas F, Neyzi O. Body mass index references for Turkish children. Acta Paediatr 2006; 95: 194-8.
12. Jetté M, Sidney K, Blümchen G. Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. Clin Cardiol 1990; 13: 555-65.
13. Tapiero H, Gate L, Tew K. Iron: deficiencies and requirements. Biomed Pharmacother 2001; 55: 324-32.
14. World Health Organization (WHO). Assessing The Iron Status Of Populations: Report of a Joint World Health Organization/ Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level Geneva 2004. Accessed: January 7, 2024. Available from: https://www.who.int/publications/i/item/9789241596107.
15. Ortiz Perez M, Vazquez Lopez MA, Ibanez Alcalde M, Galera Martinez R, Martin Gonzalez M, Lendinez Molinos F, et al. Relationship between obesity and iron deficiency in healthy adolescents. Child Obes 2020; 16: 440-7.
16. Atrushi AM, Armishty FS, Saleh SA, Abdulrahman MS. The Association Between Iron Deficiency Anemia and Obesity in Children. J Contemp Med Sci 2022; 8: 220-3.
17. Panichsillaphakit E, Suteerojntrakool O, Pancharoen C, Nuchprayoon I, Chomtho S. The Association between hepcidin and iron status in children and adolescents with obesity. J Nutr Metab 2021; 2021: 9944035.
18. Larsen B, Baller EB, Boucher AA, Calkins ME, Laney N, Moore TM, et al. Development of Iron Status Measures during Youth: Associations with Sex, Neighborhood Socioeconomic Status, Cognitive Performance, and Brain Structure. Am J Clin Nutr 2023; 118: 121-31.
19. Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M. Overweight children and adolescents: a risk group for iron deficiency. Pediatrics 2004; 114: 104-8.
20. Castillo-Valenzuela O, Duarte L, Arredondo M, Iñiguez G, Villarroel L, Pérez-Bravo F. Childhood Obesity and Plasma Micronutrient Deficit of Chilean Children between 4 and 14 Years Old. Nutrients 2023; 15: 1707.
21. Yardim M, Özcebe L, Araz Ö, Üner S, Li S, Ünlü H, et al. Prevalence of childhood obesity and related parental factors across socioeconomic strata in Ankara, Turkey. East Mediterr Health J 2019; 25: 374-84.
22. Gökler ME, Buğrul N, Metintaş S, Kalyoncu C. Adolescent obesity and associated cardiovascular risk factors of rural and urban life (Eskisehir, Turkey). Cent Eur J Public Health 2015; 23: 20-5.

Varicella Seroprevalence in Pediatric Populations: Results from a Single-Center Study

Pediatrik Popülasyonlarda Varisella Seroprevalansı: Tek Merkezli Bir Çalışmanın Sonuçları

Serhat KILIÇ[1], Betül ORHAN KILIÇ[1], Dilek KONUKSEVER[2], Esra BASKIN[3], İsmail Zafer ECEVİT[4]

[1]Department of Pediatrics, Başkent University Faculty of Medicine, Ankara, Türkiye
[2]Department of Pediatrics, Ankara Bilkent City Hospital, Ankara, Türkiye
[3]Department of Pediatric Nephrology, Başkent University Faculty of Medicine, Ankara, Türkiye
[4]Department of Pediatric Infectious Diseases, Gülhane Training and Research Hospital, Ankara, Türkiye

ABSTRACT

Objective: Varicella is a highly contagious illness with potentially severe complications, especially in young children. In Turkey, the varicella vaccine was integrated into the Universal Varicella Vaccination program in 2013, targeting 12-month-old infants. While officially reported varicella cases have decreased considerably in the past two decades, underreporting remains a challenge. This study aimed to investigate varicella seropositivity in a tertiary center.

Material and Methods: A qualitative immunoassay, the enzyme-linked immunosorbent assay (ELISA), was employed to detect anti-varicella antibodies. Serum samples were collected from individuals aged 4 to 18 residing in Turkey. Information on vaccination records, varicella history, and disease notification was also collected.

Results: The varicella IgG antibody records were accessed for a total of 90 children. The overall prevalence of positive varicella antibodies was 58.0% (n=47) in the study population. Seropositivity rates were 67.2% for the 4-6 age group and 30% for the 7-12 age group. Notably, the 4-6 age group showed a significant association between seropositivity and age [p=0.005; OR=4.85; 95%CI (1.614-14.569)].

Conclusion: The seropositivity rate of 58% for chickenpox is concerning. In light of this, conducting more extensive studies will provide valuable guidance. It may be worth considering the administration of an additional dose of the varicella vaccine within the age range of 4-6 years. Further research is necessary to assess the potential benefits and feasibility of implementing such a vaccination strategy.

Key Words: Pediatric, Seropositivity, Turkey, Varicella

ÖZ

Amaç: Varisella, özellikle küçük çocuklarda potansiyel olarak ciddi komplikasyonları olan oldukça bulaşıcı bir hastalıktır. Türkiye'de su çiçeği aşısı, 2013 yılında 12 aylık bebekleri hedef alan Ulusal Suçiçeği Aşısı programına entegre edildi. Resmi olarak rapor edilen su çiçeği vakaları son yirmi yılda önemli ölçüde azalmış olsa da, eksik raporlama hala bir sorun olmaya devam ediyor. Bu çalışmada üçüncü basamak bir merkezde suçiçeği seropozitifliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Kalitatif bir immünolojik test olan enzim bağlantılı immünosorbent testi (ELISA), anti-varisella antikorlarını tespit etmek için kullanılmıştır. Türkiye'de ikamet eden 4-18 yaş arası bireylerden serum örnekleri toplandı. Aşı kayıtları, su çiçeği geçmişi ve hastalık bildirimine ilişkin bilgiler de toplanmıştır.

0000-0003-3705-4312 : KILIÇ S
0000-0002-9949-0470 : ORHAN KILIÇ B
0000-0003-2334-9590 : KONUKSEVER D
0000-0003-4361-8508 : BASKIN E
0000-0001-6216-8480 : ECEVİT İZ

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study received approval from the Başkent University Faculty of Medicine Ethics Committee (project number: KA 23/244-19.07.2023) before its commencement.

Contribution of the Authors / Yazarların katkısı*: *KILIÇ S: Conceptualization, formal analysis, software, writing-original draft, data curation. ***ORHAN KILIÇ B***: Conceptualization, formal analysis, software, writing-original draft, data curation, investigation, formal analysis, project administration, writing-review & editing. ***KONUKSEVER D:*** Conceptualization, formal analysis, software, writing-original draft, data curation ***BASKIN E:*** Conceptualization, supervision, writing-review & editing. ***ECEVİT İZ:*** Conceptualization, formal analysis, methodology, resources, supervision, writing-review & editing.

How to cite / *Atıf yazım şekli* : Kılıç S, Orhan Kılıç B, Konuksever D, Baskın E and Ecevit İZ. Varicella Seroprevalence in Pediatric Populations: Results from a Single-Center Study. Turkish J Pediatr Dis 2024;18:192-195.

Additional information / *Ek bilgi*: We would like to thank the study participants for their valuable contributions to this research.

Correspondence Address / Yazışma Adresi:
İsmail Zafer ECEVİT
Department of Pediatrics,
Başkent University Faculty of Medicine, Ankara, Türkiye
E-posta: zaferecevit@hotmail.com

Received / Geliş tarihi : 10.01.2024
Accepted / Kabul tarihi : 12.03.2024
Online published : 16.04.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1417507

Bulgular: Toplam 90 çocuğun su çiçeği IgG antikoru kayıtlarına erişildi. Çalışma popülasyonunda pozitif su çiçeği antikorlarının genel prevalansı %58.0 (n=47)'di. Seropozitiflik oranları 4-6 yaş grubunda %67.2, 7-12 yaş grubunda ise %30 olarak belirlendi. Özellikle 4-6 yaş grubu seropozitiflik ile yaş arasında anlamlı bir ilişki gösterdi [p=0.005; OR=4.85; 95%CI (1.614-14.569)].

Sonuç: Suçiçeği için %58'lik seropozitiflik oranı endişe vericidir. Bunun ışığında daha kapsamlı çalışmaların yapılması değerli bir yol gösterici olacaktır. 4-6 yaş aralığında ek doz su çiçeği aşısı yapılması düşünülebilir. Böyle bir aşılama stratejisinin uygulanmasının potansiyel faydalarını ve fizibilitesini değerlendirmek için daha fazla araştırma yapılması gerekmektedir.

Anahtar Sözcükler: Pediatrik, Seropozitiflik, Türkiye, Suçiçeği

INTRODUCTION

Varicella-zoster virus (VZV) is a member of the herpesvirus family responsible for causing the itchy rash of chickenpox (varicella) in children and the painful blistering rash called herpes zoster (HZ) or shingles in older or immunocompromised individuals. After infection, the virus can remain dormant in the dorsal root ganglia for the individual's entire life until it is reactivated (1).

The administration of the varicella vaccine is an effective preventive measure to significantly reduce the incidence of varicella. Before the widespread availability of the vaccine, Europe reported approximately 5.5 million varicella cases annually, with 80% of unvaccinated children and adolescents showing positive serum anti-VZV IgG (2,3). Similarly, in the United States, approximately 4 million cases were reported each year before the vaccine became widely accessible (4). However, since the introduction of a single-dose vaccination for children aged 12-18 months in 1996, the incidence of varicella decreased by about 90% by 2005 (5).

According to the World Health Organization (WHO), VZV infects at least 140 million people worldwide annually, imposing significant social and economic burdens (6). To address this, the WHO has recommended the inclusion of varicella vaccines in routine childhood immunization programs. In 1995, The Centers for Disease Control and Prevention (CDC) also advised that individuals of all ages, including children, adolescents, and adults, receive two doses of the varicella vaccine as a precautionary measure against the disease (7). The first vaccine dose is typically administered to children between 12 and 15 months of age, with the second dose given to children aged 4 to 6 years.

Despite the success of varicella vaccination programs in reducing the incidence of the disease, cases of varicella infection continue to occur both domestically and internationally, as reported in several studies (6,8). These studies have demonstrated the protective effect of both one-dose and two-dose varicella vaccines. The findings indicate that the protection rates for one-dose varicella vaccine are 72.98%, while the protection rates for two-dose varicella vaccine are 100 (9). Although a single dose of the vaccine has shown to provide approximately 97% protection against severe and moderate chickenpox infections, its efficacy against any degree of severity is lower, ranging from 80-85%. Therefore, it is possible for children who receive a single dose of the vaccine to still contract a chickenpox infection. On the other hand, administering two doses of the chickenpox vaccine has been reported to significantly reduce the incidence of chickenpox cases (9).

The objective of the present study was to assess the prevalence of varicella antibody seropositivity in healthy children aged 4-12 years who had a single dose of varicella vaccine at 12 months of age.

MATERIALS and METHODS

In this study, a retrospective evaluation was conducted on the records of a total of 90 children who underwent testing for varicella IgG at Başkent University. Due to incomplete vaccination records for five children and chronic illnesses in four others, these nine children were excluded from the study. A total of 81 children have been vaccinated with only one dose of varicella vaccine at 12 months of age. These 81 children had attended the clinic for routine well-child check-ups and had received a single-dose varicella vaccine according to the standard vaccination schedule implemented in our country.

The study received approval from the Başkent University Faculty of Medicine Ethics Committee (project number: KA 23/244-19.07.2023) before its commencement.

Varicella Vaccination Strategy in Turkey

In Turkey, the varicella vaccination strategy involves including the varicella vaccine in the national vaccination calendar since 2013. According to the vaccination schedule, children in Turkey receive the varicella vaccine at the age of one year.

