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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.

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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: up to 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 the text as superscripts 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)

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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 çok 40.

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 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*

Comparison of Toxoplasma gondii IgG Antibody Levels in Children and Adolescents with Obsessive-Compulsive Disorder and Attention-Deficit Hyperactivity Disorder with Healthy Controls: A Cross-Sectional Study

Obsesif Kompulsif Bozukluk ve Dikkat Eksikliği Hiperaktivite Bozukluğu Olan Çocuk ve Ergenlerde Toxoplasma Gondii IgG Antikor Düzeylerinin Sağlıklı Kontrollerle Karşılaştırılması: Kesitsel Bir Çalışma

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ABSTRACT

Objective: Obsessive-compulsive disorder (OCD) and Attention-deficit hyperactivity disorder (ADHD) are frequently seen disorders during childhood. One of the etiological factors for both disorders is infectious diseases and T. gondii is one of them. This study was aimed to examine if there is a relation between IgG levels of T. gondii and OCD or ADHD symptoms.

Material and Methods: Of 42 children with OCD, 31 with ADHD and 28 healthy control were included. Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS), Moudsley Obession-Compulsion Inventory (MOCI), Child Depression Inventory (CDI), and Screen of Children for anxiety related disorders (SCARED), The Turgay DSM-IV-Based Child and Adolescent Behavioral Disorders Screening and Rating Scale (T-DSM-IV-S) and The Conners' Parent Rating Scale-48 (CPRS-48) were applied. Toxo gondii IgG values ≥3.0 IU/mL were considered to be reactive. SPSS 17.0 was used for analysis. $p<0.050$ was accepted as significant.

Results: The mean age was 12.13 years. Of 56.40% (n=57) were boys. Depression and anxiety symptoms were similar in OCD and controls, but were significantly lower in ADHD. All children with OCD had negative (100%) for IgG levels of T. gondii, whereas 78.60% of controls and 90.30% of children with ADHD had negative for IgG levels of T. gondii. Toxo IgG seropositivity of the control was significantly higher than that of the OCD.

Toxo IgG levels were positively correlated with Turgay's ADHD-Conduct disorder subscale scores in ADHD group ($r=0.650$, $p<0.001$). In the OCD and the control group, there was no correlation between IgG levels and scale scores (for all variables, $p>0.050$).

Conclusion: This study did not verify a relationship between the seropositivity of T. gondii with OCD and ADHD. Further studies are needed with longitudinal follow-up and extended series of patients.

Key Words: ADHD, Children, Toxoplasma gondii, OCD

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ÖZ

Amaç: Obsesif kompulsif bozukluk (OKB) ve Dikkat eksikliği hiperaktivite bozukluğu (DEHB) çocukluk çağında sık görülen bozukluklardır. Her iki bozukluğun da etiyolojik faktörlerinden biri bulaşıcı hastalıklardır ve T. gondii de bunlardan biridir. Bu çalışma, T. gondii'nin IgG düzeyleri ile OKB veya DEHB belirtileri arasında bir ilişki olup olmadığını incelemek amacıyla yapılmıştır.

Gereç ve Yöntemler: Çalışmaya OKB'si olan 42 çocuk, DEHB'si olan 31 çocuk ile 28 sağlıklı kontrol dahil edildi. Çocuklar için Yale-Brown Obsesif-Kompulsif Ölçeği (YB-OKB), Moudsley Obsesyon-Kompulsiyon Envanteri (MOCI), Çocuk Depresyon Ölçeği (CDI) ve Çocuklarda Anksiyete İlişkili Bozuklukları Tarama Ölçeği (ÇATÖ), Turgay DSM-IV Tabanlı Çocuk Ergen Davranış Bozuklukları Tarama ve Derecelendirme Ölçeği (T-DSM-IV-S) ve Conners Ebeveyn Değerlendirme Ölçeği-48 (CPRS-48) uygulandı. Toxo gondii IgG değerleri ≥3.0 IU/mL reaktif kabul edildi. Analiz için SPSS 17.0 kullanıldı. $p<0.050$ anlamlı kabul edildi.

Bulgular: Ortalama yaş 12,13 yıl ve olguların %56.40'ı (n = 57) erkekti. Depresyon ve anksiyete belirtileri OKB ve kontrollerde benzerdi, ancak DEHB'de anlamlı derecede düşüktü. OKB'si olan tüm çocukların T. gondii'nin IgG seviyeleri negatifken (%100), kontrollerin %78.60'ı ve DEHB'si olan çocukların %90.30'u T. gondii'nin IgG seviyeleri için negatifti. Kontrolün Toxo IgG seropozitifliği, OKB'ninkinden anlamlı derecede yüksekti. DEHB grubunda Toxo IgG düzeyleri ile Turgay DEHB-Davranış Bozukluğu alt ölçek puanları arasında pozitif korelasyon bulundu ($r=0.650$, $p<0.001$). OKB ve kontrol gruplarında IgG düzeyleri ile CDI, SCARED, DEHB ölçekleri arasında korelasyon saptanmadı (tüm değişkenler için $p>0.050$).

Sonuç: Bu çalışma T. gondii'nin seropozitifliği ile OKB ve DEHB arasındaki ilişkiyi doğrulamamıştır. Uzunlamasına takip ve genişletilmiş hasta serileri ile daha ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: DEHB, Çocuk, Toxoplasma gondii, OKB

INTRODUCTION

Toxoplasma gondii infects about 25-30% of the world population, only a very small proportion of them cause clear clinical findings. This infectious agent causes latent infection in many organs in the body, including the brain. Asymptomatic toxoplasma infection is not considered to be any harm before; but a lot of studies show that toxoplasma-infected patients have higher incidences of especially neuropsychiatric disorders (1,2). Schizophrenia is the most strongly proven disease associated with toxoplasma infection among neuropsychiatric disorders (3-5).

Obsessive-compulsive disorder (OCD) is a common disorder designated by uncontrollable thoughts and compulsive behaviors which cause considerable deterioration in the childs academic, social and family functioning. OCD prevelance in childhood is 1-3% and the majority of cases are symptomatic (6). Etiology of OCD has not been fully understood. It is well-known that obsessive-compulsive disorder (OCD) is influenced by genetic factors that account for about 45-65% of the variance in OCD symptoms in young people (7). There are also environmental risk factors in the etiology of OCD. Some studies have shown that there is association OCD with infectious illness. Not only streptococcocal infection thats results Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS) or Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) but also other infectious pathogens may be involved OCD etiology. Toxoplasma gondii infection may play a role in the etyopathogenesis of OCD (8,9). Toxoplasma defined as a neurotropic agent, may conduce directly by affecting cognitive function and neurotransmitter activity. Neuroimmune reaction has also been recommended another etiological cause of OCD. Antibodies of toxoplasma affecting neurons in globus pallidus, caudate and putamen with in basal ganglions indicates local immune reactions. Increased brain levels of dopamine by causing a disturbance in serotonin concentration is to play an important etiologic mechanism not only schizophrenia but also OCD (10).

Attention-deficit hyperactivity disorder (ADHD) is common disorder characterized by a inattention and/or hyperactivity-impulsivity symptomps that impairs academic performance, quality of life and interpersonal relationships. The worldwide pooled prevalence was found to be roughly 5% (11). Although genetic factors have priority in the etiology of ADHD, which is a neurodevelopmental disorder, it may play a role in many environmental factors (12,13). The study about relationship of ADHD and infectious agents are scarce (14). Evidence suggests that dysregulation of dopamine and norepinephrine, are involved in the pathophysiology of ADHD. Toxoplasma genome affects genes for rate-limiting enzymes of dopamine synthesis may play a role ADHD (15). Another explanation is that toxoplasma infection becomes latent neurons and glial cell. Higher levels of cysts were reported in some brain area that are known to contain dopamine that results altered dopamine could have negatory consequences for some brain functions. Limited number of studies investigating the relationship between toxoplasma infection and ADHD resulting in the toxoplasmosis seropositivity has not significant difference between ADHD and control group (16,17). A recent meta-analysis conducted by Nayeri et al. (18) also showed that there was not a significant association between the T. Gondii IgG Ab levels and an increased risk of ADHD. Another study showed that in ADHD subjects with toxoplasma gondii IgG positivity had higher levels of severe ADHD compared to the toxoplasma gondii IgG negative children with ADHD (19). Our hypothesis is that the clinical features of ADHD, rather than an etiological relationship between ADHD and toxoplasma seropositivity, might pose a risk for toxoplasma infection. It is thought that symptoms of ADHD, such as hyperactivity and impulsivity, may predispose a risk for this infection.

OCD and ADHD are both mostly seen neuropsychiatric disturbances in pediatric populations. There is a neurobiological link between OCD and ADHD in terms of neurochemical circuitry, neuropsychological and neuroimaging findings to examine etiological factors underlying the disorders (20). There is, however, there was no study in the literature examining the both disorder at the same time to compare in terms of toxoplasmosis gondii seropositivity. In this study, we aimed to investigate association between Toxoplasma gondii IgG antibody levels in both OCD and ADHD in children and adolescents. Our hypotheses are the following; 1) Toxoplasma gondii seropositivity is higher in OCD and ADHD grup than that of the controls. 2) The OCD and the ADHD samples' seropositivities are comparable.

MATERIAL and METHODS

Patients were recruited from the Outpatient Clinic for Ankara Training and Research Hospital, Department of Child and Adolescent Psychiatry. The following exclusion criteria were applied: Presence of psychiatric disorders such as mental retardation, autism spectrum disorder, schizophrenia, bipolar disorder, major depression, major physical (such as diabetes mellitus, cancer) or neurological (neurodegenerative) diseases, such as epilepsy and using corticosteroids or drugs that affect the immune system in the last 6 months. In this terms, among 52 subjects with OCD, 10 children were excluded because of the 7 of them had major depressive disorder and another 3 had mental retardation. During study time-span, 94 children had ADHD. Among them, 36 of subjects had oppositional defiant disorder, 14 had specific learning disorders, 12 had anxiety disorders, 8 had major depressive disorder and 3 had mental retardation. These subjects were excluded from the study due to the comorbidities they had.

The control group was drawn from of healthy volunteers that were applied for General Pediatric Clinics of Ankara Training and Research Hospital for rutine health examination. Inclusion criteria was that having no any medical problem and normal intelligence level. Healthy subjects who agreed to participate in this study were evaluated by the same hospital's child psychiatrists to carry out their psychiatric examination. The study was approved by Ankara Child Health and Diseases Hematology Oncology SUAM, Clinical Research Ethics Committee (11.06.1018-2018-062).

Psychological assessment and tools used

Clinical and sociodemographic data was recorded in a form prepared by the researchers. OCD and ADHD diagnoses were made according to the DSM-5 criteria by child and adolescent psychiatrist.

Children's Yale–Brown Obsessive–Compulsive Scale (CY-BOCS): The severity of the OCD was assigned using the CY-BOCS, which is a semistructured interview applied by the clinician (21). This scale has a 10-item administered by the clinician to assess OCD obsessions (scores ranging from 0 to 20), compulsions (scores ranging from 0 to 20), and total (scores ranging from 0 to 40) symptom severity in children. Higher total and subscale scores indicate greater or more severe obsessions and/or compulsions.

Maudsley Obsessive Compulsive Questionnaire (MOCI): The OCD symptoms' severity was also assessed by the MOCI, which was a self-reported questionnaire. This tool consists of 30 items involving the obsessional-compulsive complaints with a dichotomous rated as "Yes" which is scored 1 point and "No" answers 0 point. Its Turkish version, which is conducted by Erol et al. (22) was used in this study.

Child Depression Inventory (CDI): This is a 27-item self-report scale that can be applied to children between ages 6-17. Its Turkish validity and reliability study was carried out by Oy (23). The test-retest reliability of the scale was 0.80, and the Cronbach alpha internal consistency coefficient was 0.77. The cut-off point of the scale is 19.

Screen for Child Anxiety and Related Disorders (SCARED): This scale consists of 41 items evaluating the child's anxiety. With a self-report design, each item is given 0, 1 or 2 points, depending on the severity of the symptom. Cut-off point of the total score suggested is 25 points and is thought to indicate the presence of an anxiety disorder. Its Turkish validation study was carried out by Çakmakçı (24). The scales's Cronbach's alpha reliability values for the general scale and subscales ranged from .88 to .91 (24).

The Turgay DSM-IV-Based Child and Adolescent Behavioral Disorders Screening and Rating Scale (T-DSM-IV-S): The T-DSM-IV-S scale was developed by Turgay (25) and consists of 42 items that measure attention-deficit, hyperactivity, impulsivity, and disruptive behaviors. In the present study a shorter version of the scale was used that included 9 attention-deficit items, 6 hyperactivity items, and 3 impulsivity items (25). Parents of children filled out this scale to evaluate the severity of the child's ADHD symptoms as well as to determine concomitant disruptive behavior disorders.

Conners' Parent Rating Scale-48 (CPRS-48): This scale is a 48-item Likert-type scale used to assess problematic behaviors in children. In addition to a total score, there are 5 subscale scores as the following; Conduct problems, impulsivity and hyperactivity, learning problems, anxiety and psychosomatic problems. Dereboy et al. (26) studied this scale's Turkish validity. Similar with T-DSM-IV-S scale, parents of children filled out this scale to evaluate the child's ADHD symptoms as well as other problems as aforementioned.

Sample collection/Serological analysis:

Blood samples were collected from the pediatric patients and healthy children. Separated sera stored at +4°C and analysed within 24 hours. Quantitative determination of IgG antibodies to

T. gondii in patients' was performed using the Architect Toxo IgG assay; which is an automated chemiluminescent microparticle immunoassay, in accordance with the manufacturer's instructions (Abbott, ABD). Samples with concentration values ≥3.0 IU/mL were considered reactive for IgG antibodies to T. gondii, concentration values from 1.60 to 2.90 IU/mL were considered gray zone, and concentration values <1.6 IU/mL were considered nonreactive.

Statistical Analyses

Statistical analyses were performed by SPSS 17 Statistical Analysis program (Chicago Inc., 2008). Continuous variables were expressed as mean and standart deviation and categorical variables as frequency (n) and percentage (%). The OCD, the ADHD and the control groups' continuous variables were compared via ANOVA and Kruskal Wallis tests and categorical ones were analyzed by Fisher's exact and Pearson χ2 tests. Spearman correlation test was used to analyse should there was a relation between the IgG levels and CDI, SCARED and ADHD total scores of the groups. p<0.017 was considered significant in triple comparisons and p<0.050 was considered significant in dual comparisons.

RESULTS

The study consisted of 42 OCD, 31 ADHD and 28 healthy controls. The mean age was 12.13 years (SD=3.37, range 6-17 years). Mean age of the ADHD group was smaller than the other two groups (F(2) = 13.01, p <0.001). Of 56.40% (n = 57) of the cases were boys and 43.60% (n=44) were girls. Male gender was found to be significantly higher in the ADHD group (83.90% vs. 40.50% and 50.0%, respectively, when compared with male rates in the OCD and the control groups; $\chi^2(2)=14.313$, p=0.001). The rate of the patients with ADHD (87.10%) in primary education was significantly higher than the other two groups ($\chi^2(2)=10.094$, p=0.006). Three groups were similar in terms of family structure, number of siblings and socioeconomic level (Table I).

In OCD group (n = 42), 88.1% (n=37) of the children had "contamination" obsessions and 95.2% (n=40) of them had "cleaning-washing" compulsions. As regards the symptoms evaluation, depression and anxiety symptoms examined by the CDI and the SCARED were similar in the OCD and the control groups, but were significantly lower in the ADHD group compared to these two groups. The mean CDI scale score was significantly lower in the ADHD group than in the OCD and control group (F(2) = 138.27, p <0.001). Similar with this, the mean SCARED score was significantly lower in the ADHD group compared to the OCD and the healthy subjects (F(2) = 52.721, p <0.001). Conners 'and Turgay scale scores were naturally higher in the ADHD group compared to the control group (see Table II).

The serology findings revealed that all children with OCD had negative (100%) for T. Gondi IgG, whereas 78.6% of controls and 90.3% of children with ADHD had negative for IgG Ab. While the distribution of Toxoplasma gondii IgG seropositivity rates were similar between the control and the ADHD group (p=0.117), and between the OCD and the ADHD group (p =0.072), T. gondii IgG seropositivity of the control group was found to be significantly higher than the OCD group (p = 0.003) (Table II).

Risk factors for toxoplasma gondii infection such as contact with the cat, eating vegetables and fruits without being washed, contacting the soil, drinking water of unknown origin, undercooked meats consumption were also examined. In our study, these variables that posed a risk for toxoplasma infection were questioned in OCD, ADHD and control groups. They showed similar distribution among the three groups (see Table II).

Table I: Sociodemographic and clinical features of the participants

	Total n = 101	OCD n = 42	ADHD n = 31	Control n = 28	Statistics t, z, F or χ^2	p
Age (year)*	12.13 (3.37)	13.0 (3.26)	9.83 (2.91)	13.39 (2.79)	13.010	0.000
Gender †					14.313	0.001
Girls	44 (43.60)	25 (59.50)	5 (16.10)	14 (50.0)		
Boys	57 (56.40)	17 (40.50)	26 (83.90)	14 (50.0)		
Education†					10.094	0.006
Elemantary	65 (64.40)	23 (54.80)	27 (87.10)	15 (53.60)		
High	36 (35.60)	19 (45.20)	4 (12.90)	13 (46.40)		
Family†					6.270‡	0.129
Nuclear	86 (85.10)	32 (76.20)	28 (90.30)	26 (92.90)		
Large	11 (10.90)	8 (19.0)	1 (3.20)	2 (7.10)		
Single parent	4 (4.0)	2 (4.80)	2 (6.50)	0		
SES†					2.369‡	0.692
Low	26 (25.70)	12 (28.60)	7 (22.60)	7 (25.0)		
Moderate	68 (67.30)	28 (66.70)	20 (64.50)	20 (71.40)		
High	7 (6.90)	2 (4.80)	4 (12.90)	1 (3.60)		

:** *Mean (Standard deviation),* **†:** *n (%),* **‡** *Fisher's exact test* ***n: *Frequency,* ***SES:*** *Socioeconomic status*

Table II: Comparison of the distribution of applied scale scores between the three groups

	OCD n = 42	ADHD n = 31	Control n = 28	Statistics t, F or χ^2	p
Scales*					
CDI	25.09 (3.72)	1.35 (5.34)	21.85 (9.57)	138.27	0.000
SCARED	34.14 (16.55)	1.74 (6.76)	22.28 (13.41)	52.721	0.000
MOCI	49.78 (13.30)	NA	NA	NA	NA
CY-BOCS-Total	21.13 (6.91)	NA	NA	NA	NA
Obsession score	10.54 (3.80)	NA	NA	NA	NA
Compulsion score	10.64 (3.51)	NA	NA	NA	NA
CPRS-48					
CP	NA	16.19 (5.80)	12.42 (4.52)	2.757	0.008
I-HA	NA	10.96 (4.34)	8.03 (3.38)	2.869	0.006
LP	NA	13.83 (4.71)	9.71 (2.92)	3.981	0.000
OB	NA	7.96 (2.56)	5.71 (1.78)	3.881	0.000
Anxiety	NA	16.30 (5.15)	13.89 (4.13)	1.954	0.056
Psychosomatic	NA	8.41 (3.44)	8.64 (3.37)	-.251	0.803
T-DSM-IV-S*					
ADHD-Total	NA	34.0 (10.53)	13.42 (9.41)	7.872	0.000
IA	NA	16.51 (6.19)	7.21 (5.15)	6.232	0.000
HA	NA	17.48 (5.34)	6.21 (5.33)	8.092	0.000
ODD	NA	12.64 (4.72)	6.96 (5.31)	4.347	0.000
CD	NA	5.93 (6.53)	1.82 (2.49)	3.131	0.003
Laboratory					
Toxo-IgG‡				12.018†	0.001
Positive	0	3 (9.70)	2 (7.10)	ADHD vs. Control	0.117
Gray zone	0	0	4 (14.30)	ADHD vs. OCD	0.072
Negative	42 (100.0)	28 (90.30)	22 (78.60)	OCD vs. Control	0.003
T. gondii related factors					
Residency‡				11.841†	0.009§
City	30 (71.40)	25 (80.60)	81 (80.20)		
Rural area	1 (2.40)	3 (9.70)	2 (7.10)		
NA	11 (26.20)	3 (9.70)	0		
Contact with cat‡				15.218†	0.003§
Yes	4 (9.50)	9 (29.0)	3 (10.70)		
No	27 (64.30)	19 (61.30)	25 (89.30)		
NA	11 (26.20)	3 (9.70)	0		
Eating raw meat‡				14.306†	0.005§
Yes	8 (19.0)	6 (19.40)	2 (7.10)		
No	23 (54.80)	22 (71.0)	26 (92.90)		
NA	11 (26.20)	3 (9.7)	0		
Soil contact‡				13.125†	0.006§
Yes	26 (61.90)	27 (87.10)	26 (92.90)		
No	5 (11.90)	1 (3.20)	2 (7.10)		
NA	11 (26.20)	3 (9.70)	0		
Eating vegetable without washing‡				13.988†	0.006§
Yes	16 (38.10)	16 (51.60)	11 (39.30)		
No	14 (33.30)	12 (38.70)	17 (60.70)		
NA	12 (28.60)	3 (9.70)	0		
Drinking water of unknown origin‡				10.354†	0.031§
Yes	20 (48.80)	21 (67.70)	18 (64.30)		
No	11 (26.80)	7 (22.60)	10 (35.70)		
NA	10 (24.40)	3 (9.70)	0		

:** *Mean (standard deviation),* **†:** *Fisher's exact test,* **‡:** *n (%),* **§:** *Not-significant after controlling NA,* ***CDI: *Child depression inventory,* ***SCARED:*** *Screen for childhood anxiety related disorders,* ***MOCI:*** *Maudsley Obsessive-Compulsive,* ***CY-BOCS:*** *Children's Yale-Brown Obsessive-Compulsive Scale,* ***CPRS-48:*** *Conners' Parent Rating Scale-48,* ***T-DSM-IV-S :*** *Turgay DSM-IV-Based Child and Adolescent Behavioral Disorders Screening and Rating Scale,* ***CP:*** *Conduct problem,* ***I-HA:*** *Impulsivity-hyperactivity,* ***LP:*** *Learning problem,* ***OB:*** *Oppositional behavior,* ***IA:*** *Inattention,* ***HA:*** *Hyperactivity,* ***ODD:*** *Oppositional defiant disorder,* ***CD:*** *Conduct disorder.* ***NA:*** *not-applicable.*

Table III: Correlation between IgG levels (IU/ml) and CDI, SCARED and ADHD total scores of the groups

	IgG levels (IU/ml)					
	ADHD (n = 31)		OCD (n = 42)		Control (n = 28)	
	Spearman r	p	Spearman r	p	Spearman r	p
CDI	-0.051	0.785	-0.092	0.560	0.009	0.963
SCARED	-0.054	0.775	-0.269	0.085	0.048	0.808
T-DSM-IV-S						
ADHD-Total	-0.093	0.617	NA	-	0.323	0.093
ADHD-IA	-0.061	0.743	NA	-	0.279	0.150
ADHD-HA	-0.113	0.545	NA	-	0.302	0.119
ADHD-ODD	0.039	0.835	NA	-	0.136	0.491
ADHD-CD	0.650	0.000	NA	-	0.157	0.425
CPRS-48						
CPRS-CP	0.083	0.657	NA	-	0.370	0.052
CPRS-I-HA	-0.248	0.179	NA	-	0.000	0.999
CPRS-LP	-0.092	0.621	NA	-	0.289	0.135
CPRS-OB	-0.124	0.507	NA	-	-0.077	0.699
CPRS-Anxiety	-0.242	0.197	NA	-	0.057	0.772
Psychosomatic	-0.152	0.413	NA	-	0.191	0.330
MOCI-Total	NA	-	0.200	0.204	NA	-
CY-BOCS-Total	NA	-	0.154	0.330	NA	-
Obsession	NA	-	0.112	0.481	NA	-
Compulsion	NA	-	0.184	0.242	NA	-
OCD symptoms						
Contamination (n=37)	NA	-	0.010	0.952	NA	-
Cleaning-washing (n = 40)	NA	-	0.135	0.394	NA	-

CDI: *Child depression inventory,* ***SCARED:*** *Screen for childhood anxiety related disorders,* ***T-DSM-IV-S:*** *Turgay's DSM-IV-Based Child and Adolescent Behavioral Disorders Screening and Rating Scale,* ***ADHD-Total:*** *Turgay's ADHD scale-total,* ***HA:*** *Hyperactivity,* ***ODD:*** *Oppositional defiant disorder,* ***CD:*** *Conduct disorder,* ***CPRS-48:*** *Conners' Parent Rating Scale-48,* ***CP:*** *Conduct problem,* ***I-HA:*** *Impulsivity-hyperactivity,* ***LP:*** *Learning problem,* ***OB:*** *Oppositional behavior,* ***MOCI:*** *Maudsley Obsessive-Compulsive,* ***CY-BOCS:*** *Children's Yale-Brown Obsessive-Compulsive Scale,* ***NA:*** *not-applicable,* ***r:*** *Spearman rho correlation coefficient*

The Spearman correlation analysis revealed that in the ADHD group (n = 31), Toxo IgG levels were positively correlated with Turgay's ADHD-Conduct disorder subscale scores (Spearman r = 0.650, p <0.001). In the OCD (n = 42) and the control (n = 28) groups, there was no correlation between IgG levels and the CDI, the SCARED, the ADHD scales (for all variables, p>0.050). In the OCD group, there was also not any correlation found in terms of obsession or compulsion symptom dominancy and IgG levels. Neither the contamination obsessions nor the cleaning-washing compulsions was correlated with T. gondii IgG Ab levels (for both, p>0.050). Interestingly, there was a positive correlation between CPRS-48 conduct problems (CPRS-CP) scores and IgG levels in borderline- terms (Spearman rho = 0.370, p = 0.052) (see Table III).

DISCUSSION

This study aimed to evaluate T. gondii IgG seropositivity in children with OCD and ADHD and to compare with healthy counterparts. Although there was no association between OCD and T. gondii IgG seropositivity, an association between IgG Ab levels and conduct disorder symptoms in children with ADHD was found. This intruiging result is worth further studying, since conduct disorder is an externalizing problem. Supporting this issue, there was found a significant low level of the CDI and the SCARED scores of children with ADHD compared to the OCD and the healthy subjects. It might be ADHD is, as a whole, an externalizing disorder compared to the other internalizing disturbances including the OCD.

Although recent studies have attempted to elucidate the relationship between different type of infectious agents and psychiatric disorders, data are particularly limited in pediatric age group and there are conflicting results (27-29). Among the different type of pathogens associated with psychiatric disorders, the plurality of attention is T. gondii infectious, that has a lifelong asymptomatic latent phase in patients after a short acute period. In the recent meta-analysis and systematic review show that a toxoplasma infection is an associated factor for bipolar, schizophrenia epilepsy, but not for depression (30-33). Few studies have been conducted to investigate the possible relationship between OCD and T. gondii infection both adulthood and childhood. The first studies on this subject in the literature were case reports. Two children with toxoplasmosis and OCD showed significant reduction in the symptoms of OCD with antiprotozoal drugs (34). Similarly, there is one reports of adult case with antiprotozoal medications that show a decrease in OCD symptoms (35). The association between T.

Gondi and OCD is not clearly identified and there are conflicting results in researchs. In some studies, seroposivity rate for T. Gondi infection among OCD are considerably higher than the control group (36,37). On the other hands in many studies, no differences were found between toxoplasma seropositivity between OCD patients and control group (38-40). In a recent meta-analysis, conducted Chegeni et al. (41) which compiled 11 studies on the subject, a quarter of 389 OCD patients and roughly 17% of 9484 controls were positive for toxoplasmosis. This systematic review revealed that toxoplasma infection could be as an associated factor for OCD (OR = 1.96). Different results of investigation appraised the relationship between various variables including sex, age, education and socio-economic level, and place of residence. In our study we asked the patient and the control group should they contact with the cat, eating vegetables and fruits without being washed so forth. Although these variables have been determined according to patients' declaration; the risk factors for toxoplasma infection did not differ in the patient and control groups contributed to the exclusion of confounding factors in our study. Conflicting results in studies may have an impact on changes in specificity and sensitivity ELISA test kits and different cut-off rate are factors that affect the infection prevelance. In our study, T. Gondi Ig G seropositivity was significantly higher in the control group compared to the OCD group in contrast to previous studies. It has been shown that the most common obsessions in children are rituals for contamination and aggression, and the most common compulsions are washing and avoiding harm. Similarly, in our study, 88.1% (n=37) of the patients had contamination obsessions and 95.2% (n=40) of the cleaning-washing compulsions.

