ISSN: 2146-2372 **e-ISSN:** 2822-4469

OFFICIAL JOURNAL OF THE IZMIR CHILDREN'S HEALTH SOCIETY
AND IZMIR DR. BEHCET UZ CHILDREN'S HOSPITAL

JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

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behcetuzdergisi.com

Volume: 13
Issue: 1
APRIL '23

JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

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Web: www.galenos.com.tr
Publisher Certificate Number: 14521

Online Publishing Date: March 2023

ISSN: 2146-2372 **e-ISSN:** 2822-4469

International periodical journal published three times in a year.

JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

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AIM AND SCOPE

Journal of Dr. Behcet Uz Children's Hospital is a peer-reviewed open-access official scientific publication of the Izmir Children's Health Society and Izmir Dr. Behcet Uz Children's Hospital. The publication frequency of the journal is 3 times a year (April, August, November). Journal of Dr. Behcet Uz Children's Hospital accepts publications in English as of 2020 and published electronically.

Aims and Scope

The journal of Dr. Behcet Uz Children's Hospital is devoted to the continuing education of national and international practicing pediatrics and pediatric surgeons, and to provide a forum for social and scientific communication in the field. Studies that emphasize these aims provide the basis for publication, including original articles, case reports, reviews, annual meetings' abstracts, letters to the editor, review of the recently published books, biographies, and social articles. The journal of Dr. Behcet Uz Children's Hospital accepts only invited review articles.

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2023
Volume: 13
Issue: 1

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Journal of Dr. Behcet Uz Children's Hospital is a double-blind peer-reviewed journal which has been started to be published in 2011.

Articles in the journal are published in content pages and article title pages, as classified according to their types (research, case report, short report, review, letter to editor etc.)

Journal of Dr. Behcet Uz Children's Hospital does not charge any article submission or processing fees, and reviews are prepared due to the invitation of editor.

All manuscripts submitted to the Journal of Dr. Behcet Uz Children's Hospital must screened for plagiarism and proofreading by the authors' themselves.

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The manuscripts sent to Journal of Dr. Behcet Uz Children's Hospital are firstly evaluated by the editor. Editor checks up every manuscript whether they are worth to evaluate or not, and assigns an assistant for each. If editor and the assistant find the manuscript worth to evaluate, they send it to two reviewers or one reviewer with one editorial board member for evaluation. The manuscript is not under evaluation if it is does not require the evaluation of the reviewer or editorial board members because that it has no scientific value and not original, or it does not fit to the reader population. The author(s) should screen their article for plagiarism and share the suicide report with the journal. In addition to the plagiarism, proofreading responsibility lies with the authors.

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Original Research Articles: References and English summary are required (see writing preparation section). At most 5000 words (20 double spaced pages), 7 tables and/or figures, additionally abstract and references in English. Ethics committee approval should be added in the study.

Case Reports: For the manuscripts sent to this part, we are looking for the clinical cases that are infrequently reported in scientific literature previously, unreported clinical reflections or complications of a well known disease, unknown adverse reactions of known treatments, or case reports including scientific message that might trigger further new research, preferably. Case reports should include abstract, case and discussion. It should include 2000 words (8 double spaced pages), 15 or less references, three tables or pictures.

Abstract Reports: Researches with small numbers that have preliminary study data and findings which require further studies. References and English abstract required (see Manuscript Preparation section). At most 3000 words in length (8 double spaced pages), additionally English abstract, 15 or less references, 3 tables and/or figures. Ethics committee approval required.

Concepts: Clinical or non-clinical manuscripts about improvement of this field. References and English abstract required. At most 4000 words (16 double spaced pages), additionally Englishabstract (each less than 150 words) and references must be included.

Review Articles: Extent investigation writings including latest national and worldwide literature about public health issues. Journal of Dr. Behcet Uz Children's Hospital publishes invited review articles. A contact with the editor should be provided before the submission of uninvited reviews. At most 5000 words (20 double spaced pages). There is no limitation about number of references. Related information is available in the following article; Burney RF, Tintinalli JE: How to write a collective review. Ann Emerg Med 1987;16:1402.

Evidence based Information: Articles that could answer to the problems of clinical and medical applications. The article should include these sections; clinical vignette, questions and problems, research and selection of the best evidence, detailed examination of the evidence and implementation of the evidence. At most 4000 words (15 double spaced pages), additional English abstract. Authors should also send the copies of the articles to the editor.

Letter to Editor: These are the articles that include opinions and solution advises about the medicine and public health issues, comments about the articles published in journal of Journal of Dr. Behcet Uz Children's Hospital or other journals. At most 1500 words (6 double spaced pages), additionally references should be included.

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Cover Letter: Author, in this letter, should imply the short explanation of his research or writing, type of the study (random, double-blind, controlled etc.), the category it is sent for, whether it had been presented in a scientific meeting or not, in details. Additionally, the address, phone and fax numbers and e-mail address of the person for contact about the writing should be present at the lower pole of the letter.

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

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Original articles should contain English abstracts. Abstracts must be no longer than 250 words. The structured abstract should include objective, materials and methods, results and conclusions in original articles. Case reports should also include a structured abstract [objective, case report(s), and conclusion]. Abbreviations should not be used in the abstract.

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References should be provided at the end of the article, under the title "References" and should be numbered and listed according to their order in the text. They should be referred to in parentheses within the text. Complete author citation is required ("et al" is not acceptable). The author(s) are responsible for the accuracy of the references. Journal titles should be abbreviated according to Index Medicus. Refer to the "List of Journals Indexed in Index Medicus" for abbreviations of journal names, or access the list at http://www.nlm.nih.gov/tsd/serials/lji.html. Abbreviations are not used for journals not in the Index Medicus. Only published articles or articles "in press" can be used in references. Authors must add the DOI and/or PMID numbers to the end of each citation. Example of references are given below:

Journal:

Hull ML, Escareno CR, Godsland JM, Doig JR, Johnson CM, Phillips SC, et al. Endometrial-peritoneal interactions during endometriotic lesion establishment. Am J Pathol. 2008;173(3):700-15. doi: 10.2353/ajpath.2008.071128.

Ferrari A, Casanova M, Bisogno G, Cecchetto G, Meazza C, Gandola L, et al. Malignant vascular tumors in children and adolescents: a report from the Italian and German Soft Tissue Sarcoma Cooperative Group. Med Pediatr Oncol. 2002;39(2):109-14. doi: 10.1002/mpo.10078.

Abstract: Heidenreich A, Olbert P, Becker T, Hofmann R. Microsurgical testicular denervation in patients with chronic testicular pain. Eur Urol 2001;39 (suppl 5):126 (abstr.)

Book: Sadler TW. Langman's Medical Embryology, 5th ed., William and Wilkins, Baltimore, 1985. p.224-226.

Book Chapter: Folkman J: Tumor angiogenesis. In Bast Jr RC, Kufe DW, Polock RE,Weichselbaum RR, Holland JF, Frei E (eds). Cancer Medicine. 5th ed. London, B.C. Decker Inc.; 2000. p.132-152.

Online articles: Abood S. Quality improvement initiative in nursing homes: the ANA acts in advisory role. Am J Nurs (serial on the Internet). 2002 Jun (cited 2002 Aug 12); 102 (6): (about 3 p.). Available from: http://www.nap.edu/books/0309074029/html/.

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

INSTRUCTIONS TO THE AUTHORS

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Journal:

If the number of authors are less than or equal to 6, then all authors are indicated..

Campbell MR, Fisher J, Anderson L, Kreppel E. Implementation of early exercise and progressive mobility: Steps to success. Crit Care Nurse. 2015;35(1):82-8. doi: 10.4037/ccn2015701.

If the number of authors are more than 6, then the first three authors are indicated.

Aiken LH, Sermeus W, Van den Heede K, Sloane MD, Busse R, McKee M, et al. Patient safety, satisfaction, and quality of hospital care: Cross sectional surveys of nurses and patients in 12 countries in Europe and the United States. BMJ. 2012;344:e1717. doi: 10.1136/bmj.e1717.

If the article has not any DOI number then internet access address (website) is noted.

Pokorny ME, Koldjeski D, Swanson M. Skin care intervention for patients having cardiac surgery. Am J Crit Care. 2003;12(3):535-44. Available from: http://ajcc.aacnjournals.org/content/12/6/535.full.pdf+html?sid=f587c6d5-92a3-4971-8367-f18cd1cd63f0

Supplement:

Ahrens T. Severe sepsis management: Are we doing enough? Crit Care Nurse. 2003;23(Suppl 5):2-15. Available from: http://ccn.aacnjournals.org/content/23/5/S2.full.pdf+html

Book:

Jarvis C. Physical Examination and Health Assessment. 3rd ed. Philadelphia: W.B. Saunders Company; 2000.

If any information about the editor is available:

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

A chapter in the book:

Finke LM. Teaching in nursing: the faculty role. In: Billing DM, Halstead JA, editors. Teaching in Nursing: A Guide for Faculty. 3rd ed. USA: Saunders & Elsevier; 2009. p. 3-17.

Translated book:

Ferry DR. ECG in Ten Days [On Günde Temel Elektrokardiyografi]. Kahraman M, translator. İstanbul: Ekbil A.Ş.; 2001.

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Tolay E. Planlamanın temelleri. In: Robbins SP, Decenzo DA, Coulter M. editors. Yönetimin Esasları: Temel Kavramlar ve Uygulamalar. Öğüt A, translator. Ankara: Nobel Akademik Yayıncılık; 2013. p. 104-29.

Electronic book:

Akdag R. The Progress So Far Health Transformation Program in Turkey. Ankara, Turkey: Ministry of Health; 2009. Available from: http://ekutuphane.tusak.gov.tr/kitap.php?id=174&k=progress_report_health_transformation_program_in_turkey_january_2009

Aminoff MJ, Greenberg DA, Simon RP. Clinical Neurology. 9th ed. New York: McGraw Hill Medical; 2015. Available from: http://accessmedicine.mhmedical.com/book.aspx?bookID=1194

Electronic report/document:

World Health Organization. World Alliance for Patient Safety Forward Programme 2008-2009. 1st ed. France; 2008. Available from: http://apps.who.int/iris/bitstream/10665/70460/1/WHO_IER_PSP_2008.04_eng.pdf

İzmir Halk Sağlığı Müdürlüğü. Sağlık Bakanlığı Yoğun Bakım Ünitelerinin Standartları. İzmir; 2007. Available from: http://www.ihsm.gov.tr/indir/mevzuat/genelgeler/G_13082007_1.pdf

Dissertations/Theses:

Bayram TY. Üniversitelerde örgütsel sessizlik [master's thesis]. Bolu: Abant İzzet Baysal Üniversitesi, Sosyal Bilimler Enstitüsü; 2010.

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

INSTRUCTIONS TO THE AUTHORS

ones are sent to consultants (editor assistants) for preliminary assessment. The writings that are sent from the editor of journal directly to the writer can not be printed in Journal of Dr. Behcet Uz Children's Hospital. The duration period between the application and the preliminary assessment time is maximum 15 days. Letter informing the status about writing is reported by editor to the author, in this period. The articles which are found inappropriate are not sent back.

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

PEER REVIEW, PUBLICATION ETHICS AND MALPRACTICE STATEMENT

Peer-review

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Submitted manuscripts are subjected to double-blinded peer-review. The scientific board guiding the selection of the papers to be published in the journal consists of elected specialists of the journal and, if necessary, selected from national and international experts in the relevant field of research. All manuscripts are reviewed by the editor, section associate editors and at least three internal and external expert reviewers. All research articles are interpreted by a statistical editor as well.

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For the experimental, clinical and drug human studies, approval by ethical committee and a statement on the adherence of the study protocol to the international agreements (Helsinki Declaration revised 2008 (www.wma.net/e/policy/b3.html) are required. In experimental animal studies, the authors should indicate that the procedures followed were by animal rights (Guide for the care and use of laboratory animals, www.nap.edu.catalog/5140.html), and they should obtain animal ethics committee approval. The Ethics Committee approval document should be submitted to the Journal of Dr. Behcet Uz Children's Hospital together with the manuscript.