Detection of Anti-Varicella-Zoster Virus (VZV) IgG Antibodies

The Enzyme-linked immunosorbent assay (ELISA) test kit, ARCHITECT-in, was used to determine the presence of anti-VZV IgG antibodies in the samples. The manufacturer's instructions were followed, and established threshold values were used to interpret the results. Each test result was considered independent. Samples with a ΔA value below the threshold were classified as negative, and those above the threshold were classified as positive. The manufacturer's information indicated that anti-VZV IgG concentrations of ≥11 NTU, ≥ 9 to <11 NTU, and <9 NTU were classified as positive, equivocal, and negative results, respectively. It was observed that all patients who had borderline or negative Varicella IgG levels had received the varicella vaccine.

Statistical Analysis

Data obtained from questionnaires were analyzed using IBM SPSS Statistics version 22.0 (SPSS Inc., Armonk, NY, IBM Corp., USA) software package. Descriptive statistics such as frequency, percentage, mean, and standard deviation were used to present the data. The normal distribution of variables was evaluated using the Shapiro-Wilk test, with p-values below 0.050 considered statistically significant. To assess differences between groups, the Mann-Whitney U test was employed. For categorical data, either the Chi-square significance test or Fisher's exact test was used. Additionally, univariate and multivariate logistic regression analysis was conducted to investigate the factors influencing viral seropositivity. Results were considered statistically significant if they achieved a confidence level of 95% or a margin of error of 0.05."

RESULTS

The study included 81 healthy children with a mean age of 6.0 ± 2.1 years. Of the participants, 43.2% were female (n=41), 75.3% (n=61) were aged between 4-6 years, and 24.7% (n=20) were aged between 7-12 years. The distribution of boys and girls was similar across both age groups (p=0.843).

Upon analyzing the varicella seropositivity rates, it was found that 58% (n=47) of the participants tested positive for varicella IgG.

When comparing varicella seropositive and seronegative groups, no significant difference was found in terms of gender (p=0.831). However, a statistically significant difference was observed between the two groups in terms of age distribution (p=0.003) (Table I). The varicella seropositivity rate in the 4-6 age group was found to be 67.2%, while the rate in the 7-12 age group was 30%.

Table II presents the results of the logistic regression analysis, which aimed to identify the variables that predict varicella seropositivity rates. The analysis was conducted using two predictor variables: age group and gender. The results show that the age group variable was a significant predictor of varicella seropositivity rates [p=0.005; OR=4.85; 95%CI (1.614-14.569)]. Children in the 4-6 age group were 4.85 times more likely to be seropositive for varicella than those in the 7-12 age group. On the other hand, gender was not a significant predictor of varicella seropositivity rates [p=0.636; OR=1.24; 95%CI (0.511-2.999)].

Table I: Comparison of the participant feautures according to varicella seropositivity

	Varicella Seronegative	Varicella Seropositive	p
Age (m±SD) (years)	7±3	5±1	0.105
Age group*			
4-6 years	20 (32.8)	41 (67.2)	0.003†
7-12 years	14 (70.0)	6 (30.0)	
Gender*			
Female	17 (39.5)	26 (60.5)	0.831
Male	17 (44.7)	21 (55.3)	

:** *n(%),* **†:** *p<0.050 is significant,* ***m: *mean,* ***SD:*** *standard deviation*

DISCUSSION

Varicella, commonly known as chickenpox, is a viral infection that can lead to severe and life-threatening complications in children (10). To prevent the spread of this infection, it is crucial to be aware of its potential risks and take appropriate measures, such as vaccination and isolation of infected individuals. The present study found that the varicella seropositivity rate was 58% among healthy children in a tertiary center.

Bollaerts et al. (11) conducted a systematic review of 43 studies across 16 European countries to assess varicella seroprevalence before the implementation of universal childhood immunization. The results showed considerable variation in varicella seroprevalence among European countries during childhood, highlighting the need for tailored vaccination policies. In a study among adults in our country, Sac et al. (12) found a high seroprevalence of varicella (93%), indicating a relatively high level of immunity against varicella in the adult age group due to asymptomatic or undiagnosed infection. This information is crucial in developing vaccination strategies for maintaining and enhancing population immunity. Hu et al. (6) investigated varicella vaccination status among Chinese children aged 6-11 and found low two-dose vaccination rates among those with a history of varicella infection. The researchers recommended increasing the coverage of the varicella vaccine and including a two-dose regimen in the National Immunization Program of China.

Gabutti et al. (13) conducted a study in Italy to evaluate varicella seroprevalence and the impact of mandatory varicella vaccination for newborns. The findings indicated a high overall seroprevalence of varicella in Italy, with increasing seropositivity observed in younger age groups since the implementation of mandatory varicella vaccination for newborns in 2017. Taken together, the studies reviewed here reveal that varicella seropositivity rates are influenced by various factors, such as age, gender, vaccination status, and geographical location. The findings also demonstrate considerable variation in varicella seroprevalence across different countries and regions, indicating the need for tailored vaccination policies based on local epidemiological data. These insights are crucial in understanding the complex nature of varicella infection and developing effective strategies for preventing its spread and minimizing its associated risks. Further research is needed to explore the factors that contribute to the heterogeneity of varicella seroprevalence and to identify optimal vaccination strategies in different populations.

Our study revealed a significant difference in varicella IgG positivity rate between the 4-7 age group and the 7-12 age group,

Table II: Univariate and multivariate logistic regression of the varicella seropositivity

	Univariate Logistic Regression Analysis				Multivariate Logistic Regression Analysis			
	OR	CI 95%	beta	p	OR	CI 95%	beta	p
4-6 years	4.78	1.599-14.307	1.56	0.005	4.85	1.614-14.569	1.57	0.005
Gender	1.24	0.511-2.999	0.21	0.636	1.61	0.514-3.352	0.27	0.570

OR: *Odds ratio,* ***CI:*** *confidence interval*

with a higher rate observed in the younger age group. Zhang et al. (9) evaluated varicella seropositivity rates and protective effects of varicella vaccination in children aged 3-6 years. The study found a significant increase in positive antibodies with an increasing number of vaccine doses administered and a higher protection rate with two-dose vaccination compared to one-dose vaccination. Luan et al. (14) found a higher seroprevalence of anti-VZV IgG antibodies in Chinese children who had received varicella vaccination, indicating a positive impact of vaccination on the development of immunity against varicella. However, vaccination rates differed between genders, highlighting the need for targeted vaccination strategies.

Although our study had some limitations, such as a small sample size and the lack of examination of antibody levels in healthy children aged 12-18, we believe that our findings provide a valuable contribution to the field of varicella seroprevalence research in healthy children. By identifying the varicella seropositivity rates in our study population, we were able to shed light on the epidemiology of varicella in our community and provide insights into the potential impact of vaccination programs in our region.

In conclusion, while we acknowledge the limitations of our study, we believe that our findings provide important insights into the seroprevalence of varicella in healthy children and can serve as a starting point for further research in this area. We hope that our study will encourage continued investigation into the epidemiology of varicella and inform the development of effective vaccination programs to reduce the burden of this infection in our community and beyond.

REFERENCES

1. Steiner I, Kennedy PG, Pachner AR. The neurotropic herpes viruses: herpes simplex and varicella-zoster. Lancet Neurol 2007;6:1015–28.
2. Riera-Montes M, Bollaerts K, Heininger U, Hens N, Gabutti G, Gil A, et al. Estimation of the burden of varicella in Europe before the introduction of universal childhood immunization. BMC Infect Dis 2017;17:353.
3. Wiese-Posselt M, Siedler A, Mankertz A, Sauerbrei A, Hengel H, Wichmann O, Poethko-Müller C. Varicella-zoster virus seroprevalence in children and adolescents in the pre-varicella vaccine era, Germany. BMC Infec Dis 2017;17:356.
4. Seward JF, Watson BM, Peterson CL, Mascola L, Pelosi JW, Zhang JX, Wharton M. Varicella disease after introduction of varicella vaccine in the United States, 1995-2000. JAMA 2002;287:606-11.
5. Guris D, Jumaan AO, Mascola L, Watson BM, Zhang JX, Chaves SS, Gargiullo P et al. Changing varicella epidemiology in active surveillance sites—United States, 1995–2005. J Infec Dis 2008;197Suppl:S71-S75.
6. Hu P, Yang F, Li X, Wang Y, Xiao T, Li H, et al. Effectiveness of one-dose versus two-dose varicella vaccine in children in Qingdao, China: a matched case-control study. Hum Vaccin Immunother 2021;17:5311-5.
7. Marin M, Seward JF, Gershon AA. 25 Years of Varicella Vaccination in the United States. J Infect Dis 2022;226 (suppl 4): S375-9.
8. Almis H, Hakan Bucak I, Emre Kilic F, Kayak D, Geyik M, Tekin M, et al. The evaluation of chickenpox in the post-vaccination period in Turkey. Ann Med Res 2021;26: 833–6.
9. Zhang Z, Zhang Y, Yu J, Dong C, Zhang J, Liu N, et al. Seroprevalence rates in children aged 3-6 years after implementing a two-dose varicella vaccination: A observational study. Hum Vaccin Immunother 2023;19:2211465.
10. Modderman SC, de Kleijn ED, Hartwig NG. Serious complications of chickenpox: Healthy children are at risk as well. Ned Tijdschr Geneeskund 2021;165:D5422.
11. Bollaerts K, Riera-Montes M, Heininger U, Hens N, Souverain A, Verstraeten T, et al. A systematic review of varicella seroprevalence in European countries before universal childhood immunization: deriving incidence from seroprevalence data. Epidemiol Infect 2017;145: 2666-77.
12. Saç R, Taşar MA, Yalaki Z, Güneylioğlu MM, Özsoy G, Karadağlı S, et al. Hepatitis A, hepatitis B, measles, mumps, rubella and varicella seroprevalence in Turkish adolescent nursing students. Nobel Med 2019;15:33–40.
13. Gabutti G, Grassi T, Bagordo F, Savio M, Rota MC, Castiglia P, Baldovin T, et al. Sero-Epidemiological Study of Varicella in the Italian General Population. Vaccines 2023;11: 306.
14. Luan L, Shen X, Qiu J, Jing Y, Zhang J, Wang J, et al. Seroprevalence and molecular characteristics of varicella-zoster virus infection in Chinese children. BMC Infect Dis 2019;19:643.

Turkish Journal of Pediatric Disease
Türkiye Çocuk Hastalıkları Dergisi

A Rare Cause of Upper Extremity Thrombosis: Paget-Schroetter Syndrome

Üst Ekstremite Trombozunun Nadir Bir Nedeni: Paget-Schroetter Sendromu

Zeliha GÜZELKÜÇÜK[1], Mehmet Dalokay KILIÇ[2], Arzu YAZAL ERDEM[1], Hasan Fatih ÇAKMAKLI[3], Namık Yaşar ÖZBEK[1]

[1]Department of Pediatric Hematology-Oncology, Ankara Bilkent City Hospital,Ankara, Türkiye
[2]Department of Thoracic Surgery, Başkent University Faculty of Medicine,Ankara, Türkiye
[3]Department of Pediatric Hematology, Ankara University Faculty of Medicine,Ankara, Türkiye

ABSTRACT

Upper extremity deep vein thrombosis (UEDVT) is extremely rare in children and adolescents. Paget - Schroetter Syndrome (PSS) is the primary, spontaneous thrombosis of the subclavian venous (SV) axillary tract. Herein, we report on PSS in two children who did regular body building exercises. 16-year-old two male patients admitted with complaints of swelling, coldness and pain on the right upper extremity. Their recent history disclosed that they have been doing advanced body building exercises for the last year. Physical examination revealed a difference of 4 cm and 5 cm in the diameter of each arm, as well as coldness and venous engourgement in the upper extremities. An acute thrombus was present in the proximal right SV lumen and no flow was detected on upper extremity venous doppler ultrasaund. Surgical treatment was performed in addition to medical thrombosis treatment. Early diagnosis and treatment of PSS is important for better outcomes. PSS should be considered in young patients with UEDVT, especially those with a history of vigorious exercise.