In a study, conducted by Shehataa et al. (42) that examined the relationship between neurodevelopmental disorders and toxoplasma, the only related factor was age and seropositivity increased with age. Miman et al. (36) investigated toxoplasma infection in adult OCD patients, toxoplasma was a risk factor for OCD, but the same researcher did not show the same relationship in a later study in children and adolescents (43). Authors stated that this difference in adulthood and childhood may be related to the different nature of adulthood onset and childhood onset OCD in these periods. Even in our study, T. gondii IgG seropositivity of the control group was significantly higher than the OCD group. Considering the symptomatology of OCD, the most common type of contamination obsessions in children may decrease the risk of children becoming infected with toxoplasma. In our study, the presence of either contamination obsessions or cleaning-washing compulsions were not found correlated with T. gondii IgG Ab levels. This is result is might be a result of relatively limited number of OCD subjects.

Although the relationship between many psychiatric diseases and toxoplasma infection has been examined before, a few studies investigating the toxoplasma infection as a risk factor for ADHD (16,17). Toxoplasmosis seropositivity has not significant difference between ADHD and control group in two study as in our study. These two studies included children and adolescents. In another study, conducted by Shehataa et al. (41) seropositivity of anti-Toxoplasma IgG was significantly associated with non-schizophrenic neurodevelopmental disorders including ADHD, autism, speech and language development delay. But this study includes both child-adolescent and adult age groups and it was a statistically significant difference between toxoplasma IgG seropositivity regarding the age of the patients. If the group divided into to be less than 20 and greater than 20, 20 years or older were twice more likely exposed to T. Gondii than those younger than 20 years old. In addition, the number of ADHD cases in this study (n=14) is very low. It was thought that the clinical features of ADHD like impulsivity might pose a risk for toxoplasma infection. But in our study, no relationship was found between toxoplasmosis and any clinical signs of ADHD (attention, hyperactivity, impulsivity). On the other hands, Toxo IgG levels were positively correlated with Turgay's ADHD-Conduct disorder subscale scores. It may be related to the low level of socioeconomic level and less compliance with hygiene rules of the group with conduct disorder. It is thought that larger sample and follow-up studies are needed to demonstrate this causal relationship. Also in the literature, there is no study showing the relationship between toxoplasmosis infection and adulthood ADHD. We suggest that in the future research are need larger amount of individuals and adult age group.

This study's strenght point is to examine T. gondii IgG seropositivity levels in children with ADHD and the OCD and compare with healthy subjects. Including these two mostly seen disturbances in childhood period at the same study is a strength. There is, however, some limitations. First, this was a clinical study and findings could not be generated. Second, although three groups were included, sample numbers were relatively limited.

To conclude, this study did not verify a relationship between the T. gondii and OCD. There is however, findings suggest that there might be an association between IgG Ab levels and depressive or anxiety symptoms in children with ADHD and, even, there may be a relation between hyperactivity symptoms and IgG levels in healthy children. Further studies are needed longitudinal follow-up and extended series of patients.

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Türkiye Çocuk Hastalıkları Dergisi

Impact of Lockdown and Visiting Restrictions for COVID-19 on Breast Milk and Short-Term Morbidities in a Tertiary Neonatal Intensive Care Unit in Türkiye

COVİD-19 Nedeniyle Sokağa Çıkma Yasakları ve Ziyaret Kısıtlamasının Türkiye'de Üçüncü Basamak Bir Yenidoğan Yoğun Bakım Ünitesinde Anne Sütü ve Kısa Dönem Morbiditeler Üzerine Etkisi

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ABSTRACT

Objective: In the COVID-19 pandemic era, visiting restrictions and lockdown measures have been leaded to serious concerns in breastfeeding and maternal-infant interaction. We aimed to evaluate the effects of visiting restrictions as no physical visits allowed period (March 13th and June 20th, 2020) on feeding with breast milk, breastfeeding and associated morbidities.

Material and Methods: Neonates admitted to neonatal intensive care unit in pre-COVID-19 pandemic and during strict visiting restrictions constituted control and study groups.

Results: Study and control groups included 197 and 193 mother-baby dyads. Study group had insignificant lower gestational age, birthweight and higher prematurity rate. Median first enteral feeding and first breast milk days were similar. First enteral feeding with breast milk was insignificantly higher in control group. Median breast milk percentage at full enteral feeding (FEF) did not differ. Median time of FEF, FEF with only breast milk and intravenous fluid duration were higher in study group (p<0.050). Full enteral feeding with breast milk during NICU stay was less in study group (78.1 vs 87%, p<0.050) while at discharge there was no difference (78.1% vs 81.3%). Feeding intolerance was insignificantly higher in study group (23.8% vs 14.6%). Median duration of NICU stay was higher in study group but not significant (13.5 vs 12 d, p>0.050).

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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 Etlik Zübeyde Hanım Training and Research Hospital (22.06.2022, 2022/93).

Contribution of the Authors / Yazarların katkısı*: *ÇELİK İH: 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. ***DURUKAN TOSUN M:*** 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. ***UZLU SE:*** 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. ***BAŞ AY:*** Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Reviewing the article before submission scientifically besides spelling and grammar. ***DEMİREL N:*** Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Reviewing the article before submission scientifically besides spelling and grammar.

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Conclusion: No visits allowed period leaded to impaired breastfeeding, breast milk supply and associated morbidities. Parents should be informed about breastfeeding and breast milk in prenatal period, after birth and during NICU stay in COVID-19 era as before and precautions should be taken.

Key Words: Breastfeeding, Breast milk, COVID-19, Neonatal morbidities, Visiting restrictions

ÖZ

Amaç: COVİD-19 pandemi sürecinde ziyaret kısıtlamaları ve sokağa çıkma yasaklarının anne sütü ile beslenme ve anne-bebek ilişkisinde sorunlar yaratacağından endişe edildi. Bu çalışmanın amacı ülkemizde ziyaret yasağı sürecinin (13 Mart 2020-20 Haziran 2020) anne sütü ile beslenme, emzirme ve ilişkili morbiditeler üzerine etkisini değerlendirmektir.

Gereç ve Yöntemler: Yenidoğan yoğun bakım ünitesinde (YYBÜ) COVİD-19 pandemisi öncesi ve ziyaret yasağının uygulandığı dönemde yatan bebekler kontrol ve çalışma gruplarını oluşturdu.

Bulgular: Çalışma ve kontrol gruplarını 197 ve 193 anne-bebek çifti oluşturdu. Çalışma grubu daha düşük gestasyonel yaş, doğum ağırlığı ve daha yüksek prematürite oranına sahipti ancak istatistiksel anlamlı değildi. Ortanca ilk enteral beslenme ve anne sütü alma günleri benzerdi. Kontrol grubunda ilk beslenmenin anne sütü ile yapılma oranı daha fazlaydı ancak istatistiksel anlamlı değildi. Tam enteral beslenmeye geçişteki anne sütü oranı benzerdi. Ortanca tam enteral beslenmeye geçiş zamanı, tam enteral beslenmede anne sütü oranı ve intravenöz sıvı süresi çalışma grubunda daha yüksekti ($p<0.050$). Tam enteral beslenmenin anne sütü ile yapılması yatış sürecinde çalışma grubunda daha azken (78.1 ve %87, $p<0.050$) taburculuk sırasında benzerdi (%78.1 vs %81.3). Beslenme intoleransı (%23.8 vs %14.6) ve yatış süresi (13.5 ve 12 gün) çalışma grubunda daha yüksekken istatistiksel anlamlı değildi.

Sonuç: Ziyaretin yasaklanmasının emzirme, anne sütü sağlanması ve ilişkili morbiditeler üzerine olumsuz etkileri mevcuttur. Aileler COVİD-19 pandemisi öncesinde olduğu gibi anne sütü ve emzirmenin önemi hakkında gebelik süreci, doğum ve YYBÜ yatış sürecinde bilgilendirilmeli ve önlemler alınmalıdır.

Anahtar Sözcükler: Emzirme, Anne sütü, COVİD-19, Neonatal morbiditeler, Ziyaret kısıtlamaları

INTRODUCTION

COVID-19 was described as pandemic by the World Health Organization (WHO) on January 30th, 2020 that has still devastating effects on public health due to its mortality and morbidity. The first COVID-19 case was reported on March 11th, 2020 in Turkey and number of cases had been increasing. One of the first recommendations by WHO was the restriction of visitors and visiting periods in hospitals (1). This recomendation was applied firmly at the beginning of pandemic but evolved during the pandemic as different protocols in different countries even differences in the same country due to different properties of facilities (2, 3). In Turkey, restriction of visitors was applied as no physical visits allowed between March 13th and June 20th, 2020. During this period various lockdown measures affecting daily life were also taken.

Breastfeeding, breast milk recruitments, kangaroo mother care (KMC) and maternal/family bonding are some of the mainstay approaches for the short- and long-term outcomes in neonatal intensive care unit (NICU) (4). In a projection study, Minckas et al. (5) estimated that 50% reduction of KMC could result in 12.570 incremental deaths across 127 low- and middle-income countries. Separation of child and parents especially mothers were reported to be associated with decreased breastfeeding, less bonding, and worse parental relationship in the COVID-19 era (6,7). World Health Organization recommends to start exclusive breastfeeding as soon as after birth for the first 6 months and carry on to 2 years and later due to several short- and long-term benefits (8-10). Preterm infants received breast milk have less necrotizing enterocolitis (NEC), sepsis, respiratory infections, retinopathy of prematurity (ROP), and higher maternal bonding and neurocognitive scores (11,12).

In the COVID-19 pandemic era, visiting restrictions and lockdown measures have been leaded to serious concerns in breastfeeding and interaction between mothers and infants. In this study, we aimed to evaluate the effect of visiting restrictions during the lockdown on the feeding with breast milk, breastfeeding and short-term morbidities associated with feeding practices, and other neonatal morbidities.

MATERIALS and METHODS

This retrospective study was carried out in Etlik Zübeyde Hanım Training and Research Hospital NICU. Ethics committee approval was obtained from the same hospital (22.06.2022, 2022/93).

Inclusion criteria were 1) Study group: Neonates admitted to NICU during lockdown period between March 13th and June 20th, 2020. 2) Control group: Neonates admitted to NICU between October 1st, 2019, and February 1st, 2020 and discharged before lockdown period. 3) NICU stay ≥ 5 days to evaluate breast milk supply in association with visiting restrictions and the effect of discharge of mothers that is usually in 2 days after birth 4) No COVID-19 infection in mother and neonate.

The demographic and clinical characteristics were recorded from patients' medical records. We recorded gestational age (GA), birthweight (BW), prematurity (<37 week of GA), gender, maternal age, maternal or gestational disease status,

mode of delivery and being small for GA (SGA). Neonatal morbidities were defined as respiratory distress syndrome (RDS), transient tachypnea of newborn, sepsis, patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH, Grade 1 to 4 according to Volpe's classification), hydrocephalus, periventricular leucomalacia, bronchopulmonary dysplasia (BPD, phototherapy, feeding intolerance (emesis, abdominal distension/tenderness, increased/no bowel sounds, increased gastric residual, color change of gastric residual, bloody stool), NEC (modified Bell's staging criteria above stage II A) (13-16). Mechanical ventilation status, medical treatment of PDA, and duration of hospitalization were also recorded. Enteral feeding status were evaluated by time and type of first enteral feeding and full enteral feeding (FEF), mother-infant stay before discharge, breast milk/formula feeding at discharge. Our study included refugee patients from mainly Syria and other countries including Iraq, Afghanistan, Pakistan, and Central Asian countries.

Before COVID-19 pandemic both mothers and fathers were allowed to visit their children daily. Skin to skin contact (SSC), KMC and breastfeeding have been started soon after birth if clinically possible and continued until discharge from NICU on a daily basis. Adjustment room stay was a routine practice before COVID-19 pandemic. Breast milk was received whenever parents bring in both periods.

During visiting restrictions, mothers were allowed to visit, breastfeed their children and perform KMC during hospital stay and parents were not allowed to visit after discharge of mother. Both mothers and fathers were informed about the importance of breast milk, how to express their breast milk, store and bring to NICU before discharge. Families were informed by phone call about the status of their children and breast milk on a daily basis, regularly. Mothers and infants without COVID-19 clinical symptoms were stayed at adjustment room before discharged for a few days if needed.

Statistical Analysis

Statistical analyses were performed using the SPSS statistical package (v. 20.0 for MAC). Categorical variables between groups were analyzed using the χ^2 test. Comparison of means between two groups was examined by using a t test, where the data fit a normal distribution, and by Mann- Whitney U test, where the data were nonnormal distributions. A p value of <0.050 was deemed to indicate statistical significance.

RESULTS

Study and control groups were consisted of 197 and 193 patients, respectively. Demographic characteristics are listed in Table I. Median GA and BW were insignificantly lower in study group (33 (23-41) vs 33 (23-41) w, p=0.050, and 1854 (480-4600) vs 1935 (525-4435) g, p>0.050, respectively). Male gender was higher in study group than control group (64% vs 53.4%, p<0.050). Control group had higher full course antenatal steroid (AS) than study group (44% vs 32%, p<0.050). Prematurity and being <32 weeks of GA rates were higher in study group but statistically insignificant (88.8% vs 82.9% and 66 (33.5%) 50 vs (25.9%), p>0.050). Other demographic characteristics such as SGA, delivery route, being refugee and maternal age were similar between groups.

Table I: Sociodemographic characteristics of patients

	Study group (n:197)	Control group (n:193)	p
Gestational week, w*	33 (23-41)	33 (23-41)	0.050
Birthweight, g*	1847 (480-4600)	1935 (525-4435)	0.33
Birthweight <2000 g†	114 (58.3)	107 (55.7)	0.60
SGA, n†	35 (17.8)	46 (23.8)	0.260
Male gender†	126 (64)	103 (53.4%)	0.030
Cesarean sectio†	162 (82.2)	157 (81.3)	0.82
APGAR at 1/5 min*	7 (0-10)/9 (1-10)	7 (2-9)/8 (4-10)	0.33/0.7
Resuscitation†	26 (13.2)	36 (18.6)	0.130
Multiple pregnancy†	43 (21.8)	35 (18.1)	0.360
Refugee†	41 (20.8)	46 (23.8)	0.470
Prematurity†	175 (88.8)	160 (82.9)	0.090
GA <32 weeks	66 (33.5)	50 (25.9)	0.100
Antenatal steroid, full course†	63 (32)	85 (44)	0.010
Maternal age*	27 (16-43)	28 (16-43)	0.48
Chorioamnionitis†	8 (4.1)	4 (2.1)	0.250

median (minimum-maximum), †:n(%), **SGA: small for gestational ag*

Clinical characteristics are listed in Table II. Respiratory distress was the main admission complaint in both groups (72.6% vs 80.3%, p>0.050). Respiratory distress syndrome, surfactant use, PDA, grade 3-4 IVH rates were similar between groups while sepsis, BPD and ROP rates were insignificantly higher in study group (p>0.050). Median first enteral feeding and first breast milk days were similar in groups (p>0.050). First enteral feeding with breast milk was insignificantly higher in control group (76.6% vs 70.9%, p>0.050). Median breast milk percentage at FEF did not differ between groups (p>0.050). Median time of FEF, FEF with breast milk and intravenous fluid duration were higher in study group (p=0.001, 0.03, 0.01, respectively). Full enteral feeding with breast milk during NICU stay was less in study group (78.1 vs 87%, p<0.050) while at discharge there was no difference (78.1% vs 81.3%, p>0.050). Feeding intolerance was higher in study group but not statistically significant (23.8% vs 14.6%, p>0.050). Necrotizing enterocolitis was diagnosed in 2 patients in each group (p>0.050). Phototherapy was given to more patients in study group but not statistically different (78.1% vs 72.4%, p>0.050) while median duration of phototherapy was similar. Mother infant stay at adjustment room rate and duration did not differ between groups (p>0.050). Median duration of NICU stay was insignificantly higher in study group (13.5 vs 12 d, p>0.050). Refugee patients in study group had less FEF

Table II: Clinical characteristic of patients

	Study group (n:197)	Control group (n:193)	p
Respiratory distress*	143 (72.6)	155 (80.3)	0.070
RDS*	89 (45)	86 (44.5)	0.220
Surfactant*	24 (12.2)	27 (14)	0.590
PDA,*	17 (8.6)	20 (10.4)	0.550
Medical treatment of PDA*	14 (7.1)	11 (5.7)	0.570
Grade 3-4 IVH*	2 (1)	1 (0.5)	100
Hydrocephalus*	1 (0.5)	2 (1)	0.620
Sepsis*	24 (12.2)	16 (8.3)	0.190
Early/late onset sepsis*	5/19 (20.8)	3/13 (18.8)	1.000
BPD*	11 (5.6)	6 (3.1)	0.220
ROP (any stage)*	17 (8.6)	10 (5.2)	0.180
ROP treatment (laser or IVB)*	3 (1.5)	1 (0.5)	0.620
First enteral feeding, day†	2 (1-4)	2 (1-4)	0.7
First enteral feeding with breastmilk*	139 (70.9)	147 (76.6)	0.20
First breastmilk, day†	2 (1-13)	2 (1-12)	0.6
Breastmilk percentage at full enteral feeding, % †	100 (0-100)	100 (0-100)	0.15
Time to reach full enteral feeding, day†	6 (2-26)	5 (2-100)	0.001
Time to reach full enteral feeding with breastmilk if possible, day†	7 (3-50)	6 (3-49)	0.03
Full enteral feeding with breastmilk during NICU stay*	153 (78.1)	168 (87)	0.020
Full enteral feeding with breastmilk at NICU discharge*	154 (78.1)	157 (81.3)	0.360
IV fluid duration, day†	6 (0-26)	5 (1-49)	0.01
Feeding intolerance*	43 (23.8)	28 (14.6)	0.060
Breastmilk/formula/both at feeding intolerance*	27/6/10	16/5/7	0.800
Feeding intolerance, day†	4 (0-42)	4 (2-14)	0.65
NEC*	2 (1)	2 (1)	100
NEC, day†	6.5 (6-7)	4.5 (4-5)	0.12
First meconium, day†	1 (1-6)	1 (1-5)	0.23
NIMV, day†	2 (0-41)	1 (0-67)	0.48
IMV, day†	0 (0-60)	0 (0-21)	0.58
Oxygen, day†	1 (0-76)	1 (0-75)	0.03
Phototherapy*	153 (78.1)	139 (72.4)	0.060
Phototherapy duration, hours†	36 (6-226)	39 (6-169)	0.8
Isoimmunisation*	24 (15.7)	22 (15.2)	0.920
Visiting days of mother†	3 (0-76)	9 (1-100)	<0.001
Adjustment room stay together†	175 (89.3)	175 (93.1)	0.190
Adjustment room stay, duration, day†	1 (1-5)	1 (1-3)	0.23
NICU stay, day†	13.5 (5-128)	12 (5-165)	0.07
Mortality*	1 (0.5)	4 (2.1)	0.210

:** *n(%),* **†:** *median (minimum-maximum,* ***RDS: *respiratory distress syndrome,* ***PDA:*** *patent ductus arteriosus,* ***BPD:*** *bronchopulmonary dysplasia,* ***IVH:*** *intraventricular hemorrhage,* ***ROP:*** *retinopathy of prematurity,* ***IVB:*** *intravitreal bevacizumab,* ***NEC:*** *necrotizing enterocolitis,* ***NIMV:*** *noninvasive mechanical ventilation,* ***IMV:*** *invasive mechanical ventilation.*

with breast milk than Turkish patients at discharge (67.5% vs 81.9%, p<0.050). In both groups, breast milk percentage at FEF was lower in refugees (p<0.050). There was no statistically difference in feeding intolerance according to being refugee in groups.

DISCUSSION

In this study we evaluated the effects of visiting restrictions and found that time of FEF, FEF with breast milk and IV fluid duration were higher in study group while rate of FEF with breast milk

during NICU stay was lower. Despite these results, rate of FEF with breast milk at NICU discharge became better than NICU stay.

In a study by Gunes et al.(17) from Turkey, they asked mothers not to bring their expressed breast milk (EBM) to NICU during the first month of lockdown and then they let EBM after informed consent. Rates of EBM in pre-COVID-19 era, first month and later were 100%, 0% and 79%, respectively (p<0.050). Their study group included infants ≥ 36 w of GA and median duration of hospitalization were 9, 10 and 10 days, respectively (p>0.050) while we found that duration of hospitalization was prolonged in lockdown period. This may be associated with our patients had lower GA, BW and higher time to FEF with breast milk and increased feeding intolerance. They evaluated the EBM rates between discharge and at the end of 1th month and found 90%, 89.1% and 75.9, respectively (p>0.050). There was no difference in demographic characteristics of patients. The most important difference of our study was to let mothers to bring their breast milk in lockdown period. Full enteral feeding with breast milk rates of study and control groups at discharge were 78.1% and 81.3% in our study and similar EBM rates were reported by Gunes et al.(17) after discharge. We also evaluated the role of being refugee on breast milk supply and found less feeding with breast milk among refugees. This may be associated with economic conditions of refugees because difficulties in public transport and job options were more prominent for refugees in lockdown period.

Yi et al. (18) evaluated the outcomes of term and near-term infants in terms of family centered care management during lockdown in China. Median duration of NICU stay for study and control groups were 4 and 3 days, respectively (p<0.050). Neonates were feed with EBW and breastfeeding rate at discharge was decreased as 6% (74% vs 80%) but not significant. In our study, we also had similar breastfeeding rates at discharge despite more preterm infants in our study. Muniraman et al. (7) evaluated parental perceptions in 6 neonatal units from United Kingdom and USA with different visiting limitations. Restrictive policies were one or two parents at the cotside with restricted visit duration and one parent at the cotside with unrestricted visit duration. Most restricted group reported that less often visiting and bonding, unable to receive updates and bring breast milk; and also mild to severe impact on breastfeeding was reported in 36% of parents. We did not evaluate bonding but less bonding in study group could be predicted because of strict visitation restriction policy. Bringing expressed breast milk was negatively affected in our study group such as Muniraman's study but at NICU discharge FEF with breast milk rate became similar with pre-COVID-19 era. This may be explained by understanding the importance of breast milk by our parents.

Breastfeeding has been negatively affected by prelabour and labour restrictions of a partner or support, visiting restrictions to NICU, decreased skin to skin contact and KMC in NICU in COVID-19 era (19). The WHO has been suggesting breastfeeding and SSC even in COVID-19 positive mother or infant from the start of pandemic (20). There are studies evaluating the association between lockdown and breastfeeding after discharge from maternity ward and found conflicting results including better or worse breastfeeding in lockdown period (21-23). These studies concluded that maternal support as face to face for breastfeeding and mental health is needed for better maternal and infant health but in COVID-19 era maternal support has become limited and mostly done by telephone or video interview. In our study group, we performed face to face support during hospital stay of mother and continued as telephone interview up to NICU discharge. We think that face to face support, SSC in the first days of life and adjustment room stay before discharge contributed the high breastfeeding rate in study group.

Visiting restrictions have been affecting breastfeeding, breast milk supply, SSC, bonding and the role of families as family integrated care; and short- and long-term effects of these changes to both newborn and mother are unknown. Neurodevelopment of the infant and maternal mental health are supposed to be negatively affected. The role of parents especially mothers have changed visitors to an integral and essential part of care provision (24). In a cross-sectional study from USA evaluating restrictions on parental presence in 277 NICUs in April, 2020 reported that both parental presence during 24 h and parental participation in round decreased significantly as 83-52% and 71-32%, respectively (25). The authors also reported that reductions of lactation medicine and/or social work support in 43% of NICUs. The risks mentioned above will be faced along with these measures if precautions are not taken.

Retrospective design of the study is one of the limitations of our study. We could not evaluate the mental health status of the mothers and breastfeeding status after discharge. Strength of the study is evaluating short term morbidities and association with breast milk feeding status in NICU because there are limited studies about these issues.

In our study, breastfeeding, breast milk supply and neonatal outcomes were impaired due to lockdown period at the beginning of pandemic. Understanding of importance of these factors by parents leaded to improvement of breast milk status at discharge. In conclusion, parents should be informed about breastfeeding in prenatal period, after birth and during NICU stay in COVID-19 era as before and precautions should be taken.

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The Association of Parents Feeding Practices and Eating Behaviors of Children Between 2 to 6 Ages in Türkiye

Türkiye'de 2-6 Yaş Arası Çocukların Yeme Davranışları ile Ebeveynlerinin Beslenme Uygulamalarının İlişkisi

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ABSTRACT

Objective: To evaluate the relationship between the feeding practices of parents of preschool children and the eating behaviors of children.

Material and Methods: The parents who had 2 to 6 years of age children without chronic disease and had completed the transition to supplementary foods were included (n=315). Family demographic, socioeconomic information and children's anthropometric measurements were recorded. The parents completed the "Child Feeding Questionnaire (CFQ)", and "Children's Eating Behavior Questionnaire (CEBQ)".

Results: The mean age of the children participating were 46±14.63 months and 46.7% (147/315) of the children were girls. As parents' perceived responsibility for feeding increased, the children's food responsiveness tended to decrease. In parents who had concerns about their child's weight, their children's eating behavior was associated with higher food responsiveness and enjoyment of food and lower satiety responsiveness, slowness in eating, and emotional undereating.

Conclusion: Parents' healthy eating attitudes can be part of a process that encourages children to model healthy eating behaviors. 'Satiety responsiveness', 'slowness in eating', and 'emotional under-eating behaviors were observed more frequently with the attitude of restriction and pressure for eating.

Key Words: Children, eating behavior, feeding, parents

ÖZ

Amaç: Okul öncesi dönemdeki çocukların ebeveynlerinin beslenme uygulamaları ile çocukların yeme davranışları arasındaki ilişkinin değerlendirilmesi.

Gereç ve Yöntemler: Çalışmaya 2-6 yaş arası kronik hastalığı olmayan ve ek gıdaya geçişi tamamlamış çocukları olan ebeveynler dahil edildi (n=315). Ailenin demografik, sosyoekonomik bilgileri ve çocukların antropometrik ölçümleri kaydedildi. Ebeveynler tarafından "Çocuk Besleme Anketi (CFQ)" ve "Çocukların Yeme Davranışı Anketi (CEBQ)" doldurduldu.

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Bulgular: Araştırmaya katılan çocukların yaş ortalaması 46±14.63 ay olup, çocukların %46.7'si (147/315) kız cinsiyettir. Ebeveynlerin beslenme konusundaki algılanan sorumluluğu arttıkça, çocukların gıdaya duyarlılığı azalma eğilimindeydi. Çocuklarının kilosu hakkında endişeleri olan ebeveynlerde, çocuklarının yeme davranışı, daha yüksek gıda duyarlılığı ve yemekten zevk alma ve daha düşük tokluk duyarlılığı, yemede yavaşlık ve duygusal yetersiz beslenme ile ilişkiliydi.

Sonuç: Ebeveynlerin sağlıklı beslenme tutumları; çocukların sağlıklı beslenme davranışlarını modellemesini teşvik eden bir sürecin parçası olabilir. Yeme konusunda kısıtlama ve baskı tutumuyla birlikte 'doymaya duyarlılık', 'yemede yavaşlama' ve 'duygusal yetersiz yeme' davranışları daha sık gözlendi.

Anahtar Sözcükler: Çocuk, yeme davranışı, beslenme, ebeveyn

INTRODUCTION

The first five years of life are a time of rapid physical growth and change. During these early years, children are learning what, when, and how much to eat based on the transmission of cultural and familial beliefs, attitudes, and practices surrounding food and eating (1,2). Previous studies have indicated that a positive family system may be part of a process that establishes and promotes beneficial health behaviors through role modeling, provision of healthy foods, and support for engaging in healthy eating behaviors (3-6).

Parental feeding attitudes can be basically categorized as child-centered and parent-centered. In child-centered feeding attitudes, parents consider their children's expectations, needs, and behaviors while meeting their age-appropriate and tangible needs during meal times. In parent-centered feeding attitudes, there is a high level of parental control and low responsiveness to the children's intangible needs while meeting their tangible needs (7).

As far as we know, children's eating behaviors and their associations with parents feeding practice has investigated in few studies in Türkiye. Two studies conducted in Türkiye have studied the effect of children's eating behaviors and parental feeding style on association with childhood obesity. In the first study, the age group has chosen primary school children and the second was conducted in a small sample of preschool children. A relationship between parents' eating pressure and obesity has been found in both studies (8,9). We aimed to present the parents feeding practices and eating behaviors of preschool children in a larger sample by applying two nutritional attitude questionnaires in the urban location of Türkiye.

MATERIALS and METHODS

This prospective, cross-sectional study was conducted between May and December 2019 in Ankara city in Türkiye. The parents of children who presented to the 'General Pediatrics' and 'Social Pediatrics' outpatient clinics of our hospital were invited to participate. A total of 315 children between the ages of 2 and 6 years were included in the study. Children who were still breastfeeding, had any comorbid diseases or had a parent with a psychiatric illness were excluded. The parents who were eligible and whose written consent was obtained were included in the study. Information forms, questionnaires completion and anthropometric measurements were made by the same researcher.