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2023
Volume: 13
Issue: 1

CONTENTS

ORIGINAL ARTICLES

1 COVID-19 Infection in Children with Leukemia: A Single-center Retrospective Study
Lösemili Çocuklarda COVID-19 Enfeksiyonu: Tek Merkezli Retrospektif Çalışma
Elif Güler Kazancı, Deniz Güven, Rana Yılmaz; Bursa, Ankara, Turkey

9 Antifungal Combination Therapy for Invasive Fungal Infections in Pediatric Leukemia Patients: An Observational Cohort Study
Pediatrik Lösemi Hastalarında İnvaziv Mantar Enfeksiyonları için Antifungal Kombinasyon Tedavisi: Gözlemsel Bir Kohort Çalışması
Saliha Kanık Yüksek, Aslınur Özkaya Parlakay, Belgin Gülhan, Neşe Yaralı, Namık Yaşar Özbek, Hasan Tezer; Ankara, Turkey

16 The Frequency of Smoking Use and Factors Related to Alcohol and Substance Use Among High School Students in İzmir
İzmir İlinde Lise Öğrencilerinde Sigara Kullanım Sıklığı ve Alkol-Madde Kullanımı ile İlişkili Faktörler
Osman Hasan Tahsin Kılıç, İhsan Aksoy, Afra Sevde Çetin, Güven Güvendi, İstemihan Çoban, Berna Dirim Mete; İzmir, Ankara, Turkey

23 Retrospective Analysis of Children with Chronic Non-bacterial Osteomyelitis
Kronik Non-bakteriyel Osteomiyelitli Çocukların Retrospektif Analizi
Ümmüşen Kaya Akca, Yağmur Bayındır, Yelda Bilginer; Ankara, Turkey

30 The Use of Mean Platelet Volume, Plateletcrit, and N-terminal Brain Natriuretic Peptide as Biomarkers of Coronary Artery Involvement in Atypical Kawasaki Disease
Atipik Kawasaki Hastalığında Koroner Arter Tutulumunun Biyobelirteçleri Olarak Ortalama Trombosit Hacmi, Plateletkrit ve N-terminal Beyin Natriüretik Peptid Kullanımı
Eser Doğan, Caner Turan, Ali Yurtseven, Gamze Eman, Eylem Ulaş Saz; İzmir, Turkey

37 Evaluation of Long-term Results and Stool Incontinence of Patients with Operated Anorectal Malformations
Opere Anorektal Malformasyonlu Hastaların Uzun Dönem Sonuçlarının ve Fekal İnkontinanslarının Değerlendirilmesi
Binali Fırıncı, Ahmet Bedii Salman; Erzurum, Turkey

43 Can High Mobility Group Box 1 Protein Predict Ongoing Subclinical Inflammation in Patients With Familial Mediterranean Fever?
High Mobility Group Box 1 Proteini, Ailevi Akdeniz Ateşi Hastalarında Süregelen Subklinik Enflamasyonu Öngerebilir mi?
Betül Öztürk, Esra Baskın, Kaan Gülleroğlu, Begüm Avcı, Nilüfer Bayraktar, Feride İffet Şahin; Ankara, Turkey

49 Food Protein-induced Enterocolitis Syndrome: A Single-center Experience
Besin Proteini İlişkili Enterokolit Sendromu: Tek Merkez Deneyimi
Semiha Bahçeci, Pınar Kuyum Töz, Murat Ayar; İzmir, Turkey

54 The Association of Screen Time with Isometric Back and Leg Muscle Strength in School-aged Children
Okul Çağı Çocuklarda Ekran Süresinin İzometrik Sırt ve Bacak Kas Gücü ile İlişkisi
Sibğatullah Ali Orak, Aslı Çelebi Tayfur, Işıl Zinnuroğlu, Alev Yücel, Deniz Yılmaz, Sacit Günbey; Ankara, Turkey

61 The Most Common Causes of Morbidity and Mortality in Late Preterm Infants: 8-year Single-center Experience
Geç Prematüre İnfantlarda En Yaygın Morbidite ve Mortalite Nedenleri: 8 Yıllık Tek Merkez Deneyimi
Ela Cem, Rahmi Örs; İzmir, Konya, Turkey

CASE REPORT

70 A Newborn with Arhinia: Suspected BAM Syndrome
Burunsuz Bir Yenidoğan: BAM Sendromu
Coşkun Armağan, Tuğba Üçüncü Egeli, Can Akyıldız, Kadri Murat Erdoğan, Funda Erdoğan, Nuray Duman, Hasan Özkan; İzmir, Turkey

DOI: 10.4274/buchd.galenos.2022.39297
J Dr Behcet Uz Child Hosp 2023;13(1):1-8

Original Article

COVID-19 Infection in Children with Leukemia: A Single-center Retrospective Study

Lösemili Çocuklarda COVID-19 Enfeksiyonu: Tek Merkezli Retrospektif Çalışma

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ABSTRACT

Objective: Children were less likely than adults to develop severe illness from coronavirus disease-2019 (COVID-19) infection, whereas children with leukemia had compromised immunity and may be at increased risk of severe COVID-19 infection. The aim of this study is examine the characteristics and outcomes of COVID-19 in children with leukemia.

Method: Between March 2020 and February 2021, patients on active leukemia treatment who were diagnosed with severe acute respiratory syndrome coronavirus-2 infection were enrolled in the study. Clinical, laboratory, and radiological characteristics, as well as infection severity and prognosis, were all assessed.

Results: The children's median age was 9.6 years, and 66.7 percent of them were male. The majority of patients with COVID-19 infection were in the early stages of leukemia treatment and had severe or critical COVID-19 infection. Six patients were treated for COVID-19. Five patients required oxygen, six were in the intensive care unit, and three were intubated. Twelve patients were fully recovered, and three died. Two of the patients were re-infected with COVID-19. The disease status of re-infected patients was worse than the first infection, and the duration of polymerase chain reaction positivity was much longer.

Conclusion: Children with leukemia who have COVID-19 infection may have severe/critical illness. The type and character of primer malignancy, as well as the prognostic factors of COVID-19 infection, may all have an impact on clinical outcomes. It is critical to take the most stringent precautions to prevent infection from spreading to these patients.

Keywords: Children, COVID-19, leukemia, severity, prognosis

ÖZ

Amaç: Çocukların koronavirüs hastalığı-2019 (COVID-19) enfeksiyonundan ciddi hastalık geliştirme olasılığı yetişkinlerden daha düşükken, lösemili çocukların bağışıklığı zayıf olması nedeniyle ciddi COVID-19 enfeksiyonu geçirme riski daha yüksek olabilir. Bu çalışmanın amacı, lösemili çocuklarda COVID-19 enfeksiyonu özelliklerini ve prognozunu incelemektir.

Yöntem: Mart 2020 ile Şubat 2021 arasında aktif lösemi tedavisi gören ve şiddetli akut solunum yolu enfeksiyonu sendromu-koronavirüs-2 enfeksiyonu tanısı alan hastalar çalışmaya alındı. Klinik, laboratuvar ve radyolojik özelliklerin yanı sıra enfeksiyon şiddeti ve prognozu da değerlendirildi.

Bulgular: Çocukların ortanca yaşı 9,6 idi ve yüzde 66,7'si erkekti. COVID-19 enfeksiyonu olan hastaların çoğu lösemi tedavisinin erken aşamalarındaydı. Hastaların çoğunda ciddi veya kritik COVID-19 enfeksiyonu mevcuttu. Altı hastaya COVID-19 için spesifik ilaç tedavisi uygulandı. Beş hastanın oksijen ihtiyacı oldu, altı hasta yoğun bakım ünitesinde takip edildi, bunlarda üçü entübasyona gereksinim duydu. On iki hasta tamamen iyileşti ve üç hasta öldü. İki hastada COVID-19 ile re-enfeksiyon gelişti. Re-enfekte olan hastaların hastalık durumu ilk enfeksiyondan daha kötüydü ve polimeraz zincir reaksiyonu pozitiflik süresi çok daha uzundu.

Sonuç: COVID-19 enfeksiyonu olan lösemili çocuklarda ciddi/kritik hastalığa yol açabilir. Primer malignitenin tipi ve karakterinin yanı sıra COVID-19 enfeksiyonunun prognostik faktörleri klinik sonuçlar üzerinde etkilidir. Enfeksiyonun bu hastalara yayılmasını önlemek için en katı önlemleri almak çok önemlidir.

Anahtar kelimeler: Çocuklar, COVID-19, lösemi, şiddet, prognoz

Received: 07.02.2022
Accepted: 25.07.2022

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Cite as: Güler Kazancı E, Güven D, Yılmaz R. COVID-19 Infection in Children with Leukemia: A Single-center Retrospective Study. J Dr Behcet Uz Child Hosp. 2023;13(1):1-8

INTRODUCTION

The coronavirus disease-2019 (COVID-19) pandemic has affected people of all ages, with children accounting for 1-5% of total number of pediatric patients with infections[1]. The severity and mortality of patients infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) were directly related to age[2]. As a result, children were less likely to develop severe illness from COVID-19 infection than adults. Indeed, 16% of them did not show any relevant signs or symptoms, while 1.8% of them required intensive care[3]. Infants and children younger than 5 years old were more likely to develop severe illness than older children due to their smaller age and immaturity of their immune system[4]. The risks for pediatric leukemia patients have not been understood very well. COVID-19 infection was found to be more common in adult cancer patients than in the general population[5-8]. Children being treated for leukemia who have become immune-compromised have been thought to be suffering from a severe illness[9]. In addition, some reports suggested that this was not the case[10]. The aim of this study is to look at the progression of COVID-19 infection in children with leukemia.

MATERIALS and METHODS

Between March 2020 and February 2021, we conducted a retrospective study to examine the characteristics and outcomes of hospitalized leukemia patients with COVID-9 infection. Patients on active anticancer treatment who were diagnosed with SARS-CoV-2 infection via reverse-transcription polymerase chain reaction (RT-PCR) testing of nasopharyngeal swabs were included in the study. The presence of any respiratory or gastrointestinal symptoms or signs in a patient who tested positive for SARS-CoV-2 by RT-PCR was defined as novel COVID-19. The guidelines of Republic of Turkey Ministry of Health (MoH) and World Health Organization (WHO) were used for diagnosis[11,12]. Clinical, laboratory, and radiological characteristics were used to classify infection severity as mild, moderate, severe, or critical according to the WHO and MoH disease severity classification[11,12]. Severe disease was defined based on the presence of dyspnea, central cyanosis, and an oxygen saturation of less than 92 percent. The diagnosis of critical disease necessitated the presence of respiratory failure, sometimes with acute respiratory distress syndrome, shock, and signs of multi-organ failure such as encephalopathy, heart failure, abnormal coagulation, and acute renal failure. Patients were treated according to recommendations of the MoH. Approval for the study was obtained from MoH and Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee (decision number: 2011-KAEK-25 2020/12-19, date: 23.12.2020) and informed consent from parents and/or patients.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0.

RESULTS

Fifteen pediatric leukemia patients had also SARS-CoV-2 positivity. Eleven of them had acute lymphoblastic leukemia (pre-B-ALL: 10, ALL trans pre-B with myeloid markers: 1) and four had acute myeloid leukemia (AML) (M3:2, M4:1, M1:1) (Table 1). The children's median age ranged from 2 to 18 years, with a male preponderance (66.7%). Patients under the age of ten accounted for 66.6 percent of all patients, and the ages of the highest percentage (33.3%) of patients ranged between 9-12 years. The indicated percentages of patients were receiving induction therapy (53.3%), consolidation therapy (40%) or undergoing diagnostic tests (6.6%). Most of the patients with COVID-19 infection (53.3%) were receiving (0-3 months) induction chemotherapy. The patients were asymptomatic or had mild (26.7%), moderate (26.7%), severe (13.3%) and critically severe COVID-19 infection (33.3%). All of the patients who had severe or critically severe COVID-19 infection were in their first 3 month of cancer treatment.

The most common presenting signs were fever in 73% and cough in 33% of the patients, while 7% of the patients were asymptomatic. COVID-19 pneumonia was detected in 93% of the patients and five of them had hypoxemia. Most frequently respiratory system involvement was seen in 93% of the patients, followed by involvement of central nervous system (CNS) (33%), gastrointestinal system (GIS) (27%) cardiovascular system (CVS) (27%), urinary system (20%), musculoskeletal system (7%) and skin (7%). CNS involvement presented with blurred vision in four, altered consciousness in two and cranial infarct in one patient. GIS involvement presented with diarrhea in two, vomiting in one and pancreatitis in one patient. CVS involvement manifested itself with hypotension and shock in four patients (Table 1).