Key Words: Exercises, Paget - Schroetter Syndrome, Thrombosis

ÖZ

Üst ekstremite derin ven trombozu (UEDVT) çocuklarda ve ergenlerde oldukça nadir görülür. Paget-Schroetter sendromu (PSS), subklavyen venöz (SV) aksiller sistemin primer, spontan trombozudur. Burada; düzenli vücut geliştirme egzersizleri yapan PSS tanısı alan iki hastayı bildirmekteyiz.16 yaşında iki erkek hasta sağ üst ekstremitede şişlik, soğukluk ve ağrı şikayetiyle başvurdu. Yakın zamanda, vücut geliştirme egzersizleri yaptıkları öğrenildi. Fizik muayenede her iki kol çaplarında arasında fark olduğu, üst ekstremitelerde soğukluk ve üst ekstremite toplar damarlarında belirginleşme dikkat çekmişti. Her iki olguda da üst ekstremite venöz doppler ultrasonografide sağ SV proksimal lümeninde akut trombüs saptandı. Medikal tromboz tedavisine ek olarak cerrahi tedavi uygulandı. Tromboz sonrası iyileşmenin optimal olabilmesi için PSS'nin erken tanı ve tedavisi önemlidir. Bu nedenle ağır egzersiz öyküsü olan genç erkeklerde üst ekstremiteyi ilgilendiren şişlik, soğukluk, ağrı yakınmalarında PSS düşünülmelidir.

Anahtar Kelimeler: Egzersiz, Paget-Schroetter Sendromu, Tromboz

INTRODUCTION

Upper extremity deep vein thrombosis (UEDVT) is extremely rare in children and adolescents (1). The underlying conditions include mediastinal mass, central venous catheter, or idiopathic (1,2). Paget - Schroetter Syndrome (PSS) is the primary, spontaneous thrombosis of the subclavian venous (SV) axillary tract (2). Herein, we report PSS in two children who did regular body building exercises and discuss treatment strategies in the light of the current literature. We also aimed to draw attention to PSS which is rare in childhood, but the clinical presentation is easy to define and the management is peculiar.

0000-0003-1462-6867 : GÜZELKÜÇÜK Z
0000-0001-7813-5317 : KILIÇ MD
0000-0003-1043-8471 : YAZAL ERDEM A
0000-0003-0152-1564 : ÇAKMAKLI HF
0000-0003-0152-1564 : ÖZBEK NY

Conflict of Interest /*Çıkar Çatışması:* *On behalf of all authors, the corresponding author states that there is no conflict of interest.*
Financial Disclosure / *Finansal Destek*: The authors declared that this case has received no financial support.
***Confirmation* / *Onay*:** The written consent was received from the patient who was presented in this study.
How to cite / *Atıf Yazım Şekli* : Güzelküçük Z, Kılıç MD, Yazal Erdem A, Çakmaklı HF and Özbek NY. A rare cause of upper extremity thrombosis: Paget-Schroetter Syndrome. Turkish J Pediatr Dis 2024;18:196-198.

Correspondence Address / Yazışma Adresi :
Zeliha GÜZELKÜÇÜK
Department of Pediatric Hematology-Oncology,
Ankara Bilkent City Hospital, Ankara, Türkiye
E-posta: drguzelkucuk@hotmail.com

Received / Geliş tarihi : 21.11.2023
Accepted / Kabul Tarihi : 10.01.2024
Online published : 15.02.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1387074

CASE REPORT

Case 1: A 16-year-old male patient presented with complaints of swelling, coldness and pain in the right upper extremity. His recent history disclosed that he had been doing advanced body-building exercises for the last year. Physical examination revealed a 4 cm difference in the diameter of his arms, as well as coldness and venous engourgement the right upper extremity (Figure 1). A thrombus extending from the right proximal of the subclavian vein to the axillary vein was detected via doppler ultrasonography. Enoxaparin was initially started subcutaneously 1 mg/kg/ twice daily. Enoxaparin was discontinued after obtaining INR values over 2 were achieved with warfarin treatment. Then, INR values were adjusted to be between 2-3. Genetic risk factors for thrombosis including Factor V Leiden, and Prothrombin G20210A revealed no mutation. Anticoagulant treatment was stopped after 6 months. However, the swelling, numbness and diameter difference in the right arm recurred two months after the warfarin withdrawal. Partial thrombosis was again detected in the right SV. The patient's computed tomography (CT) angiography was compatible with PSS. Oral anticoagulation treatment was restarted. After 5 months there was a 1 cm difference in diameter, mild drowsiness and pain in the right arm of the patient. These findings were attributed to post-thrombotic syndrome. Control doppler ultrasound revealed that the right subclavian venous calibration was minimally reduced compared to the other branches, the vessel surface was irregular due to the thrombus, venous flow was markedly reduced in the compulsive movements of the arm, and several venous collaterals were found in the right axillary region. To the decompress of the PSS, the first right costa resection and scalenectomy were performed with a right transaxillary intervention. The patient was treated with warfarin for 3 months postoperatvely. Pain and drowsiness were relieved.

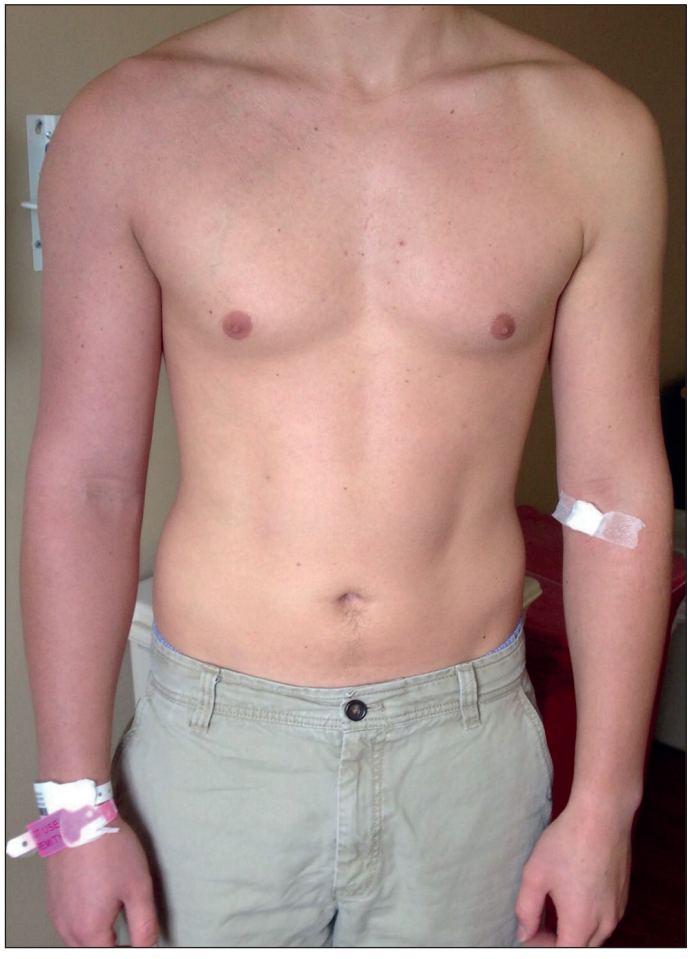

Figure 1: Swelling of right upper extremity, discoloration due to circulatory disorder (Case 1).

Case 2: Another 16-year-old male patient presented with a complaint of swelling, coldness and pain on the right side of the body. His recent history disclosed that he was doing advanced body building exercises. Physical examination revealed a 5 cm diameter difference, and cyanosis of the right arm. An acute thrombus was present in the proximal lumen of the right SV and venous doppler ultrasonography of the upper extremity showed no flow. The first 10 cm of the proximal portion of the cephalic vein was found to be completely occluded by thrombus. There was no family history of thrombosis. Enoxaparin was initially started subcutaneously 1 mg/kg/ twice daily and switched to warfarin as in Patient 1. Genetic testing revealed a heterozygous MTHFR C677T mutation and a homozygous PAI-1 4G/4G polymorphism. The patient was consulted with thoracic surgery for decompression. For the decompression of the PSS, a right first costa resection and scalenectomy were performed with a right transaxillary intervention. He did well after the operation with relief of symptoms. However, he lost follow-up one month after surgery.

DISCUSSION

Upper extremity DVT due to a primary cause is referred to as PSS that is associated with subclavian and axillary venous compression. In young, healthy patients presenting with UEDVTs, it is important to consider the primary causes (1,2). Early recognition of UEDVT's is important to reduce life-threatening complications such as pulmonary embolism (5). In PSS, thrombus formation is usually triggered by the subclavian venous exertion (2). The subclavian vein passes through the clavicle, first rib and subclavian muscles. Endothelial damage caused by recurrent retroversion, hyperabduction and enlargement movements can activate the coagulation system (2,3). It has usually been reported in young men involved in tennis, wrestling, swimming, billiards, hockey, baseball (3). Both of our patients were healthy young adolescents who were doing body building.

Upper extremity DVT usually presents with severe and sudden pain, swelling, numbness, and discolouration after a heavy

exercise or work. The right upper extremity is more likely to be affected because the majority of patients, like our patients, are right-handed.

Imaging is required to confirm the diagnosis. Doppler ultrasonography has been proposed as the first diagnostic tool because of its high sensitivity and specificity (4). The diagnoses of our patients were quickly confirmed by doppler USG.

Treatment is aims to prevent complications and obstructive symptoms (6). The main treatment approaches should be discussed depending on the absence of clinical trials to guide the treatment plan (5). More aggressive treatment approaches including thrombolysis and surgery may be required in addition to anticoagulation to prevent symptoms and post-thrombotic complications (6). In our patients, anticoagulant treatment together with surgery was successful. Therefore, we believe that thrombolysis was not always necessary in the treatment.

In conclusion; PSS should be considered in young patients with upper extremity thrombosis, especially in young men with a history of heavy exercise. Early diagnosis and treatment of PSS is important for better outcomes.

REFERENCES

1. Yunce M, Sharma A, Braunstein E, Streiff MB, Lum YW. A case report on 2 unique presentations of upper extremity deep vein thrombosis. Medicine (Baltimore) 2018;97:e9944.
2. Huang CY, Wu YH, Yeh IJ, Chen YY, Kung FY. Spontaneous bilateral subclavianNvein thrombosis in a 40-year-old man: A case report. Medicine (Baltimore) 2018;97:e0327.
3. Urschel HC Jr, Patel AN. Surgery remains the most effective treatment for Paget-Schroetter syndrome: 50 years' experience. Ann Thorac Surg 2008;86:254-60.
4. Hangge P, Rotellini-Coltvet L, Deipolyi AR, Albadawi H, Oklu R. Paget-Schroetter syndrome: treatment of venous thrombosis and outcomes. Cardiovasc Diagn Ther 2017;7:S285-S290.
5. Glavich G, Gourley J, Fong V. Paget-Schroetter syndrome with bilateral pulmonary emboli. Radiol Case Rep 2017;31:13:28-31.
6. El-Attrache A, Kephart E. Paget-Schroetter Syndrome: a case report of diagnosis, treatment, and outcome in a healthy 18-year-old athletic swimmer. Phys Sportsmed 2020;48:358-62.