Assessment Tools

Parents who participated in the study completed forms providing informed consent, family demographic and socioeconomic information (monthly family income), and their children's age and anthropometric measurements. In addition, the parents also completed the CFQ and the CEBQ, respectively. The questionnaire form was completed with a face to face interview. The CFQ was developed by Birch et al. (10) and the Turkish validation and reliability study was conducted by Camci et al. (7) Turkish validation indicated strong support for the dominant seven-factor structure originally proposed by Birch et al. (10) with the resultant seven factors explaining 73.1% of the variance.

The CEBQ was developed by Wardle et al. (11) and the Turkish validation and reliability study was conducted by Yilmaz et al. (12). For the CEBQ Turkish validation according to exploratory factor analysis, eight subscales explain 58.2% of the variance. Reliability coefficients (Cronbach Alphas) ranged from 0.61 to 0.84. Confirmatory factor analysis was calculated as 0.049 according to the Root Mean Square Error of Approximation (RMSEA) index of fitness and this analysis revealed suitability of the scale for the Turkish population. Factor structure, internal reliability, and subscale correlations were similar to the original CEBQ. The CFQ and CEBQ both use Likert-type scales with 5 response options.

The CFQ consists of 21 statements in 5 subscales including 'perceived responsibility', 'monitoring', 'concern about child weight', 'restriction' and 'pressure to eat'. The statements in the questionnaire are answered from one of five sets of response options (scored from 1 to 5 respectively): 'never', 'seldom', 'half of the time', 'most of the time', 'always'. The CEBQ consists of 35 statements in 8 subscales including 'food responsiveness', 'emotional overeating', 'enjoyment of food', 'desire to drink', 'satiety responsiveness', 'slowness in eating', 'emotional undereating', and 'food fussiness'. Response options for all statements are 'never', 'seldom', 'sometimes', 'often', 'always' (scored from 1 to 5 respectively).

Body mass index (BMI) was calculated using the formula: body weight (kg)/(height (m))^2. BMI standard deviation scores

(SDSs) were determined using age- and sex-appropriate growth percentile curves from the Centers for Disease Control and Prevention for children over 2 years of age. Children with BMI SDS below -2 were regarded as thin or underweight, those between -2 and +2 as normal weight, and those above +2 were regarded as overweight or obese (13).

The participants' income level data were compared using hunger and poverty line data announced monthly by the 'Confederation of Turkish Trade Unions'. As the study covered a period of 6 months, comparisons were based on data from June 2019 (14).

Statistical analyses

Based on data presented in the literature, power analysis calculation using e-picos (https://www.e-picos.com/) software showed that for 90% power with 5% probability of type I error, a sample size of 300 was needed.

Data obtained from questionnaires were analyzed using IBM SPSS Statistics version 22.0 software package. Descriptive data were presented using frequency, percentage and mean with standard deviation. Normal distribution of the variables was evaluated using Shapiro–Wilk test. P-values below 0.050 were considered statistically significant. When evaluating the differences between the groups, Mann–Whitney U and Kruskal–Wallis H tests were used for non-normally distributed variables. Standardized z values were given for the Mann–Whitney U test. If significant results were observed in the Kruskal–Wallis H test, the group responsible for the difference was identified with a post-hoc multiple comparison test. Spearman's correlation coefficient (R) was used when analyzing relationships between non-normally distributed variables. Cronbach's alpha reliability coefficient was used to evaluate the reliability of the scales.

The study was planned under the principles of the Declaration of Helsinki and was approved by the Local Ethics Committee of Research Hospital.

RESULTS

315 children (147 girls, 46.7%) were included in the study. The children's mean age was 46.18±14.63 months (median age 44 months, min-max: 26-60), with no significant sex-based difference (p=0.562). The anthropometric values of the participants are presented in table I. The mean BMI of the children participating in the study was 15.28±2.10 (9.83-30.61) kg/m^2. BMI SDS was above +2 in 5.12% (n=16) of the children and below -2 in 8.96% (n=28).

The mother alone was the primary caregiver for 52.1% (n=164) of the children, while both the mother and father were primary caregivers for 41.9% (n=132).

Maternal education level was high school for 30.6% (n=96) and university for 20.4% (n=64) of the mothers; paternal education level was high school for 35.4% (n=111) and university for 22.6% (n=71) of the fathers.

Twenty-four point eight percent of the parents who participated in the study stated that they found their child thin. Among these parents, the BMI value of the child of 23.1% of those who perceive their child as extremely thin is below -2 SDS, while the 76.9% is between -2 and +2 that found to be normal. Similarly, while only 17.9% of the parents who perceive their child as thin has a BMI value below -2 SDS, 82.1% of the child's BMI SDS value is between -2 and +2. Among the parents who participated in the study, 57.1% of those who perceived their children as fat were found to have a BMI SDS value between -2 and +2, while 42.9% of their children had a BMI SDS value above + 2.

The participating parents' responses to the CFQ and CEBQ are shown in Tables II and III, respectively.

In correlation analyses of the relationships between CFQ subscales, 'perceived responsibility' showed weak but statistically significant positive correlations with 'concern about child weight' and 'pressure to eat' (r=0.189 and r=0.234, respectively). A weak positive correlation was also detected between 'restriction' and 'concern about child weight' (r=0.185) and there was a moderate positive correlation between 'restriction' and 'pressure to eat' (r=0.359).

In correlation analyses of the CEBQ subscales are given in Table IV. The children's 'food responsiveness' showed weak to moderate positively correlation with their 'emotional overeating', 'enjoyment of food', 'desire to drink', and 'food fussiness' (r=0.329, r=0.485, r=0.178, and r=0.232, respectively) and was

Table I: Age, gender, weight, height, BMI mean and median values of the children participating in the study

Number (n)	Mean-Median	Minimum-Maximum	SD
Total (n=315) Age (Month)	46.18-44	26-60	14.63
Female (n=147) Age (Month)	46.2-44	24-58	14.96
Male (n=168)	46.15-46	26-54	14.39
Height (cm) (n=315)	102.29-102	70-133	10.67
Weight (kg) (n=315)	16.35-16	9-32	3.83
BMI (kg/m^2) (n=315)	15.53-15.28	9.83-30.61	2.10

SD: *Standard Deviation,* ***BMI:*** *Body Mass Index*

Table II: Distribution of CFQ responses of parents who participated in the study

Perceived responsibility	Always n(%)	Most of the time n(%)	Half of the time n(%)	Seldom n(%)	Never n(%)
When your child is at home, how often are you responsible for feeding her?	97 (30.8)	98 (31.1)	64 (20.3)	37 (11.7)	19 (6)
How often are you responsible for deciding what your child's portion sizes are?	80 (25.4)	120 (38.1)	69 (21.9)	33 (10.5)	13 (4.1)
How often are you responsible for deciding if your child has eaten the right kind of foods?	153 (48.6)	128 (40.6)	25 (7.9)	7 (2.2)	2 (0.6)
Monitoring					
How much do you keep track of the sweets (candy, ice cream cake, pies, pastries) that your child eats?	234 (74.3)	69 (21.9)	7 (2.2)	4 (1.3)	1 (0.3)
How much do you keep track of the snack food (potato chips, Doritos, cheese puffs) that your child eats?	235 (74.6)	66 (21)	5 (1.6)	8 (2.5)	1 (0.3)
How much do you keep track of the high-fat foods that your child eats?	234 (74.3)	65 (20.6)	8 (2.5)	5 (1.6)	3 (1)
Concern about child weight					
How concerned are you about your child eating too much when you are not around her?	75 (23.8)	64 (20.3)	53 (16.8)	45 (14.3)	78 (24.8)
How concerned are you about your child having to diet to maintain a desirable weight?	72 (22.9)	41 (13)	39 (12.4)	34 (10.8)	129 (41)
How concerned are you about your child becoming over weight?	97 (30.8)	48 (15.2)	51 (16.2)	29 (9.2)	90 (28.6)
Restriction					
I have to be sure that my child does not eat too many sweets (candy, icecream, cake or pastries)	268 (85.1)	24 (7.6)	13 (4.1)	4 (1.3)	6 (1.9)
I have to be sure that my child does not eat too many high-fat foods	262 (83.2)	30 (9.5)	9 (2.9)	6 (1.9)	8 (2.5)
I have to be sure that my child does not eat too much of her favorite foods	226 (71.7)	45 (14.3)	22 (7)	7 (2.2)	15 (4.8)
I intentionally keep some foods out of my child's reach	210 (66.7)	43 (13.7)	15 (4.8)	14 (4.4)	33 (10.5)
I offer sweets (candy, ice cream, cake, pastries) to my child as a reward for good behavior	58 (18.4)	96 (30.5)	25 (7.9)	47 (14.9)	89 (28.3)
I offer my child her favorite foods in exchange for good behavior	64 (20.3)	89 (28.3)	18 (5.7)	42 (13.3)	102 (32.4)
If I did not guide or regulate my child's eating, she would eat too many junk foods	165 (52.4)	65 (20.6)	17 (5.4)	20 (6.3)	48 (15.2)
If I did not guide or regulate my child's eating, she would eat too much of her favorite foods	179 (56.8)	68 (21.6)	15 (4.8)	16 (5.1)	37 (11.7)
Pressure to eat					
My child should always eat all of the food on her plate	98 (31.1)	108 (34.3)	38 (12.1)	15 (4.8)	56 (17.8)
I have to be especially careful to make sure my child eats enough	184 (58.4)	78 (24.8)	17 (5.4)	13 (4.1)	23 (7.3)
If my child says `I'm not hungry", I try to get her to eat anyway	58 (18.4)	72 (22.9)	27 (8.6)	30 (9.5)	128 (40.6)
If I did not guide or regulate my child's eating, she would eat much less than she should	158 (50.2)	59 (18.7)	20 (6.3)	19 (6)	59 (18.7)

weakly negatively correlated with their 'satiety responsiveness' and 'slowness in eating' behaviors (r=-0.270 and r=-0.217, respectively). The children's 'satiety responsiveness' was moderately positively correlated with their 'slowness in eating', 'desire to drink' and 'emotional undereating' behaviors (r=0.383, r=0.251 and r=0.330, respectively) and negatively correlated with their 'food fussiness' and 'enjoyment of food' (r=-0.368 and r=-398 respectively).

Correlation analyses of the relationships between CFQ and CEBQ subscales revealed that parental 'concern about child weight' showed weak positive correlation with children's 'food responsiveness' and 'enjoyment of food' (r=0.138 and r=0.210, respectively) and weak negative correlation with their 'satiety responsiveness', 'slowness in eating', and 'emotional undereating' behaviors (r=-0.140, r=-0.129, and r=-0.160, respectively). Parental 'pressure to eat' attitudes were weakly negatively correlated with the children's 'enjoyment of food' (r=-0.191) and weakly positively correlated with the children's 'satiety responsiveness', 'slowness in eating', and 'emotional undereating' behaviors (r=0.172, r=0.204, and r=0.204, respectively). There were also weak positive correlations between parental 'restriction' and the children's 'food responsiveness', 'satiety responsiveness', and 'emotional undereating' behaviors (r=0.194, r=0.130, and r=0.165, respectively). Correlation analyses of the relationships between CFQ and CEBQ subscales are given in table V.

When the CFQ and CBEQ subscales were compared based on family income level, 'pressure to eat' was less common among parents with a monthly income 2.100 TL or lower (hunger line: 2067 TL) compared to parents with a monthly income of 6.800 TL or higher (poverty line: 6733 TL) (chi-square=9.787, p=0.007).

Table III: Distribution of CEBQ responses of parents who participated in the study

	Never n(%)	Seldom n(%)	Sometimes n(%)	Often n(%)	Always n(%)
Food responsiveness					
My child's always asking for food	35 (11.1)	104 (33)	114 (36.2)	46 (14.6)	16 (5.1)
If given the chance, my child would always have food in his/her mouth	142 (45.1)	84 (26.7)	54 (17.1)	25 (7.9)	10 (3.2)
Even if my child is full up, s/he finds room to eat his/her favourite food	96 (30.5)	96 (30.5)	63 (20)	32 (10.2)	28 (8.9)
If allowed to, my child would eat too much	251 (79.7)	39 (12.4)	13 (4.1)	7 (2.2)	5 (1.6)
Given the choice, my child would eat most of the time	147 (46.7)	80 (25.4)	61 (19.4)	17 (5.4)	10 (3.2)
Emotional overeating					
My child eats more when worried	252 (80)	48 (15.2)	12 (3.8)	2 (0.6)	1 (0.3)
My child eats more when annoyed	265 (84.1)	38 (12.1)	6 (1.9)	6 (1.9)	0 (0)
My child eats more when anxious	259 (82.2)	40 (12.7)	13 (4.1)	3 (1)	0 (0)
My child eats more when s/he has nothing else to do	209 (66.3)	52 (16.5)	35 (11.1)	16 (5.1)	3 (1)
Enjoyment of food					
My child enjoys eating	43 (13.7)	64 (20.3)	111 (35.2)	46 (14.6)	51 (16.2)
My child loves food	80 (25.4)	79 (25.1)	101 (32.1)	33 (10.5)	22 (7)
My child finishes his/her meal quickly	123 (39)	88 (27.9)	65 (20.6)	20 (6.3)	19 (6)
My child looks forward to mealtimes	83 (26.3)	92 (29.2)	76 (24.1)	35 (11.1)	29 (9.2)
My child is interested in food	57 (18.1)	71 (22.5)	103 (32.7)	43 (13.7)	41 (13)
Desire to drink					
If given the chance, my child would always be having a drink	40 (12.7)	95 (30.2)	74 (23.5)	57 (18.1)	49 (15.6)
If given the chance, my child would drink contimously throughout the day	79 (25.1)	95 (30.2)	62 (19.7)	42 (13.3)	37 (11.7)
My child is always asking for a drink	102 (32.4)	78 (24.8)	62 (19.7)	43 (13.7)	30 (9.5)
Satiety responsiveness					
My child decides that s/he doesn't like food, even without tasting it	67 (21.3)	63 (20)	87 (27.6)	54 (17.1)	44 (14)
My child refuses new foods at first	47 (14.9)	69 (21.9)	84 (26.7)	63 (20)	52 (16.5)
My child leaves food on his/her plate at the end of a meal	27 (8.6)	67 (21.3)	119 (37.8)	69 (21.9)	33 (10.5)
My child is difficult to please with meals	51 (16.2)	91 (28.9)	81 (25.7)	41 (13)	51 (16.2)
My child gets full up easily	21 (6.7)	39 (12.4)	96 (30.5)	84 (26.7)	75 (23.8)
My child gets full before his/her meal finished	26 (8.3)	52 (16.5)	97 (30.8)	75 (23.8)	65 (20.6)
My child cannot eat a meal if s/he has had a snack just before	30 (9.5)	50 (15.9)	83 (26.3)	68 (21.6)	84 (26.7)
Slowness in eating					
My child takes more than 30 minutes to finish a meal	76 (24.1)	92 (29.2)	67 (21.3)	43 (13.7)	37 (11.7)
My child eats slowly	40 (12.7)	66 (21)	78 (24.8)	63 (20)	68 (21.6)
My child eats more and more slowly during the course of a meal	63 (20)	79 (25.1)	66 (21)	53 (16.8)	54 (17.1)
Emotional undereating					
My child eats less when s/he is tired	37 (11.7)	50 (15.9)	100 (31.7)	68 (21.6)	60 (19)
My child eats less when s/he is angry	56 (17.8)	50 (15.9)	90 (28.6)	58 (18.4)	61 (19.4)
My child eats less when s/he is upset	48 (15.2)	53 (16.8)	93 (29.5)	56 (17.8)	65 (20.6)
My child eats more when s/he is happy	61 (19.4)	61 (19.4)	91 (28.9)	46 (14.6)	56 (17.8)
Fussiness					
My child enjoys tasting new foods	74 (23.5)	85 (27)	97 (30.8)	26 (8.3)	33 (10.5)
My child enjoys a wide vairety of foods	75 (23.8)	90 (28.6)	76 (24.1)	39 (12.4)	35 (11.1)
My child is interested in tasting food s/he hasn't tasted before	74 (23.5)	96 (30.5)	90 (28.6)	26 (8.3)	29 (9.2)

When CFQ and CEBQ subscales were compared based on the BMI SDSs of the children, 'concern about child weight' was lower among the parents of children with a BMI SDS of +2 compared to the other groups (p=0.006). Greater 'food responsiveness' was detected in children with a BMI SDS above +2 compared to those with a BMI SDS below -2 (p=0.040). 'Enjoyment of food' was significantly higher among children with a BMI SDS above +2 compared to the other groups (p=0.001).

In reliability analyses of the scales, the Cronbach's alpha values were above 0.7 for both (0.724 for CFQ, 0.744 for CEBQ).

Table IV: Correlation analysis results of CEBQ subgroups' relations with each other.

	Emotional overeating	Enjoyment of food	Desire to drink	Satiety reponsiveness	Slowness in eating	Emotional undereating	Food fussiness
Food responsiveness							
r	0.329	0.485	0.178	-0.270	-0.217	-0.005	0.232
p	0.001	0.001	0.001	0.001	0.001	0.927	<0.001
n	315	315	315	315	315	315	315
Emotional overeating							
r		0.246	0.065	-0.124	-0.066	0.000	0.188
p		0.001	0.252	0.028	0.245	0.994	0.001
n		315	315	315	315	315	315
Enjoyment of food							
r			0.034	-0.398	-0.377	-0.104	0.409
p			0.545	0.001	0.001	0.065	0.001
n			315	315	315	315	315
Desire to drink							
r				0.251	0.019	0.163	-0.045
p				0.001	0.734	0.004	0.425
n				315	315	315	315
Satiety reponsiveness							
r					0.383	0.330	-0.368
p					0.001	0.001	0.001
n					315	315	315
Slowness in eating							
r						0.291	-0.138
p						0.001	0.014
n						315	315
Emotional undereating							
r							-0.107
p							0.057
n							315

r: *Correlation coefficient,* ***p:*** *Value,* ***n:*** *Number*

DISCUSSIONS

In this study investigating the relationship between parents' child feeding practices and the eating behaviors of their preschool children, it was found that as parents' perceived responsibility for feeding increased, the children's food responsiveness tended to decrease; greater parent concern about child weight was associated with higher food responsiveness and enjoyment of food and lower satiety responsiveness, slowness in eating, and emotional undereating in the children; with more parental restriction, the children's food responsiveness, satiety responsiveness, and emotional undereating behaviors tended to increase; and more pressure to eat from parents was associated with a tendency for higher satiety responsiveness, slowness in eating, and emotional undereating but less enjoyment of food in the children.

Feeding and growth of the child and the expectations of mothers often do not coincide. It is observed that most of the children whose mothers think that they have no appetite or even do not eat at all grow in accordance with their age. We determined that most of the children who were considered underweight by their parents were within normal range for their age too. Also, it was found that the parents of children with obesity had less concern about their child weight, while these children exhibited more food responsiveness and enjoyment of food. It has been reported in several studies that between 32% and 90% of parents misjudge their child's weight (15,16).

The CFQ responses of the parents who participated in our study indicated that more than 70% of parents monitored what their children eat. In a study from the United States, it was observed that in the home environment, parents provided food without asking their children what they want most of the time, and that 85% of parents struggled to get their children to eat more (17).

The use of pressuring feeding practices may provoke or worsen child fussiness, but these practices could equally be a parent's response to child fussy eating. Parental pressure to eat has been shown to increase fussy eating in children at older ages (18). In a study from Sweden, parental restrictive behavior was found to be associated with their concern about their children's weight rather than their children's food preferences (19). In the present study, we found that parents with high perceived responsibility regarding child feeding also had higher concern about child weight and exerted more pressure to eat, and that parents with high concern about child weight and those who exerted high pressure to eat showed more restrictive behavior. Similarly, in a study from Spain conducted to evaluate the validity and reliability of the CFQ, concern about child weight was positively correlated with restriction and pressure to eat (20).

Tablo V: Correlation analysis results regarding the relationship between CFQ and CEBQ subgroups

	Perceived responsibility	Monitoring	Concern about child weight	Restriction	Preassure to eat
Food responsiveness					
r	-0.121	0.053	0.138	0.194	0.029
p	0.032	0.350	0.014	0.001	0.604
n	315	315	315	315	315
Emotional overeating					
r	0.043	-0.004	0.046	0.048	-0.030
p	0.442	0.949	0.411	0.399	0.593
n	315	315	315	315	315
Enjoyment of food					
r	-0.015	0.041	0.210	0.027	-0.191
p	0.788	0.472	0.001	0.637	0.001
n	315	315	315	315	315
Desire to drink					
r	-0.030	-0.029	-0.025	0.071	0.072
p	0.591	0.602	0.665	0.209	0.200
n	315	315	315	315	315
Satiety responsiveness					
r	0.037	-0.006	-0.140	0.130	0.172
p	0.511	0.916	0.013	0.021	0.002
n	315	315	315	315	315
Slowness in eating					
r	0.036	-0.002	-0.129	0.042	0.204
p	0.523	0.973	0.022	0.453	<0.001
n	315	315	315	315	315
Emotional undereating					
r	-0.030	0.075	-0.160	0.165	0.175
p	0.593	0.183	0.004	0.003	0.002
n	315	315	315	315	315
Food fussiness					
r	-0.079	0.003	0.029	-0.039	-0.027
p	0.162	0.959	0.609	0.495	0.637
n	315	315	315	315	315

r: *Correlation coefficient,* ***p:*** *Value,* ***n:*** *Number*

Food responsiveness scale assess children's general appetite for food or desire to eat. Food fussiness scale assess the frequent rejection of both familiar and unfamiliar foods. These eating scales are associated with high energy intake and low nutritional quality. These conditions may be related to obesity. 'Food responsiveness' was positively correlated with emotional overeating, enjoyment of food, and food fussiness, and negatively correlated with satiety responsiveness and slowness in eating were determined. There was positive correlation between satiety responsiveness, slowness in eating, desire to drink, and emotional undereating, behaviors that may be related to low appetite, while these behaviors were negatively correlated with food responsiveness, emotional overeating, and enjoyment of food, behaviors that may be related to strong appetite. In a study from Iceland using confirmatory factor analyses to compare the 4 different models of the CEBQ used in various parts of the world, the strongest correlations detected were the positive correlations between satiety responsiveness and enjoyment of food and between food responsiveness and emotional overeating, as well as the negative correlation between food fussiness and enjoyment of food (21).

Parents are often worried when their child eats very little, does not eat healthy foods like fruits and vegetables, or refuses a meal completely. Often parents find themselves using pressure, force or coercion to try and get their child to finish their meal. This stuation can be the opposite effect to what was intended. The act of being pressured into eating can lead to the development of negative associations with the food, and ultimately dislike and avoidance. İn contrast ıt can also stop children from recognising and responding appropriately to internal signals of hunger and fullness, which can make them more likely to overeat in later life. In our study parental 'pressure to eat' attitudes were weakly negatively correlated with the children's 'enjoyment of food' and weakly positively correlated with the children's 'satiety responsiveness', 'slowness in eating', and 'emotional undereating' behaviors. It has been found that satiety responsiveness, slowness in eating, and emotional undereating are more common and enjoyment of food is less common among the children of parents who pressure them to eat (22,23). In another study, high pressure to eat from mothers was associated with lower enjoyment of food and food responsiveness in children. In the same study, children whose

parents pressured them to eat showed a higher prevalence of eating disorders and were pickier eaters (24).

In a study from the Netherlands that included 4987 children, emotional undereating, satiety responsiveness, food fussiness, and pressure to eat were found to be associated with lower BMI values, while enjoyment of food, food responsiveness, emotional overeating, and restriction were associated with higher BMI values (23). Similarly, in a study from the United Kingdom involving 482 parents with 3-year-old children, satiety responsiveness, slowness in eating, food fussiness, and emotional undereating were associated with lower BMI values, while emotional overeating, desire to drink, food responsiveness, and enjoyment of food were associated with higher BMI values (25). In the present study, it was found that children with obesity exhibited more food responsiveness and enjoyment of food.

We observed that pressuring children to eat was less common among parents with low income compared to parents with high income. In contrast, another study reported that restriction and pressure to eat were less common among parents of higher socioeconomic status (26).

The strengths of our study are as follows; large sample size and in terms of evaluating the data of two feeding questionnaires. This study has certain limitations. Firstly, this was a cross-sectional, situation analysis study. Due to the high number of questions in the questionnaire forms used in our study, although a possible decrease in the respondents' interest level was observed, the forms completed.

Parents' children's attitudes towards eating may affect their children's eating behaviors. As the parental restriction increases, the children's food responsiveness, satiety responsiveness, and emotional undereating behaviors tended to increase. Higher the pressure to eat from parents was associated with a tendency for higher 'satiety responsiveness', 'slowness in eating', and 'emotional undereating' but less enjoyment of food in the children. The data revealing the culturally parents children's eating attitude and behaviors could promote potential early intervention approaches for healthier intake patterns during infancy and very early childhood.

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Evaluation of Epidemiological, Clinical, and Laboratory Findings in Pediatric Patients with IgA Vasculitis (Henoch-Schönlein Purpura)

IgA Vasküliti (Henoch-Schönlein Purpurası) Olan Pediatrik Hastaların Epidemiyolojik, Klinik ve Laboratuvar Bulgularının Değerlendirilmesi

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ABSTRACT

Objective: Immunoglobulin A vasculitis (Henoch-Schönlein Purpura) is the most common systemic vasculitis of childhood involving the skin, joints, gastrointestinal tract, and kidneys, and less frequently affects other systems. In this study, we aimed to evaluate the epidemiologic, clinical, and laboratory findings of pediatric patients with IgA vasculitis.

Material and Methods: In this study, 366 patients diagnosed with IgA vasculitis (Henoch-Schönlein Purpura) in the pediatric nephrology clinic were retrospectively analyzed. Demographic characteristics, clinical findings, system involvement, and laboratory findings were recorded.

Results: Of the patients in the study, 57.9% (212) were male and the male-to-female ratio was 1.37. The most common age group was found to be between 5-9 years of age. A statistically significant correlation existed between age and renal involvement ($p<0.001$). It was found that renal involvement increased with increasing age. Gastrointestinal system involvement was statistically significantly higher in the male gender ($p=0.003$). A statistically significant correlation existed between increased leukocyte counts, gastrointestinal system involvement, and renal involvement ($p=0.001$, $p=0.009$, respectively).

Conclusion: Age and increased leukocyte count were found to be risk factors for renal involvement. Male gender and increased leukocyte count were found to be risk factors for gastrointestinal system involvement.

Key Words: Child, IgA Vasculitis, Gastrointestinal involvement, Henoch-Schönlein Purpura, Renal involvement

ÖZ

Amaç: IgA vasküliti (Henoch-Schönlein purpurası) çocukluk çağının en sık görülen sistemik vasküliti olup deri, eklemler, gastrointestinal sistem ve böbrekleri tutar ve daha az sıklıkla diğer sistemleri etkiler. Bu çalışmada, IgA vaskülitli çocuk hastaların epidemiyolojik, klinik ve laboratuvar bulgularını değerlendirmeyi amaçladık.

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Gereç ve Yöntemler: Bu çalışmada, pediatrik nefroloji kliniğinde IgA vasküliti tanısı alan 366 hasta retrospektif olarak analiz edildi. Demografik özellikler, klinik bulgular, sistem tutulumu ve laboratuvar bulguları kaydedildi.

Bulgular: Çalışmaya katılan hastaların %57.9'u (212) erkekti ve erkek/kadın oranı 1.37'di. En sık görülen yaş grubu 5-9 yaş arası olarak saptandı. Yaş ile böbrek tutulumu arasında istatistiksel olarak anlamlı bir korelasyon vardı (p<0.001). Yaş arttıkça böbrek tutulumunun arttığı saptandı. Gastrointestinal sistem tutulumu erkek cinsiyette istatistiksel olarak anlamlı derecede yüksekti (p=0.003). Lökosit sayısındaki artış ile gastrointestinal sistem tutulumu ve böbrek tutulumu arasında istatistiksel olarak anlamlı bir korelasyon vardı (sırasıyla p=0.001, p=0.009).

Sonuç: Yaş ve artmış lökosit sayısı böbrek tutulumu için risk faktörü olarak bulunmuştur. Erkek cinsiyet ve artmış lökosit sayısı gastrointestinal sistem tutulumu için risk faktörü olarak bulunmuştur.

Anahtar Sözcükler: Çocuk, IgA vasküliti, Gastrointestinal tutulum, Henoch-Schönlein Purpurası, Renal tutulum

INTRODUCTION

Henoch-Schönlein purpura (HSP), newly defined as Immunoglobulin A (IgA) vasculitis, is the most common systemic vasculitis in childhood, involving the skin, joints, gastrointestinal (GI) tract, and kidneys, and less commonly affecting other systems. It is characterized by IgA1 deposition in small-diameter blood vessels, especially in postcapillary venules (1). The cause is not known for certain. It is frequently seen in children between the ages of 3 and 15, and seasonally it is more common in the fall and winter months (2,3). Classification criteria include palpable purpura, arthritis or arthralgia, abdominal pain, renal involvement, or a skin biopsy with predominantly IgA deposition. Palpable purpura is a "sine qua non" among these criteria and is often the first finding (4). In addition, central nervous system (CNS) involvement, cardiac involvement, and pulmonary involvement can also be seen more rarely during the disease. Although IgA vasculitis is generally a self-limiting disease with a good prognosis, in the presence of renal involvement, morbidity, and mortality may increase about the severity of involvement.