At the time of presentation, laboratory tests revealed neutropenia in 66.6%, lymphopenia in 80%, and thrombocytopenia in 66.6% of our patients. C-reactive protein (CRP) (73.3%), D-dimer (80%), ferritin (93.3%), fibrinogen (20%), and lactate dehydrogenase (LDH)

Table 1. Demographic and clinical characteristics of leukemia patients with COVID-19		
Total number of cancer patients with cancer	**n=15**	**%**
Median age (range) (years)	9.4 (2-18)	
Sex		
Male	10	66.7
Female	5	33.3
Type of cancer		
AML	4	26.6
ALL	11	73.3
Disease status		
At diagnose	1	6.6
Induction	8	53.3
Consolidation	6	40
Diagnosis		
PCR, clinic and radiologic positivity	6	40
PCR positivity only (contact with positive person)	1	6.6
PCR negative, clinic and radiologic positivity	8	53.3
Contact history	9	60
Most frequent symptoms and findings		
Asymptomatic	1	6.6
Fever	11	73.3
Cough	5	33.3
Other respiratory system findings (tachypnea, dyspnea, hypoxemia)	5	33.3
Organs and system involvement		
Respiratory system (upper respiratory tract infection + pneumonia)	14	93.3
Gastrointestinal system	4	26.6
Diarrhea	2	13.3
Vomiting	1	6.6
Pancreatitis	1	6.6
Central nervous system	5	33.3
Blurred vision	4	26.6
Change in consciousness	2	13.3
Cranial infarct	1	6.6
Musculoskeletal system	1	6.6
Skin	1	6.6
Cardiovascular system (Hypotension and shock)	4	26.6
Urinary system	3	20
Radiologic findings		
Normal	1	6.6
Bilateral diffuse or patchy ground glass opacity	5	33.3
Diffuse or patchy pneumonic infiltration or consolidation	7	46.6
Pneumonic infiltration and ground glass opacity	2	13.3
COVID-19: Coronavirus disease-2019, AML: Acute myeloid leukemia, ALL: Acute lymphoblastic leukemia, PCR: Polymerase chain reaction		

(33.3%) levels increased in respective percentages of patients (Table 1). After treatment, CRP and ferritin levels decreased while hemoglobin, white blood cell (WBC), platelet (PLT), and lymphocyte counts increased. Patients coded as P1, P2, and P10 were critically ill children with greatest alterations in laboratory test results (Table 2). COVID-19 infection was discovered after four patients had received steroids as part of their leukemia treatment protocol. Patients suffering from febrile neutropenia received empirical treatment with broad-spectrum antibiotics. Six patients were given hydroxychloroquine, azitromycine, remdesevir, and lopinavir to treat COVID-19 infection. COVID-19 was untreated in nine patients. Mortality rates of patients who did and did not receive COVID-19 treatment did not differ statistically significantly.

The patients were all hospitalized for a median of 17 (6 to 53) days. Five patients (33%) required oxygen treatment. Six patients (40%) were admitted to the intensive care unit (ICU), and three (20%) were intubated (Table 2). Twelve patients recovered completely, and three (20%) patients died. One of the deceased patients had recently been diagnosed with AML, and the other two were in the first and third months of ALL treatment, respectively. AML patient had an intracranial infarct, diffuse intravascular coagulation, and fungal infection (*Candida albicans* was detected in blood culture). After contracting COVID-19 infection, all three patients died of cardiovascular shock. At a median of 11 (2 to 25) days, the PCR tests became negative. Two ALL patients were re-infected with COVID-19, with the second PCR positivity lasting longer than the first (Table 2).

DISCUSSION

The incidence of COVID-19 infection was estimated to be 0.8% in the general pediatric population, 1.3% in pediatric cancer patients, and 2.5% in pediatric oncology patients[13,14]. Children with cancer appear to be more vulnerable to COVID-19 than the general pediatric population.

Our patients' median age was 9.4 years (average 9.6), with the greatest percentage of age range being between 9 and 12 years (33.3%). In our study, as in the literature, there was a male preponderance (66.7%)[15-19].

In our study, a significant difference in the detection rate of COVID-19 infection was found between the patients in the early and late stages of leukemia treatment. The majority of patients (53.3%) who were diagnosed with COVID-19 infection were in the early stages of treatment (0-3 months) which could be related to the patient's weaker immunosuppression status at the start of treatment.

COVID-19 in pediatric patients leads generally asymptomatic, mild, or moderate course[14-21]. In our study, the percentage of patients with severe or critical COVID-19 infection (46.6%) were higher when compared with children having asymptomatic, mild, or moderate COVID-19 infection. The first three months of treatment were found to be the most critical period for pediatric leukemia patients with COVID-19 infection, as all of the patients who had critical or severe disease were in their first three months of treatment.

Most common presenting symptoms of our patients were fever and cough, which was consistent with the findings of COVID-19 infection reported in other cited studies[14-16,17,20-24]. Except for one study[25], other respiratory system findings (tachypnea, dyspnea, and hypoxemia) were more common than those reported in referenced studies[14,15,17,24]. The rates of involvement of the respiratory system were generally comparable to the literature data, whereas the involvement of the CNS and GSI was observed in greater number of our patients than reported in the literature[16,19,20-23]. CNS symptoms were the most common clinical symptoms in our critically ill patients with fatal outcomes. Other referenced studies[13,14,24,25] reported radiologic abnormalities at average incidence rates of 30%, 40%, 50%, and 57%, respectively, whereas radiologic abnormalities were found in 93% of our patients.

Significantly elevated CRP, fibrinogen, D-dimer, LDH, ferritin levels, as well as lymphopenia, thrombocytopenia, and neutropenia, were found in our critically ill patients comparable to those previously reported relevant laboratory values[13,14,17,24,26-29]. Following treatment, there was a decrease in CRP and ferritin levels, as well as an increase in hemoglobin, WBC, PLT, and lymphocyte levels. Patients with the greatest changes in these laboratory variables were generally severely/critically ill (P1, P2, and P10) children with fatal outcomes.

In the largest pediatric case series, more than 90% of children diagnosed with COVID-19 had asymptomatic, mild or moderate disease[30-34]. Children who required intensive care (1.8%) and having higher mortality rate (2.2%) had other underlying diseases [30-34]. Presumably, in pediatric immunosuppressive cancer patients with COVID-19 disease may have much more severe illness than in healthy children [35-37]. The link between immunosuppression and severe COVID-19 disease is not

Table 2. Laboratory characteristics, treatment, hospitalization days and prognosis of patients		
Total number of cancer patients with cancer	**n=15**	**%**
Laboratory findings		
Neutropenia	10	66.6
Lymphopenia	12	80
Anemia	15	100
Thrombocytopenia	10	66.6
CRP (mg/dL) median (range)	16.5 (3-182)	
Increased CRP	11	73.3
D-dimer (Mg/mL) median (range)	1.6 (0.4-35)	
Increased D-dimer	12	80
Ferritin (mL/ng) median (range)	1155 (349-10000)	
Increased ferritin	14	93.3
Fibrinogen (median) (range)	358 (62-694)	
Increased fibrinogen	3	20
LDH (median) (range)	253 (96-608)	
Increased LDH	5	33.3
Severity of disease		
Asymptomatic/mild	4	26.6
Moderate	4	26.6
Severe	2	13.3
Number of hospitalized	15	100
Length of hospitalization (median, range) day	17 (6-53)	
Necessity of intensive care unit	6	40
Necessity of oxygen treatment	5	33.3
Necessity of intubation	3	20
Treatment for COVID-19		
No treatment	9	60
Hydroxychloroquine	2	13.3
Hydroxychloroquine + Azitromycine	1	6.6
Lopinavir	2	13.3
Lopinavir + Remdesevir	1	6.6
Steroid treatment	4	26.6
Length of PCR positivity (median, range) days	11 (2-25)	
Outcome of disease		
Recovery	12	80
Death	3	20
Reinfection	2	13.3
Length of PCR positivity (median, range) at reinfection, days	21 (8-35)	
CRP: C-reactive protein, LDH: Lactate dehydrogenase, PCR: Polymerase chain reaction, COVID-19: Coronavirus disease-2019		

well established. In some studies children who received chemotherapy and immunosuppressive therapy had a mild/asymptomatic disease course and favorable clinic outcomes of COVID-19[13,18,38-40]. COVID-19 appeared to lead a milder course in the immune-compromised pediatric population, possibly due to non-smoking status, the presence of fewer comorbidities, lower expression of ACE2 receptors, and having a higher number of B and T regulator cells, resulting in a "lesser inflammatory" immune response[41,42]. Although the

proportion of children with cancer who had critical illness with progressive respiratory failure and required ICU (8.5-17.6%) was higher than in the general pediatric population[14,17], a systematic review could not find any correlation between COVID-19 disease and an increase in associated mortality in children with cancer. The types of tumors have no effect on the outcome[43]. Children in our study required ICU care (40%), were intubated (20%), and died (20%) at significantly higher rates than those previously reported in the literature[14,17]. These higher rates may be associated with several causative factors as follows: 1) greater number of children in our study had severe/critical (46.6%) COVID-19 disease; 2) the majority of them were still in the early stages of induction treatment; 3) two of three deceased patients had newly diagnosed leukemia with aggressive prognostic factors; and finally all patients had unfavorable prognostic laboratory findings regarding COVID-19 infection (higher CRP, fibrinogen, D-dimer, LDH, ferritin levels, lymphopenia, thrombocytopenia, and neutropenia). The prognosis of all re-infected patients is an important point to be considered in our study. The disease status of re-infected patients was worse relative to those with novel COVID-9 infection with longer duration of PCR positivity.

Study limitation

The limitations of our study are that it is a single-center study and the number of patients is low.

CONCLUSION

As a result, children with leukemia may have severe/critical COVID-19 infection. When diagnosis of leukemia is made, the course of chemotherapy, and the prognostic factors of COVID-19 infection may all become important criteria for clinic outcomes. In these children, extreme caution is required.

Ethics

Ethics Committee Approval: Approval for the study was obtained from MoH and Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee (decision number: 2011-KAEK-25 2020/12-19, date: 23.12.2020).

Informed Consent: Informed consent was received from parents and/or patients.

Peer-review: Internally peer reviewed.

Author Contributions

Surgical and Medical Practices: E.G.K., Concept: E.G.K., Design: E.G.K., Data Collection and/or Processing: D.G., R.Y., Analysis and/ or Interpretation: D.G., R.Y., Literature Search: D.G., Writing: D.G.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.92489
J Dr Behcet Uz Child Hosp 2023;13(1):9-15

Original Article

Antifungal Combination Therapy for Invasive Fungal Infections in Pediatric Leukemia Patients: An Observational Cohort Study

Pediatrik Lösemi Hastalarında İnvaziv Mantar Enfeksiyonları için Antifungal Kombinasyon Tedavisi: Gözlemsel Bir Kohort Çalışması

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ABSTRACT

Objective: The role of combination regimens in the treatment of invasive fungal infections (IFIs) in hematologic malignancies remains unclear. We aimed to demonstrate data about combined antifungal therapy (CAT) in pediatric leukemia patients with IFI.

Method: Between January 2014 and December 2018, a total of 33 IFI episodes in 28 leukemia patients were analyzed retrospectively.

Results: The study patients had acute lymphoblastic leukemia (n=19), acute myeloblastic leukemia (n=9), leukemia relapse (n=21; 75%) and remission (n=7; 25%). The patients were classified as having possible (n=26; 78.8%), probable (n=5; 15.1%) and proven IFI (n=2; 6.1%). Liposomal amphotericin B (LamB) was the most preferred agent (50%) in monotherapy. Mean duration of monotherapy was 12.84±4.28 (5-24) days. LamB plus voriconazole (54.5%) was the most commonly preferred CAT. Mean duration of CAT was 42.36±36.4 days, and this combination regimen was not changed throughout the treatment period (p=0.571). Total and IFI-related mortality rates were 60.7% vs 46.4%, respectively. Mortality rates were significantly higher in patients with relapse (p=0.006). Complete response was obtained in 81.8% of surviving patients. Side effects of CAT were observed at quite a low level.

Conclusion: CAT has been found to be safe in the treatment of IFI episodes of pediatric leukemia. Uncontrolled underlying disease is the most important factor affecting the mortality rates in IFI.

Keywords: Pediatric, leukemia, invasive fungal infection, combined antifungal therapy

ÖZ

Amaç: Hematolojik malignitelerde invaziv fungal enfeksiyonların (İFE) tedavisinde kombinasyon rejimlerinin rolü belirsizliğini korumaktadır. Bu çalışmada, lösemili pediyatrik İFE hastalarında kombine antifungal tedavi (KAT) ile ilgili verilerin sunulması amaçlamıştır.

Yöntem: Ocak 2014 ile Aralık 2018 arasında, lösemili 28 hastada toplam 33 İFE atağı geriye dönük olarak analiz edildi.

Bulgular: Hastaların (19'u akut lenfoblastik lösemili ve 9'u akut miyeloblastik lösemili), 21'inde (%75) lösemi relapsı ve 7'sinde (%25) remisyon mevcuttu. İFE, 26 (%78,8) atakta mümkün, 5 (%15,1) atakta olası ve 2 (%6,1) atakta kanıtlı idi. Monoterapide en çok tercih edilen ajan (%50) lipozomal amfoterisin B (LamB) idi. Ortalama monoterapi süresi 12,84±4,28 (5-24) gündü. KAT'da en sık kombinasyon tercihi LamB artı vorikonazol (%54,5) idi. Ortalama KAT süresi 42,36±36,4 gündü ve kombinasyon rejimi tipine göre değişmiyor idi (p=0,571). Toplam mortalite oranı ve İFE'ye atfedilebilir ölüm oranı %60,7'ye karşılık %46,4 idi. Relaps olan hastalarda mortalite oranı anlamlı olarak daha yüksekti (p=0,006). Hayatta kalan hastaların %81,8'inde tam yanıt alındı. KAT kullanımına bağlı yan etkiler oldukça düşük düzeyde gözlendi.