Unexpected Hyperkalemia During Kawasaki Disease

Kawasaki Hastalığının Seyri Sırasında Beklenmedik Şekilde Gelişen Hiperkalemi

Gülsüm İclal BAYHAN[1,3], Muhammed Yasin GÖKDÖL[2], Abdullah Talha ÖCAL[2], Tuğba ERAT[3], Utku PAMUK[4], Vildan GÜNGÖRER[5], Aslınur ÖZKAYA PARLAKAY[1,3]

[1,3]Department of Pediatric Infectious Disease, Ankara Yıldırım Beyazıt University, Faculty of Medicine, Ankara City Hospital, Ankara, Türkiye
[2]Department of Child Health and Diseases, Ankara Bilkent City Hospital, Ankara, Türkiye
[3]Department of Pediatric Infectious Disease, Ankara Bilkent City Hospital, Ankara, Türkiye
[4]Department of Pediatric Cardiology, Ankara Bilkent City Hospital, Ankara, Türkiye
[5]Department of Pediatric Romatology, Ankara Bilkent City Hospital, Ankara, Türkiye

ABSTRACT

Pseudohyperkalemia is defined as a markedly elevated serum potassium level with a normal plasma potassium concentration. It is mainly caused by leucocytosis and thrombocytosis. Here we report an infant treated with aspirin for Kawasaki disease who developed hyperkalemia during follow-up. He was finally diagnosed with pseudohyperkalemia based on a normal whole blood potassium level measured with a blood gas analyser.

Key Words: Hyperkalemia, Kawasaki disease, Pseudohyperkalemia

ÖZ

Psödohiperkalemi, plazma potasyum konsantrasyonu normal iken serum potasyum seviyesinin belirgin şekilde yüksek bulunması olarak tanımlanır. Esas olarak lökositoz ve trombositozdan kaynaklanır. Burada, Kawasaki hastalığı için aspirin tedavisi alan ve takip sırasında hiperkalemisi gelişen bir bebek bildirilmektedir. Kan gazı cihazı ile ölçülen tam kan potasyum seviyesinin normal bulunması ile psödohiperkalemi tanısı konulmuştur.

Anahtar Kelimeler: Hiperkalemi, Kawasaki hastalığı, Psödohiperkalemi

INTRODUCTION

Hyperkalemia is defined as a potassium level greater than 5.5 mmol/L (mEq/L) (1). Haemolysis should be investigated first when the potassium level is unexpectedly high and inconsistent with the patient's clinical status and treatments received. Fist clench during venipuncture, prolonged use of tourniquets, squeezing the extremity during blood collection and holding the sample for more than 30 minutes before testing all lead to haemolysis. Several drugs can cause hyperkalemia (1,3). Hyperkalemia associated with non-steroidal anti-inflammatory drug (NSAID) has rarely been reported (4,5).

Another cause is pseudohyperkalemia. Pseudohyperkalemia is defined as a markedly elevated serum potassium level with a normal plasma potassium concentration. It is mainly caused by leucocytosis and thrombocytosis (2). Here, we report an infant who was treated with aspirin for Kawasaki disease and developed hyperkalaemia during follow-up.

CASE

A 7-month-old male patient was admitted with a 5-day history of fever and generalized maculopapular rash. The patient was born

0000-0002-1423-4348 : BAYHAN Gİ
0000-0002-6541-8550 : GÖKDÖL MY
0009-0003-9458-5336 : ÖCAL AT
0000-0003-2434-4134 : ERAT T
0000-0001-7046-8571 : PAMUK U
0000-0002-9838-2603 : GÜNGÖRER V
0000-0001-5691-2461 : ÖZKAYA PARLAKAY A

Conflict of Interest /*Çıkar Çatışması:* *On behalf of all authors, the corresponding author states that there is no conflict of interest.*

Financial Disclosure / *Finansal Destek*: The authors declared that this case has received no financial support.

***Confirmation* / *Onay*:** The written consent was received from the patient who was presented in this study.

How to cite / *Atıf Yazım Şekli* : Bayhan Gİ, Gökdöl MY, Öcal AT, Erat T, Pamuk U, Güngörer V et al. Unexpected Hyperkalemia During Kawasaki Disease. Turkish J Pediatr Dis 2024;18:199-202.

Correspondence Address / Yazışma Adresi :
Gülsüm İclal BAYHAN
Department of Pediatric Infectious Disease, Ankara Yıldırım Beyazıt University, Faculty of Medicine, Ankara City Hospital, Ankara, Türkiye
E-posta: gibayhan@ybu.edu.tr

Received / Geliş tarihi : 21.11.2023
Accepted / Kabul Tarihi : 20.02.2024
Online published : 27.02.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1393779

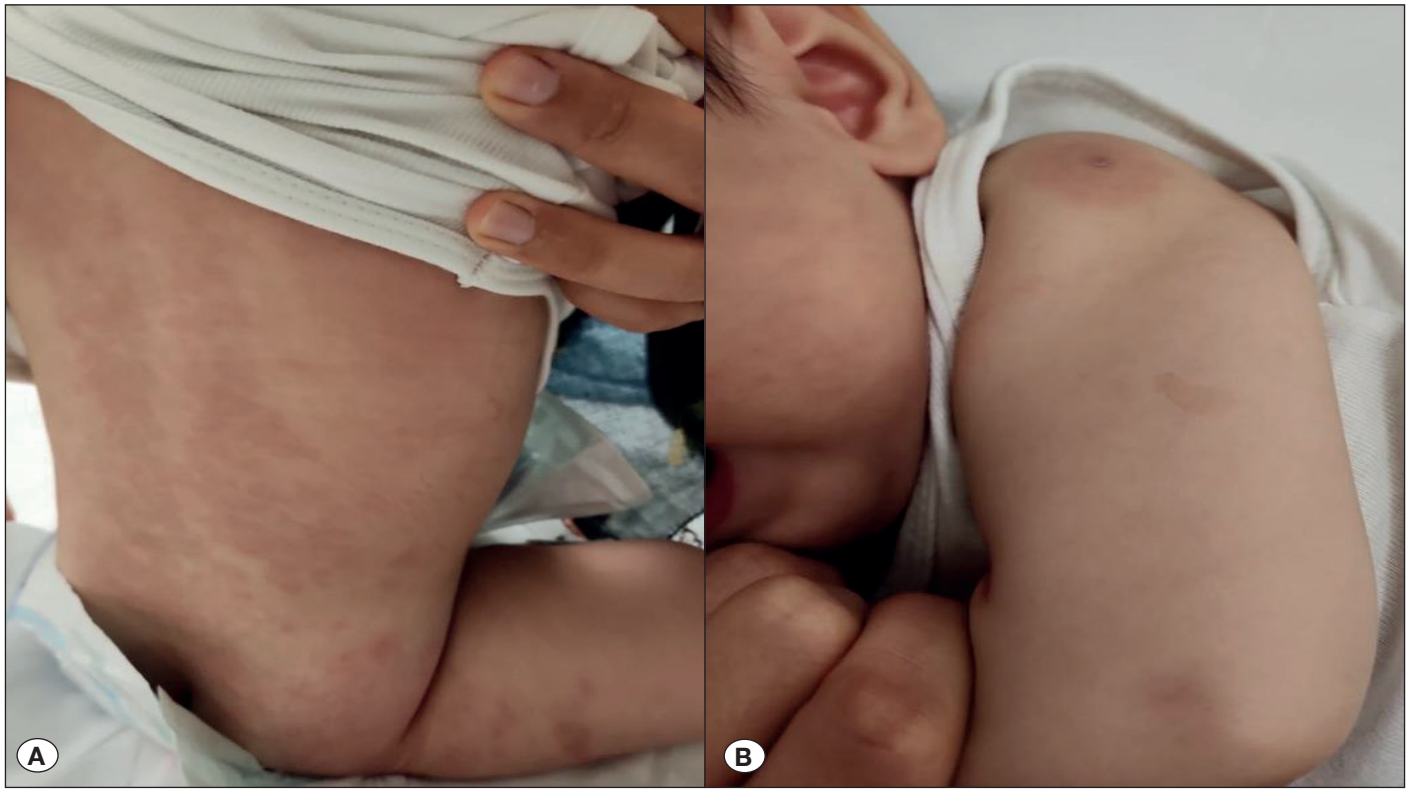

Figure 1: A) There was a maculopapular rash predominantly on the arms and legs and in the lumbar region, **B)** Hyperemia around the BCG vaccination scar

at 40 weeks with a weight of 3800 g and has been completely healthy until now. On physical examination, his temperature was 38.6°C, a 1.5x1 cm cervical lymphadenopathy was palpated in the right cervical region. The patient had bilateral hyperemic conjunctiva, hyperemic lips, non-pitting edema of the hands and feet; the scrotum was edematous. There was a maculopapular rash predominantly on the arms and legs and in the lumbar region and hyperemia around the BCG vaccination scar (Figure 1). Laboratory tests showed hemoglobin 7.9 g/dL (10.2–13.4 g/dL), white blood cell count (WBC) 19.460x10^6/L (6.600-15.600x10^6/L), platelets 574.000x10^6/L (240.000-520.000x10^6/L), C-reactive protein (CRP) 301 mg/L (normal range 0-5). An echocardiogram performed with the diagnosis of Kawasaki's disease showed small pericardial effusion of surrounding the heart. IVIG at a dose of 2 g/kg was infused over 12 hours and acetylsalicylic acid (ASA) at a dose of 50 mg/kg/day and ceftriaxone was started. On day 5 of ASA treatment, the dose was reduced to 5 mg/kg because the patient remained afebrile. Serologic tests for common viral infections associated with maculopapular rash were negative. Ceftriaxone was stopped because there was no growth in blood and urine cultures. On the same day, laboratory tests revealed a potassium level of 5.9 mEq/L (Table I). The patient had not received any intravenous fluids for last days. The patient's nurse was interviewed and asked if there were any difficulties in taking the blood. It was noted that the patient's blood was easily drawn without any external pressure and after the blood sample was taken, it was immediately sent to the laboratory. There was no hyperpotassemic changes were seen on the ECG. A pneumatic tube system was used to transport the sample to laboratory. While this system allows the sample to reach the laboratory faster, it also increases the risk of haemolysis. However, visual inspection of the serum sample that reached the laboratory showed no haemolysis. The venous blood sample collected in a lithium heparin-containing syringe and analyzed on a blood gas analyser immediately after collection was normokalemic and potassium levels were found to be 4.9 mEq/L. On the following day, the serum potassium level was found to be 6.1 mEq/L. Potassium level measured on blood gas analyser was 3.7 mEq/L. The diagnosis of NSAII-associated hyperkalemia was ruled out as the potassium level was normal on blood gas analyser. As hyperkalemia developed simultaneously with thrombocytosis, the patient was considered to have pseudohyperkalemia secondary to thrombocytosis (Table I). The patient's echocardiographic findings were normal at follow-up and the patient discharged at the 11th day of hospitalisation.