This study aimed to retrospectively evaluate the epidemiologic, clinical, and laboratory findings of patients diagnosed with IgA vasculitis.

MATERIALS and METHODS

In this study, the files of 366 patients who were admitted to the Pediatric Nephrology outpatient clinic of Dr. Sami Ulus Obstetrics, Gynecology, Pediatrics Training, and Research Hospital between January 2009 and January 2013 and diagnosed with HSP according to EULAR/PRINTO/PRES criteria were retrospectively reviewed. This Study Dr. Approved by the academic board of Sami Ulus Child Health and Diseases Training and Research Hospital (E-73799008-799-222825118). Patients were identified from automation records and analyzed retrospectively. Patients with insufficient file data were not included in the study. Demographic data (age, gender, anthropometric measurements), physical examination findings at the time of diagnosis, laboratory findings, and treatments administered were evaluated. Age groups are categorized as 2-5 years, 5-9 years, 10-14 years and 15 years and above. Triggering factors (such as previous infection, vaccination, insect bites), region of residence, and season of the disease were investigated. Blood urea nitrogen (BUN), creatinine, complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), antistreptolysin O titer (ASO), complete urinalysis and urine microscopic examination, fecal occult blood values were evaluated. Normal values of leukocyte count according to age, platelet count 150.000-450.000/mm^3, ASO value 0-200 IU/ml, CRP 0-8 mg/l, ESR 0-20 mm/h were considered normal. Skin involvement was determined as palpable purpura, petechiae and ecchymosis. Joint involvement was classified as arthralgia and arthritis. Abdominal pain, including pain occurring within 2 weeks before the onset of palpable purpura, vomiting, hematemesis, hematochezia, melena, or occult blood presence in the feces, acute abdomen, or increased intestinal wall thickness on ultrasonographic examination, is considered significant for GI involvement. Renal involvement includes microscopic hematuria (more than 5 erythrocytes in a centrifuged urine sample at 40 magnification), macroscopic hematuria, proteinuria (spot urine protein/creatine ratio >0.2 mg/mg or >4mg/m^2/hour), nephrotic syndrome (spot urine protein/creatine ratio >2 mg/mg or protein in 24-hour urine >40mg/m^2/hour hypoalbuminemia: 2.5 g/dl, hyperlipidemia and edema), nephritic syndrome (hematuria and/or proteinuria, edema, hypertension, oliguria and azotemia), presence of nephritic-nephrotic syndrome. Indications for renal biopsy were nephrotic proteinuria, persistent hematuria or persistent nephritic proteinuria, and acute kidney injury according to KDIGO (Kidney Disease: Improving Global Outcomes) criteria (7). In order to assess system involvement, the study investigated the influence of age and gender. The relationship between baseline acute phase markers (leukocyte count, ESR, CRP) and system involvement was analyzed. The relationship between patients with GI involvement and other system involvement was evaluated. The type, dose, and duration of treatment were recorded. The treatments administered were grouped as antihistamine treatment, nonsteroidal anti-inflammatory drug (NSAID) treatment, steroid treatment (oral and/or intravenous (IV) bolus), and steroid and other immunosuppressive treatments.

SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were given as numbers and percentages for categorical variables and mean and standard

deviation for numerical variables. For independent categorical variables, Chi-Square was used for pairwise and multiple group comparisons, Fisher's Exact Test was used for pairwise comparisons when the Chi-Square condition was not met, and Monte Carlo Simulation was used for multiple comparisons. Logistic Regression Analysis was used to determine the risk factors affecting involvement. The statistical significance level was accepted as a p-value less than 0.050.

RESULTS

In our study, 57.9% (212) of the patients were male and 42.1% (154) were female, with a male-to-female ratio of 1.37. The most common age group was found to be between 5-9 years of age (n=212, 57.9%). Approximately two-thirds of the patients were diagnosed in the fall and winter months. Possible factors that may play a role as a triggering factor in the etiology of IgA vasculitis were analyzed and upper respiratory tract infection was observed in 168 patients (45.9%). Elevated ASO was found in 50 (32.7%) patients and group A β-hemolytic streptococcus was grown in the throat cultures of 28 (10.1%) patients. Demographic and etiologic characteristics of the patients are summarized in Table I.

All patients had skin involvement. When other system involvement was analyzed, it was observed that joint involvement was the most common (59.7%). This was followed by GI involvement (25.4%), renal involvement (19.9%), scrotal involvement (6.8%), and CNS involvement (3.8%). System involvements are summarized in Table II. Purpura was found in all patients. The rashes were classified according to their anatomical localization. The majority of patients had rashes on the lower extremities (56.8%) and lower extremities and buttocks (30.3%). Joint involvement was present in 59.7% of patients. Arthritis was present in 70.6% of patients with joint involvement. Approximately 75% of the patients had single joint involvement. Ankle joint involvement was most common (64.9%), followed by knee joint (20.1%). Multiple joint involvement was found in 7.8% of patients. GI involvement was present in 25.4% of patients. Fecal occult blood positivity was detected in 59.1% of these patients. The main complaint of patients in this group was abdominal pain. Among patients with GI involvement, 11.8% had intussusception and 1.1% had intestinal perforation. Renal involvement was present in 19.9% of patients. Among patients with renal involvement, 83.5% had hematuria (80.8% microscopic and 2.7% macroscopic hematuria). Proteinuria was present in 66.0 of these patients. Mild proteinuria was found in 41.1%, isolated microscopic hematuria in 27.3%, isolated macroscopic hematuria in 2.7%, nephritic in 17.8%, nephrotic in 8.2% and mixed nephritic and nephrotic syndrome in 2.7%. Renal biopsy was performed in 5.8% of patients with renal involvement. In all of these patients, diffuse mesangial proliferation was detected on light microscopy, and IgA precipitates were detected in the mesangium on immunofluorescence examination. Crescentric involvement was not detected. The characteristics of patients with renal involvement are summarized in Table III. All patients with CNS findings had prolonged headaches. Convulsions were not observed in any of the patients.

Table I: Demographic and Etiologic Distribution of Patients

Patients' Characteristics	n (%)
Sex	
Female	154 (42.1)
Male	212 (57.9)
Age	
2-5 years	48 (13.1)
5-9 years	212 (57.9)
10-14 years	89 (24.3)
15 years and older	17 (4.6)
Season	
Spring	67 (18.3)
Summer	62 (16.9)
Autumn	158 (43.2)
Winter	79 (21.6)
Possible trigger factors	
Upper Respiratory tract infection	168 (45.9)
Surgery	8 (2.2)
Unknown	190 (51.9)

Table II: System involvement of patients

System involvement	n (%)
Skin involvement	366 (100)
Gastrointestinal involvement	93 (25.4)
Scrotal involvement	25 (6.8)
Central nervous system involvement	14 (3.8)
Renal involvement	73 (19.9)
Joint involvement	218 (59.7)

Table III: Distribution of patients with renal involvement

Characteristics of renal involvement	n (%)
Isolated microscopic hematuria	20 (27.3)
Isolated macroscopic hematuria	2 (2.7)
Mild proteinuria	30 (41.1)
Nephritic syndrome	13 (17.8)
Nephrotic syndrome	6 (8.2)
Mixed nephritic/nephrotic syndrome	2 (2.7)

No statistically significant relationship was found between age and joint involvement, GI involvement, scrotal involvement, and CNS involvement. The rates of involvement were similar in all age groups (p=0.448, p=0.103, p=0.577, respectively). However, there was a statistically significant relationship between age and renal involvement, and renal involvement increased with increasing age (p<0.001) (Table IV). The relationship between gender and system attitudes was evaluated and GI involvement was statistically significantly higher in males than in females (p=0.003). The effects of baseline white blood cell counts on system involvement were analyzed. A statistically significant

Table V: The relationship between age and system involvement

System involvement	2-5 years*	5-9 years*	10-14 years*	15 years and older*	p
Gastrointestinal system involvement	1 (11.1)	38 (23.6)	40 (26.0)	14 (33.3)	0.448
Scrotal involvement	2 (22.2)	14 (8.7)	8 (5.2)	1 (2.4)	0.103
Renal involvement	2 (22.2)	14 (8.7)	41 (26.6)	16 (38.1)	<0.001
Central nervous system involvement	0 (0.0)	5 (3.1)	6 (3.9)	3 (7.1)	0.577
Joint involvement	2 (22.2)	99 (61.9)	89 (57.8)	28 (66.7)	0.084

***:** *n(%)*

association was found between patients with increased leukocyte counts and GI involvement and renal involvement (p=0.001, p=0.009, respectively). No significant correlation was found between other system involvement and increased leukocyte values. When the relationship between baseline ESR and CRP values and system involvement was evaluated, no statistically significant relationship was found. The rate of renal involvement was statistically significantly higher in patients with GI involvement (p=0.012). No significant correlation was found between GI involvement and other system involvement.

In terms of the treatments administered, 68.3% of the patients received supportive treatment consisting of anti-inflammatory drugs and/or antihistamines. Oral steroids or IV steroids were administered to 27.1% of the patients. Steroid treatment was administered in patients with severe GI symptoms, significant testicular involvement and renal involvement presenting with nephrotic syndrome. The preferred steroid type was prednisolone. Steroid treatment was given to 73.0% of patients with GI involvement, 60.0% of patients with scrotal involvement, and 29% of patients with renal involvement. IV pulse steroid treatment was given to 3.0% of the patients who were steroid-refractory and continued to have proteinuria. Cyclophosphamide and/or azathioprine treatment was given to 4 patients who were steroid-refractory and continued to have proteinuria after pulse steroid treatment was stopped and interrupted. The results of the renal biopsy were also evaluated.

All patient's recovered with survival. In terms of renal involvement, nephritic proteinuria persisted in only two patients. None of the patients developed chronic kidney disease.

DISCUSSION

HSP, now also known as IgA vasculitis, is the most common type of systemic blood vessel inflammation in children. It is identified by a rash with palpable purpura on the skin, joint pain, stomach issues, and kidney problems, often seen in the lower parts of the body (6,7). In this study, we aimed to evaluate the demographic, clinical, and laboratory findings, treatments administered, and follow-up results of 366 IgA vasculitis cases in our center. As a result of our study, we showed that increased leukocyte count and the presence of GI involvement were risk factors for renal involvement, and the risk of renal involvement increased with increasing age. In terms of GI involvement, we found that increased leukocyte count and male gender increased the risk of GI involvement.

It has been reported that IgA vasculitis is generally observed 1.5-2 times more frequently in males than in females (8). Yang et al. (9) from Taiwan reported a male/female ratio of 1.11 in their study including 2759 children with IgA vasculitis. In our study, 154 (42.1%) of the patients were females and 212 (57.9%) were males and the male/female ratio was similar to that reported in the literature.

The most common age range in which IgA vasculitis is observed is reported to be between 3 and 15 years (10). In our study, the mean age of our patients ranged between 7.95±3.27 years by these data. In studies reported from our country, IgA vasculitis was reported to vary mostly between the ages of 7-10 years and our findings are compatible with the data from our country (11). In the literature, it has been reported that IgA vasculitis is observed more frequently in the fall and winter months (12). However, different seasonal distributions have also been reported; there are also publications reporting that the disease is most frequently seen in spring (13,14). In our study, it was found that the cases were most frequently seen in the fall months (43.2%), followed by the winter months (21.6%).

Although nearly 2 centuries have passed since the description of HSP, now called IgA vasculitis, the etiology is still unclear. Many agents have been blamed for the etiology of IgA vasculitis. The common opinion is that the disease may start after an upper respiratory tract infection and group A beta-hemolytic streptococcal infection (15). The fact that the disease is observed more frequently in the fall and winter months is attributed to the increase in the frequency of upper respiratory tract infections in these seasons (12). In our study, it was found that 45.9% of our patients had a history of upper respiratory tract infection before the diagnosis of IgA vasculitis and seasonally, it was observed mostly in the fall and winter months as previously mentioned. Elevated ASO was found in 50 (32.7%) of the patients with a history of upper respiratory tract infection, and group A β-hemolytic streptococcus was grown in the throat culture of 28 (10.1%) patients. However, no significant relationship was found between this growth and the disease. In the literature, there are studies indicating that streptococcal infections play a triggering role in the etiology of

IgA vasculitis but a definite cause-effect relationship could not be determined (16).

All patients (100.0%) had rash at presentation. Skin biopsy was performed in 19 (5.2%) of the patients at presentation due to atypical clinical findings and atypical rash. Histopathologic examination of the skin biopsy revealed leukocytoclasis on light microscopy and a typical image of IgA deposition on immunofluorescence microscopy. Our findings of skin involvement were similar to the literature (15,17). Joint involvement was found in 59.7% of the patients. Of these patients, 70.6% had arthritis. Joint involvement was transient in all patients and left no sequelae. Our findings regarding the frequency of joint involvement and involved joints were compatible with the literature (8). In our study, the rate of GI involvement was 25.4%. Intussusception was observed in 11 patients with GI involvement and intestinal perforation developed in 1 patient. In the literature, the rate of GI tract involvement is reported to be 50-76% (18). One of the reasons why the number of patients with GI involvement was lower in our study compared to the literature may be that complaints such as abdominal pain, nausea, and vomiting were not questioned sufficiently when GI involvement was questioned. It has been shown in the literature that GI involvement is more common in boys. Karadağ et al.(11) from our country also showed that GI bleeding was more common in boys than in girls. In our study, it was shown that the frequency of GI involvement was higher in boys by the literature. In some studies, the presence of abdominal symptoms and GI involvement were found to be risk factors for renal involvement (11,19, 20). In our study, renal involvement was statistically significantly higher in patients with GI involvement. We think that patients with GI involvement should be followed up more closely in terms of renal involvement.

Scrotal involvement in IgA vasculitis has been reported with a rate of 2.0-38.0% (21). This rate was found to be 6.8% in our study. IgA vasculitis with scrotal involvement constitutes 3.0% of all acute scrotum cases. The acute scrotum is an urgent clinical picture and is in the differential diagnosis of testicular torsion requiring urgent surgical intervention. In acute scrotal involvement associated with IgA vasculitis, treatment is symptomatic. Since the treatment approaches are completely different from each other, scrotal involvement should be evaluated very carefully in boys with IgA vasculitis to avoid unnecessary surgical interventions (22).

Although IgA vasculitis is usually a self-limiting disease with a good prognosis, the most important factor determining prognosis is renal involvement. In our study, renal involvement was observed in 73 patients (19.9%). It has been reported that renal involvement is observed in approximately 20-80% of pediatric IgA vasculitis cases (23,24). The reason for this wide distribution may be related to the differences in the definition of renal involvement in various studies. Microscopic hematuria was found in 80.8% of our patients, macroscopic hematuria in 2.7%, and proteinuria in 66%. Renal biopsy was performed in 21 (5.8%) patients with nephrotic proteinuria, persistent hematuria, and persistent proteinuria. In all patients who underwent renal biopsy, diffuse mesangial proliferation was detected on light microscopy, and IgA precipitates were detected in the mesenchyma on immunofluorescence. In our study, the persistence of proteinuria at nephritic level was found in two patients and no patient developed end-stage renal failure.

Central nervous system involvement has been reported with a rate of 2- 32% in IgA vasculitis; CNS symptoms develop secondary to vasculitis, metabolic changes, bleeding disorders and hypertension developing as a result of renal involvement. Headache, changes in consciousness and convulsions are the most common neurologic findings (25). In our study, the number of patients with CNS symptoms was 14 (3.8%). All patients had headaches that started with the symptoms of HSP and regressed after the active phase of the disease. Convulsions and altered consciousness did not occur.

IgA vasculitis has no specific laboratory findings. Moderate leukocytosis and left shift may be observed in some cases. In our study, leukocytosis was found in 32.2% of the patients. When we investigated the relationship between leukocytosis and system involvement, the relationship between leukocytosis and renal involvement and GI involvement was found to be significant. In the literature, there are studies showing that decreased lymphocyte, increased platelet and increased leukocyte values are associated with the risk of GI bleeding in patients with IgA vasculitis (18,26). Ekinci et al. (27) showed that increased neutrophil count may be associated with severe GI involvement and nephritis. In all these studies, it has been suggested that especially increased neutrophil count may be associated with a more severe immune system response and therefore may be associated with more severe involvement such as GI and nephritis (18,26,27).

In our study, the relationship between the age of the patients and the presence of system involvement was investigated and it was shown that the frequency of renal involvement increased with increasing age and this was statistically significant. In a study reporting multivariate analyses of clinical findings and renal morphologies of IgA vasculitis at the initial stage of the disease, it was reported that renal involvement was higher in older children and adults (28). In another study comparing pediatric and adult patients with IgA vasculitis, it was found that chronic renal failure developed with a frequency of 15.8% in adults and 7.0% in children (9). In another study in which clinical and laboratory findings of IgA vasculitis in adults and children were compared, it was reported that renal involvement was more frequent and more severe in adults (29).

The most important limitation of this study is that it was performed retrospectively and the follow-up period of some of our patients was limited. We think that the most important strength of this study is that it provides important information

due to the high number of patients for a single center and the wide patient distribution profile.

CONCLUSION

In conclusion, we found that GI involvement and advanced age were risk factors for renal involvement in IgA vasculitis. At the same time, a significant correlation was found between the increase in leukocyte count at baseline and GI involvement and renal involvement. We believe that these results will contribute to the definition of clinical and laboratory findings in IgA vasculitis in terms of system involvement, especially GI and renal involvement, and to the evaluation of risk factors.

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Long-Term Effects of COVID-19 on Respiratory Symptoms and Asthma Control in Pediatric Patients with Asthma

COVİD-19 Enfeksiyonu Olan Pediatrik Astımlı Hastaların Uzun Vadede Değerlendirilmesi

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ABSTRACT

Objective: After Covid-19 infection, many patients complain of persistent symptoms. There are limited studies evaluating the long-term consequences of COVID-19, in pediatric patients with asthma. The aim of this study is to evaluate the persistence of symptoms and asthma control in asthmatic children during follow-up after COVID-19 infection.

Material and Methods: Children with asthma who were admitted to our hospital between March 11, 2020, and August 31, 2021, for COVID-19 infection were included. Patients were evaluated for long- term symptoms and asthma control through phone interviews at least 6 months after infection.

Results: Eighty-five children with asthma were evaluated. The median duration of follow-up was 20 months. Patients experiencing symptoms after 1 month, 3 month and 6 month of COVID were 45.8% (n:39), 30.6% (n:26), and 23.5% (n:20) respectively. The most common symptom was cough. No significant relationship was found between time period from the date of COVID-19 to phone call and symptom persistence. Patients adopting a new pet and having stress were significantly higher in group of patients having symptoms at first month. Frequency of being well controlled was not different before and at 1 and 6 month of infection.

Conclusion: This study suggests that respiratory symptoms may persist in pediatric asthma patients for an extended period after COVID-19 infection.

Key Words: Asthma, Child, COVID-19

ÖZ

Amaç: Çalışmanın amacı, COVİD-19 enfeksiyonu sonrası astımlı çocukların persistan semptomlarını ve astım control durumlarını değerlendirmektir.

Gereç ve Yöntemler: Çalışmaya 11 Mart 2020 ve 31 Ağustos 2021 arasında astım tanısı olan ve COVİD-19 enfeksiyonu olan çocuklar alınmıştır. Hastalara telefon görüşmesi aracılığıyla, COVİD-19 enfeksiyonundan en az 6 ay sonra, uzun dönem semptom ve astım kontrolü için değerlendirildi.

Bulgular: Astımlı 58 çocuk değerlendirildi. Ortanca izlem süresi 20 aydı. COVİD-19 enfeksiyonundan 1, 3 ve 6 ay sonra halen semptomu olan hastalar sırasıyla %45.8 (n:39), %30.6 (n:26), ve %23.5 (n:20) olarak saptandı. En sık saptanan semptom öksürüktü. COVİD-19 enfeksiyonu geçirdikleri tarihten telefon görüşmesine kadar olan süre ile semptom persistansı arasında anlamlı ilişki saptanmadı. COVİD-19 enfeksiyonu sonrası 1.ayında semptomu sebat edenlerde anlamlı olarak yeni evcil hayvan sahiplenme ve stresli olma daha fazla saptandı.

Astımda iyi kontrollü olma durumu enfeksiyon öncesi dönem, enfeksiyon sonrası 1. ve 6.ayda anlamlı olarak farklı olmadığı saptandı.

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Contribution of the Authors / Yazarların katkısı*: *TOYRAN M: Conceived of the presented idea. ***CİVELEK E, TOYRAN M and DİBEK MISIRLIOĞLU E:*** developed the theory and performed the computations. ***METBULUT AP, YILMAZ D and KÜLHAŞ ÇELİK İ:*** chose and directed the appropriate patients for the study. ***METBULUT AP and TOYRAN M:*** carried out literature review and verified the analytical methods. ***METBULUT AP:*** mainly wrote the article. ***METBULUT AP* and *TOYRAN M:*** supervised the findings of this study. All authors discussed the results and contributed to the final article.

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Sonuç: Bu çalışma, pediatrik astım tanısı olan çocuklarda semptomların uzun süre devam edebileceğini göstermektedir.
Anahtar Sözcükler: Asthma, Çocuk, COVİD-19

INTRODUCTION

SARS-CoV-2 is a coronavirus responsible for Coronavirus Disease 2019 (COVID-19), which HAS led to a global pandemic (1). COVID-19 causes serious acute respiratory syndromes that can cause significant morbidity and mortality. The spectrum of diseases caused by the coronavirus can range from the common cold to severe acute respiratory syndrome. Asystematic review reported that 1%–5% of individuals diagnosed with COVID-19 were children, and clinical findings were milder in children than in adults (2). According to the Centers for Disease Control and Prevention (CDC), 8.1% of patients infected with COVID-19 are children, and their mortality rate is <0.1% (3). Comorbidities, such as hypertension, chronic obstructive pulmonary disease, diabetes mellitus, and obesity, affect the prognosis of COVID-19 (4).

Asthma is one of the most common chronic conditions among children, and its prevalence in the US was increasing until recently (5). In two studies from Turkey, the prevalence of asthma in children was found to be 8.6%–12.6% between 1994 and 2004, and between March and June 1997 respectively (6,7). In a study conducted at our hospital, 54 (0.87%) of 6205 pediatric patients diagnosed with COVID-19 were found to have asthma (8). According to the CDC, individuals with moderate and severe asthma have an increased risk of being hospitalized for COVID-19 (9). COVID-19 may cause asthma attacks, pneumonia, and acute respiratory disease due to its effects on the nose, throat, and lungs (10).

Few studies have evaluated pediatric patients with asthma during follow-up after COVID-19 infection. As there is a lack of data on the long-term effects of COVID-19 infection on asthma control status and persistent symptoms among children with asthma, we believe that our data are important and can shed some light on this subject. Accordingly, the aim of this study is to evaluate persistence of symptoms and asthma control state of asthmatic children during follow up after Covid-19 infection.

MATERIALS and METHODS

This retrospective study included children with asthma who were admitted to our hospital between March 11, 2020, and August 31, 2021, and were diagnosed with COVID-19 based on reverse transcription-polymerase chain reaction (RT-PCR) tests using nasopharyngeal and throat swabs. The inclusion criteria were having asthma diagnosis, having COVID-19 and being below 18 years of ages.

We collected data from patients' medical records, including medical history, demographic information, asthma evaluation data, asthma medication usage during COVID-19, and asthma control according to the Global Initiative for Asthma Main Report (GINA) criteria (12). Partially controlled and uncontrolled patients were classified as not well controlled for better statistical analysis, and asthma maintenance was evaluated according to GINA criteria (12). Patients' symptoms and treatment at the time of COVID-19 infection were collected from hospital files. Patients who had undergone COVID-19 infection at least 6 months before the study were chosen and evaluation was done according to data at 1st month and data at 6th month, all patients had these data. Follow-up interviews were evaluated by interviewing the patient's parents. A questionnaire created by the authors was used however more detailed questions were asked when needed for a better definition of symptoms.The questions were open-ended. Symptoms occurring in a short time period as a worsening of asthma were defined as "asthma exacerbation" but other symptoms ongoing in most of the days were defined as "symptoms". They were asked whether they had persistent symptoms after their COVID-19 infection (Fever, nausea, headache, vomiting, weakness, abdominal pain, cough, diarrhea, shortness of breath, joint pain, sore throat, runny nose), how long their symptoms had lasted, whether they had any symptoms at the time of polyclinic control, if there were any changes in their asthma treatment following infection, whether they experienced asthma exacerbation, if they had undergone systemic steroid use, or if they had an emergency department visit or hospitalization for asthma after infection or within six months prior to infection. The presence of other possible risk factors for developing symptoms or exacerbation, such as exposure to tobacco smoke, new pet ownership, and differences in home and school environments, were also determined.

The study was approved by Ankara Bilkent City Hospital Ethics Committee (E2-22-1900). Parental consent was received.

Statistical analysis

SPSS 22 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Results were expressed as percentiles (absolute numbers), means and standard deviations, or as medians and interquartile ranges (IQRs) as required. A chi-square test was performed to compare the categorical variables. p-values < 0.050 were considered to be statistically significant.

Patients symptoms after one month, three months, six months, and at the time of the phone call were gathered from these data. Patients who did and did not have symptoms one month following infection were compared for possible risk factors. Asthma characteristics in the periods six months before and six months after COVID-19 infection were also compared. The Mac–Nemar test was used to determine if the patients had well-controlled or non-well- controlled asthma before COVID-19

infection, one month after infection, and at the time of the phone call. The relation of symptom presence and duration between the infection and phone interview were determined using a box-plot test.

RESULTS

Characteristics of the patients

There were 85 COVID-19 patients with asthma in our hospital between March 2020 and August 2021, who we could be reached by phone call for the study. Patients who still had symptoms were invited to the clinic; 35 (41.2%) of them came for evaluation and were examined and treated for their symptoms.

Of the 85 patients, 44 (51.8%) were male. The female/male ratio was 0.93. The median age of the patients was 13 years (IQR: 8–17 years). Forty-four (51.8%) of the patients had concomitant allergic disease, and allergic rhinitis (n:36, 42.4%) and atopy (n:42, 49.4%) were particularly common. The most commonly detected allergen was pollen (n:30, 35.3%). All of the patients had a follow-up period of at least six months.

COVID-19 symptoms and management of these patients during infection

The median age at the first diagnosis of COVID-19 was 12 years (IQR 7–15.5 years). Seventy-nine patients were diagnosed with COVID-19 once, five were diagnosed twice, and only one patient was diagnosed three times. The symptoms of the patients are shown in Table I. Of the patients, 12 (14.1%) were hospitalized, but none required treatment in the intensive care unit. The mean duration of hospitalization was four days (min–max:1–6 days). Only three (3.5%) patients needed oxygen treatment, and 18 (21.2%) were treated with antibiotics and/or antivirals. All of the patients have recovered.

Before contracting COVID-19, 69 (81.2%) of the patients had well-controlled asthma. Twenty-nine patients (34.1%) were on asthma maintenance therapy at step 1, 34 (40%) were at step 2, and 22 (25.9%) were at step 3.

Persistent symptoms after COVID-19 infection

The patients' symptoms after one month, three months, and six months are shown at Table I.

After one month of COVID-19

One month after the COVID-19 diagnosis, 39 (45.9%) of the patients still had symptoms: 29 (93.5%) had respiratory symptoms, while 17 (54.8%) had non-respiratory symptoms. The most common symptom was cough (n:20). The patients' symptoms one month after their COVID-19 diagnosis are shown in Table I.

Comparing patients who did and did not have symptoms one month after infection, the frequency of adopting a new pet and experiencing stress (e.g. due to an important exam) was higher in the patients with persistent symptoms (p=0.003 and p=0.021, respectively). There were no differences in age, gender, atopy status, concomitant allergic disease, asthma control status before infection, asthma treatment before COVID-19, and regular daily asthma maintenance between the groups (Table II).