Sonuç: Pediyatrik löseminin İFE ataklarında KAT güvenli bulunmuştur. İFE'de mortalite oranını etkileyen en önemli faktör kontrolsüz altta yatan hastalıktır.

Anahtar kelimeler: Pediyatrik, lösemi, invaziv mantar enfeksiyonu, kombine antifungal tedavi

Received: 28.03.2022
Accepted: 25.07.2022

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Cite as: Kanık Yüksek S, Özkaya Parlakay A, Gülhan B, Yaralı N, Özbek NY, Tezer H. Antifungal Combination Therapy for Invasive Fungal Infections in Pediatric Leukemia Patients: An Observational Cohort Study. J Dr Behcet Uz Child Hosp. 2023;13(1):9-15

INTRODUCTION

Although many novel antifungal agents are in use, invasive fungal infections (IFI) continue to cause high morbidity and mortality rates in pediatric patients with hematologic malignancies[1,2]. Successful treatment of IFI is required to ensure the survival of this pediatric population. Currently, there are four classes (azoles, polyenes, pyrimidine analogues, and echinocandins) of drugs used in the treatment of IFI in children[3]. Appropriate use of available antifungals in this vulnerable population is important for the treatment of IFI[2]. However, safety and efficacy data including antifungal activity, pharmacokinetic properties, and toxicity of the antifungal agents in children still needs to be reinforced by trials[3,4]. Monotherapy is often preferred for the treatment of fungal infections in pediatric patients[4]. In some serious fungal infections where monotherapy is insufficient, the combination of antifungals remains on the agenda as a potential treatment strategy[5]. Since serious fungal infections need to be cured during the treatment of primary diseases, children with hematologic malignancies are the group of patients in whom combination regimens are frequently considered and tried to be applied[4,5].

Antifungal combination therapies are used in patients with hematologic malignancies in consideration of potential gains such as preventing resistance problems, increasing treatment efficacy and reducing side effects[5,6]. Combined antifungal therapy (CAT) is not a new notion, it is even used effectively in the treatment of some well-defined infections[7]. Unfortunately, the role of combination regimens in the treatment of IFI in patients with hematologic malignancies remains controversial[8]. Despite insufficient evidence, there are preclinical studies indicating that combination regimens are effective in treatment-resistant fungal infections[9-11]. However, these studies could not be transferred to the clinical practice, and quite few data are available for their clinical use. The limited data on this treatment regimen with scarce number of relevant prospective studies, and even more scanty data in pediatric patients, are obtained as a result of clinical experience[6,8,12-15]. In a few recent pediatric reports, it has been stated that CAT is preferred as a treatment option in progressive IFI or as a salvage treatment in patients with poor prognosis with anticipated achievement of satisfactory results[16-19]. The combinations of four groups of antifugal agents, acting through different molecular pathways and different cellular targets have been customized to the preference and priority of clinicians as there is no definitive accepted recommendation[8,9]. In the absence of sufficient evidence and suggestions; we aimed to demostrate experimental data on the use of CAT in pediatric IFI patients with hematologic malignancies, including the results of its efficacy and toxicity.

MATERIALS and METHODS

A retrospective study was conducted at University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital from January 2014 until December 2018. Patients under 18 years of age with a hematologic malignancy (acute lymphoblastic or myeloblastic leukemia) diagnosed as having IFI were enrolled in the study. Basic demographic data, underlying conditions, duration of neutropenia (absolute neutrophil count <500 cells/μL), radiological findings, antifungal medications, treatment-related clinical and laboratory side effects, and outcomes were noted. Galactomannan (GM) enzyme immunoassay (Platelia Aspergillus, BioRad, France) analyzes with plasma samples were performed twice weekly in the presence neutropenic fever and IFI episodes, and serum GM levels of ≥0.5 ng/mL were considered positive for IFI. All patients received prophylactic antifungal agents such as fluconazole or voriconazole according to their primary disease protocol (ALL IC BFM 2009, AML-BFM 2004 INTERIM, and ALL-REZ BFM 2002), and the risk or history of IFI. Diagnosis of IFI was defined according to the criteria of the European Organization for Research and Treatment of Cancer/IFIs Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG)[20]. Patients were grouped according to the proven, probable, or possible diagnosis of IFI. Empirical antifungal treatment was initiated if fever persisted for more than 96 hours after initiation of empirical antibacterial treatment in consideration of relevant guidelines[21,22]. If there were symptoms, signs, laboratory or radiological findings suggestive of IFI, antifungal therapy was initiated earlier than the onset of the above-mentioned treatment. CAT was initiated based on the severity of patient's IFI and clinician's own subjective decision. The day of diagnosis was defined as the day that the clinician confirmed diagnosis of IFI, and initiated the antifungal treatment. Response to treatment was defined according to criteria of EORTC/MSG[20]. Overall response and survival rates estimated for the duration of 12 weeks were recorded. Research ethics committee approval was obtained for the study

from the University of Health Sciences, Ankara Child Health and Diseases Hematology-Oncology Training and Research Hospital Clinical Research Ethics Committee of the institution (decision number: 2013-057, date: 19.11.2013).

Statistical Analysis

Statistical analysis was performed using SPSS v25.0 (IBM Corp., Armonk, New York, USA) statistical package. Categorical variables were compared by chi-square or Fisher exact tests, and summarized with frequencies. For continuous variables, median and interquartile range were calculated. Group comparisons were carried out using independent samples t-test or Mann-Whitney U tests, and Kruskal-Wallis test wherever appropriate. All tests were 2-sided with a significance level of 0.05.

RESULTS

Thirty-three IFI episodes in 28 patients were examined. Two or more episodes were recorded in four patients. Demographic and clinical characteristics of the patients, and data for IFI were listed in Table 1. None of the patients underwent hematopoietic stem cell transplantation prior to the onset of IFI. GM analysis was performed in all patients at the beginning of a febrile neutropenia attack and afterwards in case of need. During 10 (32.3%) episodes, GM analyzes yielded positive results. The median GM index of the positive episodes was 1 (0.74-9). The most common radiological findings were ground-glass opacities (75.8%), nodules (54.5%) and consolidations (24.3%). Cavitation and/or halo sign were recorded in 7 episodes. Radiological examination performed revealed the presence of pulmonary IFI in 27, hepatosplenic IFI in 2, central nervous system IFI in 1 and paranasal IFI in 1 episode.

Liposomal amphotericin B (LAmB) was the first antifungal agent used for monotherapy in 16 (50%), caspofungin in 11 (34.4%), and voriconazole in 5 (15.6%) episodes. Mean duration of monotherapy was 12.84±4.28 (5-24) days. The second antifungal was added because of insufficient response at all episodes, and voriconazole was preferred in 22 (68.7%), LAmB in 7 (21.8%), caspofungin in 2 (6.4%), and posaconazole in 1 (3.2%) patient. CAT was initiated directly in only one episode as voriconazole plus caspofungin. Preferred combination regimens, duration of therapy, treatment responses, and mortality rates are summarized in Table 2. Combination treatment with antifungal agents lasted between 15, and 67 days, and in surviving patients transition from combined regimen to monotherapy took 28-67 days. In both of the proven IFI episodes, the causative agents were *Candida* spp.

The 12-week overall and survival rates of the patients were 75% and 39.2%, respectively. The cause of death was progression of IFI in 13 patients, and the remaining 4 patients did not respond to the treatment of the underlying disease. IFI- related mortality rate was 46.4%. The IFI-related mortality rate was 76.5%. Mortality rates were significantly higher in patients with relapse, despite being treated with a specific relapse protocol, (χ^2=7.47; p=0.006). The effect of the combination regimen used on mortality was found to be statistically insignificant (z=1.3; p=0.192). Complete response (CR) to the treatment was obtained in 9 (81.8%) of 11 surviving patients. Median recovery time in patients with CR was 120 (28-210) days. Duration of neutropenia (z=0.39; p=0.695), CAT (z=1.37; p=0.173), and time to recovery (z=0.768; p=0.443) were not statistically different in the episodes with/without fatal outcomes. The effect of the selected primary antifungal agent could not be evaluated due to insufficient number of cases. Duration of neutropenia (z=0.22; p=0.821), CAT (t=0.795; p=0.446), and time to recovery (t=0.991; p=0.355) were not statistically significantly different according to relapse or remission status. There was no statistically significant relationship between IFI classification and the episodes with/without fatal outcomes (χ^2=3.726; p=0.293). Among the surviving patients, voriconazole was preferably used in 9 and posaconazole in two patients as the secondary prophylactic antifungal agents.

CAT was well tolerated in most patients. Renal dysfunction accompanied by electrolyte disturbances in one patient and increased transaminase levels in two patients were seen as adverse effects of CAT, and discontinuation of treatment was required in only one patient because of side effects. Monitoring plasma levels of voriconazole was not implemented in our center. IFI-induced surgical procedure was not applied to any patient.

DISCUSSION

IFI-related mortality rates range from 45% to 90%, and treatment success for IFI is reported to be around 60% in various reports[1,5,23,24]. The advantages and disadvantages of CAT in the treatment of IFI have not been determined compared to monotherapy. However, thanks to increased drug synergy and efficacy, and decreased resistance to antifungal drugs, the expectation that CAT may improve the outcome of the patients is still a valid assumption. Due to the absence

Table 1. Demographic and clinical characteristics of the patients, and data for IFI episodes	
Total cases	28
Age (years)	
Mean ± SD	8.79±5.03
Median (range)	7 (3.5-18)
Gender, n (%)	
Male	15 (53.6%)
Female	13 (46.4%)
Underlying diseases, n (%)	
ALL	19 (67.9%)
AML	9 (32.1%)
Status of underlying disease at the time of IFI episode, n (%)	
Remission	7 (25%)
Relapse	21 (75%)
Prophylactic agent before onset of IFI episode, n (%)	
Flucanazole	26 (92.8%)
Voriconazole	2 (7.2%)
Chemotherapy phase during IFI	
Induction	33 (100%)
Consolidation	0
Duration of prophylaxis before onset of IFI episodes (days)	
Mean ± SD (range)	47±24.04 (30-84)
Duration of neutropenia at the onset of IFI (days)*	
Mean ± SD (range)	42.87±35.5 (7-140)
in ALL	42.18±40.1 (7-140)
in AML	44.75±20 (20-64)
IFI classification, episode (%)	
Possible	26 (78.8%)
Probable	5 (15.1%)
Proven	2 (6.1%)
No statistical difference was found according to the underlying disease (p=0.18)	
ALL: Acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, IFI: Invasive fungal infection, SD: Standard deviation	

Table 2. Preferred combination regimens; duration of therapy, treatment responses, and mortality rates					
Combination regimens*	**n (%)**	**Duration (day) of therapy† Median (min-max)**	**Treatment response rates (%)**		**Mortality rates (%)**
			CR	**OR**	
LAmB plus Caspofungin	4 (12.1%)	23.5 (10-67)	25	50	50
LAmB plus Voriconazole	18 (54.5%)	32 (10-300)	33.4	50	50
Voriconazole plus Caspofungin	10 (30.3%)	65 (7-450)	20	20	80
LAmB plus Posaconazole	1 (3)	20	100	100	0
Total	33 (100%)	32 (7-450)	45	55	60.7
CR: Complete response, LAmB: Liposomal amphotericin B, OR: Overall response					
*All antifungals applied during CAT were used at the recommended doses for monotherapy.					
†was not statistically significant according to the combination regimen used (p=0.571)					

of prospective and randomized controlled clinical trials with an adequate statistical power, combined antifungal use in the treatment of IFI is included in current international guidelines as having a low level of evidence and recommendation[21,22]. Although the CAT approach in IFI has weak foundations, it is frequently applied in daily practice[14], and there are even reports emphasizing that this alternative has been used in up to 90% of the cases[25]. In a multicenter point prevalence survey from Turkey, Çağlar et al.[26] revealed that CAT was preferred in 8.4% and 61.5% of pediatric hematology, and oncology patients, respectively.

In our study, IFI-related mortality rates were not significantly different from the expected IFI-related mortality rates (46.4%). In previously published studies, overall treatment response to CAT was reported at rates ranging from 35% to 60%[8]. Generally higher 12-week survival rates were reported when compared with the overall response rates[8-12,16]. Previous studies reported that CAT appears helpful especially in patients with poor prognostic features[8,16-19]. Based on our results, considering that our patient population generally consisted of children with poor prognostic factors such as prolonged neutropenia and relapsed leukemia, despite lack of any control group, the overall response to CAT can be interpreted as favourable. However, ultimately the IFI-related mortality rates were quite high. Neutropenia is an important risk factor for IFI, and the duration of neutropenia affects the treatment response and mortality rates[2]. In our patient group, the mean duration of neutropenia was found to be 42.87±35.5 days which was longer than reported in previous studies[13,23]. Although the duration of neutropenia was not statistically different in the episodes with/without fatal outcomes, we thought that it was the main determinant in the higher IFI-related mortality rates.