DISCUSSION

The most common cause of hyperkalemia in children is mechanical haemolysis during difficult blood draws from small veins. Once the possibility of mechanical haemolysis has been ruled out, the first step in investigating the etiology of

Table I: The laboratory findings of the patient during hospitalisation

Hospitalisation day	1	6	7	9	10	11	12	13
K (mEq/L)	4.2	5.9/5.8	6.1	5.8	5.5	4.5	4.6	4.9
K (mEq/L) (measured by blood gas analyser)		4.9 /4.2	3.7	4.2	-	-	-	-
PLT (x 10^6/L)	574.000	631.000		1.232.000		837.000	752.000	360.000
Hgb (g/dL)	7.9	10.5		10		9.0	9.3	10.9
WBC (x 10^6/L)	19.460	13.490		12.970		6.750	6.090	9.450
Na (mEq/L)	135	133	134	136		137	136	134
Glu (mg/dL)	115		80					90
Urea (mg/dL)	13	17	23	15				17
Krea (mg/dL)	0.09	0.27	0.22	0.27				0.24
CK (U/L)	26							
GFR (mL/min/1.73 m^2)	340	113	139	113				127
UA (mg/dL)	2.1	2.6	2.4	2.5				
ALB (g/L)	31	38	41					
AST (U/L)	24	25	24	22				24
ALT (U/L)	31	15	14	16				14
ALP (U/L)	85		104	121				
LDH (U/L)	404	357	302					
Ca (mg/dL)	9.1	10.6	9.9	10.5				
Mg(mg/dL)	2	2.2		2.3				
P (mg/dL)	3.2	6.2	4.7					
CRP (mg/L)	301	22						
Ph		7.53	7.44	7.43				
HCO3		24	24	24				
pCO_2		29	36	36				
BE		1.7	0.4	0.0				
Lactate		1.98	1.87	1.29				

Glu: *glucose,* ***US:*** *uric acid,* ***ALB:*** *albumin,* ***AST:*** *aspartate aminotransferase,* ***ALT:*** *alanine aminotransferase,* ***ALP:*** *alkaline phosphatase,* ***LDH:*** *lactate dehydrogenase,* ***Ca:*** *calcium,* ***P:*** *phosphorus,* ***Na:*** *sodium,* ***K:*** *potassium,* ***GFR:*** *glomerular filtration rate,* ***Hgb:*** *haemoglobin,* ***WBC:*** *white blood cell count,* ***PLT:*** *platelets,* ***CRP:*** *C-reactive protein,* ***ASA:*** *aspirin,* ***CLO:*** *clopidogrel*

hyperkalemia should be to check for oral or parenteral potassium intake (6). Secondly, it is important to check whether the patient is taking medication that may increase serum potassium levels. The commonly used drugs that may increase potassium levels are potassium-sparing diuretics, angiotensin-converting enzyme inhibitors, cyclosporine, tacrolimus, trimethoprim, mannitol and NSAIDs (3). Hyperkalemia associated with NSAIDs has been reported in elderly patients with diabetes and signs of diabetic nephropathy or type IV renal tubular acidosis and underlying renal insufficiency as well as in young and nondiabetic patients (5).

Since 98% of body potassium is intracellular, a small release of potassium from the inside to the outside of the cell can significantly affect the serum potassium concentration (7). Potassium is the major cation in the intracellular fluid of platelets, leukocytes, and erythrocytes. Therefore, when these cells are lysed during clotting, potassium is released, and the serum potassium concentration rises. The increase in serum potassium levels is not significant when platelet or leukocyte counts are normal but can be significant when platelet or leukocyte counts are markedly elevated (8).

Although it is more prominent in thrombocytosis, serum potassium levels can be higher than plasma potassium levels even when platelet counts are normal (9). This is probably due to the fact that serum contains activated platelets and plasma contains non-activated platelets. The platelet activation, which occurs during clotting, is associated with potassium release from platelets (10). The mean difference between serum and plasma is 0.36 ± 0.18 mmol/L (2). It has been reported that there is a significant positive correlation between serum potassium level and platelet count, and that serum potassium increases by 0.11 mmol/L for every 100x10^9/L increase in platelet count (10). Another study found a significant correlation between serum potassium and platelet count, with serum potassium increasing by 0.27 mEq/L per 100 x 10^9/L platelets ($r = 0.640$; $p<0.001$) (11). Serum potassium levels and platelet counts are not entirely directly proportional. While the potassium level

initially increased linearly with the platelet count, it decreased at higher platelet counts. This decrease has probably been interpreted as a decrease due to the re-entry of potassium into the erythrocytes to maintain homeostasis (7). As there is a positive but poor correlation between platelet count and serum potassium, correction of serum potassium by formulating according to platelet count has not been recommended (12).

In a study conducted in 16 patients with Kawasaki disease, both serum and plasma potassium levels were measured in the same blood sample. Serum potassium levels were higher than plasma potassium levels in all patients. There was a strong positive correlation between platelet count and the difference between serum and plasma potassium levels (8). Our patient had thrombocytosis as expected during Kawasaki disease. Pseudohyperkalemia associated with thrombocytosis was the most likely diagnosis in our patient because the whole blood potassium was normal.

It was reported that when serum, plasma and whole blood potassium levels were analysed in the same patient, serum potassium level was found higher than plasma and plasma was higher than whole blood. For whole blood potassium, blood was drawn with a lithium heparin injector and was analysed in the blood gas analyser. When potassium was measured in plasma, it was thought that the centrifugation performed to separate plasma from cells could lead to cell destruction and consequently an increase in plasma potassium. Therefore, plasma potassium is higher than whole blood (13). In our patient, we analysed whole blood potassium with a blood gas analyser. Plasma potassium is not a test that is done very often in our hospital. However, it is the most appropriate method to accurately determine potassium levels in patients with pseudohyperkalemia.

With this case we would like to draw attention to pseudohyperkalemia. In a patient with inappropriately high serum potassium levels, the possibility of pseudohyperkalemia should be considered. This will avoid unnecessary laboratory evaluations and unnecessary changes in treatment. As plasma potassium measurement is not widely used, it may not be considered by clinicians, as in this case. Pseudohyperkalemia can be practically excluded by plasma potassium measurement.

REFERENCES

1. Lehnhardt A, Kemper MJ. Pathogenesis, diagnosis and management of hyperkalemia. Pediatr Nephrol 2011;26:377-84.
2. Meng QH, Wagar EA. Pseudohyperkalemia: A new twist on an old phenomenon. Crit Rev Clin Lab Sci 2015;52:45-55.
3. Daly K, Farrington E. Hypokalemia and hyperkalemia in infants and children: pathophysiology and treatment. J Pediatr Health Care 2013;27:486-96.
4. Rotenberg FA, Giannini VS. Hyperkalemia associated with ketorolac. Ann Pharmacother 1992;26:778-9.
5. Tan SY, Shapiro R, Franco R, Stockard H, Mulrow PJ. Indomethacin-induced prostaglandin inhibition with hyperkalemia. A reversible cause of hyporeninemic hypoaldosteronism. Ann Intern Med 1979;90:783-5.
6. Janjua H. Management of hyperkalemia in children. Curr Opin Pediatr 2023 Nov 27. doi: 10.1097/MOP.0000000000001321.
7. Asirvatham JR, Moses V, Bjornson L. Errors in potassium measurement: a laboratory perspective for the clinician. N Am J Med Sci 2013;5:255-9.
8. Shimizu T, Yamashiro Y, Yabuta K. Pseudohyperkalaemia in Kawasaki disease. Eur J Pediatr 1992;151:497-8.
9. Sevastos N, Theodossiades G, Archimandritis AJ. Pseudohyperkalemia in serum: a new insight into an old phenomenon. Clin Med Res 2008;6:30-2.
10. Robson WL, Kabani AM, Leung AK, Fick GH, McKenna AI. The relationship of the serum potassium to the platelet count in children. Clin Pediatr (Phila) 1992 ;31:123-5.
11. Graber M, Subramani K, Corish D, Schwab A. Thrombocytosis elevates serum potassium. Am J Kidney Dis 1988;12:116-20.
12. Mäkelä K V Kairisto, O Peltola, T Hollmén, A Virtanen, K Pulkki, V Näntö. Effect of platelet count on serum and plasma potassium: evaluation using database information from two hospitals. Scand J Clin Lab Invest Suppl 1995;222:95-100.
13. Šálek T. Pseudohyperkalemia - Potassium released from cells due to clotting and centrifugation - a case report. Biochem Med (Zagreb) 2018;28:011002.

Mesenchymal Stem Cell Applications in Graft Versus Host Disease

Graft Versus Host Hastalığı'nda Mezenkimal Kök Hücre Uygulamaları

Gamze GÜRSOY[1,2], Dilek GÜRLEK GÖKÇEBAY[3], Namık Yaşar ÖZBEK[3]

[1]Department of Child Health and Disease, Ankara Bilkent City Hospital, Ankara, Türkiye
[2]Graduate School of Health Science, Ankara University, Ankara, Türkiye
[3]Department of Child Health and Disease, Pediatric Hematology and Oncology, Ankara Bilkent City Hospital, Ankara, Türkiye

ABSTRACT

Allogeneic hematopoietic stem cell transplantation stands as a promising cure for a variety of diseases. However, the potential of acute or chronic graft-versus-host disease (GvHD), which leads to significant morbidity and mortality, remains a cause for concern. GvHD occurs due to the complex interactions of immune cells from the graft and the host cells. Despite the existence of prophylactic treatments, GvHD may still occur, and the resistance to conventional therapies necessitates novel approaches and treatments.

Mesenchymal stem cells, which are pluripotent stem cells capable of self-renewal and multilineage differentiation, have gained attention for their low immunogenicity and ability to be sourced from various origins. They have shown promise as therapeutic tools for the cell-based treatment of inflammatory, immune-mediated, and degenerative diseases owing to their remarkable abilities in immunomodulation, immunosuppression, and tissue regeneration. In GvHD, MSCs have demonstrated therapeutic potential through paracrine activity and organelle transfer via nanotubes, microvesicles, or exosomes.

The emergence of MSCs as a treatment for severe steroid-resistant GvHD gained attention in the early 2000s. While initial studies have demonstrated encouraging results in the use of MSCs for the prevention of GvHD, there is still a need for further investigation. Therefore, in this current review, we aim to delve deeper into MSC's features and their clinical applications in the case of GvHD treatment.

Key Words: Mesenchymal stem cell, Hematopoietic stem cell, Immunomodulation, Stem cell transplantation, Graft-versus-host disease

ÖZ

Allojenik hematopoetik kök hücre transplantasyonu, pek çok hastalık için umut verici bir tedavi yöntemidir. Ancak tedavinin bir komplikasyonu olabilen akut veya kronik greft-versus-host hastalığı (GvHH) mortalite ve morbidite riskini önemli ölçüde artırabilmektedir. GvHH, donörden gelen bağışıklık hücreleri ile konak hücreleri arasındaki uygunsuz immün yanıttan kaynaklanmaktadır. Profilaktik tedavilerin varlığına rağmen, GvHH hala görülebilmekte olup konvansiyonel tedavilere direnç, yeni tedavi çalışmalarının gerekliliğini ortaya koymaktadır. Mezenkimal kök hücreler (MKH), kendini yenileyebilme, farklı doku hücrelerine farklılaşma, düşük immunojenite özelliklerine sahip olup çeşitli dokulardan elde edilebilirler. İmmünomodülasyon, immünsüpresyon ve doku rejenerasyonu özelliklerinden dolayı, inflamatuar, immün aracılı, dejeneratif hastalıkların tedavisinde umut vaat etmektedirler. GvHH'de, MKH'ler, parakrin aktivite ve nanotüpler,

0000-0001-8304-6546 : GÜRSOY G
0000-0001-8097-3950 : GÜRLEK GÖKÇEBAY D:
0000-0001-6857-0681 : ÖZBEK NY

Conflict of Interest /Çıkar Çatışması: *On behalf of all authors, the corresponding author states that there is no conflict of interest.*

Contribution of the Authors / Yazarın Katkısı: GÜRSOY G: Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literatu-re review for the study, Taking responsibility in the writing of the whole or important parts of the study. ***GÜRLEK GÖKÇEBAY D:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literatu-re review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***ÖZBEK NY:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / Atıf Yazım Şekli **:** Gürsoy G, Gürlek Gökçebay D and Özbek NY. Mesenchymal Stem Cell Applications in Graft Versus Host Disease. Turkish J Pediatr Dis 2024;18:203-210.