After six months of COVID-19

Six months after the COVID-19 diagnosis, 20 (23.5%) patients still had symptoms: 19 (95%) had respiratory symptoms, and 12 (60%) had non-respiratory symptoms. The most common

Table I: Reported symptoms by duration of follow-up after COVID-19 diagnosis

Symptoms	During COVID-19 diagnosis, n(%) n:85	After 1st month of COVID-19, n(%) n:39 (45.8)	After 3th month of COVID-19, n(%) n:26 (30.6)	After 6th month of COVID-19, n:(%) n:20 (23.5)
Fever	39 (45.9)	0	0	0
Cough	48 (56.5)	22 (25.9)	16 (18.8)	11 (12.9)
Dyspnea	19 (22.4)	20 (23.5)	15 (17.6)	11 (12.9)
Chest pain	5 (5.9)	11 (12.9)	7 (8.2)	7 (8.2)
Chest palpitation	NA	5 (5.9)	5 (5.9)	4 (4.7)
Activity limiting symptom	NA	11 (12.9)	8 (9.4)	6 (7.1)
Headache	6 (7.1)	12 (14.1)	9 (10.6)	7 (8.2)
Fatigue	15 (17.6)	12 (14.1)	9 (10.6)	7 (8.2)
Loss of smell and taste	3 (3.5)	4 (4.7)	3 (3.5)	3 (3.5)
Anxiety	NA	3 (3.5)	3 (3.5)	2 (2.4)
Vertigo	1 (1.2)	2 (2.4)	1 (1.2)	1 (1.2)
Joint pain	11 (12.9)	1 (1.2)	1 (1.2)	1 (1.2)
Amnesia	NA	1 (1.2)	1 (1.2)	1 (1.2)
Nausea	8 (9.4)	1 (1.2)	1 (1.2)	1 (1.2)

Table II: Evaluating the risk factors in terms of having symptoms after 1 and 6 month of COVID-19

	Having symptoms after 1 month of COVID-19	Not having symptoms after 1 month of COVID-19	p	Having symptoms after 6 month of COVID-19	Not having symptoms after 6 month of COVID-19	p
Age of the patients, mean, years	13.8	10.8	0.148	12.9	10.2	0.599
Male gender, n(%)	17 (43.6)	27 (58.7)	0.195	9 (45)	35 (53.8)	0.489
Having atopy, n(%)	22 (56.4)	20 (43.5)	0.542	9 (45)	33 (50.8)	0.662
Having additional allergic disease, n(%)	22 (56.4)	22 (47.8)	0.822	10 (50)	34 (52.3)	0.857
Patients using asthma maintenance therapy every day regularly, n(%)	17 (43.6)	24 (52.2)	0.321	9 (45)	32 (49.2)	0.926
Patients GINA control assessment before COVID-19, n(%)						
Well controlled asthma	29 (74.4)	40 (87)	0.139	16 (80)	53 (81.5)	1.00
Not well controlled asthma	10 (25.6)	6 (13)		4 (20)	12 (18.5)	
Asthma treatment Step before COVID-19						
Step 1	12 (30.8)	17 (37)	0.147	6 (30)	23 (35.4)	0.002*
Step 2	13 (33.3)	21 (45.7)		3 (15)	31 (47.7)	
Step 3	14 (35.9)	8 (17.4)		11 (55)	11 (16.9)	
Additional risk factors, n(%)						
Patients having school's closed	25 (64.1)	34 (73.9)	0.418	3 (15)	5 (7.7)	0.385*
Patients changing their homes	2 (5.1)	3 (6.5)	0.461*	1 (5)	3 (4.6)	1.00*
Patients adopting new pet	7 (17.9)	0	0.003*	1 (5)	6 (9.2)	0.682*
Patients having a family member smoking at home	7 (17.9)	2 (4.3)	0.073*	4 (20)	5 (7.7)	0.205*
Patients having increased family member living at home	2 (5.1)	1 (2.2)	0.591*	2 (20)	1 (1.5)	0.137*
Patients changing home heating system	1 (2.6)	0	0.459*	1 (5)	0	0.235*
Patients changing school	6 (15.4)	3 (6.5)	0.290*	2 (10)	7 (10.8)	1.00*
Patients changing class at school	6 (15.4)	3 (6.5)	0.290*	2 (10)	7 (10.8)	1.00*
Patients having stress	7 (17.9)	1 (2.2)	0.021*	5 (25)	3 (4.6)	0.16*

*Chi-square test, *Fisher test*

symptoms were cough and dyspnea (n:11). The patients' symptoms six month after their COVID-19 diagnosis are shown in Table I.

Comparing patients who did and did not have symptoms six months after infection, the frequency of experiencing stress (e.g. due to an important exam) and treatment at step 3 before COVID were higher in the group of patients with persistent symptoms (p=0.160 and p=0.002, respectively). There were no differences in age, gender, atopy status, concomitant allergic disease, adopting a pet, asthma control status before infection, asthma treatment step before COVID-19, and regular daily asthma maintenance between the groups (Table II).

Evaluation of persistent symptoms in terms of time between time of assessment and diagnosis of COVID-19 were shown in Figure 1. 25 patient's schools were open during pandemic. Patients having persistent symptomw after 1 and 6 month of COVID-19, were 11 (44%) and 7 (28%) of them respectively. There was no significant differences in terms of having symptoms after 1 and 6 month of COVID-19, and school attendance (p=0.302 and 0.557 respectively).

Assessment of asthma control after COVID-19

Before COVID-19, and one month after COVID-19 infection, there were 69 (81.2%), and 60 (70.6%) well-controlled patients, respectively. Meanwhile, there were 16 (18.8%), and 15 (29.4%) non-well-controlled patients before COVID-19, and one month after infection, respectively.

Of the 69 patients who were well controlled before infection, 71% were still well controlled one month after infection, and 84.1% were still well controlled six months after infection. All of the patients who were not well controlled before COVID-19 were well controlled at the final assessment (p=0.012) (Table III).

Evaluation of the patients' characteristics six months before and after COVID-19

Regular asthma maintenance treatment was more frequent in the six months following COVID-19 infection than in the six months preceding it (p=<0.001). There were no significant differences in terms of hospitalization due to asthma, asthma attacks, or receiving systemic steroid treatment between the groups (Table IV).

Table III: Evaluating the GINA assesment of patients at 1st, 6th and at last assesment

GINA assesment	After 1 month of COVID *Well controlled n(%)	After 1 month of COVID *Not well-controlled n(%)	Total n(%)	p	After 6 month of COVID *Well controlled n (%)	After 6 month of COVID *Not well-controlled n (%)	Total n (%)	p	At phone call at last follow-up of COVID *Well controlled n(%)	At phone call at last follow-up of COVID * Not well-controlled n(%)	Total n(%)	p
Before COVID diagnosis *Well-controlled n(%)	49 (71)	20 (29)	69	0.150***	58 (84.1)	11 (15.9)	69	0.557***	65 (94.2)	4 (5.8)	69	0.012***
Before COVID diagnosis *Not well controlled n(%)	11(68.8)	5 (31.3)	16	0.150***	15 (93.8)	1 (6.1)	16	0.557***	16 (100)	0 (0)	16	0.012***
Total, n(%)	60 (70.6)	25 (29.4)	85	0.150***	73 (85.9)	12 (14.1)	85	0.557***	81 (95.3)	4 (4.7)	85	0.012***

***McNemar test*

Table IV: Comparison of characteristics of patients 6 months before and after the diagnosis of COVID-19

	6 month before COVID-19	6 month After COVID-19	p
Having hospitalization due to asthma, n(%)	12 (14.1)	13 (15.3)	0.585*
Having asthma attack, n(%)	8 (9.4)	14 (16.3)	0.494
Having systemic steroid treatment, n(%)	3 (3.5)	1 (1.2)	0.964*
Patients taking asthma maintenance theraphy regularly everyday, n(%)	41 (48.2)	46 (54.1)	<0.001

*Chi-square test, *Fisher test*

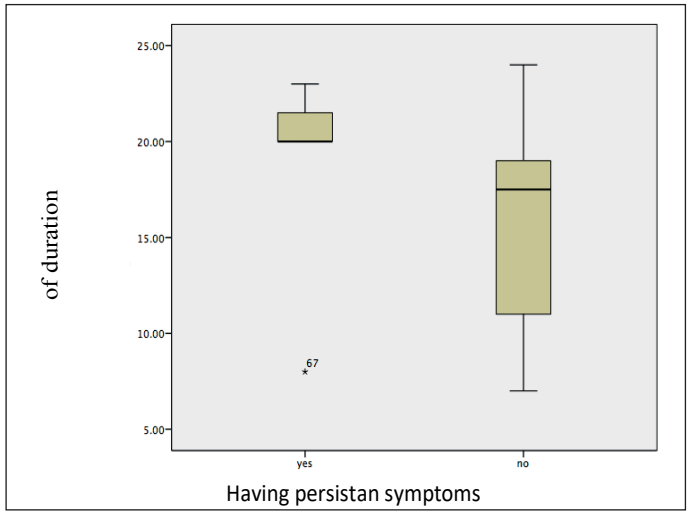

Figure 1: Evaluation of persistent symptoms in terms of time between time of assessment and diagnosis of COVID-19

DISCUSSION

In our study, 85 pediatric COVID-19 patients with asthma were evaluated for persistent symptoms after COVID-19 infection. The mean symptom duration was 4.1 months. Twenty (23.5%) of the patients were observed to have persistent symptoms at the sixth month of COVID-19 recovery. The most common symptom was cough and dyspnea. The frequency of well-controlled asthma did not differ before and one month after infection.

Fatigue, dyspnea, cough, and chest pain have been observed to persist two to six months after COVID-19 infection (13-15). Although it is well known that many patients have persistent symptoms after COVID-19 infection, data on symptom prolongation and asthma control after COVID-19 infection among asthmatic patients are scarce. As far as we could find, Dobkin et al. (15) conducted the only relevant study on children with asthma. They evaluated 29 pediatric patients, 11 of whom were asthmatic, 1.3–6.7 months after post-acute COVID-19 infection (15). Dyspnea, cough, and exercise intolerance were observed in 96.6%, 51.7%, and 48.3% of the patients respectively (16). In our study, 31 (36.5%) patients had persistent symptoms one month after infection, 20 (23.5%) had symptoms six months after infection.

Pet ownership and stress have been found to be higher during the COVID-19 pandemic (16,17). In our study, the numbers of patients who adopted a new pet and experienced stress were significantly higher in the group with symptoms one month after COVID-19 infection. Psychological effects can worsen asthma, and thus life stresses added to pandemic- related stress could negatively affect asthma. The number of patients experiencing additional stress in this study was limited, but individuals with additional stressors and pet owners should be monitored more closely.

COVID-19-related coughing may result from involvement of vagal sensory neurons and/or a neuro-inflammatory response to SARS-CoV-2, resulting in peripheral and central hypersensitivity

of the cough pathways. It is hypothesized that post-COVID syndrome results from a neuro-inflammatory response that affects various regions of the brain, causing chronic fatigue, pain, shortness of breath, and cough (18). A few studies have observed an association between mast cell activation syndrome and the resultant cytokine storm in long COVID. The organ damage caused by such excessive inflammatory response takes much longer to heal and is responsible for the symptoms of long COVID (14,19). The respiratory symptoms of children with asthma after infection may also be exacerbated by the infection.

In a meta-analysis of pediatric patients conducted by Yang et al. (20) , the level of asthma control according to ACT was observed to be significantly improved after COVID-19 infection. Further, a systematic review found increased therapeutic compliance of pediatric asthma patients during the COVID-19 pandemic (21). In our study, the number of well-controlled patients (according to GINA assessment) was observed to be significantly higher at the last follow-up than before COVID-19 infection. Further, regular asthma maintenance treatment was more frequent in the six months following COVID-19 infection than in the six months preceding it. This is likely due to the fact that patients tend to take their medications more regularly during the pandemic because they fear developing a more severe infection as a result of their chronic illness. It is thought that the increased use of masks by these patients and the decreased frequency of exacerbations due to the decrease in viral infections related to compliance with social isolation rules also contribute to the improvement of the control conditions. In our study, while there was no statistically significant difference one month after COVID-19 infection, the status of one-third of the patients changed from well controlled to not

well controlled. Although most children with asthma appear to tolerate COVID-19 infection well in terms of asthma control, this one-third must be considered, and it is crucial to monitor children with asthma closely following COVID-19 infection. This may be due to the long-term effects of COVID-19. More studies are needed on this subject.

The main limitation of this study is that it was a retrospective study. This may have caused errors due to patients' memory. However, during the pandemic, patients were reluctant to come to the hospital for follow-up. Other infections that patients may have had during this period and the symptoms that develop due to these may be confused with post-COVID period symptoms are also the limiting factor of our study. Since there is a limited data on this subject in the literature, our study will contribute to the literature.

CONCLUSIONS

Physicians must be aware of the long-term effects of COVID-19. This study shows that respiratory symptoms may persist for pediatric patients following COVID-19 infection, and one-third of children with asthma may lose control of their asthma. However, with close follow-up these patients can achieve better GINA control than before COVID-19 infection due to improved adherence to their asthma maintenance therapy. Further studies are needed to establish the prognosis of COVID-19 in pediatric asthma patients.

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The Role of Professional Support in Toilet Training

Tuvalet Eğitiminde Profesyonel Desteğin Rolü

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ABSTRACT

Objective: Toilet training is an important step in the development of children. It was aimed to examine the factors affecting the toilet training of children aged 18-36 months and the effect of professional support on training process.

Material and Methods: In the study, data was collected through a questionnaire. Quantitative method was used as the research method in the questionnaire. 214 children were divided into 2 groups (study group: 90, control group: 124) and included in the study. Toilet training in the study group was provided with professional support. In the control group, training was given by the families.

Results: The age of training onset was 25.60 months in the study group and 24.19 months in the control group. The duration of bladder, bowel control and toilet training completion in the study group was spread over time, and it was frequently between 1-30 days in the control group (p=0.001).

Conclusion: Our study showed that it would be appropriate to start training around 24 months and the process would be completed within 2 months. Especially in families that received professional support, the training processes of children were more positive.

Key Words: Child, Social-emotional development, Toilet training

ÖZ

Amaç: Tuvalet eğitimi çocukların gelişiminde önemli bir adımdır. Bu çalışmada 18-36 ay arası çocukların tuvalet eğitimini etkileyen faktörlerin ve profesyonel desteğin eğitim sürecine etkisinin incelenmesi amaçlandı.

Gereç ve Yöntemler: Araştırmada veriler anket aracılığıyla toplanmıştır. Ankette araştırma yöntemi olarak nicel yöntem kullanılmıştır. 214 çocuk 2 gruba ayrılarak (çalışma grubu: 90, kontrol grubu: 124) çalışmaya dahil edilmiştir. Çalışma grubuna tuvalet eğitimi profesyonel destek verilerek sağlanmıştır. Kontrol grubunda ise aileler tarafından eğitim verilmiştir.

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Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Ankara Child Health and Diseases Training and Research Hospital, Clinical Research Ethics Committee with decision number 04.02.2019/ 2019-011.

***Contribution of the Authors / Yazarların katkısı*: KAÇMAZ HC:** 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. **KARA UZUN A:** 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, Reviewing the article before submission scientifically besides spelling and grammar. **YILDIZ AKKUŞ S:** 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. **ÇUHACI ÇAKIR B:** Organizing, supervising the course of progress and taking the responsibility of the research/study, Reviewing the article before submission scientifically besides spelling and grammar. **ÖZAYDIN B:** Organizing, supervising the course of progress and taking the responsibility of the research/study, Reviewing the article before submission scientifically besides spelling and grammar.

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Bulgular: Eğitime başlama yaşı çalışma grubunda 25.60 ay, kontrol grubunda ise 24.19 ay olarak bulunmuştur. Çalışma grubunda mesane, bağırsak kontrolü ve tuvalet eğitimini tamamlama süresi zamana yayılmış olup, kontrol grubunda bu sürelerin sıklıkla 1-30 gün arasında olduğu saptanmıştır (p=0.001).

Sonuç: Çalışmamız eğitime 24 ay civarında başlamanın uygun olacağını ve sürecin 2 ay içerisinde tamamlanacağını göstermektedir. Özellikle profesyonel destek alan ailelerde çocukların eğitim süreçleri daha olumlu geçmektedir.

Anahtar Sözcükler: Çocuk, Sosyal-duygusal gelişim, Tuvalet eğitimi

INTRODUCTION

Toilet training (TT) is the child's ability to independently control the bladder and bowel. This sensitive period is affected by many factors related to the child, family and environment (1). In order to start TT, not only the bladder and bowel control should develop, but the child should also be ready for training in terms of cognitive and psychological aspects. Although this state of readiness varies, it is usually between 18-24 months (2). In this critical period, the attitudes and expectations of the family may affect the development of the child (1). While early and strict training was preferred in the past, two methods particularly preferred today are; they stand out as Brazelton's child-oriented approach and Azrin-Foxx's family-oriented approach. Although both have given successful results, comparative studies have not been conducted. The child-oriented method is recommended by the American Academy of Pediatrics. Other rare methods include Dr. Spock approach, assisted infant toilet training and elimination method (2). Traditional methods such as rewarding, punishing and being a role model are mostly used in Türkiye (3).

Toilet training is affected by many factors such as gender, cultural and socioeconomic characteristics. It has been shown that the age of readiness, onset and completion of TT is earlier in girls. Studies have shown that the readiness time for TT ranges from 22 to 30 months. A relationship between early onset and early completion of education has not been demonstrated (2–4). With cultural differences, the age to training onset is mostly between 18-36 months (5). According to Freud, this period is the anal period and the child uses feces to socialize, so problems in TT negatively affect personality development (6). In addition to age, physiological, psychological and cognitive development are also important factors for initiating TT (7). Although there is no consensus, it is recommended to start training when signs of readiness are observed (1,2,7). Among these signs; development of motor movements, acquisition of coordination, age-appropriate cognitive and language development, explaining the need for the toilet, and signs of socialization (2). From this perspective, social-emotional development becomes even more important (8). Parental readiness is also important for TT. Parents should not directly associate success or failure in TT with child intelligence, should support the child in this process and should not see possible negative results as an attack on their own authority. The educational process should be seen by the family as a process carried out by the child's own interest and motivation. In this respect, it is argued that TT should be discussed with parents starting from the 12th month during child health visits (1).

Many problems can negatively affect TT. Common problems include enuresis, encopresis, constipation, refusal to use the toilet, and turning back (1,8). These are more common in children who are not yet ready for training and who have rigid TT approaches (7,8). Environmental factors such as new sibling, new home, change of caregiver and family conflicts also negatively affect TT (2).

TT studies in Türkiye and analyzes of bladder, bowel control and TT completion times in these studies are limited. It was seen that other studies mostly focused only on TT onset and completion time. In our study, TT processes were examined separately, and the factors that were not studied frequently until today were investigated, and the effects of the professional support provided to children who did not have problems in their social-emotional development on the process were examined. This research is a study that mainly aims to investigate such factors with less research.

MATERIAL and METHODS

This study is a prospectively designed study. The research was carried out in the Social Pediatrics Polyclinic between 01.01.2019 and 31.12.2019. The study was approved by Ankara Child Health and Diseases Training and Research Hospital, Clinical Research Ethics Committee with decision number 04.02.2019/ 2019-011.

In the study, the factors affecting the TT of 18-36 months old children who have no problems in their social-emotional development and the effect of professional support on the process of acquiring toilet training were examined. Inclusion criteria for the study; It was determined as not having received TT, age of the child between 18-36 months, social-emotional development scores within the normal range as a result of the Brief Infant-Toddler Social and Emotional Assessment (BITSEA) scale, and no known chronic disease. Toilet training was given by a child development specialist in the study group, and in the control group in line with the families' own knowledge levels. 90 children constituted the study group and 124 children formed the control group. A total of 214 children were included in the study. Participants volunteered to participate in the study by signing a consent form.

General Information Form and BITSEA was used during the data collection phase. General Information Form is a survey

form consisting of three parts. First part; It is the section that includes socio-demographic characteristics of the family. The second part was asked one month after TT started. In this section, TT onset period (day and night) and the problems in the process were questioned. The third part was administered three months after TT onset. The time to gain bladder and bowel control, and the TT completion time were evaluated in this section. BITSEA was used to evaluate social-emotional development. BITSEA was developed by Briggs-Gowan in 2004, and its Turkish translation and adaptation were made by Karabekiroğlu in 2009 (9).

In study protocol, the first part of the general information form was applied to the participants. After this stage, the participants were divided into study and control groups. This distinction is made according to whether the families want to carry out the TT process with the support of experts. Of the participants, 90 children formed the study group and 124 children formed the control group. Children included in the study group were directed to the child development unit to receive TT. After the first interview, the families were called for control one month and three months after the start of TT and the second and third sections of the general information form were filled. The control group consisted of families who wanted to manage training process themselves. The point to be noted in the control group is that the mothers gave TT in line with their own level of knowledge without any training or support from us. As in the study group, the participants in the control group were contacted by phone in the 1st and 3rd months after the start of TT, and the necessary information for the second and third sections of the general information form was obtained.

The data obtained from the study were evaluated in the SPSS version 22 statistical program. Analyzes were performed at 95% confidence intervals and $p<0.050$ was accepted for statistical significance.

RESULT

A total of 214 children, 90 in the study group and 124 in the control group, were included in the study. The sociodemographic characteristics of the participants are given in table I. The mean age of the children was calculated as 25.81 months in the study group and 27.38 months in the control group. 45.6% of the children in the study group were girls, 54.4% were boys, 48.4% of the children in the control group were girls and 51.6% were boys.

The mean TT onset age was 25.60 months in the study group and 24.19 months in the control group. There is no significant difference between the groups. There was no significant difference between the TT onset age and gender, having a younger sibling, educational status and age of the parents, monthly family income, working status of the mother, and family type. Having problems in the TT process was 13.3% in the study group and 29.1% in the control group. In the study group, the frequency of having problems was found to be significantly higher in those with a high TT onset age ($p=0.042$). There was no significant difference in this area in the control group.

In the study group, those who completed bladder control earlier had a significantly higher TT onset age ($p=0.001$). No similar association was found in bowel control and TT completion times. There was no significant difference between the relevant parameters in the control group. Relevant data are given in table II.

There was no significant relationship between gender, having younger siblings, parental age and education level, monthly income, family type, house type, toilet type, potty use, warming type, time of day/season factors and bladder, bowel control and TT completion times. It was determined that 25% of the families in the control group applied reward, 2.42% applied punishment, and 5.64% applied both punishment and reward. TT completion times were found to be significantly shorter for the participants who received the award ($p <0.050$).

A significant difference was found between the study and control groups in terms of bladder and bowel control times ($p=0.001$). It was observed that the relevant periods were spread over a longer period of time in the study group, while the accumulation was greater between 1-30 days in the control group. In addition, sagging over 60 days was observed to be higher in the control group table III.

DISCUSSION

Toilet training is an important developmental step for children. Every successful step supports the child's self-confidence in this process (10). Many factors affecting TT have been investigated in studies. In our study, besides the factors affecting TT, the effect of professional support on the process was also investigated. There are currently no standardized training models for toilet training. There are differences in the training onset age between countries and sociocultural groups (5). With these differences, it is suggested that the appropriate time to start training is after 18 months, and this approach has been shifted to 24-36 months recently (2,7). The mean TT onset age in Türkiye is 22 months, and training starts at an earlier age compared to developed countries (3). In our study, the mean TT onset age was found to be 24 months. According to this result, it can be thought that the training onset age has increased in our country.

The TT onset age and the educational process are affected by many factors. Studies have shown that signs of readiness for TT develop earlier in girls and there is a significant correlation between late onset of TT and male gender (8). Various studies have not found a significant difference between the TT onset age and gender in Türkiye (11). In our study, while the TT onset age in the control group did not change according to gender, the mean TT onset age in the girls in the study group was

Table I: Socio-demographic characteristics of the participants

	Study group (n:90)	Control group (n:124)	p
Child Age*	25.81±5.51; 25 (18-36)	27.38±4.88; 27 (19-36)	0.032
Gender†			
Girl	41 (45.6)	60 (48.4)	0.333
Boy	49 (54.4)	64 (51.6)	
Siblings*			
Yes	9 (10)	10 (8.1)	0.816
No	81 (90)	114 (91.9)	
Mother Age*	30.21±5.05; 29.5 (17-43)	30.32±4.67; 30 (21-42)	0.870
Father Age*	33.51±4.78; 33(25-47)	34.18±5.99; 33 (24-57)	0.367
Mother Education Status†			
Noneducated	2 (2.2)	3 (2.4)	
Primary	25 (27.8)	37 (29.9)	0.200
Highschool	29 (32.2)	43 (34.6)	
University	34 (37.8)	41 (33.1)	
Father Education Status†			
Noneducated	0	2 (1.6)	
Primary	18 (20)	29 (23.3)	0.583
Highschool	33 (36.6)	59 (47.6)	
University	39 (43.4)	34 (27.5)	
Family Type†			
Nuclear	78 (86.7)	102 (82.3)	0.775
Extended	12 (13.3)	22 (17.7)	
Home Type†			
Apartment	90 (100)	109 (87.9)	-
Detached	0	15 (12.1)	
Warming Type†			
Heater	90 (100)	116 (93.5)	-
Stove	0	8 (6.5)	

***:** *Mean ±SD; Median (min-max) (**Min:** minimum, **Max:** maximum, **SD:** standard deviation),* **†:** *n(%),*

Table II: Relationship between TT onset age and bladder / bowel control and TT completion times

	1-30 day*	30-45 day*	45-60 day*	60 days or more*	Total*	p
Bladder Control Time						0.001
Study Group	27.57±5.17	23.58±4.61	22.40±4.72	0	25.60±5.29	0.264
Control Group	24.64±4.37	22.92±3.80	24.50±3.78	23±3.82	24.19±4.22	
Bowel Control Time						
Study Group	26.94±4.98	25.32±5.49	22.86±4.37	26.33±7.64	25.60±5.29	0.097
Control Group	24.08±4.19	24.64 ±4.79	25.00 ±2.65	23.20 ±3.56	24.19 ±4.22	0.785
TT Completion Time						
Study Group	26.94±4.98	25.32 ±5.49	22.86 ±4.37	26.33 ±7.64	25.60 ±5.29	0.097
Control Group	24.68±4.32	23.67 ±4.30	24.10 ±3.81	23.00 ±3.57	24.19 ±4.22	0.515

***** Mean±SD (***SD:** standard deviation),* ***TT:** toilet training*

found 2 months earlier, but this difference was not statistically significant. The results are similar to other studies conducted in our country on this subject.

Parental variables can affect the TT process. Studies have shown that as the maternal education level increases, the TT onset age also increases. Similar relationship could not demonstrated with paternal education (12). Although there was no significant relationship between parental education levels and TT onset age in our study, it was observed that mothers who had at least 12 years of education in the control and study groups started TT 1.5 months later on average than other mothers. In our study, it was found that the higher education level, the higher the level of knowledge about TT. From this perspective, it can be understood that parents with high education level do not rush the education of their children by following the developmental stages, as suggested. Supporting this, van Nunen et al also stated that as the education level increases, mothers expect signals from children to start TT, and they start later (13).

Socioeconomic factors can also affect TT process (1,2). Studies have determined that as the monthly income decreases, the TT onset age decreases. This relationship has been attributed to the low-income families' desire to avoid diaper costs earlier (14). In our study, no significant relationship was found between monthly income and TT onset age. Most of the participants have middle monthly income is thought to cause this result.

Table III: Comparison of study and control groups in terms of bladder / bowel control and TT completion times

	1-30 day n(%)	30-45 day n(%)	45-60 day n(%)	60 days or more n(%)	Total n(%)	p
Bladder Control Time						
Study Group	47 (35.9)	38 (61.3)	5 (38.5)	0	90 (42.1)	0.001
Control Group	84 (64.1)	24 (38.7)	8 (61.5)	8 (100)	124 (57.9)	0.001
Bowel Control Time						
Study Group	35 (28.2)	38 (60.3)	14 (73.7)	3 (37.5)	90 (42.1)	0.001
Control Group	89(71.8)	25(39.7)	5 (26.3)	5 (62.5)	124 (57.9)	
TT Completion Time						
Study Group	35 (34.7)	38 (49.4)	14 (58.3)	3 (25)	90 (42.1)	0.046
Control Group	66(65.3)	39 (50.6)	10 (41.7)	9 (75)	124 (57.9)	

TT: *Toilet training*

Each child has a unique developmental pattern, so choosing a fixed age to start training or starting training early or late can bring various problems (13). Most of the participants in our study stated that they did not experience any problems in TT processes. In the control group, there was no significant relationship between TT onset age and having problems during TT. In the study group, the participants who had problems during TT started training 3 months later on average than those who did not have any problems. The low number of participants (n: 12, 13%) who had problems in this subject in the study group can be thought to lead to this result. These data should be supported by larger studies.

Methods such as punishment, rewarding and role modeling are preferred among the methods frequently used in Türkiye (2,3). In our study, the children who received a reward had significantly shorter TT completion times than the others. In addition, it was found that TT processes for children who were not awarded were delayed more than 60 days. These findings show that positive reinforcements to the child during training make the process easier and provide a comfort zone for the child. In our study, the mean TT onset age was found 24 months. Although this age is higher than similar studies in Türkiye before, it is similar to that of developed countries. Studies have shown that starting training early can extend the TT completion time (10). In our study, it was observed that the TT onset age was significantly higher in the participants with shorter bladder control time in the study group. In addition, although not significant, the increase in TT onset age, a shortened bowel control time and TT completion times were also observed.