Based on our results, LAmB was found to be the mostly preferred first-line agent to be used in monotherapy due to the inability to distinguish invasive aspergillosis from mucormycosis on the day of diagnosis. Monotherapy was switched to CAT within approximately two weeks in all IFI episodes and voriconazole-based combination regimen was applied most frequently. Although in vitro studies have reported that caspofungin and voriconazole have a synergistic effect against *Aspergillus* spp.[27,28] and this combination reduces fungal burden in animal tissues compared to single echinocandin or triazole administration[11], the combination regimen preferences may vary in practice[8,14,15]. In the literature, it is stated that LampB is often preferred as the first-line agent and voriconazole is included as a second agent in CAT regimens[16,18,19]. In our experience, the choice of antifungal combination was found to vary according to the first antifungal chosen in monotherapy, and LAmB plus voriconazole was the most common preference. The total mortality rate in combination regimens was around 60% in our patients, and the highest mortality rate was seen in the voriconazole plus caspofungin combination at a rate of 80%. When evaluating this result, it should be kept in mind that our study did not contain a control group, and a patient group that received monotherapy for the purpose of comparison. In response to the expectation of improvement in the outcomes of the patients, some authors have claimed that CAT is ineffective in improving patient outcomes in general[6,13,14,29], despite clinical trials with favourable results[6,12]. In our study, CAT could not be compared with monotherapy due to the absence of a control group, but the type of combination regimen used had not any impact on mortality. This study has demonstrated once again that the uncontrolled underlying disease was one of the most important factors affecting the mortality rates[1,2].

When the previously performed relevant studies are evaluated, it is seen that both the transition time from monotherapy to CAT and the duration of CAT have not been standardized, and varies widely[6,8,10,12-15]. In this study, the mean duration of therapy was 42.36±36.4 days, and remained unchanged according to the type of combination treatment regimen. The main determinant of the duration of CAT apparently is specified in consideration of the combined clinical, radiological and microbiological response of the patient. Due to individual differences in determining the treatment alternative, duration of CAT can be very short or very long[6,12-15,17].

CAT has been generally reported in association with an increased risk of adverse events[13,14]. Although adverse effects vary with the type of antifungal drug used, the most common side effects of CAT were reported as hepatic, renal, and neurologic toxicity[12,13]. Despite reports indicating increased risk of side effects, there are also studies reporting that CAT with proven efficacy does not cause significant side effects other than mild or moderate adverse events[6,16,17,30]. Based on our experience, treatment results of CAT are generally well tolerated. In other words, CAT has been found to have a favourable safety profile for use in the treatment of IFI episodes of pediatric leukemia.

Study Limitations

Our retrospective study has reported data coming from only one institute. Due to the small number of patients, the power of statistical comparisons decreased. Furthermore, due to quite limited number of cases with proven IFI, it is not clear which combined antifungal regimen was administered for IFI, which fungal agent was the causative pathogen. Despite the limitations, we think that the data of this study may contribute to the analysis of combined antifungal use in daily clinical practice, tolerability and treatment outcomes of CAT in IFI patients with hematologic malignancies.

CONCLUSIONS

Optimal therapy for IFI in patients with pediatric hematologic malignancies is unknown, while some clinicians use the CAT approach as an alternative to improve the outcome of these critically ill patients. However, though various preclinical studies suggest the possibility of using this preference, there is no definite accepted recommendation yet. Well-designed and randomized trials are required to define the role of combined antifungal use in pediatric patients with hematologic malignancies.

Ethics

Ethics Committee Approval: Research ethics committee approval was obtained for the study from the Ankara Child Health and Diseases Hematology-Oncology Training and Research Hospital Clinical Research Ethics Committee of the institution (decision number: 2013-057, date: 19.11.2013).

Informed Consent: Retrospective study.

Peer-review: Internally peer reviewed.

Author Contributions

Surgical and Medical Practices: S.K.Y., Concept: S.K.Y., Design: A.Ö.P., Data Collection and/or Processing: B.G., Analysis and/or Interpretation: N.Y., N.Y.Ö., H.T., Literature Search: S.K.Y., B.G., Writing: S.K.Y.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.92259
J Dr Behcet Uz Child Hosp 2023;13(1):16-22

Original Article

The Frequency of Smoking Use and Factors Related to Alcohol and Substance Use Among High School Students in İzmir

İzmir İlinde Lise Öğrencilerinde Sigara Kullanım Sıklığı ve Alkol-Madde Kullanımı ile İlişkili Faktörler

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ABSTRACT

Objective: To determine the frequency of cigarette use and the sociodemographic and psychological factors that may be related to alcohol and substance use and in all public high schools in Karabağlar district of İzmir province of Turkey.

Method: Sociodemographic variables, the self-confidence scale (SCS) and Multidimensional Scale of Perceived Social Support (MSPSS) were applied to 1,697 high school students online and their results were analyzed.

Results: Of the students, 159 (9.4%) were using cigarettes. The mean score of SCS was found to be 3.85±0.63. The mean score of MSPSS was found to be 64.51±16.71. The total MSPSS score of the students who smoke (61.51±17.30) was found to be significantly lower than non-smokers (64.82±16.62). Absenteeism rate and disciplinary punishment were found to be higher in smoking students. There was no significant relationship between students' SCS scores and smoking. The MSPSS scores of students who had friends who use alcohol or substances were lower than those who did not.

Conclusion: We have found that presence of any type of whether alcohol, substance or cigarette user in family incresed the risk of cigarrette use in students. MSPSS scores of cigarette users and who had alcohol or substance user friend was found lower in our study. These findings shows us strong relationship with addictive behaviours and social support. It is important to know factors associated with alcohol-substance use disorder and related factors in order to prevent this psychosocial problem.

Keywords: Cigarette, alcohol, substance, adolescent

ÖZ

Amaç: Bu çalışmanın amacı, İzmir ili Karabağlar ilçesinde yer alan tüm devlet liselerinde sigara kullanım sıklığı ve alkol-madde kullanımı ile ilişkili olabilecek sosyodemografik ve psikolojik faktörleri belirlemektir.

Yöntem: Sosyodemografik veri formu, Özgüven Ölçeği (SCS) ve Çok Boyutlu Algılanan Sosyal Destek Ölçeği (MSPSS), 1.697 lise öğrencisine online olarak uygulanmış ve sonuçları analiz edilmiştir.

Bulgular: Öğrencilerin 159'u (%9,4) sigara kullanmaktaydı. SCS puan ortalaması 3,85±0,63 olarak bulundu. MSPSS puan ortalaması 64,51±16,71 olarak bulundu. Sigara içen öğrencilerin toplam MSPSS puanı (61,51±17,30), içmeyenlere (64,82±16,62) göre anlamlı derecede düşük bulundu. Sigara içen öğrencilerde devamsızlık oranı ve disiplin cezası daha yüksek bulundu. Öğrencilerin SCS puanları ile sigara içme arasında anlamlı bir ilişki yoktu. Alkol veya madde kullanan arkadaşları olan öğrencilerin MSPSS puanları kullanmayanlara göre daha düşüktü.

Sonuç: Ailede veya arkadaşlarda her türlü sigara alkol ya da madde kullanıcısının bulunmasının öğrencilerde sigara kullanım riskini artırdığını saptadık. Çalışmamızda sigara ve alkol ya da madde kullanan arkadaşları olan öğrencilerin MSPSS puanları daha düşük bulundu. Bu bulgular bize bağımlılık yapan davranışlar ve sosyal destek ile güçlü bir ilişki olduğunu göstermektedir. Alkol-madde kullanım bozukluğu ile ilişkili faktörlerin ve ilişkili faktörlerin bilinmesi bu psikososyal sorunu önlemek için önemlidir.

Anahtar kelimeler: Sigara, alkol, madde, adölesan

Received: 20.04.2022
Accepted: 27.07.2022

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Cite as: Kılıç OHT, Aksoy İ, Çetin AS, Güvendi G, Çoban İ, Dirim Mete B. The Frequency of Smoking Use and Factors Related to Alcohol and Substance Use Among High School Students in İzmir. J Dr Behcet Uz Child Hosp. 2023;13(1):16-22

INTRODUCTION

Adolescence is the transition period extending from childhood to adulthood. During this period when brain maturation takes place; functions of the brain such as impulse control, reasoning, and the ability to evaluate the consequences of behavior develop. Hence adolescence, when these functions are not fully mature, is a risky period for alcohol and substance use. Moreover, during this period, alcohol and substance use impairs cortical maturation, leading to deterioration in executive functions, the perpetuation of impulsive behaviors, and a further increase in the risk of addiction[1].

Cigarette, alcohol, and substance use is a growing global public health concern, especially in developing countries. According to the 2008 parliamentary research commission data, respective percentages of high school students use cigarettes and alcohol (19.2%), cigarettes and substances (5.7%), alcohol and substances (5.6%), and all three of them (4.9%) in Turkey[2]. European School Survey Project on Alcohol and Other Drugs (ESPAD) has been collecting data since 1995 and the latest update was provided in 2019 by 99,647 students from 35 European countries including Turkey which released the last data in 2003. The rates of daily cigarette smoking ranged from 1.9% in Iceland and Norway to 22% in Bulgaria. Over half of the students stated that they had used alcohol at least once in their life. The rates of cannabis climbed up to 16% whereas use of other illicit substances was reported by 3.4% of the study participants[3].

In our country, there are not enough studies on the frequency and related causative factors of cigarette, alcohol, and substance use. Systematic studies are even more difficult to perform, especially among high school students, due to the drawbacks of school administrators. In our study, we aimed to determine the frequency of cigarette, alcohol, and substance use and the possibly relevant sociodemographic and psychological factors prevalent in all public high schools in the Karabağlar district of İzmir province of Turkey.

MATERIALS and METHODS

Population and Sampling

The population of the research consisted of 17,689 students studying at state high schools in Karabağlar District of İzmir Province of Turkey where a total of 5 vocational technical high schools (including 1 multi-program high school), 6 religious vocational high schools, and 12 Anatolian high schools (1 multi-program high school) exist.

We established contact with the school principals and counselors where the students were educated through Karabağlar District National Education Directorate and Karabağlar Guidance Research Center Directorate, and an online meeting was held on Wednesday, March 24, 2021, with the participation of school principals and guidance teachers, and information was given about the purpose of the research and how to deliver the questionnaires to the students. The questionnaire, consisting of questions about sociodemographic variables (n=20), multidimensional perceived social support (n=12), and self-confidence (n=33) was prepared through the online survey portal SurveyMonkey® (www.tr.surveymonkey.com). The survey link was sent to the WhatsApp® accounts of the parents of a total of 1,697 students and the answers were collected after informed consent the of students, and their parents participating in the study were obtained digitally. The ethical approval was obtained from İzmir Democrasy University Non-invasive Clinical Research Ethics Committee (number: 2020/07, date: 27.02.2020).

Data Collection Tools

Sociodemographic Data Form

This questionnaire was prepared by the researchers and consisted of 20 questions inquiring the students' age, gender, and grade, the type of school they attended; whom they lived with; the monthly total income of the family; the employment status and the education level of the parents; whether they have received any psychiatric or psychological treatment and/or disciplinary punishment before; their rates of absenteeism; whether they have friends and/or family members who were / had been using alcohol, substance or cigarettes, and their opinions about the ease of access to substances. Due to the drawback of the school administrators, only responses to the questions related to smoking status were included in the data form, and frequencies of alcohol-substance use were not.

Self-Confidence Scale

The self-confidence scale (SCS) was developed by Akın[4]. The 33-item scale is a 5-point Likert-type scale with responses categorized as "1=never, 2=rarely, 3=often, 4=generally, and 5=always". Validity and reliability analyzes of the scale were performed and the Cronbach alpha value was calculated as 0.89. The highest score that can be obtained from the scale is 165 and the lowest score is 33 points. A high score on the scale indicates higher level of self-confidence. A person's self-

confidence level is calculated by dividing the total score obtained from the scale by the number of items on the scale. Very low (1.00-1.79), low (1.80-2.59), moderate (2.60-3.39), high (3.40-4.19), and very high (4.20-5.00) levels of self-confidence are categorized based on the scores obtained by the responders.

Multidimensional Scale of Perceived Social Support

The 12-question Multidimensional Scale of Perceived Social Support (MSPSS) which has been developed by Zimet et al.[5] in 1998 was used for the subjective assessment of social support. MSPSS is a 7-point Likert-type scale with answers graded between "1= absolutely no, and 7= absolutely yes". The validity and reliability analysis of the scale in Turkish was performed by Eker and Arkar[6] in 1995 and the reliability coefficient was calculated as 0.89. The scale has three subdimensions as family, friend, and special person support which represent the support sources and each subdimension has four items. The subdimension score is obtained by adding the scores of four items in each subdimension, and the total score of the scale is obtained by summing all subdimension scores. Higher total scores indicate higher perceived social support.