Correspondence Address / Yazışma Adresi:
Gamze GÜRSOY
Department of Child Health and Disease,
Ankara Bilkent City Hospital, Ankara, Türkiye
E-posta: grsoygamze@hotmail.com

Received / Geliş tarihi : 08.01.2024
Accepted / Kabul tarihi : 16.02.2024
Online published : 19.02.2024
Elektronik yayın tarihi
DOI: 10.12956/tchd.1415924

mikroveziküller veya eksozomlar yoluyla terapötik potansiyel gösterebilmektedirler. Steroid tedavisine dirençli GvHH'nin tedavisi için MKH'ler 2000'lerin başında kullanılmaya başlanmış olup, yapılan çalışmalar MKH'lerin etkili bir teröpötik araç olduğunu göstermiştir, ancak daha fazla araştırmaya ihtiyaç duyulmaktadır. Bu derlemede MKH'lerin özelliklerini ve GvHH tedavisindeki klinik uygulamalarını incelemeyi amaçladık.

Anahtar Kelimeler: Mezenkimal Kök Hücre, Graft Versus Host Hastalığı, Kök Hücre Nakli, Hematopoetik Kök Hücre, Immunmodülasyon

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) provides a potential cure for various diseases, including hematological and non-hematological diseases. Despite significant advances in transplantation technologies, including high-resolution HLA typing and the advancement of immunosuppressive medications, poor treatment-related survival remains a problem, with disease relapse, graft rejection, and acute or chronic graft-versus-host disease (GvHD) (1, 2).

Systemic inflammation arises from donor lymphocytes within the transplanted tissue recognizing the recipient as foreign. This triggers an immune response where activated T cells strive to eliminate antigen-carrying cells in the host, leading to severe organ damage. This condition can be classified into acute or chronic forms, traditionally distinguished by the onset time; if it occurs within the first 100 days, it is classified as acute GvHD (1). Nevertheless, acute GvHD can extend beyond the first 100 days, leading to an overlap between the acute and chronic syndromes (3).

Currently, 30–50% of patients undergoing allogeneic stem cell transplantation develop acute GvHD, while 30–70% develop chronic GvHD. Despite the prophylactic use of calcineurin inhibitors and methotrexate, GvHD still occurs, and corticosteroids are the mainstay of treatment (4). The increasing resistance to steroids necessitates the development of new therapeutics (5).

Mesenchymal stem cells (MSCs) are pluripotent stem cells which can be derived from various sources like bone marrow, adipose or placental tissue (5,6). These cells have been shown to have an immunosuppressive effect on GvHD by inhibiting T cell and natural killer cell proliferation, inhibiting Th17 and B cell differentiation, increasing the number of regulatory T cells, interfering with the maturation of antigen-presenting cells, and increasing the secretion of immunomodulatory molecules , including prostaglandin E2, transforming growth factor-β (TGF-β), Interleukin-10, and heme oxygenase (6-8). They can reduce the incidence of acute GvHD and decrease the severity of both acute and chronic GvHD after HSCT (9, 10).

Within the past twent years, there has been significant interest in exploring the potential therapeutic applications of MSCs in various clinical scenarios, including their role in supporting hematopoietic stem cell transplantation (HSCT) and their use in treating GvHD (11-13). This review focuses on using MSCs in GvHD.

What is GvHD?

The development of GvHD is influenced by the intricate interactions of immune cells from the graft and pro-inflammatory cytokines, which are affected by differences in tissue histocompatibility between donor and recipient. B and T lymphocytes have vital functions in identifying and eliminating host cells. Inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and platelet-derived growth factor (PDGF) are released due to tissue injury by the pre-transplantation conditioning regimen. These cytokines activate donor alloreactive T cells along with antigen-presenting cells, causing host tissue damage through the expansion of donor helper T cells (including Th17), cytotoxic T lymphocytes, and natural killer cells. Inflammatory immune responses against these cells lead to significant tissue harm, affecting various organs and body areas such as the skin, oral cavity, eyes, digestive system, liver, lungs, joints, and reproductive tract and promoting GvHD (14-17).

Patients receive high doses of immunosuppressive drugs during allogeneic HSCT to enable the transplantation and prevent GvHD. Dysregulation of lymphocyte reconstitution can compromise self-tolerance, leading to an increase in self-reactive B and T cells. This situation may result in the overproduction of autoantibodies by self-reactive B cells, causing immune complexes to accumulate in healthy tissues and blood. Activation of inflammatory cells can also lead to collagen production and fibrosis while promoting chronic GvHD (15).

Treatment options for GvHD include the use of broad-spectrum immunosuppressives, but these treatments may be ineffective and could potentially increase the risk of cancer recurrence (14, 15, 17). The standard treatment depends on the affected organ or site and can be either local or systemic, with more adverse effects associated with systemic treatments. Steroids such as prednisone (2 mg/kg per day) are recommended by NIH guidelines for their lymphopenic and anti-inflammatory properties when used alone or combined with calcineurin inhibitors (18).

What are MSCs and their abilities?

Mesenchymal stem cells, alternatively known as multipotent stromal cells or mesenchymal stromal cells (MSCs), have been the focus of extensive scientific research since their initial identification in the late 1960s (19). Mesenchymal stem cells are postnatal stem cells that can self-renew and maintain a versatile capacity to differentiate into multiple lineages, such as

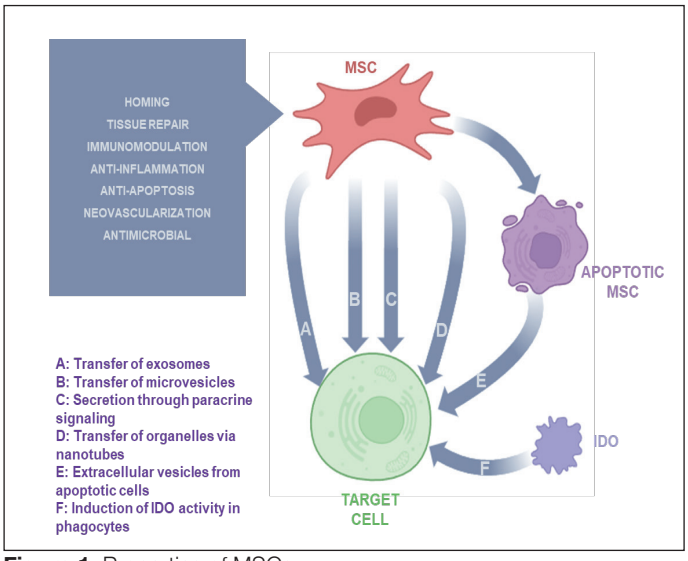

Figure 1: Properties of MSCs

osteoblasts, adipocytes, and chondroblasts (20). Mesenchymal stem cells typically display low immunogenicity. They exhibit only moderate levels of major histocompatibility complex I (MHC) expression and show little to no expression of MHC II antigens and co-stimulatory molecules (21).

In addition, they have several unique characteristics, which will be explained in this review and given in Figure 1.

Homing Capacity:

Homing is the process of MSCs selectively migrating towards the sites of injury, facilitated by specific receptors or ligands expressed by damaged tissues, enabling MSC's to adhere and infiltrate. This process involves three major steps: First chemoattraction to sites of inflammation, achieved by chemotaxis, through the accumulation of chemokines and cytokines such as epidermal growth factor (EGF), insulin-like growth factor (IGF), PDGF, vascular endothelial growth factor (VEGF), TNF-α, interleukin (IL)-1, IL-6 and IL-8; than adhesion to injured cells with molecules such as selectins and integrins; finally infiltration into sites of inflammation aided by enzymes such as matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) (22).

Tissue Regeneration:

Several functional properties of MSCs make them suitable for tissue regeneration and repair. These properties include differentiating into various cell types, migrating to injured tissues, promoting angiogenesis, exhibiting anti-apoptotic activity, and releasing bioactive soluble factors. The secretion of paracrine factors by MSCs alters the tissue microenvironment, significantly influencing proliferation, antioxidant activity, and differentiation. Paracrine signaling recruits macrophages and endothelial cells and likely stimulates resident stem cells to assist in the tissue repair process. Another vital function of MSCs is activating local stem cells through the secretion of growth factors. The trophic factors secreted by MSCs have been linked to the stimulation and expansion of internal stem cell populations, emphasizing the intricate paracrine and cell-to-cell communication essential for tissue recovery and restoration (22,23).

Regulation of the immune system:

Mesenchymal stem cells create an immunosuppressive and immunoregulatory environment through the secretion of cytokines, chemokines, growth factors, and extracellular vesicles (24, 25)

Several factors, such as IL10, transforming growth factor beta (TGF-β), prostaglandin E2, indoleamine 2,3-dioxygenase (IDO), nitric oxide, and FAS/ FAS ligand, play a role in the immunomodulatory features of MSCs by inhibiting the proliferation and function of various immune cells. Mesenchymal stem cells can impact B and T lymphocytes, dendritic cells, natural killer (NK) cells, monocytes, neutrophils, and macrophages. They may suppress B-cell proliferation while enhancing IgG secretion; inhibit chemotaxis; upregulate antibody secretion; decrease pro-inflammatory cytokine secretion by Th1 cells; increase IL-4 secretion by Th2 cells; inhibit T cell proliferation; decrease cytotoxic effects of cytotoxic T cells; suppress dendritic cell differentiation, antigen presentation to T cells and NK cell activation. Additionally, they may decrease local infiltration and activation of neutrophils while upregulating genes responsible for phagocytosis in macrophages to improve bacterial clearance (23, 26).

Anti-Inflammatory effect:

Utilizing their immunomodulatory abilities, MSCs have been shown to have a systemic anti-inflammatory effect, by reducing the levels of pro-inflammatory cytokines and procalcitonin (27).

The immunomodulatory characteristics of MSCs efficiently regulate adaptive and innate immune responses. Focusing specifically on T cells, MSCs can suppress the proliferation of activated CD4+ and CD8+ T cells and hinder the differentiation of CD8+ cytotoxic T cells by preventing T cell proliferation and arresting them in the G0/G1 phases of the cell cycle and potentially inducing T cell apoptosis (28,29). Substantial evidence supports that MSCs prompt the polarization of T cells towards a regulatory phenotype, potentially contributing to the suppression of inflammation (30).

Mesenchymal stem cells have been associated with the inhibition of B lymphocytes. In vitro studies consistently demonstrate that human MSCs suppress B-cell proliferation in the presence of anti-immunoglobulin antibodies, soluble CD40 Ligand, and various cytokines like IFN-γ (31-33).

In addition, MSCs exhibit constitutive secretion of IDO, and when activated by inflammatory cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), IDO secretion is upregulated (33,34). IDO, in turn, plays a crucial role in suppressing the proliferation of allogeneic T cells (35).

The apoptosis of MSCs in vivo also contributes to their immunomodulatory effects. The production and release of apoptotic extracellular vesicles potentially mediate this. During apoptosis, MSCs also induce the production of IDO in recipient phagocytes, adding another layer to their multifaceted immunoregulatory capabilities (19, 36, 37).