In Türkiye, mean TT completion time was found 6.60 months, and no gender difference was observed in TT completion time (3). In our study, no significant effect of gender was found in the groups in terms of bladder, bowel control and TT completion times. Most of the participants completed the training within 60 days. It is thought that the gain of TT in a shorter time may be related to starting the age range suitable for education.

We found that bladder, bowel control and TT completion time showed a significant difference between the groups. Participants in the study group were more evenly distributed in terms of time, while in the control group there were mostly accumulations between 1-30 days. It can be said that the families who provide education with professional help in the study group expect the child's participation and readiness signs, and do not take a hasty attitude. In addition, more sagging was observed in the control group for 60 days or more. When it was examined in detail, it was seen that half of this delayed group had negative factors such as the presence of new sibling and not wanting to leave the diaper. In addition, the fact that the process was uncomplicated and comfortable in children who received professional support may have made this difference. The importance of professional support and raising the awareness of parents can be understood from the findings.

CONCLUSION

Our study shows that the TT process is not much affected by environmental factors, is a part of the child's normal development and can be gained spontaneously over time. First of all, it was determined that mothers were responsible for the basic care of children in most of the participants. When we look at the maternal education levels, it is striking that the maternal education levels in the control group is higher than the study group. Today is described as the age of technology. Access to information and resources becomes much easier. Particularly, mothers' sharing about the development processes of their children through social media applications, which have recently become a trend, and being in contact with other mothers and expectant mothers, also positively / negatively affected the knowledge level of mothers. These include many issues such as toy selection, supplementary food processes of babies, problems encountered in TT, sleep disorders. Mothers with a high level of education and awareness use this information they have acquired through environmental processes through certain filters. In our study, we did not question the knowledge level of mothers included in the control group about TT, but we left them to the mothers' individual competence. This situation showed us that mothers were able to provide TT to their children even without professional support. In addition, raising the awareness of families with the professional support given to both the family and the child in the working group makes the process more comfortable by responding to the needs of the child more appropriately. Determining the distinctive points

on the variables with wider and detailed researches may cause the content of professional support for TT to be reshaped in the future. Adopting appropriate approaches to children's developmental stages is critical in terms of development and personality traits. Healthcare professionals and parents have a duty to continue this process consciously. In this study, children who received professional support were given education in accordance with their developmental level and thus the child was provided with a more accurate education. On the other hand, we think that the possible oppressive attitudes and wrong practices in the families of children who do not receive support may play a negative role in the psychosocial development of children in the future. According to the results of our study, it is seen that TT are more comfortable and positive, especially by raising the awareness of parents. More comprehensive studies are needed on this subject.

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Toxicological Analysis of a New Fibrin-Derived Dermal Scaffold (Dermoturk); Acellular and Combined with Stem Cells Forms

Fibrinden Türetilmiş Yeni Bir Dermal matriksin (DermoTürk) Aselüler ve Kök Hücreler ile Kombine Formlarının Toksikolojik Analizi

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ABSTRACT

Objective: We aimed to reveal the toxicological analysis of the newly developed fibrin-derived scaffold forms (DermoTurk) before human studies.

Material and Methods: 42 male Albino Wistar rats were used. Two of them were used to produce mesenchymal and epidermal stem cells. Forty rats were divided into five groups, each consisting of 8 rats; the acellular scaffold applied group as Group-1, the mesenchymal stem cells added scaffold used group as Group-2, the MSCs and epidermal stem cells-added scaffold applied group as Group-3, MSCs- and epidermal stem cells-added scaffold applied outbred group as Group-4 and control as Group-5. The changing of laboratory tests in the groups was evaluated five days before application and on the 7th and 40th days. After the autopsy performed on the 40th day of the study, rats' organs and scaffold implanted skin area were evaluated histologically. All the results of the groups were compared. SPSS 22.0 was used for analyses. $p < 0.050$ was accepted as statistically significant.

Results: There were no differences between the groups in terms of laboratory results. Histologically, a mild-grade foreign body reaction against the DermoTurk was found in all groups; this reaction was less in groups 3 and 4 with the richest stem cells.

Conclusion: This study revealed that DermoTurk is safe in rats. It could be an important alternative to skin substitutes, with stem cells or alone. Human studies for clinical efficacy should be carried out.

Key Words: Burn, Fibrin-derived dermal scaffold, Epidermal stem cell, Mesenchymal stem cell, Tissue engineering

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ÖZ

Amaç: Bu çalışmamızda yeni geliştirdiğimiz fibrinden türetilmiş dermal matriksin (DermoTürk) değişik formlarının insan çalışmalarından önce yaptığımız toksikoloji testlerinin sonuçlarını paylaşmayı amaçladık.

Gereç ve Yöntemler: Çalışmada 42 adet erkek Albino-Wistar rat kullanıldı. Bunlardan ikisi mezenkimal ve epidermal kök hücre üretmek için kullanıldı. Kırk tane rat ise her biri 8 denekten oluşan beş gruba ayrıldı; aselüler matriks uygulanan grup (Grup-1), fibrin matriks ile mezenkimal kök hücre (MKH) uygulanan grup (Grup-2), fibrin matriks ile MKH'ler ve epidermal kök hücrelerin (EKH) uygulandığı inbread grup (Grup-3), fibrin matrilks ile MKH'ler ve EKH'lerin uygulandığı outbread grup (Grup-4), kontrol ise Grup-5 olarak belirlendi. Gruplardaki laboratuvar testlerinin sonuçları uygulamadan beş gün önce ve uygulamanın 7. ve 40. günlerinde değerlendirildi. Çalışmanın 40. gününde yapılan otopsinin ardından deneklerin organları ve fibrin matriks implante edilen cilt alanı histolojik olarak değerlendirildi. Grupların tüm sonuçları karşılaştırıldı. İstatistiksel analiz SPSS 22.0 ile yapıldı. $p<0.050$ istatistiksel olarak anlamlı kabul edildi.

Bulgular: Laboratuvar sonuçları açısından gruplar arasında fark yoktu. Histolojik olarak tüm gruplarda DermoTürk'e karşı hafif derecede yabancı cisim reaksiyonu saptandı; bu reaksiyon kök hücrelerin bulunduğu 3. ve 4. gruplarda daha azdı.

Sonuç: Çalışmamız fibrinden türetilen DermoTürk'ün ratlarda güvenli olduğunu göstermiştir. Bu matriks kök hücrelerle veya tek başına diğer deri benzerlerine önemli bir alternatif olabilir. Klinik etkinliliğin gösterilmesi için insan çalışmaları yapılmalıdır.

Anahtar Sözcükler: Yanık, Fibrinden türetilmiş dermal matriks, Epidermal kök hücreler, Mezenkimal kök hücreler, Doku mühendisliği

INTRODUCTION

Despite the significant developments in the treatment methods of burn over the last decades all over the world, it continues to be one of the most important causes of death due to trauma. Still, major skin burns continue to become a health problem that has not been fully solved with the consequences of mortality and morbidity (1, 2). Especially in the case of full-thickness skin burns involving a large part of the body, there is not enough donor, and even if this is achieved, it cannot be reconstructed with all the layers and attachments of the skin. Advances in tissue engineering have increased interest in applying artificial skins obtained by attaching stem cells to different scaffolds (3).

Cellular therapies have emerged as an important development in burn treatment to ensure full-thickness skin formation. The first of these studies was carried out in 1975 when keratinocyte colonies were obtained from human epidermal cells, and it was thought that it could be a good alternative in extensive burns where the autologous skin graft option is insufficient (4). In addition to dermal cells, the recent use of other stem cells, which are essential in regenerative medicine, has revealed a new approach. It is thought that stem cells can provide many therapies for tissue engineering and regenerative medicine thanks to their ability to transform into different cells and their immunomodulatory properties (5).

Mesenchymal stem cells (MSCs) have recently been tested in the treatment of burn wounds as well as in the treatment of many diseases and have been shown to accelerate healing via their capacity to convert into different cells and their paracrine effects as well as their secreted cytokines (TGF-β, IL-10, IL-6), chemokines (CCL2, CCL5, CXCL12), growth factors (VEGF, IGF, FGF, SDF, HGF) (6). MSCs are also used in treating certain immune system diseases due to their immunomodulatory effects. MSCs can be used safely as autologous and allogeneic (7,8).

Although treatment methods using cell suspension alone are simple and reliable for minor burns, they have disadvantages such as prolonged operation time, high cost, and insufficient for extensive burns (9). In addition, the fact that tissue engineering products containing combined fibroblasts contain growth factors such as VEGF, PDGF, IGF-I, TNF, and TNF-β has paved the way for the development of tissue engineering products obtained with synthetic matrices in which human dermal cells are seeded. In animal experiments conducted with these products, an acceleration in the healing of burn wounds, a decrease in infection, and a decrease in the development of hypertrophic scars were observed (10).

Different types of natural polymers can be obtained from various sources for tissue engineering applications for regenerative medicine. Collagen and hyaluronic acid from animal sources and agarose and alginate from algae are among the examples that can be considered. The common feature of all these polymers is that they are soluble in buffer solutions and can be degraded under in-vivo conditions. Collagen, hyaluronic acid, and elastin are essential for skin regeneration (11). The fact that these effective biomaterials are not of human origin appears as an obstacle. At this point, the use of human-derived fibrin stands out. Various studies have demonstrated the regenerative capacity of the fibrin matrix. Full-thickness skin biopsy samples taken after applying the fibrin matrix showed that the fibrin matrix stimulates various cellular changes in the skin. The main changes have been reported as fibroblast activation, new collagen deposits, increased angiogenesis, and stimulation of subepidermal adipocytes (12). Fibrin matrix can have a chemotactic effect on many cell types, such as endothelial cells, epidermal cells, and keratinocytes, thanks to the various growth factors it contains, such as PDGF, TGF-β, VEGF, EGF, and IGF. In addition, these factors can trigger the production of collagen and fibronectin and accelerate angiogenesis (13). These properties allow fibrin-based structures to be combined with skin cells or as fillers or graft holders.

Our group is working on an effective tissue engineering product for the treatment of deep burns, considering the positive effects of stem cells and fibrin matrix on skin regeneration. This study aims to reveal the necessary toxicological data before moving

on to human studies by examining different versions of the product we designed.

MATERIALS and METHODS

Ethics committee approval required for the study was obtained from Diskapi Yildirim Beyazit Training and Research Hospital Experimental Animals Local Ethics Committee (Decision No. 01.03.2012/2). The study was conducted in the Experimental Animals Laboratory of Diskapi Yildirim Beyazit Training and Research Hospital. Forty-two male Albino Wistar rats weighed 250-300 grams were used. Two rats were used to obtain tissue samples for use in the production of stem cells. The other 40 were used in experimental groups. One group was outbred (Group 4), and the remaining four groups were composed of inbred. The subjects were fed in an ad libitum style during the experiment with sufficient standard feed and water.

Collecting tissue samples for cell production

The groin area of one inbred rat, to which general anesthesia was applied by administering ketamine hydrochloride (35mg/kg intramuscularly) and xylazine hydrochloride (5mg/kg intramuscularly), was shaved, and this area was cleaned with 10% polyvinylpyrrolidone iodine solution (Polyod ®, Drogsan, Türkiye). An incision was made in the groin, and 1 cm of fat tissue was removed for MSC production. Then, 20 hair follicles were removed with the FUE (Follicular Unit Extraction) technique using the microsurgical motor. The tissue samples were placed in Ringer's Lactate solution containing 1% Penicillin, Streptomycin, and Amphotericin B 50 mcg/mL. Tissues were transferred to Acıbadem Labcell Istanbul laboratory on the same day in a transport bag at 2-8 ˚C temperature.

Culture of Adipose Tissue-Derived Mesenchymal Stem Cell

After the mechanical dissection of the fat tissues by splitting with scalpel, 25% collagenase (Sigma Aldrich, C6885) was added and incubated in a 37 ^{0}C medium. After incubation, PBS was added to the tube (Biological Industry, BI02-023-1A) and the resulting mixture was centrifuged at 800 G for 5 minutes to decompose the cells. The cells obtained were cultured within T-150 flasks of DMEM LG (Biological Industries, 01-050-1A) containing 1% antibiotic (Biological Industries, BI03-031-1B) and 10% fetal calf serum (FBS) (Biological Industries, 01-050-1A) with 5% CO_2, 7% O_2 and 37 ^{0}C medium. The medium was changed every 3 days and the cells were passaged when the cells covered 70% of the flask base. The cells were removed from the flask surface using trypsin (Biological Industries BI03-054-1B) and 0.5 ml of FBS was added to neutralize the enzyme. These cells were collected and transferred to a tube and added with PBS (Biological Industrires BI02-023-1A) and centrifuged at 400 G for 10 min to wash out. Once the washing procedure was repeated, the cells were trypsin free and the cells were resuspended in the same medium and under the same conditions. The medium was replaced for every 3 days with the 70% of the flask base, the passage was repeated and the cells were replicated. After second passaging, cells were removed with trypsin to be implanted into the fibrin scaffold, and after washing procedure, a sample was taken for quality control tests and flow-analysis analysis (Figure 1: A).

Preparation of epidermal stem cell suspension

The hair follicles were taken into a tube containing 25% collagenase (Sigma Aldrich, C6885) and incubated in a 37 ^{0}C medium for half an hour. After incubation, the cells were centrifuged for 5 minutes at 800 G by adding PBS (Biological Industries, BI02-023-1A). After centrifugation, cells deposited in the lower part of the tube were cultured within T-150 flasks of DMEM LG (Biological Industries, 01-050-1A) containing 1% antibiotic (Biological Industries, BI03-031-1B) and 10% fetal calf serum (FBS) (Biological Industries, 01-050-1A) with 5% CO_2, 7% O_2, and 37^{0}C medium. The medium was changed every three days, and the cells were passaged when they covered 70% of the flask base. Passages were carried out as described in the MSCs passages. After the second passaging, the sample was collected from this passage for quality control and flow analysis (Figure 1:B).

Flow cytometric analysis

Specific monoclonal antibodies were used as CD34, CD45, CD90, HLA-DR, CD105, and CD73 cell surface antigens for the mesenchymal stem cells, and CD200, CD34, CD45, CD271, CD29 (ß1 integrin), and Nestin cell surface antigens for the epidermal stem cells. The cells were taken up into the test tube, and 10 µl of the fluorescent isothiocyanate (FITC), phycoerythrin (PE), and alloficocyanine (APC) conjugated monoclonal antibodies and isotype controls were added, then was incubated for 45 minutes at room temperature in a light-protected environment. After incubation, the cells were resuspended in 400 µl wash solution and analyzed by the FACSDiva ® program in the BD FacsCanto-II flow meter (Figure 2).

Differentiation tests of mesenchymal stem cells

Separated mesenchymal stem cells were cultured with chondrocytes (ThermoFisher Scientific, StemPro™ Chondrogenesis Differentiation Kit A1007101), fat (ThermoFisher Scientific, StemPro™ Adipogenesis Differentiation Kit, A1007001) and bone (ThermoFisher Scientific, StemPro™ Osteogenesis Differentiation Kit A1007201) mediums for evaluation of differentiation capacities. A staining procedure was performed on the 14th day of culture for chondrocyte and fat differentiation and on the 21st day for bone differentiation. For the determination of chondrocyte differentiation, toluidine (Fisher Scientific, 92-31-9), for bone differentiation, alizarin-Red (Sigma Aldrich, Alizarin-Red Staining Solution, TMS-008), and for fat differentiation, oil-Red (Sigma Aldrich, O0625-25g) dyes were used (Figure 1: D,E,F).

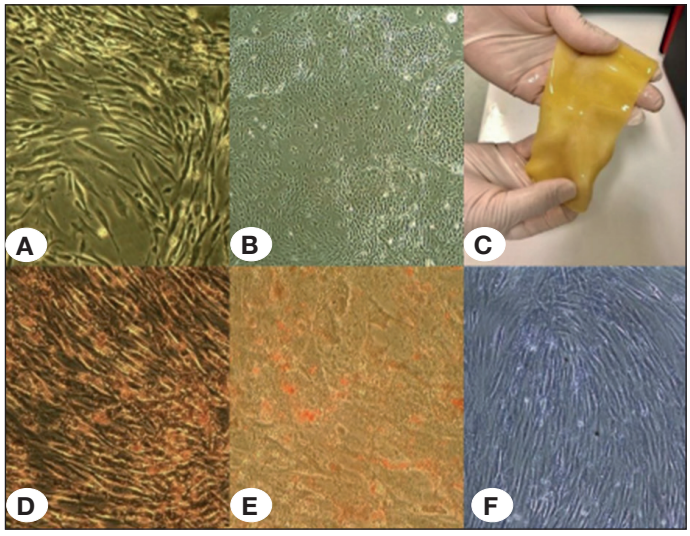

Figure 1: **A);** Appearances of the mesenchymal stem cells in culture medium at the end of the second passage (10X), **B);** Invert microscopic views of the epidermal stem cells in culture medium at the end of second passages (5X), **C);** The view of DermoTurk 00 at the end of the production. In D, E, F, the picture of the mesenchymal stem cells' differentiation tests are seen. **D);** The fat cells were shown by staining with Oilred, **E);** bone cells with Alizarin and **F);** cartilage cells with Toluidine.

Marking the cells

MSCs and ESCs were labeled with the Q-Dot technique to distinguish them from the rat's cells. Before Q-Dot labeling, the cells were sampled for flow cytometric analysis to measure labeling efficiency. Components A and B were shaken gently in the Qtracker Cell Labeling Kit (ThermoFisher Scientific Q Tracker® 605 Cell Labeling Kit). For every $1x10^6$ cell to be labeled, 10 µl of components A and B were added into a tube and incubated for 5 minutes at room temperature. Subsequently, 0.2 ml of fresh medium was added for every $1x10^6$ cells, and the mixture was vortexed for 30 seconds, then cells were counted. At the end of the one-hour incubation period, fresh medium was added to the cells and centrifuged at 400 G for 10 minutes to wash out. This process was repeated, and the samples were taken for flow cytometric analysis. Marked and unmarked cells were analyzed in flow cytometry, and the marking rate was determined.

All tissue samples were stained with DAPI (4′,6-diamidino-2-phenylindole) and then examined with a fluorescent microscope to track Q-Dot positive cells in the tissue.

Preparation of Fibrin Matrix (DermoTurk)

Five frozen human cryoprecipitates, were thawed and then distributed equally into 50 ml conical tubes. Portioned cryoprecipitates were irradiated at 25 kgy and sterilized by the gamma irradiation. Sterile cryoprecipitate was distributed as 10 ml into each of the six-well plate wells. Calcium Gluconate Levulinate (Calcium Picken 10% ampoule, Adeka Pharmaceutical Company, Türkiye) was added as 10% of the amount of cryoprecipitate into each well, and 1% of Tranexamic acid (Transamine® 10% Ampoul, Actavis Pharmaceutical Company, Türkiye) was added and incubated for 37 hours. It was incubated for approximately 5 minutes until it solidified in an environment with a temperature of 100 °C. The matrix thus obtained was treated for stabilization. After an hour of stabilization, the fibrin matrix (DermoTurk) was ready for application (Figure 1: C).

Preparation of fibrin matrices containing MSCs and epidermal stem cells

Q-Dot marked cells were planted in the fibrin matrix at $3x10^6$ cell/cm^2 in the Petri dishes (TPP, 93060). In group 1, no cells were added to the fibrin matrix. In group 2, only MSCs were added to the fibrin matrix. In group 3, MSCs and ESCs were added to the fibrin matrix and applied to inbred rats. In Group 4, a fibrin matrix loaded with MSCs and ESCs was applied to outbred rats. Phenol red-free DMEM LG (Biological Industries, 01-050-1A) containing 1% antibiotics (Biological Industries, BI03-031-1B) and 10% FBS (Biological Industries, 01-050-1A) was added. In groups 3 and 4, the height of the medium was reduced to 1 mm on the 6^{th} day. Thus, air contact was achieved by reducing the height of the medium, and keratinocyte differentiation was induced with 20% O_2 pressure.

Monitoring glucose consumption and lactic acid production

To show that the cells planted in the fibrin matrix were alive and active, samples were taken from the fibrin matrix culture medium at 0, 4, 8, and 24 hours, and glucose and lactic acid measurements were made with the ADVIA 1800 Chemistry System. Thus, glucose consumption and lactic acid production rates were obtained.

Transport of the products

All prepared fibrin matrix forms were washed three times with Ringer's Lactate solution and put into Petri dishes containing Ringer's Lactate. Finally, the products were transported at 2-8 0C in heat-sealed carrying cases, delivered to the application center within 6 hours, and applied within 24 hours.

The design of the groups

In the study, the effectiveness and toxicological effects of only fibrin matrix, MSC-loaded fibrin matrix, ESC-loaded fibrin matrix, and MSC+ESH-loaded fibrin matrices were examined. At the beginning of the study power analysis was done by a statistical expert. According to this analysis, subjects were divided into 5 groups, each consisting of 8 rats. The groups were designed as shown below

1. Group 1 (Acellular fibrin matrix scaffold): Scaffold applied without cells (DermoTurk 00).
2. Group 2 (MSCs-added fibrin matrix scaffold) (DermoTurk 01).

3. Group 3 (MSCs- and ESCs-added fibrin matrix scaffold): Inbred group in which MSCs and ESCs added (DermoTurk 02).
4. Group 4 (Outbred group): DermoTurk 02 is applied allogeneically.
5. Group 5 (Control group): The subjects were opened with 1 cm incision on their backs and closed without any procedure.

Laboratory tests

Five days before the scaffold implantation, one cc of blood was taken from the tail veins, and basal laboratory values were obtained. Similar to previous studies, 7th-day laboratory results were examined for acute effects, and 40th-day laboratory results and autopsy findings were reviewed for long-term outcomes. Therefore, blood was retaken on the 7th, and 40th days after the procedure. On the 40th day, 0.5 cc cerebrospinal fluid (CSF) was obtained. On the same day, rats were sacrificed, and samples were taken for pathological examination from the skin area where the matrix was applied and the brain, lung, heart, liver, spleen, kidney, pancreas, small intestine, and testicle.

Hemoglobin (Hb), hematocrit (Hct), white blood cell (WBC), platelet count (Plts), sodium (Na^+), potassium (K^+), calcium (Ca^{+2}), chlorine (Cl), glucose (Glu), albumin (Alb), alanine aminotransferase (ALT), creatine (Cre), alkaline phosphatase (ALP) were examined from the venous blood, and Na, Cl, glucose and protein levels from CSF samples. Results are summarized in Table I.

Histopathological examination

Following the autopsy performed on the 40th day of the study, the skin where the scaffolds were implanted was evaluated histopathologically regarding inflammation, granulation, foreign body reaction, and the status of implanted cells. The samples taken from the brain, lung, heart, liver, spleen, kidney, pancreas, small intestine, and testis were examined, and the groups were compared with the control group.

Application of DermoTurk series

Under general anesthesia (described above), the dorsum of the rats was shaved and cleaned with 10% polyvinylpyrrolidone iodine. Then, a 1 cm long vertical midline incision was made. The prepared products were implanted under the skin of the rats according to the created groups. In the rats in the control group, a 1 cm long vertical incision was made and closed without inserting anything inside. The incisions were sutured with 3/0 Prolene (Propylene ®, Doğsan, Turkey). Wound dressing was made daily with 10% polyvinylpyrrolidone iodine solution for a week (Figure 3: A).

Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences, version 22.0 (SPSS Inc., Armonk, NY, IBM Corp., USA). Categorical variables were displayed as numbers and percentages, and numerical variables were expressed as mean and standard deviation or median (IQR-Interquartile Range). The Shapiro-Wilk test was used to examine whether the data were normally distributed. Hgb, Hct, Plt, WBC, Na^+, Albumin, Calcium, and Glucose values obtained on days 0.7, and 40 were normally distributed, while the other variables were not normally distributed. Differences between groups were examined with one-way analysis of variance (ANOVA) for those with normal distribution. When a difference was found, Dunnett's t-test was applied to determine the source of the difference. For variables that were not normally distributed, the Kruskall-Wallis test and the Bonferroni test were used as post-hoc tests. $p < 0.050$ level was accepted as statistically significant.

RESULTS

Flow cytometric analysis of ESCs performed with the FACSDiva ® program, CD200, CD29 (β1 Integrin), CD271, K15, and Nestin antibodies were found to be positive, and CD34 and CD45 antibodies were negative (Figure 2: A,B,C,D,E). In viability tests, it was determined that the cells were 90% alive and 90% stained with Q-Dot. The viability of cells in MSC-loaded fibrin matrix and MSC&ESC-loaded fibrin matrices was analyzed. Following cell transplantation, glucose, and lactic acid rates were determined in the samples taken into the culture medium at intervals for 24 hours. According to glucose consumption and lactic acid production, cells planted in the fibrin matrix were shown to be alive and active.

Flow cytometric analysis of MSCs produced from the adipose tissue of rats, performed with the FACSDiva ® program on the BD Facs Canto-II flow cytometry device, CD73, CD90,

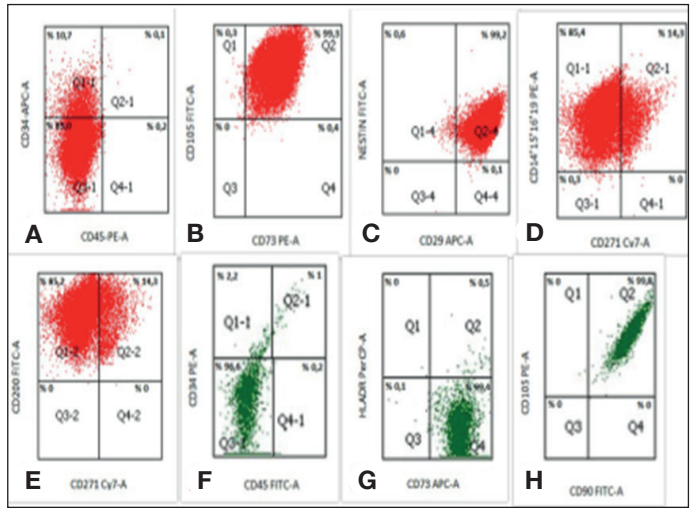

Figure 2: Histogram images showing surface antigens of epidermal stem cells and mesenchymal stem cells in flow cytometry analysis. **A, B, C, D, E);** Surface antigens belonging to epidermal stem cells including CD29 (β1 integrin), CD14, CD15, CD16, CD19, CD73, CD105, CD200, CD271 and Nestin were found positive whereas CD34 and CD 45 were negative. **F, G, H);** In mesenchymal stem cells, CD73, CD90 and CD105 surface antigens were positive whereas CD34, CD45 and HLADR surface antigens were negative.

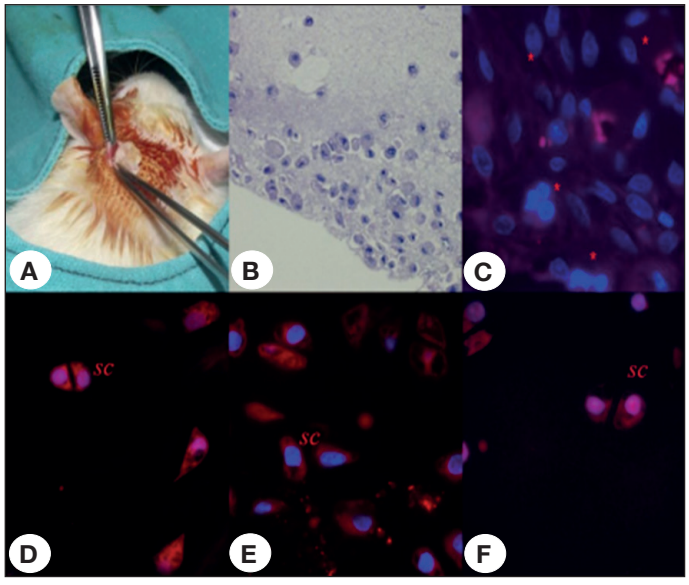

Figure 3: **A);** Implantation of the biomatrix subcutaneously to the rats' skin. **B);** Appearance of the stem cells (sc) in H&E staining within DermoTurk 02 (X20) in the sample taken immediately before application. **C, D, E, F);** In the immunofluorescence examination, it was observed that the stem cells located in the subcutaneous biomatrix were still alive at the day of 40th and were colonized there. **C);** Qdot positive cell (MSC) is not observed in the samples taken from rats in Group 1. The cells seen (*) are their own cells. Q-dot positive stem cells (sc) were seen in **D);** rats in Group 2, **E);** rats in Group 3 and **F);** rats in Group 4. Q-dot positive cells are marked with arrow.