Statistical Analysis

Collected data was analyzed using the statistical package program (SPSS 20.0) and the results were interpreted. Descriptive statistics including arithmetic mean, standard deviation, frequency, and percentage distribution were presented. The data showed normal and parametric distribution. The chi-square test and the independent t-test were applied to compare variables between smokers and non-smokers. A p-value of below 0.05 was considered to be statistically significant.

RESULTS

Sociodemographic and Education-related Characteristics

A total of 1,697 students including 1,102 (65.3%) female, and 585 (34.7%) male participants were enrolled in our study. The students were living with their parents (n=1,464; 86.6%), their mothers (n=162; 9.6%), fathers (n=34; 2.0%), and a non-family person (n=30; 1.8%). A total of 142 (8.4%) students had previously received or were receiving psychiatric or psychological treatment. The rates of smoking, alcohol, and substance use in the parents of the students were 55.7% (n=940), 19.4% (n=329), and 1.1% (n=19), respectively. The total income of families were; below 2,500 (n=486; 29.2%), 2,500-5,000 TL (n=767; 46.1%), 5,000-10,000 TL (n=337; 20.3%) and over 10,000 TL (n=72; 4.3%).

The study participants were 9th (n=734; 43.5%), 10th (n=503; 29.8%) 11th (n=282; 16.6%) and 12th (n=170; 10.1%) grade. While, they were studying in Anatolian high school (n=1,255; 74.3%), vocational technical high school (n=332; 19.6%), and religious vocational high school (n=103; 6.1%). Only 95 (5.6%) students had received disciplinary punishment before. The students exceeded the maximum days of absence rights (n=17; 1.0%), or they were very often (n=33; 2.0%) or rarely (316; 18.7%) cut classes, and 1321 (78.3%) of them stated that they did not make absenteeism unless necessary.

Perceived Social Support and Self-confidence

The mean score of SCS was found to be 3.85±0.63 points. According to the scale estimation scores, students had very low (n=6; 0.4%), low (n=63; 3.7%), moderate (n=311; 18.3%), high (n=785; 46.3%), and very high (n=532; 31.3%) levels of self-confidence.

The mean score of MSPSS was found to be 64.51±16.71. The mean scores of "family", "friend" and "special person" subdimensions of the scale were found to be 23.08±6.09, 20.67±7.33, and 20.76±7.69 points, respectively.

Data on Smoking Status of the Students

A total of 159 (9.4%) students were using cigarettes. The mean age of the students who were smoking (16.55±1.38) was found to be significantly higher than those who were not (15.85±1.18). The rate of smokers in 12th grade (21.2%) was higher when compared with 9th (6.9%), 10th (7.0%) and 11th (11.0%) grade students (p=0.001). There was no significant difference between male (8.0%) and female (9.7%) students in terms of smoking (p=0.287). Students whose families were using cigarettes, alcohol, or substance had significantly higher rates of smoking (p=0.001). Neither monthly total income of the family (p=0.063) nor maternal or paternal education levels (p=0.462, p=0.216) were related to smoking status of the students (Table 1).

The total MSPSS score of the students who smoke (61.51±17.30) was found to be significantly lower than that of non-smokers (64.82±16.62) (p=0.015). There was no significant relationship between students' SCS scores and smoking (p=0.362) (Table 2).

The smoking rate of students studying at vocational technical high school (17.5%) was higher than students

studying at religious vocational high school (10.7%), which was the lowest among students studying at Anatolian high school (%6.9) (p=0.001). Absenteeism rate and disciplinary punishment were found to be higher in smokers (p=0.004, p≤0.001) (Table 3).

Data on Alcohol or Substance Use

The students stated that accessing illicit substances was very difficult (n=47; 2.8%), difficult (n=31; 1.8%), easy (n=228; 13.5%), and very easy (n=241; 14.3%), while 1,137 (67.5%) students indicated that they did not have information about easiness of procurement of illicit substances.

Students had 1-5 (n=54; 3.18%) and more than 5 (n=15; 0.9%) 5 friends who used substance. The mean age of the students who had substance user friends was higher than those who had not (p<0.001). The rate of having friends who used substances in the 9th grade (1.8%) was found to be lower compared to the other grades (5.2% in 10th, 5.7% in 11th and 5.9% in 12th grades) (p<0.001). No significant difference was found between the rates of substance use

Table 1. Relationship between smoking and sociodemographic factors

	Smoker (n=154; 9.1%)	Non-smoker (n=1,533; 90.9%)	p-value
Gender			
Male (n=585; 34.7%)	47 (8%)	538 (92%)	0.287
Female (n=1,102; 65.3%)	107 (9.7%)	995 (90.3%)	
Age (mean ± SD)	16.55±1.38	15.89±1.18	<0.001
Smoking status of parents			
Yes (n=940)	106	834	0.001
No (n=749)	50	699	
Alcohol use of parents			
Yes (n=329)	50	279	<0.001
No (n=1362)	106	1256	
Substance use of parents			
Yes (n=19)	6	13	0.001
No (n=1673)	150	1523	
Total monthly income			
0-2,500 TL	53 (35.8%)	433 (28.6%)	0.063
2,500-5,000 TL	66 (44.6%)	701 (46.3%)	
5,000-10,000 TL	20 (13.5%)	317 (20.9%)	
Above 10,000 TL	9 (6.1%)	63 (4.2%)	
Paternal education			
Illiterate	4 (2.6%)	18 (1.2%)	0.216
Literate	4 (2.6%)	26 (1.7%)	
Primary school	52 (33.5%)	459 (30.0%)	
Middle school	40 (25.8%)	352 (23.0%)	
High school	39 (25.2%)	432 (28.2%)	
University	16 (10.3%)	245 (16.0%)	
Maternal education			
Illiterate	10 (6.4%)	70 (4.6%)	0.462
Literate	9 (5.8%)	53 (3.5%)	
Primary school	56 (35.9%)	568 (37.0%)	
Middle school	24 (15.4%)	247 (16.1%)	
High school	42 (26.9%)	392 (25.5%)	
University	15 (9.6%)	206 (13.4%)	

SD: Standard deviation

and friendliness between female (3.7%) and male (4.1%) students (p=0.692). There was no difference between school types (p=0.187) and gender (p=0.692) in terms of having friends who were using illicit substances.

Students had 1-5 (n=305; 17.9%) and more than 5 friends (n=153; 9%) who had alcohol addiction. The mean age of those who had friends who were using alcohol was higher than those who were not (p<0.001). Higher number of friends in 12th (50%) and 11th (44.7%) grades were using alcohol when compared with 10th (29.8%) graders, whereas greater number of 10th graders were using alcoholic beverages relative to 9th (12.5%) graders (p<0.001). There was no significant difference between female (27.0%) and male (26.3%) students in terms of the number of friends using alcohol (p=0.817). Higher number of friends among Anatolian high school students (29.3%) were using alcohol when compared with vocational technical high school (21.4%) and religious vocational high school (14.6%) (p<0.001) students.

The MSPSS scores of students who had friends who were using alcohol were lower than those who did not (p=0.001). Similarly, the MSPSS scores of students who had friends who were using illicit substances were lower than those who were not (p=0.001). There was no significant relationship between students' SCS scores and having friends who were using illicit substances (p=0.142).

Table 2. The relationship between smoking and self-confidence and perceived social support

		Smoker (n=159; 9.4%)	Non-smoker (n=1,538; 90.6%)	p-value
SCS levels (Total mean: 3.85±0.63)	Very low (n=6; 0.4%) Low (n=63; 3.7%) Middle (n=311; 18.3%) High (n=785; 46.3%) Very high (n=532; 31.3%)	3.79±0.67	3.86±0.63	0.362
MSPSS levels (Total mean: 64.51±16.71)		61.51±17.30	64.82±16.62	0.015

SCS: Self-confidence scale, MSPSS: Multidimensional scale of perceived social support

Table 3. The relationship between smoking and school-related factors

	Smoker	Non-smoker	p-value
Grade			
9th grade	51 (6.9%)	683 (93.1%)	<0.001
10th grade	35 (7.0%)	468 (93.0%)	
11th grade	31 (11.0%)	251 (89.0%)	
12th grade	36 (21.2%)	134 (78.8%)	
School type			
Religious vocational high school	11 (10.7%)	92 (89.3%)	0.001
Vocational technical high school	58 (17.5%)	274 (82.5%)	
Anatolian high school	87 (6.9%)	1168 (93.1%)	
Disciplinary punishment			
Yes	28 (17.9%)	67 (4.4%)	<0.001
No	128 (82.1%)	1468 (95.6%)	
Absenteeism			
No unless necessary	105 (67.3%)	1,216 (79.4%)	0.004
Rarely	42 (26.9%)	274 (17.9%)	
Often	6 (3.8%)	27 (1.8%)	
Use all limit	3 (1.9%)	14 (0.9%)	

DISCUSSION

We have found lower perceived social support levels in smokers. The smoking risk was associated with having disciplinary punishment and higher absenteeism, presence of alcohol, cigarette, or substance user(s) in the family, higher age, 12th grade, and school type. There was no relationship between smoking status and self-confidence levels, gender, family income, or education level.

In the current study, the overall smoking rate was found as 9.4% which was reaching up to 21.2% among 12th graders. Aras et al.[7] performed a similar study in a different district of İzmir province and found that 24.3% of students were smokers, which is similar to our results concerning 12th graders. Consistent with our results they also stated that the frequency of smoking was significantly higher in students with higher absenteeism and higher rates of disciplinary punishment. Adolescents are susceptible to engaging in risky and impulsive behaviors, including substance use because the brain areas associated with executive functioning, judgment, and decision-making maturate after the areas associated with emotional responses and reward systems[8]. In a study, researchers suggested that adolescents were more vulnerable to the addictive properties of nicotine because the duration of smoking and the number of cigarettes required to establish nicotine addiction is lower compared to adults[9]. Also, the dramatic increase in smoking rates in the 12th grade may be related to the stress of the Higher Education Foundations Examination. It is known that increased stress levels can increase the rates of smoking, alcohol, and substance use[10]. Absenteeism may be the result of alcohol, substance, and cigarette use, or it may lead students to become acquainted with drugs.

The influences of family, peers and school interactions are the primary sources of both encouraging and discouraging messages for the habit of smoking in the lives of adolescents[11]. In accordance with this data we have found that the presence of any type of alcohol, substance, or cigarette user in the family increased the risk of cigarette use in students.

Kokkevi et al.[12] studied the ESPAD data for 6 European countries and concluded that the self-confidence levels were not correlated with substance use in line with our results. We did not find any relationship between students' SCS scores and cigarette use or having a friend who were using illicit substances. We think that high levels of self-confidence can increase risky behaviors such as smoking, alcohol and substance use, while low self-esteem levels can increase the rates of addiction for self-medication, which means misusing them as a partially successful attempt to soothe painful emotions[13].

MSPSS scores of cigarette users and those who had alcohol or substance user friend(s) were comparatively lower in our study. These findings show a strong relationship with addictive behaviors and social support. In a study, which supports our results, researchers have found that teenagers reporting poor perceived social support showed a significantly higher frequency of cigarette smoking[14]. Social support can decrease addiction risk in students by increasing their sense of well-being, protecting them from the negative effects of stress, and contributing to personal development[15,16].

Another remarkable finding of our study is that more than one-fourth of the students stated that accessing illicit drugs was easy or very easy. Similar results were found in a study in which most of the participants stated illicit drugs were available at low cost[17]. Despite stringent laws in our country prohibiting the sale of cigarettes and alcohol to people under 18 years old, adolescents have easy access to cigarettes and alcohol either by using adults or purchasing them themselves. On the other hand, this easy accessibility to illicit drugs, and alcoholic beverages may be due to their sales on online platforms, which are relatively more difficult to control and are used more frequently by adolescents compared to the general population. These results show us the inadequacy in the fight against their procurement, which is one of the most important pillars in the fight against use of addictive substances.

Socioeconomic status and male gender are known traditional risk factors for any substance-related disorders but we didn't find any relationship between gender, income or education level of the parents with smoking status or having substance-alcohol user friends. Recent studies have shown a narrowing gender gap in substance use disorders[18]. However, lower socioeconomic status still has been linked to increased rates of substance use among youngsters[3]. In our study, the fact that all of the students were studying in a public school and in the same district may explain our results.

Study Limitations

The cross-sectional design of our study and evaluation of students based on self-reported behavioral characteristics rather than direct observation constitutes

limitations of our study and prevents generalization of our data to the whole country and different age groups.

CONCLUSION

It is important to know the factors associated with alcohol-substance use disorder and related factors to prevent this psychosocial problem. Keeping track of cigarette use among adolescents is important because use of tobacco products is usually the first step before starting to use other illicit substances, which is termed the "gateway" phenomenon.