Suppression of Apoptosis:

The anti-apoptotic activity of MSCs has been well-documented, with evidence suggesting that they can protect injured cells and maintain organ function by inhibiting programmed cell death. This mechanism involves the up-regulation of DNA repair, down-regulation of mitochondrial death pathways, and alteration of anti- and pro-apoptotic protein expression. Additionally, the secretion of specific mediators by MSCs, such as stromal cell-derived factor 1 (SDF-1), insulin-like growth factor 1 (IGF-1), nuclear factor erythroid 2-related factor 2 (Nrf2), hypoxia-inducible factor (HIF), heme oxygenase contributes to the downregulation of pro-apoptotic proteins (22,23,38).

Neo-angiogenesis:

Furthermore, MSCs have also demonstrated the ability to stimulate the neovascularization in tissue through the expression of angiogenic cytokines including, vascular endothelial growth factors, fibroblast growth factors, and angiopoietin-1. By secreting these soluble factors, MSCs can improve tissue vascularity by stimulating endothelial cell growth and inducing neo-angiogenesis (22, 23).

Antimicrobial Effect:

Mesenchymal stem cells have been found to have antimicrobial effects due to their ability to secrete antimicrobial peptides such as human antimicrobial peptide LL-37 and Lipocalin-2 in response to pathogenic stimulation. These antimicrobial factors contribute to the disruption of bacterial membranes and aid in bacterial clearance, further displaying the diverse therapeutic potential of MSCs (22, 23).

MSC treatment in GvHD

Clinical trials of MSCs for various diseases and phases have been documented on the National Institutes of Health website, including cardiovascular diseases, neurodegenerative diseases, bone and cartilage diseases, cancers, autoimmune diseases such as GvHD (22).

MSCs' self-renewal and differentiation capacity, along with their ability to prevent T cell proliferation in response to antigenic stimuli and their anti-proliferation effects on B cells, natural killer, and dendritic cells, render them well suited for immunosuppression. Mesenchymal stem cells are characterized by the absence of human leukocyte antigen (HLA) class II expression, enabling their allogeneic administration without the need for donor-recipient matching.

The therapeutic effects of MSCs are multifaceted and include paracrine activity through the secretion of proteins, peptides, and hormones. Additionally, MSCs demonstrate capabilities such as the transfer of mitochondria through tunneling nanotubes or microvesicles, as well as the transfer of exosomes or microvesicles containing RNA and various bioactive molecules. These diverse mechanisms contribute to the potential therapeutic impact of MSCs in various clinical applications, including the treatment of conditions such as GvHD (19, 39).

The effectiveness of mesenchymal stem cell treatment relies on various factors, including the origin of the cells, their expansion methods, and their capacity to migrate to the tissues affected by GvHD. While MSCs from a particular donor may work for one recipient, they may not be effective for another. Therefore, recipient factors are crucial in determining how mesenchymal stem cells function (40-42).

Since 2008, there has been a substantial increase in attention towards MSCs for the management of GvHD following promising findings (41). However, the actual clinical effectiveness of these cells is questionable (43).

Clinical Studies

In treating severe steroid-resistant acute (aGvHD) and chronic graft-versus-host disease (cGvHD), mesenchymal stem cells have gained substantial attention in research and clinical practice. Here, we share a selection of contemporary studies and their corresponding outcomes, wherein the pediatric age cohort is included.

In 2008, Le Blanc et al. (41) published their phase II clinical trial with 55 patients, including children with severe aGvHD. It was shown that most of the patients had a complete response or improvement, and the effectiveness of the MSC treatment was not linked to donor HLA-match. No adverse effect was reported during or right after the infusions. Patients with a full response showed lower transplantation-related mortality and improved overall survival compared to those with partial or no response (41).

Another study that defined steroid-resistant grade IV acute GvHD in 42 cases, including pediatric cases, observed better overall survival in patients with GvHD grade less than 4, in those who initially had MSC treatment, and in pediatric patients. No immediate or delayed toxicity or side effects were documented (44).

Ball et al. (45) conducted a study with 24 patients with aGvHD, and a complete response to MSC infusion was observed in 65% of them. Transplantation-related mortality had a cumulative incidence of 17% in patients who achieved a complete response and 69% in those who did not. Overall survival after a median follow-up of 2.9 years was 37%, with early initiation of multiple MSC infusions showing better outcomes (45).

In a research in 2014, Kurtzberg et al. (46) demonstrated that an external administration of MSCs significantly increased survival rates at day +100 following in pediatric patients with

treatment-resistant acute GvHD. This outcome was associated with patient response by day +28, and treatment was generally well received without any indication of the development of ectopic tissue (46).

Introna et al. (47) conducted a phase I multicenter study involving 40 patients with steroid-resistant grade II to IV GvHD, supplied a median cell dose of 1.5×10/kg per infusion, with an overall response rate of 67.5% at 28 days after the last MSC injection. Overall survival at 1 and 2 years was reported as 50.0% and 38.6%, respectively (47).

In 2015, Zhao et al. (48) published a study of 47 patients suffering from resistant acute GvHD and found that the group treated with MSCs had an overall response rate of 75%, while the non-MSC group had a response rate of 42.1%. The incidence and severity of chronic GvHD were reduced in the MSC group. Additionally, MSC treatment enhanced thymus function, stimulated regulatory T cells, and did not increase the risk of infections or tumor recurrence (48).

In 2016, Kuçi and colleagues reported that MSC end-products showed an overall response of 77% in GvHD patients and an overall survival rate of 71±11% at two-year follow-up in severe acute GvHD patients (49). In the same year, in another study with 25 participants (grade III, 22 patients; grade IV, 3 patients), four weeks after the initial MSC infusions, a complete response was observed in 24% of the patients, while partial response was seen in 36%. By week 24, 48% of the participants achieved a durable, complete response. At the end of week 52, patients who had shown an overall response (complete and partial response) exhibited significantly better survival rates. No adverse effects associated with MSC infusions were reported (50).

In 2017, Dotoli et al. (51) published their clinical trial, in which 46 children and adults with steroid-refractory aGvHD III/IV received MSC infusions as a salvage therapy, showing clinical improvement in half of the cases. The estimated survival rate at two years was 17.4%, and only 4.3% of patients experienced temporary side effects during the MSC infusion, suggesting this treatment's safety and potential applicability (51).

Mazic et al. (52) in 2018 reported that three patients who underwent allogeneic hematopoietic cell transplantation and later developed steroid-refractory GvHD were treated with MSC infusions, resulting in complete remission of a GvHD in two patients and partial remission in one, confirming the feasibility of using MSCs to treat severe steroid-refractory acute GvHD in clinical practice in 2018 (52).

In a retrospective study published in 2021, 25 patients who received MSCs for acute GvHD were monitored for a median of 9.3 years. Partial response to GvHD was observed in 76.0% of the cases, while complete remission was 24.0%. Patients in complete remission had no transplant-related mortality. The use of MSCs led to an average improvement of one stage in GvHD. Long-term adverse effects like secondary malignancy were not detected (53).

Another phase 3 multicenter study was conducted in 2022, involving 203 participants aged 14 to 65 with steroid-refractory acute GvHD. The inclusion of MSCs in second-line treatment improved effectiveness, decreased drug toxicity, and reduced the occurrence of chronic GvHD without increasing relapse rates (54).

In the same year, research utilizing clinical-grade MSCs administered intravenously found an overall response rate of 58.7% for acute GvHD and 65.50% for chronic GvHD. The treatment was considered effective and safe, with four adverse events reported, all resolved without complication (55).

A meta-analysis conducted by Chen et al., including 13 studies with a total of 301 patients, revealed that the application of MSCs in treating steroid-resistant aGvHD led to an overall response rate of 68.1%. Among the patients, 136 showed complete remission, while 69 had partial remission or mixed response, making 205 patients with an overall response. Patients suffering from skin aGvHD exhibited more favorable clinical responses compared to those dealing with gastrointestinal or liver aGvHD conditions. Furthermore, recipients with Grade II steroid-refractory aGvHD showed better response to MSC therapy in comparison to Grade III/IV GvHD. Moreover, a trend showed that children had superior clinical responses than adults. Another meta-analysis involving 13 non-randomized studies at moderate risk of bias and comprising a total of 336 patients reported a survival rate of 63% (outcome of 119 patients from 6 studies) at six months following MSC treatment (56-58).

The first clinical use of MSCs for the treatment of refractory aGvHD in Türkiye was reported in 2016, with 33 pediatric patients, resulting in a complete response in 18 patients, a partial response in 7, and no response in 8. The estimated probability of overall survival at the two-year was significantly different between patients with a complete response and those with a partial or no response. Additionally, the incidence of transplant-related mortality at day 100 after the first MSC infusion was higher in patients with partial/none response compared to those with complete response (59).

Finally, in a multicenter study from Türkiye in 2023, seventy-six patients with grade III-IV acute GvHD were received weekly adipose or umbilical cord-derived MSC infusions in addition to standard treatment. The study concluded that MSC treatment was safe, with no adverse effects observed during over 190 infusions in 76 patients. Notably, the late aGvHD group showed significantly higher response rates (complete response: 23.3%, partial response: 36.7%) compared to the aGvHD group. Additionally, at the 2-year follow-up, patients with late aGvHD had a lower cumulative non-relapse mortality (40%) and a higher probability of survival (59%) compared to those with acute GvHD (71% and 28%, respectively) (60).

However, some studies suggest that MSC treatment may break the graft versus host reaction cycle rather than induce immune tolerance in aGvHD due to poor long-term survival (61, 62).

Furthermore, one of the main challenges in the clinical application of MSCs is their potential risks, including immunosuppressive effects, tumorigenic potential, immune responses, pathogen transmission, adipogenic differentiation, prothrombotic events, and various acute, intermediate, and long-term problems (22). Despite the widespread use of MSCs for immunomodulation and regenerative cell therapy, current evidence from clinical trials is inconclusive, adding complexity to their practical implementation (63).

These issues encompass immune-mediated reactions, embolic phenomena, graft-versus-host disease, secondary infections, and the risk of malignancy. As a result, the cautious use of MSCs is emphasized, taking into account factors such as the type or class of stem cells used, the level of manipulation, the culture history, the handling/storage conditions, and the components of the growth medium in clinical applications (22, 64, 65).

More high-quality, large-sample clinical trials are needed to verify the association between the clinical use of MSCs and tumor recurrence and infection. The current limitations of the literature and patient samples, which are small, underscore the importance of further research to establish a more pronounced understanding of the relationship between MSC therapy and clinical outcomes.

CONCLUSION

In conclusion, mesenchymal stem cells can be highly beneficial in hematopoietic stem cell transplantation for the prevention of graft-versus-host disease. Additionally, MSCs show promise in the treatment of acute and chronic GvHD. The clinical impact of MSC infusion for the treatment of GvHD needs to be further determined with high-quality clinical trials involving large numbers of patients.