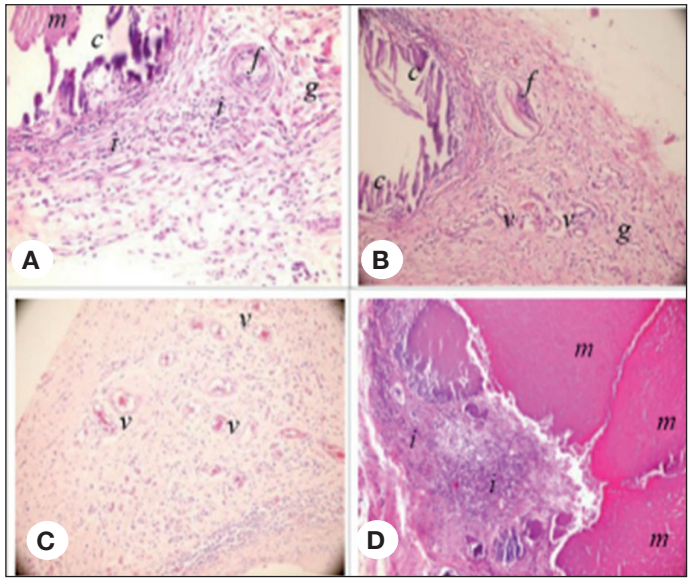

Figure 4: In the samples taken on the 40th day of the application, an increase in inflammation, granulation tissue formation, and foreign body reaction were observed in the vicinity of the biomatrix. **A, B);** Around matrix (m), calcification foci (c), diffuse inflammatory cell deposition, granulation formation (g) and foreign body reaction (f) were observed. **C);** More neovascularization (v) is observed in the groups in which the matrix given by epidermal stem cells and mesenchymal stem cells (DermoTurk 02) is applied. This indicates an increased recovery process. **D);** A more intense inflammatory cell increase (i) was observed around the non-cellular matrix (m).

Table I: Comparison of laboratory values of blood samples taken from tail veins of subjects 5 days before starting of the study

Variables	Group 1	Group 2	Group 3	Group 4	Group (Control)	Reference values	F	X^2	p
Hgb*	14.4±0.5	14.2±0.6	14.3±1.1	14.4±0.8	13.6±1.2	11-19.2	1.233	-	0.315‡
Htc*	44.9±2.1	47.4±6.7	44.9±3.9	45.4±2.1	42.3±5.1	39.6-52.5	1.400	-	0.254‡
Plt*	790000±137721	734000±300802	776375±218406	939875±133087	816625±111871	500-1.3x10^6	1.292	-	0.292‡
WBC*	8751±1703	7577±1766	10994±2127	6736±2581	8983±2488	6-18x1000	4.453	-	0.005‡
Na*	141.8±2.9	141.8±1.9	144.3±2.9	143.3±2.8	142.5±2.0	140-150	1.409	-	0.251‡
Alb*	3.3±0.2	3.4±0.1	3.3±0.2	3.2±0.1	3.3±0.2	3.8-4.8	0.862	-	0.496‡
Ca*	10.2±0.3	10.3±0.4	9.8±0.2	9.7±0.3	10.1±0.5	8-13	3.590	-	0.015‡
Glu*	303.3±49.7	321.1±65.2	249.9±38.7	324.1±54.4	260.3±51.6	50-160	3.460	-	0.017‡
K†	5.45 (0.50)	5.70 (1.63)	5.00 (0.68)	5.60 (0.93)	4.90 (1.35)	4.3-5.6	-	7.597	0.108§
Cl†	101.00 (3.75)	102.50 (5.25)	100.50 (3.75)	102.50 (6.50)	99.50 (4.50)	95-115	-	7.980	0.092§
Alt†	54.00 (14.25)	56.00 (24.75)	61.50 (21.75)	72.00 (11.75)	68.50 (24.75)	35-80	-	13.890	0.008§
Cre†	0.46 (0.07)	0.52 (0.10)	0.47 (0.10)	0.50 (0.05)	0.45 (0.13)	0.5-1	-	5.730	0.220§
Alp†	169.00 (57.25)	156.00 (22.25)	184.50 (80.00)	203.00 (86.75)	205.00 (120.75)	62-230	-	4.129	0.389§

*****$\bar{x}$±SD, †Median (IQR), ‡***One-way ANOVA test was used;*** *the Dunnett t test was used for post-hoc comparisons,* §***Kruskal-Wallis test was used;*** *the Mann-Whitney test with Bonferroni correction was used for post-hoc comparisons.*

and CD105 antibodies were found to be positive, and HLA-DR, CD45, and CD34 antibodies were negative (Figure 2: F, G, H). In viability tests, it was determined that the cells were 95% alive and 92% stained with Q-Dot. It was observed that MSCs could differentiate into chondrocytes, fat cells, and bone cells in the appropriate environment. At the end of the differentiation analysis, fat cells were stained with Oil red, bone cells with Alizarin, and cartilage cells with Toluidine (Figure 1: D, E, F).

Laboratory results

Biochemical values in all groups (5 days before the start of the study, 7th, 40th days) were evaluated. The results of the study

groups (group 1,2,3, and 4) were compared with the control group (Group 5) and then compared each other.

The biochemical values of 5th day before procedures and their comparisons within groups were shown in Table I. According to this, there was no difference between all groups in terms of Hgb,Htc, Plt, Na^{+}, Alb, K^{+}, Cl^{-}, Cre and ALP levels. However, there were statistically significant differences between the groups in terms of WBC, Ca^{+2}, Glucose and ALT values

Table II: Biochemical values obtained from rats on the 7th day of the study

Variables	Group 1	Group 2	Group 3	Group 4	Group 5 (Control)	Reference values	F	X^2	p	group number‖	p
Hgb*	14.1±0.5	13.8±0.3	13.9±1.3	13.9±0.5	13.0±1.3	11-19.2	1.840	-	0.144‡	–	–
Htc*	44.2±2.6	43.9±1.1	44.6±3.7	43.1±2.9	41.7±5.1	39.6-52.5	0.901	-	0.474‡	–	–
Plt*	814625±60469	817000±105287	677000±121604	838429±113979	778875±75971	500-1.3x10⁶	3.367	-	0.020‡	–	–
WBC*	9033.8±2535.0	7553.8±1111.5	11755.0±2740.7	7128.6±945.1	7790.0±1706.1	6-18x1000	7.335	-	<0.001‡	3	0.001‡
Na*	141.8±1.4	141.0±1.3	141.6±1.8	138.7±1.4	140.6±0.9	140-150	5.740	-	<0.001‡	4	0.039‡
Alb*	3.5±0.2	3.5±0.1	3.4±0.2	3.2±0.1	3.4±0.2	3.8-4.8	1.970	-	0.121‡	–	–
Ca*	10.5±0.4	10.7±0.3	9.9±0.4	9.5±0.2	10.2±0.5	8-13	12.697	-	<0.001‡	4	0.002‡
Glu*	N/A	N/A	N/A	N/A	N/A	50-160	N/A	-	N/A	–	–
K†	4.80 (1.05)	4.70 (0.38)	4.90 (1.78)	5.60 (0.90)	5.50 (0.55)	4.3-5.6		14.614	0.006§	2	0.016§
Cl†	102.00 (3.00)	101.50 (2.00)	102.00 (1.50)	100.00 (2.00)	100.00 (3.50)	95-115		8.517	0.074§	–	–
ALT†	49.50 (14.75)	56.50 (15.25)	62.00 (13.25)	71.00 (9.00)	72.50 (35.00)	35-80		15.044	0.005§	1	0.008§
Cre†	0.42 (0.05)	0.40 (0.06)	0.39 (0.15)	0.39 (0.02)	0.40 (0.06)	0.5-1		3.752	0.441§	–	–
ALP†	123.00 (52.50)	129.50 (34.25)	179.50 (60.75)	219.00 (74.00)	190.00 (63.25)	62-230		11.202	0.024§	–	–

*$\bar{x}$±SD, †Median (IQR), ‡**Oneway ANOVA test was used;** *the Dunnett t test was used for post-hoc comparisons,* §**Kruskal-Wallis test was use;** *the Mann-Whitney test with Bonferroni correction was used for post-hoc comparisons.* ‖ *Group compared whit the control group.*

Table III: Biochemical values obtained from rats on the 40th day of the study

Variables	Group 1	Group 2	Group 3	Group 4	Group 5 (Control)	Reference values	F	X^2	p	group number‖	p
Hgb*	14.4±1.1	14.6±1.2	16.5±1.9	14.5±1.4	13.4±2.0	11-19.2	3.567	-	0.016‡	3	0.003‡
Htc*	45.8±2.9	47.9±5.1	54.1±6.3	49.1±4.8	45.6±7.0	39.6-52.5	2.716	-	0.047‡	3	0.022‡
Plt*	696125±110988	624625±197373	588333±83691	703857±257882	723000±330419	500-1.3x10⁶	0.475	-	0.754‡	–	–
WBC*	9022.5±3078.6	5882.5±2104.5	7713.3±2799.7	6551.4±1569.1	5665.0±1578.6	6-18x1000	2.906	-	0.037‡	1	0.022‡
Na*	141.1±2.2	142.5±0.8	141.5±2.6	142.7±4.3	142.6±1.4	140-150	0.650	-	0.631‡	–	–
Alb*	3.6±0.2	3.6±0.2	3.6±0.2	3.4±0.1	3.3±0.3	3.8-4.8	3.903	-	0.011‡	1. 2	<0.050‡
Ca*	N/A	N/A	N/A	N/A	N/A	8-13	N/A	-	N/A	N/A	N/A
Glu*	278.3±62.0	288.0±91.2	269.0±36.2	275.0±101.1	215.8±84.3	50-160	1.036	-	0.404‡	–	–
K†	5.10 (3.00)	8.40 (2.35)	7.30 (4.00)	7.35 (2.55)	5.20 (3.05)	4.3-5.6	-	8.138	0.087§	–	–
Cl†	100.00 (3.00)	101.00 (1.50)	101.50 (2.50)	103.00 (2.50)	102.00 (2.75)	95-115	-	6.613	0.158§	–	–
Alt†	68.00 (44.00)	109.00 (100.25)	87.50 (51.50)	114.00(251.50)	94.50 (58.00)	35-80	-	5.371	0.251§	–	-
Cre†	0.44 (0.10)	0.50 (0.09)	0.47 (0.17)	0.49 (0.14)	0.47 (0.12)	0.5-1	-	2.412	0.661§	–	–
ALP†	103.00 (52.00)	108.00 (56.75)	126.00 (29.75)	167.00 (46.00)	183.00 (71.00)	62-230	-	14.283	0.006§	1. 2	<0.05§

*$\bar{x}$±SD, †Median (IQR), ‡**Oneway ANOVA test was used;** *the Dunnett t test was used for post-hoc comparisons,* §**Kruskal-Wallis test was used;** *the Mann-Whitney test with Bonferroni correction was used for post-hoc comparisons,* ‖ *Group compared whit the control group.*

Table IV: Biochemical values of the rats cerebrospinal fluids on the 40th day of the study

Variables	Group 1	Group 2	Group 3	Group 4	Group 5 (Control)	F	x^2	p
Cl*	124.6±1.7	123.3±2.3	122.0±1.4	123.6±2.8	123.9±2.6	1.277	-	0.299‡
Na†	156.0 (4.0)	155.5 (1.8)	154.5 (3.0)	156.0 (1.0)	156.0 (2.8)	-	4.456	0.348§
Glu†	86.5 (11.0)	81.0 (11.5)	83.5 (7.5)	85.0 (10.0)	84.5 (24.5)	-	1.255	0.869§
Protein†	42.5 (4.6)	42.6 (12.4)	38.3 (8.0)	41.0 (4.0)	40.6 (10.1)	-	3.780	0.437§

**$\bar{x}$±SD, †Median (IQR), ‡Oneway ANOVA test was used, the Dunnett t test was used for post-hoc comparisons, §Kruskal-Wallis test was used, the Mann-Whitney test with Bonferroni correction was used for post-hoc comparisons.*

Tablo V: Histological examination results of rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5 (Control)
Inflammation and granulation rates Available/n	8/8	4/8	6/8	2/8	0/8
Distribution according to the degree of inflammatory reaction					
0	1	4	1	6	8
1	3	1	3	1	–
2	2	2	2	–	–
3	2	1	2	1	–
Distribution according to the degree of granulation reaction					
0	1	4	1	6	8
1	1	3	4	–	–
2	3	1	2	1	–
3	3	–	1	1	–

(p=0.005, p=0.015, p=0.017 and p=0.008 respectively) (Table I). While the change in these laboratory values is statistically significant, the change in values is within the limits of normal references laboratory values and is clinically meaningless. These increases were attributed to the sudden increase in sympathetic activity, which resulted from acute stress.

Results of post-procedure 7th day's were shown in Table II. Compared with the control group, ALT values of group 1 (p=0.008) and the K^+ values of Group 2 were lower (p=0.016). The WBC counts of Group 3 were higher than the control group (p=0.001). Both Na+ values (p=0.039) and Ca^{+2} values (p=0.002) of Group 4 were lower than the control group. While the change in these laboratory values is statistically significant, the change in values is within the limits of normal references laboratory values and is clinically meaningless. In terms of other values, there were no statistical differences between all the other groups and the control group. When compared among themselves, there were statistically significant differences between the groups regarding Plt, WBC, Na^+, Ca^{+2}, K^+, ALT and ALP values. However, all values except for Na^+ values of Group 4 were within normal references values. The Na^+ values of Group 4 were less than the reference values.

Results of the 40th day were shown in Table III. Compared with the control group; there were statistical differences in term of WBC, Alb, and ALP values of Group 1 (p=0.022, p<0.050, p<0.050 respectively); Alb and ALP values of Group 2 (p<0.050, p<0.050 respectively) and Hgb and Htc values of Group 3 (p=0.003, p=0.022 respectively). However, the values of all groups were within acceptable limits.

The results of CSF on the 40th day were shown in Table IV. In the statistical analysis there were no statistically significant differences between the groups and the control group.

Histopathological results

MSC&ESC-loaded fibrin matrix was examined macroscopically (Figure 1: C) and microscopically with Hematoxylin&Eosin staining (Figure 3: B). The fibrin matrix maintained its integrity, and the transplanted cells maintained viability.

At the end of the study, the most typical finding in the histopathological examination was that there was a mild-grade foreign body reaction in almost all groups against the scaffolds, but this reaction was observed to be less in the stem cell-rich groups (groups 3 and 4). Increased inflammation, granulation formation, and foreign body reactions were observed in the vicinity of the biomatrix (Figure 4: A,B,C,D). It was more pronounced in allogeneic group (group 4) (Table V). In the immunofluorescence, the stem cells in the fibrin matrices implanted under the skin were still live on the 40th day and to be colonized here (Figure 3: D,E,F). No histopathological changes were observed in the other tissues. The results of the histological examination are summarized in Table V.

DISCUSSION

The ultimate goal of tissue engineering for artificial skin is to produce a self-renewable, functional skin similar to all layers of the skin and all skin appendages, including hair follicles, sweat glands, and neurosensory structures. Because conventional treatments do not produce the desired results in treating burns, scientists use tissue engineering techniques to make artificial skin. In this sense, one of the first milestones was carried out in 1981 by O'Connor et al.(14). They used cultured epithelial autografts (CEA) obtained by the proliferation of autologous keratinocytes in the laboratory on an excessively burned child (14). However, it is not preferred because the preparation of keratinocyte culture takes a long time; during this time, the risk

of infection increases, and the produced skin is very fragile; the success rate in third-degree burns is low, is an expensive method, the skin does not appear in the desired appearance (15).

The effectiveness of many cell types has been examined in tissue engineering applications. Studies have shown that MSCs positively affect wound healing and tissue regeneration thanks to their secreted trophic factors. Adipose tissue-derived MSCs have stem cell-specific surface markers CD90, CD105, CD73, CD44, and CD166 and low expression of CD45 and CD34. They have a collagen production capacity and are genetically and morphologically more stable in long-term cultures, with low senescence and high proliferation capacity (16).

Various materials have been used as scaffolds in the production of artificial skin. However, the desired result has not been obtained. Allograft skin grafts from cadavers or volunteers have long provided temporary coverage in burn patients. The most significant disadvantage is rejection due to the immunogenic structures. Another significant disadvantage is the risk of transport of some infections from the donor to the recipient (17). While many studies have been conducted with different scaffold samples in many centers, most scaffold structures studied are synthetic biomaterials, and some are allogeneic cadaveric. For these reasons, we believe that a fibrin-derived scaffold is a considerable alternative for skin loss conditions such as full-thickness burns because it can be easily, quickly, and autologously prepared.

Sclafani applied a platelet-rich plasma-derived fibrin matrix to the skin for the first time in 2010. After the application to 15 patients with deep nasolabial folds, it was reported that it stimulated fibroblasts thanks to the growth factors contained in the fibrin matrix, ensured collagen deposition in the dermal-subdermal connections, and statistically significant decreases were observed in wrinkle scores at the end of 12 weeks (18). In another study examining the effectiveness and safety of fibrin matrix application for aesthetic purposes for treating facial depressions, nasolabial folds, and scars, Sclafani followed 50 patients for ten months. He reported that the swelling in all patients disappeared within five days at most, only minimal bruising was observed, and most patients were satisfied with the treatment. It was reported that the fibrin matrix was safe and effective (19).

Although the fibrin-derived matrices have been shown to be more effective in the treatment of full thickness burns than the control group, this effect was not found to be sufficient. However, it is highly probable that fibrin matrices are more effective after being combined with stem cells because these cells increase their efficacy (20).

Gentile et al. applied a fibrin matrix in a mixture with adipose-derived stromal vascular fraction cells to patients with burn sequelae and post-traumatic scars. They compared them with the group in which they used only adipose-derived stromal vascular fraction cells. It was observed that the reconstruction rate was 69% in the fibrin matrix with the Adipose-derived stromal vascular fraction cells application group, and this rate was 39% in the adipose-derived stromal vascular fraction cells only group (21). Autologous limbal cells were seeded on fibrin structures and applied to the corneas of 152 patients with ocular burns for various reasons. The study showed that severely damaged human cornea could be successfully treated using limbal cells combined with a fibrin-derivated matrix (22). Considering all these results, our group believes that the effectiveness of a fibrin-based matrix can be increased by supporting it with autologous stem cells. A completely autologous skin substitute would be more effective.

In this study, we aimed to evaluate DermoTurk, an acellular matrix we developed, and its cellular forms in terms of toxicity before preclinical studies. Although no systemic toxicity was found in the cellular and acellular fibrin matrix forms, pathological examination revealed foreign body reaction, inflammation, and granulation in all forms in the subcutaneous area where it was applied. The most likely reason for this was thought to be excessive membrane phospholipids from the cryoprecipitate used to prepare the fibrin matrix. We considered the most likely reason of this to be the dense membrane phospholipids from the xenogeneic platelet-rich plasma used to prepare DermoTurk (23). Therefore, depending on the applied matrix, an acceptable rate of inflammation, granulation, and foreign body reaction localized to the applied area was considered reasonable. However, it is noteworthy that this reaction is less in the cellular forms of the product, especially in biomatrices richer in stem cells. We think the most likely reason for this is the immunosuppressive effect of the MSCs and skin stem cells (24).

One of the critical findings of this study is that on the 40th day after implantation, allogeneic stem cells can maintain their presence under the skin. Previous studies have shown that allogeneic MSCs can continue to be functional in the host tissue for five weeks without tissue adherence, with a small number of long-term host tissues (25,26). This finding is vital in developing fully human-derived skin substitute biomaterials in patients with sudden skin requirements.

In conclusion, the toxicological examination of DermoTurk forms revealed they are safe. Therefore, it can be considered to be used in the development of new products. It is thought that clinical efficacy studies of DermoTurk forms should be conducted.

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Multisystem Inflammatory Syndrome in a Child Presenting with Acute Hemiparesis as a Rare Neurologic Manifestation

Nadir Bir Nörolojik Bulgu Olarak Akut Hemiparezi ile Başvuran Çocukta Multisistem İnflamatuar Sendrom

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ABSTRACT

The coronavirus pandemic has emerged as one of the most significant global health crises, affecting people worldwide and resulting in the loss of millions of lives. It was officially named "Coronavirus disease 2019" (COVID-19) when it first appeared at the end of 2019. Multisystem Inflammatory Syndrome in Children (MIS-C) is a relatively new disease entity that has arisen in the wake of the COVID-19 pandemic. While MIS-C is recognized to manifest with various symptoms, our understanding of it continues to evolve as more articles and case reports are published in the scientific literature. Although MIS-C affects multiple organ systems, there have also been reported cases of neurological involvement. According to the literature, cases of hemiparesis without imaging findings in MIS-C have rarely been reported. To the best of our knowledge, this is a rare reported case of hemiparesis without intracranial and spinal pathology in the context of MIS-C reported in this article.

Key Words: Acute hemiparesis, Child, COVID-19, MIS-C, Neurologic manifestation

ÖZ

Koronavirüs pandemisi tüm dünyayı etkisi altına alan ve milyonlarca insanın ölümüne neden olan en önemli hastalıklardan biri haline gelmiştir. 2019 yılının sonunda başladığı için Koronavirüs hastalığı-2019 (COVİD-19) olarak adlandırılmış ve çocuklarda Multisistem İnflamatuar Sendromu (MIS-C), COVİD-19 pandemisi sonucu ortaya çıkan, yeni bir hastalık tanımı olarak literatürde yerini almıştır. MIS-C'nin birçok farklı semptoma neden olduğu bilinmesine rağmen, literatürde daha fazla makale ve vaka sunumu yayınlandıkça bilgimiz katlanarak artmaktadır. MIS-C birçok sistemi etkilemekle birlikte çeşitli nörolojik tutulumlar da bildirilmiştir. Literatüre göre MIS-C'de görüntüleme bulgusu olmaksızın hemiparezi olgusu nadiren bildirilmiştir. MIS-C tanısında intrakraniyal ve spinal patoloji olmaksızın nadir olarak görülen hemiparezi vakası bu yazıda bildirilmiştir.

Anahtar Kelimeler: Akut hemiparezi, Çocuk, COVİD-19, MIS-C, Nörolojik belirtiler

INTRODUCTION

Coronaviruses (CoV) are pathogens that affect both humans and animals. In late 2019, a new variant emerged in China, officially named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (1). COVID-19 in most children is typically asymptomatic and less severe than in adults (2). In children with COVID-19, the most common symptoms are cough and fever (3). As our understanding of COVID-19 continues to evolve, we now know that SARS-CoV-2 can rarely progress to MIS-C (Multisystem Inflammatory Syndrome in Children). The diagnostic criteria for MIS-C involve fever lasting at least 24 hours, laboratory evidence of inflammation, involvement of two or more organ systems, hospitalization, and exposure to a COVID-19 case within one month of symptom onset (4). Symptoms are associated with dysfunction in affected

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systems, which can include conjunctivitis, rash, gastrointestinal symptoms, myocardial dysfunction, and fever (5). While MIS-C affects various organ systems, there have been reports of neurological involvement, which has been documented in 6% to 58% of MIS-C patients (1,2). To the best of our knowledge, there have been rare reported cases of hemiparesis with MIS-C without magnetic resonance imaging findings. In this report, we describe a rare case presenting with hemiparesis without intracranial and spinal pathology in the context of MIS-C.

CASE REPORT

An 11-year-old patient was admitted to the hospital with a severe headache in the frontal region, which was not accompanied by any other findings such as photophobia, double vision, speech disturbances, dizziness, memory loss, or confusion, was unaffected by light and sound, and did not respond to analgesics. She had never experienced a similar headache before. Fundus examination and cranial Computed Tomography (CT) were normal. Three days later, the patient developed a high fever, and a COVID-19 test returned positive. Her initial symptoms included vomiting, severe headaches, conjunctival hyperemia, and maculopapular rashes on her extremities. During her illness, weakness developed in her left upper and lower extremities, leading to a neurological deficit, and her motor strength was assessed as 3/5 according to the MRS scale. Her vital signs were generally normal, except for a fever of 38.7°C. Tendon reflexes were normal and symmetrical. There was no pain, warmth, swelling, or tenderness in the extremities. No sensory deficits were found in the comprehensive neurological examination.

According to her medical history, two months prior, her uncle had tested positive for COVID-19, but the COVID-19 PCR tests for her entire family had returned negative. There was no family history of diagnosed headaches, such as migraines.

Laboratory examinations revealed the following results: Alanine transaminase is 279 U/L (0–55), and aspartate transaminase is 290 U/L (5–34). Serum electrolytes, coagulation factors, and complete blood counts were within normal ranges. Acute phase reactants (AFR) showed elevated levels: C-reactive protein 36 mg/L (0–5), procalcitonin 0.58 µg/L (<0.5), ferritin 510.52 µg/L (4.63-204), D-dimer 1595 mg/L (0–275), and fibrinogen 175 mg/dL (180-350). Immunoglobulins (Ig) were assessed before immunoglobulin therapy: IgA 1.69 g/L, IgG 29.76 g/L, IgM 1.45 g/L, and total IgE 1506 IU/mL. Cranial Magnetic Resonance Imaging (MRI), including diffusion tensor and susceptibility-weighted imaging (SWI), revealed no abnormalities to explain the hemiparesis.

A diagnosis of MIS-C was established based on several findings. The patient had ongoing fever, involvement of at least two organ systems (maculopapular rash and conjunctival hyperemia as dermatological involvement and elevated liver enzyme levels as gastrointestinal involvement), elevated AFR, and evidence of recent SARS-CoV-2 infection. Echocardiography to assess myocardial function was normal. Chest radiography and tomography showed no abnormalities. The patient was initiated on MIS-C treatments, including favipiravir, intravenous immunoglobulin (2 g/kg), aspirin (80 mg/kg), antibiotics, and corticosteroids (2 mg/kg). By the third day of follow-up, her weakness had completely resolved, and no motor deficits remained. The therapies were gradually tapered and eventually discontinued. No additional issues were observed during the three-month outpatient follow-up.

DISCUSSION

COVID-19 has rapidly spread worldwide since late 2019. While it primarily affects the pulmonary system, neurological symptoms have become increasingly recognized as part of the clinical spectrum of the disease. In addition to the well-documented symptoms of dysgeusia and anosmia in the neurological system, myalgia, headaches, and dizziness have become common in the early stages of infection (6, 7). A study revealed that 57% of adult COVID-19 patients experienced various neurological symptoms, with myalgias, headaches, dizziness, and anosmia being the most common, along with myopathy, cerebrovascular diseases, seizures, and movement disorders (7). In a retrospective case series of 214 hospitalized adult COVID-19 patients, neurologic manifestations were observed, including central nervous system manifestations (headaches, acute cerebrovascular disease, and seizures), peripheral nervous system manifestations (anosmia, nerve pain), and skeletal muscular injuries. More severe infections were associated with more life-threatening symptoms (8).

In contrast, neurological manifestations in children with COVID-19 are relatively rare. A systematic review of 3.707 pediatric patients found nonspecific neurological symptoms like fatigue, headache, and myalgia in 15.6% of patients and specific neurological manifestations, such as seizures, encephalopathy, and meningeal signs, in 1% (9).

In April 2020, reports emerged of a condition in children resembling toxic shock syndrome (5). Subsequently, MIS-C, a rare complication of COVID-19, was identified (10, 11). The main clinical manifestations of MIS-C encompass the cardiovascular, dermatological, neurological, respiratory, and renal systems (10–15). In a case series study involving 27 children with MIS-C, four had neurological manifestations, including headaches, encephalopathy, muscle weakness, cerebellar signs, and reduced reflexes, and they exhibited MRI abnormalities such as splenium signal changes (16). In a study of 1,695 patients with MIS-C, 365 (22%) showed documented neurologic involvement, with 81 having underlying neurologic

disorders. Transient manifestations occurred alongside other life-threatening symptoms, including cerebral edema, stroke, severe encephalopathy, central nervous system infection, or demyelination (17). Children with MIS-C appeared to have a higher prevalence of severe neurological symptoms than COVID-19 (17, 18). In a retrospective study, encephalopathy and left hemiparesis were reported in a 10-year-old patient with MIS-C who also had sickle cell anemia. Brain CT/MRI images revealed right frontal intraparenchymal hemorrhage and infarction (18).

However, in the case of our patient, no pathology was detected in the cranial CT and MRI, including diffusion tensor imaging Although many studies have reported weakness as a neurological involvement in MIS-C patients, a rare case of hemiparesis like ours has been reported. Unlike other patients, our patient's initial symptom was a severe headache; her MRI and tendon reflexes were normal, and motor weakness was prominent. Fortunately, her neurological problems were completely resolved.

Differential diagnosis of acute hemiparesis in children is a critical process that involves evaluating various potential underlying causes. In children, common differential diagnoses include hemiplegic migraines, stroke, infections, demyelinating disorders such as multiple sclerosis, and structural lesions such as brain tumors or vascular malformations. Precise clinical assessment, imaging studies, and laboratory tests are essential in identifying the specific cause and guiding appropriate treatment (19). Hemiplegic migraine is a severe subset of migraine with aura, with symptoms including reversible hemiparesis in addition to other aura symptoms (20). In our case, hemiparesis occurred three days after the onset of a headache, with no other clinical manifestations such as aura symptoms, such as visual disturbances, speech difficulties, or sensory changes. The MRI sequences done to exclude other diagnoses for hemiparesis, including SWI and diffusion-tensor imaging, showed normal results.