Acknowledgments: We would like to thank Karabağlar District Governorship of İzmir for their cooperation.

Ethics

Ethics Committee Approval: The ethical approval was obtained from İzmir Democrasy University Non-invasive Clinical Research Ethics Committee (number: 2020/07, date: 27.02.2020).

Informed Consent: The survey link was sent to the WhatsApp® accounts of the parents of a total of 1,697 students and the answers were collected after informed consent the of students, and their parents participating in the study were obtained digitally.

Peer-review: Internally peer reviewed.

Author Contributions

Concept: O.H.T.K., A.S.Ç., B.D.M., Design: O.H.T.K., A.S.Ç., G.G., Data Collection or Processing: O.H.T.K., A.S.Ç., İ.Ç., Analysis or Interpretation: İ.A., G.G., İ.Ç., B.D.M., Literature Search: O.H.T.K., İ.A., G.G., İ.Ç., B.D.M., Writing: O.H.T.K., İ.A., A.S.Ç.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.98475
J Dr Behcet Uz Child Hosp 2023;13(1):23-29

Original Article

Retrospective Analysis of Children with Chronic Non-bacterial Osteomyelitis

Kronik Non-bakteriyel Osteomiyelitli Çocukların Retrospektif Analizi

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ABSTRACT

Objective: Chronic non-bacterial osteomyelitis (CNO) is a rare autoinflammatory bone disorder which mainly affects children and young adolescents. In this study, we report our single-center experience with pediatric CNO patients.

Method: Children diagnosed with CNO at the Department of Pediatric Rheumatology of Hacettepe University between November 2006 and July 2021 were retrospectively reviewed. The demographics, clinical features, laboratory findings, imaging modalities, concomitant diseases, and treatments were recorded. Diagnostic delay was defined as the time interval from symptom onset to diagnosis.

Results: A total of 48 patients (52.1% male) with a median age of 13.7 (minimum-maximum: 3.3-20.4) years were included. Local bone pain was the most frequent symptom (72.9%), followed by arthralgia (52.1%), limping or difficulty in walking (43.8%), and back pain (33.3%). Elevated erythrocyte sedimentation rate (52.1%) and high C-reactive protein levels (43.8%) were the most frequently observed laboratory abnormalities. Magnetic resonance imaging (MRI) (regional MRI in 87.5% and whole-body MRI in 66.7% of patients) was widely used in the diagnosis. Non-steroidal anti-inflammatory drugs (NSAIDs) were used in all patients and six patients (12.5%) achieved complete clinical remission with NSAIDs alone. Methotrexate (MTX) (80.9%), biological agents (7.1%), and pamidronate (11.9%) were used as the second-line treatment. Also, 26.4% of patients achieved clinical remission with MTX. Biological treatment was required in a total of 27 patients (56.2%).

Conclusion: Local bone pain is a warning sign for CNO diagnosis. Complete clinical remission can be achieved in CNO patients with an escalating anti-inflammatory treatment, having NSAIDs in one end, and biological drugs and bisphosphonates on the other end of the spectrum.

Keywords: Chronic non-bacterial osteomyelitis, children, autoinflammatory bone disease

ÖZ

Amaç: Kronik nonbakteriyel osteomiyelit (KNO) nadir görülen bir otoenflamatuvar kemik hastalığıdır ve esas olarak çocukları ve genç adolesanları etkiler. Bu çalışmada pediatrik KNO hastalarına ait tek merkez deneyimimizi sunmayı amaçladık.

Yöntem: Hacettepe Üniversitesi Çocuk Romatoloji Anabilim Dalı'nda Kasım 2006-Temmuz 2021 tarihleri arasında KNO tanısı alan çocuklar geriye dönük olarak değerlendirildi.

Bulgular: Çalışmaya ortanca yaşı 13,7 (min-maks: 3,3-20,4) yıl olan toplam 48 hasta (%52,1 erkek) dahil edildi. Lokal kemik ağrısı en sık görülen semptomdu (%72,9), bunu artralji (%52,1), topallama veya yürüme güçlüğü (%43,8) ve bel ağrısı (%33,3) izledi. Artmış eritrosit sedimantasyon hızı (%52,1) ve yüksek C-reaktif protein seviyeleri (%43,8) en sık gözlenen laboratuvar anormallikleriydi. Tanıda manyetik rezonans görüntüleme (MRG) (hastaların %87,5'inde bölgesel MRG ve %66,7'sinde tüm vücut MRG) yaygın olarak kullanılmıştır. Tedavide tüm hastalara nonsteroid anti-enflamatuvar ilaçlar (NSAİİ) verildi ve altı hasta (%12,5) sadece NSAİİ tedavisi ile tam klinik remisyon sağladı. İkinci basamak tedavi olarak metotreksat (MTX) (%80,9), biyolojik ajanlar (%7,1) ve pamidronat (%11,9) kullanıldı. Hastaların %26,4'ü MTX ile klinik remisyona ulaştı. Toplam 27 hastada (%56,2) biyolojik ajan tedavisi gerekti.

Sonuç: Lokal kemik ağrısı, KNO tanısı için uyarıcı bir işarettir. Spektrumun bir ucunda NSAİİ'lerin diğer ucunda ise biyolojik ilaçlar ve bifosfonatların bulunduğu artan anti-enflamatuvar tedavi planı ile KNO hastalarında tam klinik remisyon elde edilebilir.

Anahtar kelimeler: Kronik non-bakteriyel osteomiyelit, çocuk, otoenflamatuvar kemik hastalığı

Received: 19.06.2022
Accepted: 27.07.2022

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Cite as: Kaya Akca Ü, Bayındır Y, Bilginer Y. Retrospective Analysis of Children with Chronic Non-bacterial Osteomyelitis. J Dr Behcet Uz Child Hosp. 2023;13(1):23-29

INTRODUCTION

Chronic non-bacterial osteomyelitis (CNO) is an autoinflammatory disorder characterized by sterile bone inflammation[1]. It was defined as "chronic recurrent multifocal osteomyelitis", since the first described cases were characterized by subacute and chronic symmetric multifocal bone lesions. However, considering that the disease is not always multifocal and recurrent, the term CNO is commonly used as an umbrella term for all presentations[2,3]. Although the annual incidence is estimated to be 0.4 per 100,000 children, the true incidence of CNO in childhood is still unclear[4]. The number of new cases has been increasing, as awareness raises[5]. The co-occurrence of inflammatory bowel disease (IBD), palmoplantar pustulosis, psoriasis, acne fulminans, and ankylosing spondylitis with CNO has also been reported[6-9]. Altered expression of cytokine and chemokine is considered to play a central role in the pathogenesis of CNO. Reduced interleukin-10 (IL-10) levels, high levels of pro-inflammatory cytokines [IL-1β, IL-6, tumor necrosis factor-alpha (TNF-α)] and chemokines, increased inflammasome assembly, and osteoclast differentiation and activation are involved in the pathogenesis[10,11].

The most common clinical manifestation is bone pain which is a common symptom in childhood[12]. CNO osteomyelitis may be difficult to diagnose due to the non-specific symptoms and clinical findings. Possible infective and malignant causes should be ruled out in the differential diagnosis. The fact that acute phase reactants may be normal in laboratory evaluation and no pathology can be detected in radiographs, particularly in the early stage of the disease, makes the diagnosis even more difficult. Magnetic resonance imaging (MRI) has a high sensitivity in detecting CNO lesions. In the treatment, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs including sulfasalazine and methotrexate (MTX), biological treatments (anti-TNF agents), and bisphosphonates are widely used.

In the present study, we aimed to report our single-center experience in pediatric patients with CNO.

MATERIALS and METHODS

This single-center, retrospective study was conducted at Department of Pediatric Rheumatology of Hacettepe University a tertiary care center between November 2006 and July 2021. Patients diagnosed with CNO were included. The demographics, clinical features, laboratory findings, imaging modalities, concomitant diseases, and treatments were recorded. Diagnostic delay was defined as the time interval from symptom onset to diagnosis. Since the lack of validated and accepted diagnostic criteria, the diagnosis was based on expert opinion and the exclusion of other bone pathologies such as malignancies and infections. In the presence of CNO-related symptoms, typical findings on bone imaging were helpful for diagnosis. Laboratory tests at the time of diagnosis were also noted.

The study was approved by the Hacettepe University Non-invasive Clinical Research Ethics Committee (decision no: 2022/10-04, date: 07.06.2022) and conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using the SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in median [minimum (min) - maximum (max)] or number and frequency. The normality of distribution of the variables was checked using the visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test).

RESULTS

Patient Characteristics

A total of 48 patients, 25 boys (52.1%), with a median age of 13.7 (min-max: 3.3-20.4) years were enrolled in the study. The demographics and clinical findings of the patients at the time of diagnosis are summarized in Table 1. The median age at diagnosis was 9.9 (min-max: 2.9-16.8) years and the median follow-up time was 2.3 (min-max: 0-12.9) years. The median diagnostic delay time was 1.0 (min-max: 0-6.3) year. The most common symptom observed in almost three-quarters of patients was local bone pain and most of them presented with leg pain. Other common signs and symptoms included arthralgia (52.1%), limping or difficulty in walking (43.8%), and back pain (33.3%). In addition, CNO was accompanied by psoriasis in three (6.3%), IBD in four (8.3%), and severe papulopustular lesions in one patient (2.1%). Two patients met the International League of Associations for Rheumatology classification criteria for enthesitis-related arthritis.

Parental consanguinity was present in 14.6% of patients and five patients had a family history of rheumatic disease including rheumatoid arthritis,

ankylosing spondylitis, and psoriatic arthritis. Physical examination revealed tenderness on palpation of the affected area in 28 (58.3%), swelling of the affected area in seven (14.6%), and redness in one patient (2.1%). The modified Schober's test was positive in 13 of 35 patients (37.1%).

Laboratory Features and Diagnostic Tests of Patients

In the laboratory examination, elevated erythrocyte sedimentation rate (ESR), high C-reactive protein (CRP) levels, thrombocytosis, anemia, and leukocytosis were found in decreasing order of frequency (52.1%, 43.8%, 39.6%, 27.1%, and 2.1%, respectively) (Table 2). Human leukocyte antigen-B27 positivity was detected in four of 15 patients (26.6%). During the diagnosis, regional MRI was performed in 42 (87.5%), whole-body MRI in 32 (66.7%), plain radiography in 25 (52%), and bone scintigraphy in eight patients (16.7%). Bone marrow aspiration was performed in 18 patients (37.5%) and bone biopsy in 19 patients (39.6%) to exclude malignancy. Pathological fractures were detected in seven patients (14.5%) in the imaging findings.

Table 1. Demographic and clinical features of patients with chronic non-bacterial osteitis

Median (minimum-maksimum) or n (%)	Patient number (n=48)
Gender, male	25 (52.1)
Age at study, years	13.7 (3.3-20.4)
Age at disease onset, years	8.5 (0.1-13.4)
Age at diagnosis, years	9.9 (2.9-16.8)
Diagnostic delay, years	1 (0-6.3)
Follow-up time, years	2.3 (0-12.9)
Fever	11 (22.9)
Fatigue	11 (22.9)
Weight loss	1 (2.1)
Local bone pain	35 (72.9)
Diffuse bone pain	4 (8.3)
Leg pain	33 (68.8)
Back pain	16 (33.3)
Arthritis	7 (14.6)
Arthralgia	25 (52.1)
Walking with a limp or difficulty in walking	21 (43.8)
Concomitant diseases	
Inflammatory bowel disease	4 (8.3)
Psoriasis	3 (6.3)
Severe papulopustular lesions	1 (2.1)
PFAPA syndrome	1 (2.1)
Parental consanguinity	7 (14.6)
Family history of rheumatic diseases	5 (10.4)
Swelling of affected area	7 (14.6)
Tenderness on palpation of the affected area	28 (58.3)
Redness of the affected area	1 (2.1)
Schober's test <5 cm	13/35 (37.1)

PFAPA: Periodic fever, aphthous stomatitis, pharyngitis, and adenitis

Treatments

The flowchart of treatment in our cohort is shown in Figure 1. All patients received NSAIDs in the first-line treatment, until the definitive diagnosis was made. Six patients (12.5%) achieved remission with only NSAIDs. Subsequent medical treatment was MTX in 34 patients (80.9%), biological agents in three patients (7.1%), and pamidronate in five patients (11.9%). Pamidronate was mostly preferred in patients with spinal lesions. Short courses of glucocorticoid regimen were given to nine patients (19.7%). In the MTX-treated patients, remission was achieved in nine (26.4%). The median duration of treatment was 8.1 (min-max: 2.0-10.9) months for MTX. Three patients were given pamidronate due to flares,

Table 2. Laboratory findings at diagnosis and the summary of diagnostic imaging tests in patients with chronic non-bacterial osteitis