REFERENCES

1. Garnett C, Apperley JF, Pavlů J. Treatment and management of graft-versus-host disease: improving response and survival. Ther Adv Hematol 2013;4:366-78.
2. Phelan R, Chen M, Bupp C, Bolon Y-T, Broglie L, Brunner-Grady J, et al. Updated trends in hematopoietic cell transplantation in the United States with an additional focus on adolescent and young adult transplantation activity and outcomes. Transplant Cell Ther 2022;28:409. e1-. e10.
3. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11:945-56.
4. Zeiser R, Blazar BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. N Engl J Med 2017;377:2167-79.
5. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells-current trends and future prospective. Biosci Rep 2015;35:e00191.
6. Shafeghat Z, Dorfaki M, Dehrouyeh S, Arab FL, Roozbehani M, Falak R, et al. Mesenchymal stem cell-derived exosomes for managing graft-versus-host disease: An updated view. Transpl Immunol 2023:101957.
7. Prasad VK, Lucas KG, Kleiner GI, Talano JAM, Jacobsohn D, Broadwater G, et al. Efficacy and safety of ex vivo cultured adult human mesenchymal stem cells (Prochymal™) in pediatric patients with severe refractory acute graft-versus-host disease in a compassionate use study. Biol Blood Marrow Transplant 2011;17:534-41.
8. Zheng Q, Zhang S, Guo W-Z, Li X-K. The unique immunomodulatory properties of MSC-derived exosomes in organ transplantation. Front Immunol 2021;12:659621.
9. Lai P, Chen X, Guo L, Wang Y, Liu X, Liu Y, et al. A potent immunomodulatory role of exosomes derived from mesenchymal stromal cells in preventing cGVHD. J Hematol Oncol 2018;11:1-15.
10. Li K-L, Li J-Y, Xie G-L, Ma X-Y. Exosomes released from human bone marrow–derived mesenchymal stem cell attenuate acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation in mice. Front Cell Dev Biol 2021;9:617589.
11. Crippa S, Santi L, Berti M, De Ponti G, Bernardo ME. Role of ex vivo expanded mesenchymal stromal cells in determining hematopoietic stem cell transplantation outcome. Front Cell Dev Biol 2021;9:663316.
12. Galderisi U, Peluso G, Di Bernardo G. Clinical trials based on mesenchymal stromal cells are exponentially increasing: where are we in recent years? Stem Cell Rev Rep 2022;18:23-36.
13. Introna M, Golay J. Tolerance to bone marrow transplantation: do mesenchymal stromal cells still have a future for acute or chronic GvHD? Front Immunol 2020;11:609063.
14. Kostareva I, Kirgizov K, Machneva E, Ustyuzhanina N, Nifantiev N, Skvortsova Y, et al. Novel and Promising Strategies for Therapy of Post-Transplant Chronic GVHD. Pharmaceuticals 2022;15:1100.
15. Li Z, Rubinstein SM, Thota R, Savani M, Brissot E, Shaw BE, et al. Immune-mediated complications after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2016;22:1368-75.
16. Saidu NEB, Bonini C, Dickinson A, Grce M, Inngjerdingen M, Koehl U, et al. New approaches for the treatment of chronic graft-versus-host disease: current status and future directions. Front Immunol 2020;11:578314.
17. Zeiser R, Marks R, Bertz H, Finke J. Immunopathogenesis of acute graft-versus-host disease: implications for novel preventive and therapeutic strategies. Ann Hematol 2004;83:551-65.
18. DeFilipp Z, Couriel DR, Lazaryan A, Bhatt VR, Buxbaum NP, Alousi AM, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: III. The 2020 treatment of chronic GVHD report. Transplant Cell Ther 2021;27:729-37.
19. Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Res Ther 2016;7:125.
20. Wei X, Yang X, Han Z-p, Qu F-f, Shao L, Shi Y-f. Mesenchymal stem cells: a new trend for cell therapy. Acta Pharmacol Sin 2013;34:747-54.

21. Chen C. From mesenchymal stem cell therapy to discovery of drug therapy for systemic sclerosis. University of Southern California, ProQuest Dissertations Publishing 2014; 3628136.
22. Saeedi P, Halabian R, Fooladi AAI. A revealing review of mesenchymal stem cells therapy, clinical perspectives and Modification strategies. Stem Cell Investig 2019;6:34.
23. Wannemuehler TJ, Manukyan MC, Brewster BD, Rouch J, Poynter JA, Wang Y, et al. Advances in mesenchymal stem cell research in sepsis. J Surg Res 2012;173:113-26.
24. Galipeau J, Sensébé L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell stem cell 2018;22:824-33.
25. Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. Cells 2019;8:1605.
26. Hirvonen T. Glycan binding proteins in therapeutic mesenchymal stem cell research. Helsinki, University of Helsinki, Finland 2014.
27. Kusadasi N, Groeneveld AJ. A perspective on mesenchymal stromal cell transplantation in the treatment of sepsis. Shock. 2013;40:352-7.
28. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002;99:3838-43.
29. Rasmusson I, Ringdén O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation 2003;76:1208-13.
30. Burnham AJ, Daley-Bauer LP, Horwitz EM. Mesenchymal stromal cells in hematopoietic cell transplantation. Blood Adv 2020;4:5877-87.
31. Cooke KR, Luznik L, Sarantopoulos S, Hakim FT, Jagasia M, Fowler DH, et al. The biology of chronic graft-versus-host disease: a task force report from the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. Biol Blood Marrow Transplant 2017;23:211-34.
32. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. Blood 2006;107:367-72.
33. Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. Front Immunol 2019;10:1191.
34. Martin I, Galipeau J, Kessler C, Le Blanc K, Dazzi F. Challenges for mesenchymal stromal cell therapies. Sci Transl Med 2019;11:eaat2189.
35. Terness P, Bauer TM, Röse L, Dufter C, Watzlik A, Simon H, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2, 3-dioxygenase–expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med 2002;196:447-57.
36. Fu Y, Sui B, Xiang L, Yan X, Wu D, Shi S, et al. Emerging understanding of apoptosis in mediating mesenchymal stem cell therapy. Cell Death Dis 2021;12:596.
37. Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med 2017;9:eaam7828.
38. Kiani AA, Kazemi A, Halabian R, Mohammadipour M, Jahanian-Najafabadi A, Roudkenar MH. HIF-1α confers resistance to induced stress in bone marrow-derived mesenchymal stem cells. Arch Med Res 2013;44:185-93.
39. Sharma RR, Pollock K, Hubel A, McKenna D. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. Transfusion 2014;54:1418-37.
40. Cornelissen AS, Maijenburg MW, Nolte MA, Voermans C. Organ-specific migration of mesenchymal stromal cells: Who, when, where and why? Immunol Lett 2015;168:159-69.
41. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371:1579-86.
42. Ullah M, Liu DD, Thakor AS. Mesenchymal stromal cell homing: mechanisms and strategies for improvement. Iscience 2019;15:421-38.
43. Rizk M, Monaghan M, Shorr R, Kekre N, Bredeson CN, Allan DS. Heterogeneity in studies of mesenchymal stromal cells to treat or prevent graft-versus-host disease: a scoping review of the evidence. Biol Blood Marrow Transplant 2016;22:1416-23.
44. Resnick IB, Barkats C, Shapira MY, Stepensky P, Bloom AI, Shimoni A, et al. Treatment of severe steroid resistant acute GVHD with mesenchymal stromal cells (MSC). Am J Blood Res 2013;3:225-38.
45. Ball LM, Bernardo ME, Roelofs H, van Tol MJ, Contoli B, Zwaginga JJ, et al. Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III–IV acute graft-versus-host disease. Br J Haematol 2013;163:501-9.
46. Kurtzberg J, Prockop S, Teira P, Bittencourt H, Lewis V, Chan KW, et al. Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. Biol Blood Marrow Transplant 2014;20:229-35.
47. Introna M, Lucchini G, Dander E, Galimberti S, Rovelli A, Balduzzi A, et al. Treatment of graft versus host disease with mesenchymal stromal cells: a phase I study on 40 adult and pediatric patients. Biol Blood Marrow Transplant 2014;20:375-81.
48. Zhao K, Lou R, Huang F, Peng Y, Jiang Z, Huang K, et al. Immunomodulation effects of mesenchymal stromal cells on acute graft-versus-host disease after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2015;21:97-104.
49. Kuçi Z, Bönig H, Kreyenberg H, Bunos M, Jauch A, Janssen JW, et al. Mesenchymal stromal cells from pooled mononuclear cells of multiple bone marrow donors as rescue therapy in pediatric severe steroid-refractory graft-versus-host disease: a multicenter survey. Haematologica 2016;101:985-94.
50. Muroi K, Miyamura K, Okada M, Yamashita T, Murata M, Ishikawa T, et al. Bone marrow-derived mesenchymal stem cells (JR-031) for steroid-refractory grade III or IV acute graft-versus-host disease: a phase II/III study. Int J Hematol 2016;103:243-50.
51. Dotoli G, De Santis G, Orellana M, de Lima Prata K, Caruso S, Fernandes T, et al. Mesenchymal stromal cell infusion to treat steroid-refractory acute GvHD III/IV after hematopoietic stem cell transplantation. Bone Marrow Transplant 2017;52:859-62
52. Česen Mazič M, Girandon L, Kneževič M, Avčin SL, Jazbec J. Treatment of severe steroid-refractory acute-graft-vs.-host disease with mesenchymal stem cells–single center experience. Front B ioeng Biotechnol 2018;6:93.
53. Döring M, Cabanillas Stanchi KM, Lenglinger K, Treuner C, Gieseke F, Erbacher A, et al. Long-term follow-up after the application of mesenchymal stromal cells in children and adolescents with

steroid-refractory graft-versus-host disease. Stem Cells Dev 2021;30:234-46.

54. Zhao K, Lin R, Fan Z, Chen X, Wang Y, Huang F, et al. Mesenchymal stromal cells plus basiliximab, calcineurin inhibitor as treatment of steroid-resistant acute graft-versus-host disease: a multicenter, randomized, phase 3, open-label trial. J Hematol Oncol 2022;15:22.
55. Macías-Sánchez MdM, Morata-Tarifa C, Cuende N, Cardesa-Gil A, Cuesta-Casas MÁ, Pascual-Cascon MJ, et al. Mesenchymal stromal cells for treating steroid-resistant acute and chronic graft versus host disease: A multicenter compassionate use experience. Stem Cells Transl Med 2022;11:343-55.
56. Chen X, Wang C, Yin J, Xu J, Wei J, Zhang Y. Efficacy of mesenchymal stem cell therapy for steroid-refractory acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation: a systematic review and meta-analysis. PLoS One 2015;10:e0136991.
57. Hashmi S, Ahmed M, Murad MH, Litzow MR, Adams RH, Ball LM, et al. Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. Lancet Haematol 2016;3:e45-e52.
58. Lin T, Yang Y, Chen X. A review of the application of mesenchymal stem cells in the field of hematopoietic stem cell transplantation. Eur J Med Res 2023;28:268.
59. Erbey F, Atay D, Akcay A, Ovali E, Ozturk G. Mesenchymal stem cell treatment for steroid refractory graft-versus-host disease in children: a pilot and first study from Türkiye. Stem Cells Int 2016;2016:1641402.
60. Keklik M, Deveci B, Celik S, Deniz K, Gonen ZB, Zararsiz G, et al. Safety and efficacy of mesenchymal stromal cell therapy for multi-drug-resistant acute and late-acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation. Ann Hematol 2023;102:1537-47.
61. Salmenniemi U, Itälä-Remes M, Nystedt J, Putkonen M, Niittyvuopio R, Vettenranta K, et al. Good responses but high TRM in adult patients after MSC therapy for GvHD. Bone Marrow Transplant 2017;52:606-8.
62. Zhou X, Jin N, Wang F, Chen B. Mesenchymal stem cells: a promising way in therapies of graft-versus-host disease. Cancer Cell Int 2020;20:114.
63. Stoma I, Karpov I, Krivenko S, Iskrov I, Milanovich N, Koritko A, et al. Mesenchymal stem cells transplantation in hematological patients with acute graft-versus-host disease: characteristics and risk factors for infectious complications. Ann Hematol 2018;97:885-91.
64. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. J Transl Med 2011;9:1-14.
65. Volarevic V, Nurkovic J, Arsenijevic N, Stojkovic M. Concise review: therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. Stem Cells 2014;32:2818-23.