To the best of our knowledge, this is a rare case presenting with transient hemiparesis without intracranial pathology in the diagnosis of MIS-C.

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Hemoperitoneum Related with Peritoneal Dialysis in A Female Adolescent: it is Not as Frightening as it Seems

Adolesan Bir Kız Hastada Peritoneal Diyaliz İlişkili Hemoperitoneum: Göründüğü Kadar Korkunç Değil

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ABSTRACT

Although hemoperitoneum is a benign and common complication of chronic peritoneal dialysis the apperance of the effluent will be devastating for the patients. In women of reproductive age group gynecological causes are the common etiological factors.

A 17 years old girl who had been on automated peritoneal dialysis for six months was admitted because of bloody effluent lasting for two days. At admission she described mild abdominal pain; physical examination was unremarkable and bleeding time and coagulation profile was normal. Abdominal ultrasound revealed hemorrhagic cysts on left ovary. Peritoneal effluent was cleared by using rapid exchanges with room temperature dialysate.

Hemoperitoneum is a well recognized complication of peritoneal dialysis in women of reproductive age.Abdominal ultrasound is the first, easily applicable and reliable diagnostic modality in order to detect underlying causes of hemoperitoneum. Performing rapid exchanges with cold-room temperature dialysate is the mainstay of treatment.

Key Words: Adolescent, Hemoperitoneum, Peritoneal dialysis, Ovarian cyst

ÖZ

Hemoperitoneum kronik periton diyalizinin selim ve sık görülen bir komplikasyonu olmasına rağmen periton drenaj sıvısının görünümü aileler için çoğunlukla korkutucu olmaktadır. Jinekolojik sebepler reprodüktif çağdaki kadınlarda etiyolojik faktörlerin en sık sebebi olarak karşımıza çıkmaktadır.

Altı aydır aletli periton diyaliz tedavisi almakta olan 17 yaşındaki kız hasta iki gündür devam eden kanlı periton diyaliz sıvısı olması nedeni ile başvurdu. Hastanın hafif karın ağrısı dışında eşlik eden şikayeti yoktu, fizik muayenede özellik saptanmadı, kanama zamanı ve koagülasyon testleri normal sınırlardaydı. Abdominal ultrason incelemede sol overde hemorajik kist saptandı. Oda ısısında diyalizat solusyonu ile hızlı değişimler uygulandı. Bu tedavi ile hastanın diyalizat sıvısının kısa sürede normale döndüğü saptandı.

Hemoperitoneum periton diyaliz tedavisi alan reprodüktif çağdaki kadınlarda sık rastlanılan bir komplikasyonudur. Hemoperitoneumun altta yatan nedenini saptayabilmek açısından abdominal ultrason ilk yapılması gereken, kolayca uygulanabilen ve güvenilir bir görüntüleme modalitesidir. Soğuk-oda ısısında diyalizat ile hızlı değişimler uygulanması tedavinin temelini oluşturmaktadır.

Anahtar Kelimeler: Adolsean, Hemoperitoneum, Periton diyalizi, Over kisti

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INTRODUCTION

Hemoperitoneum; which is described as the presence of blood in peritoneal dialysate (PD) effluent is a relatively common complication of PD (1,2). For women in the reproductive age receiving peritoneal dialysis common causes of hemoperitoneum are menses, endometriosis, hemorrhagic ovarian cyst and ovarian cyst rupture (1,3). Here we present an adolescent girl in whom hemoperitoneum was caused by hemorrhagic ovarian cyst and easily treated with rapid exchanges with cold dialysate.

CASE REPORT

A 17 years old girl, with end stage kidney disease secondary to focal segmental glomerulosclerosis had been on automated peritoneal dialysis for six months. She was presented to our emergency department with bloody peritoneal effluent lasting for two days. She described mild abdominal pain; and there was no abdominal trauma. She was sexually inactive, had regular menses after PD initiation lasting for 21 to 23 days and her last menstrueal period was about ten days before. The last two days all of the dialysate exchanges were bloody (Figure 1).

On physical examination she appeared generally well, heart rate was 88/min; blood pressure was 100/60 mmhg, respiratory rate was 15/minute; she was afebrile. Abdomen was soft, there was no rebound tenderness. She did not have peripheral edema. Cardiac and respiratory examinations were normal.

Laboratory examinations were normal except for renal function tests. Bleeding time and coagulation profile were in normal limits. Urine examination was normal and peritoneal dialysate culture was negative.

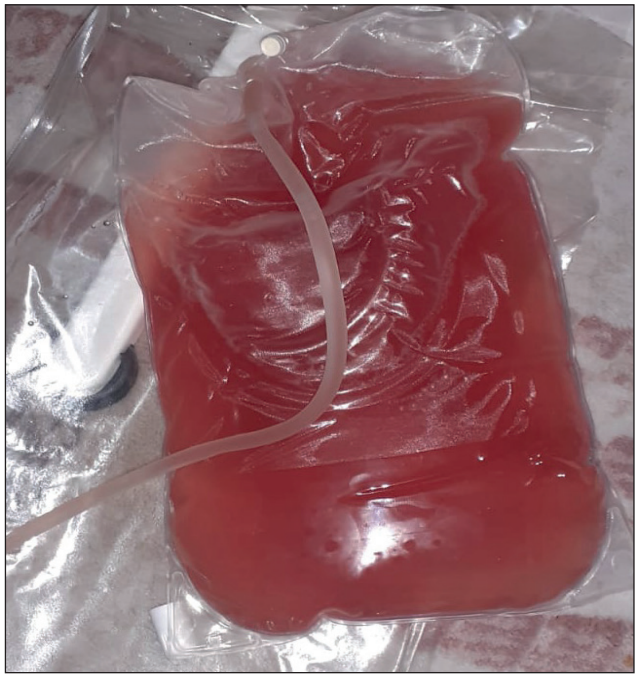

Figure 1: Bloody peritoneal effluent

The Tenckhoff catheter was detected to be in good position in the pelvis on abdominal X ray and there was no free air or dilatation of bowel segments. Abdominal ultrasound revealed hemorrhagic cysts on left ovary. Rapid exchanges with room temperature dialysate cleared peritoneal effluentin two days. Because hemoperitoneum did not recur in this patient no further evaluation was performed.

DISCUSSION

PD related hemoperitoneum is a benign complication of chronic peritoneal dialysis (1,2). Because the PD catheter provides an opening to peritoneum even small amounts of blood may cause peritoneal effluent to appear bloody (1,2). Underlying etiology is commonly evident from the patients history and peritoneal dialysate is usually cleared with several rapid exchanges (1,2).

The incidence of hemoperitoneum differs among reported studies mainly depending on the patient population (4-6). Tse et al. (4) reported that forty six among 549 patients experienced 116 episodes of hemoperitoneum in 10 years of follow-up. None of these patients developed ultrafiltration failure (4). In the study of Greenberg et al. (5) 30 episodes of hemoperitoneum occured in 26 among 424 patients who had been followed up for 11 years. Twenty four of 26 patients had benign causes of hemoperitoneum (5). Recently Aksoy et al. (6) evaluated noninfectious complications of PD in children and they demonstrated that the frequency of hemoperitoneum was 5.6 % in their study group. Seventeen among 302 patients had hemoperitoneum; fifteen were females and 14 of the episodes were menstruation related (6).

Etiological causes of hemoperitoneum vary in a broad range (1,2,4,7). The most common causes reported in literature are catheter related complications, gynecological or obstetric causes, trauma to intraabdominal organs, coagulopathies, uremic bleeding or vascular injuries (1,2,4).

Gynecological causes particularly menstruation associated bleeding are by far the most common causes among female adults and adolescents (4,6-9). Ovulation with mid cycle bleeding, hemorrhagic luteal cysts, ovarian cyst rupture and pregnancy are commonly reported causes of hemoperitoneum in women (1,4).

Hemoperitoneum may develop soon after PD catheter placement (1,2). Trauma, rupture of intraabdominal organs mostly liver and spleen are rare but severe causes of hemoperitoneum. Ruptured hepatic or renal cyst with intraperitoneal bleeding may also result with hemoperitoneum (1,2). Because uremic patients have platelet dysfunction bleeding diathesis should also be kept in mind (1,2).

In patients on chronic PD with several years, peritonitis particularly encapsulated peritoneal sclerosis is an important and devastating cause of hemoperitoneum (1,2).

Rare conditions as the erosion of mesenteric vein by Tenckhoff catheter, pericardiocentesis, radiation to an intraabdominal organ may also cause hemoperitoneum (1,2). In some cases so called idiopathic episodes of hemoperitoneum is likely that a minor Tenckhoff catheter related tear in omental venules may be the cause of bleeding (1,2).

As mentioned above detailed personal history is the mainstay of the evaluation of the patient with PD related hemoperitoneum. Abdominal X-ray may be performed to detect the proper position of Tenckhoff catheter. Abdominal ultrasound is required to detect intraabdominal pathologies (1,2). Authors suggest further investigation as CT or MRI where ultrasound is negative or inconclusive (2). If the patient has intractable bleeding isotope labeled red blood cell (RBC) scan can be performed in order to detect the exact site of bleeding. If more definitive diagnosis is needed angiography will be performed in selected cases (2). Our patient did not have a severe abdominal pain , also did not have a history of hepatic, splenic and renal cysts or a recent abdominal trauma. She had regular menses and her last period was 10 days before. She was sexually inactive and B-HCG was normal. Her initial laboratory tests were non diagnostic and abdominal X ray revealed a normal position of Tenckhoff catheter. Abdominal ultrasound revealed hemorrhagic cysts on left ovary. One of them was 15x9 mm in size and the other was 24x35 mm, the larger one did not have vascularization, was hyperechogenic with hypoechogenic areas inside. The second cyst suggested a complicated hemorrhagic cyst and in order to exclude malignant cases alpha-fetoprotein and CA-125 levels were obtained and they all came out to be negative. The underlying cause of bloody effluent in this case was attributed to these hemorrhagic ovarian cysts.

When managing a patient with PD related hemoperitoneum the extent of bleeding and the severity of symptoms are of great importance (1,2). Benign causes as menstruation-related do not require further evaluation. In this instance reassuring the patient and families that this is a transient condition is the basis of management (2).

Several and rapid exchanges with room temperature (cold) dialysate clears PD- related hemoperitoneum quickly (2). Some authors suggest installation of heparin in order to prevent catheter clotting (2). If there is a coagulopathy it should be corrected by administration of fresh frozen plasma (2). Blood transfusions may be required in severe cases (1,2).

Besides clearing the peritoneal effluent treating the underlying cause is crucial.In cases of hemorrhagic ovarian cysts as in our case; patients should be conservatively managed and carefully monitored (1-3). Recurrent hemoperitoneum requires hormonal or surgical intervention (1-3). Abdominal intervention is essential when bleeding from an intraabdominal organ is the case (1,2,5). In our patient hemoperitoneum improved within two days and did not reccur the following months so no further therapy was required. Follow up ultrasound examination performed one month after the first episode and demonstrated regression of the cysts.

Here we presented an adolescent female in whom PD related hemoperitoneum due to a hemorrhagic cyst resolved easily with conservative management. We aimed to emphasize that pediatric nephrologists should be aware of hemoperitoneum particularly when dealing with female adolescents. One should keep in mind that although the presentation is frightening for both patient and the families the underlying cases are usually benign and no complex-intervention is required and can easily be managed conservatively.

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Anaphylaxis: Current Approach

Anafilaksi: Güncel Yaklaşım

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ABSTRACT

Anaphylaxis is the most important acute systemic allergic reaction. The incidence of anaphylaxis has been increasing in recent years. Therefore, all healthcare providers should know the diagnosis and management of anaphylaxis. In this article, the current approach to anaphylaxis is presented, considering the criteria accepted by the World Allergy Organization (WAO) and the European Academy of Allergy and Clinical Immunology (EACCI). Intramuscular epinephrine is the first-line treatment for anaphylaxis. However, it is still not used at the desired level. Children at risk of anaphylaxis should be trained to use adrenaline autoinjectors. After anaphylaxis develops, children should be referred to an allergist to investigate the underlying causes.

Key Words: Anaphylaxis, Children, Current approach

ÖZ

Anafilaksi en önemli akut sistemik alerjik reaksiyondur. Anafilaksi insidansı son yıllarda giderek artmaktadır. Bu nedenle, tüm sağlık çalışanları anafilaksinin tanı ve yönetimini bilmelidir. Bu makalede, Dünya Alerji Örgütü (WAO) ve Avrupa Alerji ve Klinik İmmünoloji Akademisi (EACCI) tarafından kabul edilen kriterler göz önünde bulundurularak anafilaksiye güncel yaklaşım sunulmuştur. İntramüsküler epinefrin anafilaksi için ilk basamak tedavidir. Ancak hala istenilen düzeyde kullanılmamaktadır. Anafilaksi riski taşıyan çocuklar adrenalin otoenjektörlerini kullanmak üzere eğitilmelidir. Anafilaksi geliştikten sonra, altta yatan nedenlerin araştırılması için çocuklar bir alerji uzmanına yönlendirilmelidir.

Anahtar Kelimeler: Anafilaksi, Çocuk, Güncel yaklaşım

INTRODUCTION

Anaphylaxis is a rapid onset serious life threatening systemic hypersensitivity reaction. Although potentially life-threatening, this risk can be reduced with accurate and prompt diagnosis and treatment. In this article, the current approach to anaphylaxis is presented, considering the criteria accepted by the World Allergy Organization (WAO) and European Academy of Allergy and Clinical Immunology (EAACI) (1, 2).

Epidemiology

Although the incidence of anaphylaxis varies according to age and diagnostic criteria, the lifetime prevalence of anaphylaxis is estimated to be 0.05-2 % (3). In recent studies, the incidence of anaphylaxis in children varies between 1-761/100.000 (4). Although the incidence has increased 5-7 fold in the last decade, mortality rates are stable or show a decreasing trend due to the adoption of guidelines in diagnosis and treatment and increased awareness (5, 6). It should be kept in mind that

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26.5-54 % of anaphylaxis patients develop anaphylaxis again in 1.5-25 years of follow-up (5).

Risk factors

Several risk factors for anaphylaxis have been identified. Infants are among the risk groups due to difficulties in diagnosis due to their inability to describe their symptoms, and adolescents are among the risk groups due to their tendency to exhibit risk-taking behavior (7).

Asthma and other respiratory diseases, cardiovascular diseases, mastocytosis and clonal mast cell disorders, psychiatric diseases such as depression may increase the mortality risk of anaphylaxis. Physical exercise, infections, fever, premenstrual period, insomnia, alcohol consumption, and drug use can potentially increase the risk of anaphylaxis by lowering the allergen exposure threshold required to trigger anaphylaxis (8-11). The use of beta-blockers and angiotensin-converting enzyme (ACE) inhibitors has been shown to cause severe anaphylaxis by inhibiting the adrenaline response to treatment (12, 13).

Pathophysiology

Despite common clinical symptoms, the mechanisms underlying anaphylaxis may vary (14). The classic and most common IgE-mediated anaphylaxis is initiated by an antigen that interacts with allergen-specific IgE binding to high-affinity IgE receptors (FcεRI) on mast cells and basophils. After this binding, clinical findings occur due to mediators and cytokines secreted by activated cells (15).

Non-IgE mediated anaphylaxis is divided into immunologic and non-immunologic. Non-IgE mediated immunologic anaphylaxis may be mediated by the complement system (anaphylatoxins, C3a and C5a), contact and coagulation system activation or IgG Non-immunologic anaphylaxis (opiates, vancomycin, radiocontrast agents, etc.) develops with direct stimulation of mast cells and basophils (16).

Triggers

Food is the most common cause of anaphylaxis in children, followed by drugs and venom (7,17,18). The frequency of triggering anaphylaxis with certain foods may vary according to dietary habits, food preparation, and type of exposure. Theoretically, anaphylaxis can develop with any type of food in sensitized children. Peanuts, tree nuts, seafood, and cow's milk have been implicated as the most frequent culprits of fatal anaphylaxis (10). In studies conducted in Turkey, cow's milk was found to be the most responsible food (7,19). Cow's milk and hen's egg are the most common foods causing anaphylaxis in infants, cow's milk and tree nuts in preschoolers, and tree nuts and legumes in school children (19).

Drug-induced anaphylaxis is most common with antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs) (20). Chemotherapy drugs, biological agents such as cetuximab, rituximab, infliximab, and omalizu

mab, and enzyme replacement therapies are among the causes of drug-induced anaphylaxis (21-24). In addition, disinfectants such as chlorhexidine, and preservatives in drugs and vaccines such as polyethyleneglycol may also trigger anaphylaxis (25,26). In addition to the drugs mentioned above, anaphylaxis has been shown to develop with latex, radiocontrast agents and perioperative rocuronium, thiopental, propofol, opiates, protamine and plasma expanders (27). Anaphylaxis can also occur during skin prick tests and intradermal tests, food and drug challenge tests, as well as during immunotherapy and desensitization (28). "Idiopathic anaphylaxis" is defined as cases in which the cause of anaphylaxis has not been established despite detailed investigations and the presence of conditions such as systemic mastocytosis that may be associated with anaphylaxis has not been demonstrated (29).

Diagnosis

Anaphylaxis is diagnosed clinically by recognizing symptoms and signs that occur suddenly (within minutes to a few hours)

Table I: Signs and symptoms of anaphylaxis *

Systems	Signs and symptoms	Incidence rates %
Skin and mucous membranes	Redness, itching, urticaria, angioedema, morbiliform rash, conjunctival erythema, tearing, itching and swelling of the lips, tongue, palate and uvula	80-90
Respiration	Nose: itching, congestion, discharge, sneezing Larynx: itching, feeling of narrowness, dysphonia, coarsening of the voice, dry-hard cough, stridor Lung: shortness of breath, feeling of tightness in the chest, deep cough, wheezing, bronchospasm (reduced PEF), Cyanosis	70
Gastrointestinal	Nausea, cramping abdominal pain, vomiting, diarrhea, dysphagia	30-45
Cardiovascular	Chest pain, palpitation, tachycardia, bradycardia, dysrhythmia, feeling faint, mental change, hypotension, loss of sphincter control, shock, arrest	10-45
Neurologic	Feeling of death, restlessness, throbbing headache, dizziness, confusion; sudden behavioral changes in infants and young children (irritability, interruption of play, clinging to parents, etc.)	10-15
Others	Metallic taste in the mouth, uterine contractions (postpubertal)	

*****Table taken from 30 with permission. **PEF:** Peak expiratory flow*

Table II: Criteria for anaphylaxis according to EACCI*

Anaphylaxis is highly likely when any one of the following three criteria is fulfilled:
1.Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue or both (eg;generalized hives, pruritus or flushing, swollen lips-tongue-uvula and least one of the following
a.Respiratory compromise (eg; dyspnoea, wheeze-bronchospasm, stridor, reduced PEF and hypoxemia)
b.Reduced BP or assosicated symptoms of end-organ dysfunction (eg hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):
a.Involvement of the skin-mucosal tissue (eg; generalized hives, itch-flush, swollen lips-tongue-uvula)
b.Respiratory compromise (eg; dyspnoea, wheeze-broncohospasm, stridor, reduced PEF, hypoxemia)
c.Reduced BP or associated symptoms (eg; hypotonia [collapse], syncope, incontinence)
d.Persistent gastrointestinal symptoms (eg; crampy abdominal pain, vomiting)
3.Reduced BP after exposure to known allergen for that patient (minutes to several hours):
a.Infants and children: low systolic BP (age spesific) or >30% decrease in systolic BP†
b.Adults: systolic BP of <90 mmHg or >30% decrease from that person's baseline

Table taken from 32 with permission. †Low systolic blood pressure for children is defined as <70 mmHg from 1 month to 1 year, less than (70 mmHg + [2 x age]) from 1 to 10 years and <90 mmHg from 11 to 17 years,* ***EAACI:* *Eacci the European Academy of Allergy and Clinical Immunology* ***PEF:*** *Peak expiratory flow,* ***BP:*** *Blood pressure*

Table III: Criteria for anaphylaxis according to WAO*

Anaphylaxis is highly likely when any one of the following two criteria are fulfilled:
1. Acute onset of an illness (minutes to several hours) with simultaneous involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula) and at Least one of the following
a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
b. Reduced BP or associated symptoms of end- organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
c. Severe gastrointestinal symptoms (eg, severe crampy abdominal pain, repetitive vomiting), especially after exposure to non-food allergens
2. Acute onsef of hypotension[a] or bronchospasm[b] or laryngeal involvement[c] after exposure to a known or highly probable allergen[d] for that patient (minutes to several hours), even in the absence of typical skin involvement.

Table taken from 33 with permission.* ***WAO:* *World Allergy Organisation*. ***PEF:*** *Peak expiratory flow,* ***BP:*** *blood pressure. [a]Hypotension defined as a decrease in systolic BP greater than 30% from that person's baseline, or i.Infants and children under 10 years: systolic BP less than (70 mmHg + [2x age in years]) ii. Adults and children over 10 years: systolic BP less than <90 mmHg. [b]Excluding lower respiratory symptoms triggered by common inhalant allergens or food allergens perceived to cause "inhalational" reactions in the absence of ingestion. [c]Laryngeal symptoms include: stridor, vocal changes, odynophagia. [d]An allergen is a substance (usually a protein) capable of triggering an immune response that can result in an allergic reaction. Most allergens act through an IgE-mediated pathway, but some non-allergen triggers can act independent of IgE (for example, via direct activation of mast cells).*

after exposure to a known or possible trigger. Common findings during anaphylaxis are shown in Table I (30).

Skin manifestations are present in 80-90 % of anaphylaxis cases. However, in 10-20 % of patients, the diagnosis of anaphylaxis may be missed due to its absence (31). Anaphylaxis has a very broad spectrum. Different clinical pictures can be observed from mild clinical findings to severe form with shock. It was observed that not all cases could be diagnosed with the current diagnostic criteria because not all cases had multi-system involvement, there were patients without cutaneous findings or shock findings, and there were patients presenting with isolated respiratory system findings. For this reason, revision of the criteria was brought to the agenda and the criteria were revised by WAO in 2020 and by EAACI in 2021 (1, 2). The diagnostic criteria for anaphylaxis revised by EAACI are shown in Table II and the diagnostic criteria revised by WAO are shown in Table III (1, 2).

Laboratory

The diagnosis of anaphylaxis is based on clinical findings. Laboratory tests have a limited role in the diagnosis of anaphylaxis. Measurement of serum tryptase level may help the diagnosis in suspected cases. Serum tryptase increases within 15 minutes to 3 hours after the onset of anaphylaxis symptoms. Tryptase levels remain at peak level for 1-2 hours (34). Elevated tryptase levels support the diagnosis of anaphylaxis, whereas normal levels do not exclude anaphylaxis (35). Tryptase levels are found to be elevated more frequently in cases of severe anaphylaxis in which the allergen enters the body by injection, drug and venom anaphylaxis and hypotension and shock develop, compared to anaphylaxis induced by food and in which arterial blood pressure remains normal (36).

Even if the serum basal tryptase level measured during anaphylaxis results within normal limits, it is recommended to measure the serum basal tryptase level again 24 hours after the symptoms of anaphylaxis have completely resolved (37). A peak mast cell tryptase (MCT) >1.2 x basal tryptase + 2 ng/L has been proposed to diagnose acute mast cell activation (38). However, if both acute and baseline tryptase levels are greater than 11.4 ng/mL, the diagnosis of mastocytosis or clonal mast cell disorder should be excluded (39).

Differential Diagnosis

Clinical conditions that may be confused with anaphylaxis are shown in Table IV (1, 31).

Treatment

Anaphylaxis is an emergency that needs to be rapidly diagnosed and treated. Initially, the substance that triggers anaphylaxis, such as medication or therapeutic agent, if any, should be removed from the environment. Airway, respiration, circulation, mental status and skin should be assessed. Intramuscular adrenaline should be administered to the vastus lateralis part of the patient's quadriceps muscle and the patient should be placed in position according to the patient's findings. If there is respiratory distress, the patient should be seated. If loss of consciousness has developed, a rescue position should be given (40).

Although intramuscular adrenaline is the first drug to be administered in the treatment of anaphylaxis, its use is still not at the desired level (41-43). Adrenaline should be administered intramuscularly at a dose of 0.01 mg/kg with a maximum dose of 0.3 mg in children and 0.5 mg in adolescents. The time of adrenaline administration should be recorded and if symptoms persist despite treatment, adrenaline should be repeated every 5-15 minutes. There is a possibility of fatal arrhythmia if adrenaline is administered intravenously. Therefore, the intramuscular route should be preferred as the safest route (44). However, if there is no response to adrenaline given intramuscularly 2 times and there are signs of severe hypotension and cardiovascular shock, adrenaline can be given by infusion. In children, it is started at a dose of 0.1-1 ug/kg/min and the dose is adjusted according to blood pressure (1, 45). If stridor is present, adrenaline can be additionally nebulized (1).

Patients who develop respiratory distress or need repeated adrenaline should be given oxygen at a rate of 6-8 L/min with a non-rebreather facemask immediately until transport to hospital. An intravenous line should be opened using broad cannulas. If hypotension and collapse develop, 10 mL/kg saline should be given (1).

If bronchoconstriction occurs, a short-acting beta agonist (salbutamol/albuterol) should be given by inhalation. The patient's blood pressure, heart rate and circulation, respiration and mental status should be checked at frequent and regular intervals (1).

Antihistamines and corticosteroids are the second used drugs in the treatment of anaphylaxis. H1 antihistamines have a limited role in the treatment of anaphylaxis. They can be used to treat skin manifestations (46). Steroids are widely used, especially to prevent biphasic anaphylaxis. However, there is growing evidence that they are not useful in the acute management of anaphylaxis, may be harmful and their routine use is controversial (22, 47). Glucagon can be used when the desired response to adrenaline is not obtained, especially in patients using beta blockers (48).

Table IV: Clinical conditions that may be confused with anaphylaxis*

Common diagnostic dilemmas
Acute asthma
Syncope
Anxiety/panic attack
Acute generalized urticaria[a]
Foreign body aspiration
Cardiovascular events (myocardial infarction,[a] pulmonary embolus)
Neurologic events (seizure, cerebrovascular event)
Postprandial syndroms
Scombroidosis[b]
Pollen-food allergy syndrome[c]
Monosodium glutamate
Sulfites
Food poisoning
Excess endogenous histamine
Mastocytosis/ clonal mast cell disorders[d]
Basophilic leukemia
Flush syndromes
Peri-menopause
Carcinoid syndrome
Autonomic epilepsy
Medullary carcinoma of the thyroid
Nonorganic Disease
Vokal cord dysfunction
Hyperventilation
Psychosomatic episode
Shock
Hypovolemik
Cardiogenic
Distributive[e]
Septic
Other
Nonallergic angioedema
Hereditary angioedema types 1,2 and 3
ACE inhibitor- associated angioedema
Systemic capillary leak syndrome
Red man syndrome (vancomycin)
Pheochromocytoma (Paradoxical response)

**Table taken from 31 with permission. [a]Acute asthma symptoms, acute generalized urticaria, or myocardial infarction symptoms can also occur during an anaphylactic episode. [b]Histamine poisoning from fish, eg, tuna that has been stored at an elevated temperature; usually, more than one person eating the fish is affected. [c]Pollen-food allergy syndrome is elicited by fruits and vegetables containing various plant proteins that cross-react with airborne allergens. Typical symptoms include oral allergy symptoms (itching, tingling and angioedema of the lips, tongue, palate, thorat, and ears) after eating raw, but not cooked, fruits and vegetables, [d]In mastocytosis and clonal mast cell disorders, there is an increased risk of anaphylaxis; also, anaphylaxis may be the first manifestion of the disease. [e]Distributive shock may be due to anaphylaxis or to spinal cord injury.*

It is recommended to follow up with the patients for at least 6-8 hours after anaphylaxis and 12-24 hours for those presenting with circulatory disturbance (40). The majority of biphasic anaphylaxis reactions occur within the first 6-12 hours after anaphylaxis is treated (49). Therefore, the observation period

Table V: Adrenaline autoinjector doses according to weight

Weight	Adrenaline dose
Children <25-30 kg	0.15 mg
Adults/children ≥ 25-30 kg	0.3 mg

should be prolonged, especially in severe reactions and in patients receiving multiple doses of adrenaline (22).

Prevention

Although it is difficult to prevent anaphylaxis, it is possible to reduce its frequency and severity with preventive measures. Anaphylaxis education should be individualized according to the patient's history, age, triggers, comorbidities, and medications. A written personalized anaphylaxis emergency action plan should be prepared. Patients at risk of anaphylaxis should be prescribed two adrenaline autoinjectors. Adrenaline autoinjector doses according to weight are shown in Table V (2). They should be trained on why, when and how to use it and this training should be repeated at intervals (40). It may be protective for patients to wear markers such as name cards, bracelets and badges indicating their allergy status, the treatment to be administered and contact information (40).

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