	Patient number (n=48)
Anemia, n (%)	13 (27.1)
Hemoglobin (g/dL), median (min-max)	12.2 (9.1-15.1)
Leukocytosis, n (%)	1 (2.1)
Leukocyte count (10^9/L), median (min-max)	7.5 (4.6-19.1)
Thrombocytosis, n (%)	19 (39.6)
Platelet count (10^9/L), median (min-max)	360 (185-857)
High level of CRP, n (%)	21 (43.8)
CRP (mg/dL), median (min-max)	0.6 (0.1-16.9)
Elevated ESR, n (%)	25 (52.1)
ESR (mm/h), median (min-max)	22 (2-120)
HLA-B27 positivity, n (%)	4/15 (26.6)
Type of imaging test at diagnosis	
Plain radiography, n (%)	25 (52.0)
Regional MRI, n (%)	42 (87.5)
Whole body MRI, n (%)	32 (66.7)
Bone scintigraphy, n (%)	8 (16.7)

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, HLA: Human leukocyte antigen, MRI: Magnetic resonance imaging, min: Minimum, max: Maximum

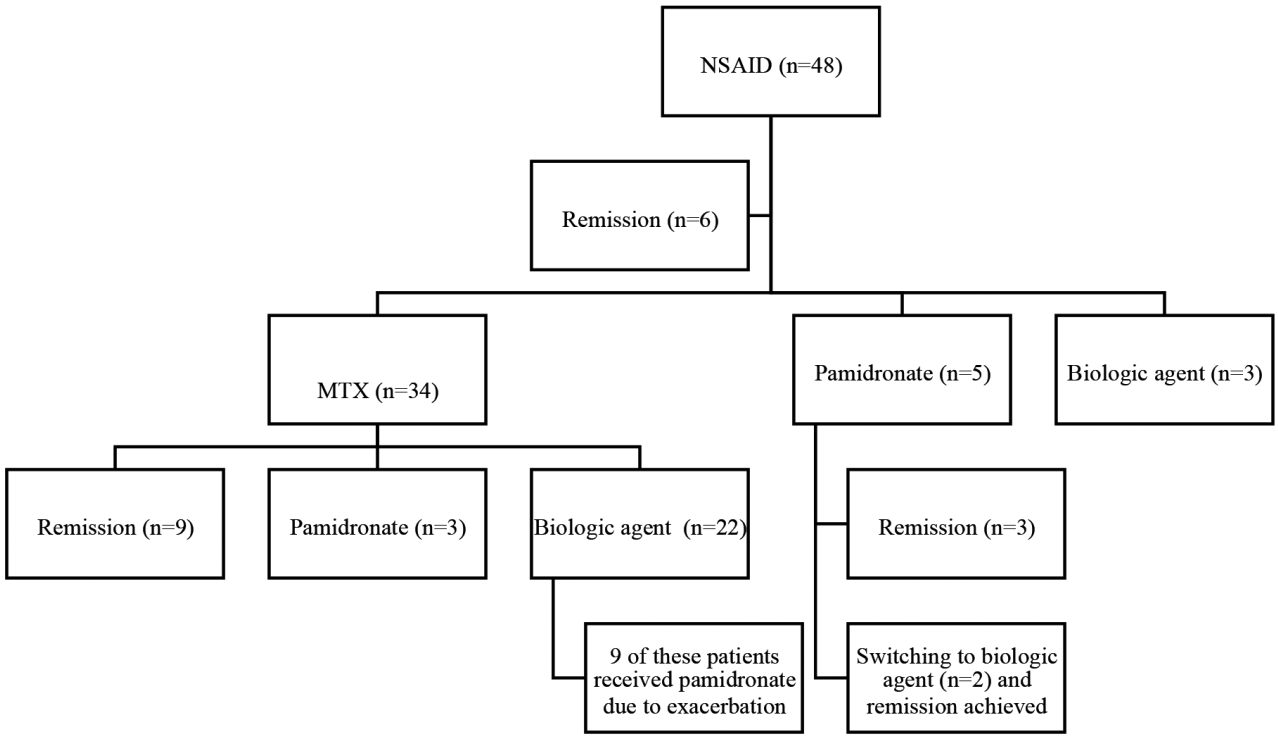

Figure 1. Flow chart of the treatment scheme in our patients with chronic non-bacterial osteitis

NSAID: Non-steroidal anti-inflammatory drugs, MTX: Methotrexate

while treatment was switched to a biological agent in 22 patients who did not respond to MTX. Nine of these patients with biological treatment also received pamidronate due to flares during the course of the disease.

Treatment with Biological Agents

A total of 27 patients (56.2%) used biological agents for refractory disease or as second-line therapy. The median duration of treatment was 8.0 months (min-max: 5.0-39.0) months for biological agents. Etanercept (ETN) was the first-choice biological in all patients, except for one with IBD who was administered adalimumab at the time of diagnosis. In four patients, ETN was switched to adalimumab due to psoriasis or IBD during follow-up. Two patients had refractory disease, despite MTX and ETN and/or pamidronate treatments. One of them achieved remission with the adalimumab treatment, while adalimumab, infliximab, and tocilizumab treatments were used in the other patient to control disease activity. In addition, ETN was restarted due to disease activity in one patient whose ETN treatment was discontinued due to long-term disease control.

DISCUSSION

In the present study, we evaluated the characteristics of children with CNO, a rare disease in children, in a large cohort in a tertiary referral center. Fifty-two percent of patients were male in our cohort. Female predominance was reported in two large CNO cohort, one was a series of 486 cases (64% female) from the Eurofever international registry and the other was the German national pediatric rheumatology database, the largest cohort of the CNO patients (n=774, 62.8% female)[13,14]. The increased awareness and increasing number of CNO patients are helpful to understand whether sex distribution is different or affects the disease occurrence. The median age at the time of diagnosis in the present study was similar to other previous reports[5,15-18]. However, the diagnostic delay time indicates a wide variety ranging from 3 to 21 months in the literature which was one year in our study group[15,19-21]. This can be attributed to varying levels of awareness and diagnostic difficulties. The data of 15 patients diagnosed with CNO between January 2008 and January 2017 in our center were published previously[22]. Between January 2017 and July 2021, 32 patients were newly diagnosed with CNO, while only one patient was diagnosed between November 2006

and January 2008. Over the years, a significant increase in the rate of CNO diagnosis was noted.

The most common symptom in our patients was local bone pain consistent with the literature, mostly presented as leg pain[23,24]. Diagnosing CNO in patients presenting with the complaint of leg pain, which is a common symptom in children, may be challenging. The lack of internationally accepted diagnostic criteria and specific laboratory markers for CNO makes the diagnosis more difficult. Normal laboratory values can be detected in some patients at diagnosis[5,25]. The rate of increased acute phase reactants in our study group was similar to the Eurofever cohort (59% vs 52.1% for ESR, 49% vs 43.8% for CRP)[14]. Thrombocytosis was also reported in approximately 30% of CNO patients, as we detected in 39.6% of patients[26]. As for the use of diagnostic imaging modalities for CNO patients, a survey was conducted by members of the Childhood Arthritis and Rheumatology Research Alliance. The frequency of use of imaging modalities among physicians was listed as plain radiographs (89%), regional MRI (78%), bone scintigraphy (43%), and whole-body MRI (36%). In the analysis of Eurofever registry, the whole-body MRI was reported to use in 34% of patients which was lower compared to our cohort (66.7%)[14]. The whole-body MRI is the most sensitive method to detect bone lesions[27]. The widespread use of whole-body MRI may ease and fasten the diagnostic process. However, it is not accessible in all centers and it may not be cost-effective to apply whole-body MRI to all patients.

To exclude malignancy and infection for patients with particularly unifocal lesions in our cohort, bone biopsy was performed in 19 patients (39.6%). In the analysis of the German National Pediatric Rheumatology database, the frequency of bone biopsy was reported as 69.1%, 49.4%, and 54.8% for the 2009-2012, 2013-2015, and 2016-2018 time periods, respectively[13]. Decreased biopsy rates over time were noted. Along the same lines, a bone biopsy was reported to conduct in 60% of CNO patients in the Eurofever registry analysis[14]. The lower rate of bone biopsy in our study group can be attributed to the widespread use of whole-body MRI over time. The chance of detecting multifocal bone lesions and/or typical sites involvement such as clavicula with the imaging may have reduced the need for biopsy.

Currently, NSAIDs are frequently used as the first-line treatment. These agents provide symptomatic relief and are effective in controlling inflammation in a limited group of patients. In our study, only 12.5% of the patients achieved remission with NSAID treatment alone. Along the same lines, in the analysis of the Irish national cohort with CNO, 13.6% of patients achieved remission with NSAIDs[28]. In general, MTX, biological agents, and pamidronate are the second-line treatment agents used in the CNO treatment. Borzutzky et al.[29] found the remission rate with MTX in their CNO cohort to be 20%. Similar to our findings (remission rate with MTX: 26.4%), it was effective in achieving remission only in a limited subset of patients. Although there are case reports and case series indicating that anti-TNF and pamidronate are effective in CNO, there are no clinical studies or treatment guidelines[30,31].

Study Limitations

Nonetheless, this study has some limitations. It is a single-center, retrospective study. Also, the possibility of spontaneous regression in CNO might have affected our results while evaluating treatment response and outcome. On the other hand, we believe that our study provides additional contribution to the literature to increase awareness of CNO and gain a better understanding of this rare disease.

CONCLUSION

In conclusion, delay in diagnosis is frequently observed in CNO. It is essential to increase awareness of this disease to prevent missing the diagnosis. The more widespread use of whole-body MRI in diagnosis can be helpful to diagnose CNO earlier. Further large-scale, prospective studies are needed to draw more reliable conclusions on this subject.

Ethics

Ethics Committee Approval: The study was approved by the Hacettepe University Non-invasive Clinical Research Ethics Committee (decision no: 2022/10-04, date: 07.06.2022) and conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Concept: Ü.K.A., Y.Bi., Design: Ü.K.A., Y.B., Y.Bi., Data Collection or Processing: Ü.K.A., Y.B., Y.Bi., Analysis or Interpretation: Ü.K.A., Y.B., Y.Bi., Literature Search: Ü.K.A., Y.B., Y.Bi., Writing: Ü.K.A., Y.Bi.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.75002
J Dr Behcet Uz Child Hosp 2023;13(1):30-36

Original Article

The Use of Mean Platelet Volume, Plateletcrit, and N-terminal Brain Natriuretic Peptide as Biomarkers of Coronary Artery Involvement in Atypical Kawasaki Disease

Atipik Kawasaki Hastalığında Koroner Arter Tutulumunun Biyobelirteçleri Olarak Ortalama Trombosit Hacmi, Plateletkrit ve N-terminal Beyin Natriüretik Peptid Kullanımı

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ABSTRACT

Objective: Coronary artery aneurysm and ectasia develop in approximately 15% to 25% of children with untreated Kawasaki disease (KD). Atypical KD has a higher incidence of coronary artery involvement compared to typical KD. Our aim in this study was to identify new markers to support early diagnosis and prevent complications associated with delayed treatment in atypical KD.

Method: The patients' demographic characteristics, presenting complaints, clinical findings, mean platelet volume (MPV), plateletcrit (PCT), and N-terminal brain natriuretic peptide (NT-proBNP) levels were analyzed. Coronary artery abnormalities were evaluated using two-dimensional echocardiography. Results are expressed as mean (± standard deviation).

Results: Sixty children between the ages of 3 and 96 months who were diagnosed with atypical KD were included. Forty consecutive normal children were included as a control group. NT-proBNP, MPV, and PCT values were 381.7 (±272.7) pg/mL, 5.8 (±0.93) fL, and 0.266% (±0.92%) in the patient group and 48.5 (±28.5) pg/mL, 8.29 (±1.12) fL, and 0.227% (±0.78%) in the control group, respectively (p<0.001 for all). In the comparison of atypical KD patients with coronary artery involvement (CAI subgroup) and without (non-CAI subgroup), the NT-proBNP values in these subgroups were 542.9 (±226.8) and 171 (±161.7) pg/mL (p<0.001), MPV values were 5.8 (±0.77) and 6.54 (±0.95) fL (p<0.005), and PCT values were 0.264% (±0.1%) and 0.269% (±0.08%), respectively.

Conclusion: The simultaneous evaluation of MPV, PCT, and NT-proBNP was useful for the diagnosis of atypical KD. NT-proBNP and MPV can be used as markers of CAI in atypical KD.

Keywords: Kawasaki disease, mean platelet volume, plateletcrit, n-terminal brain natriuretic peptide

ÖZ

Amaç: Koroner arter anevrizması ve ektazisi, tedavi edilmemiş Kawasaki hastalığı (KH) olan çocukların yaklaşık %15 ila %25'inde gelişir. Atipik KH, tipik KH'ye kıyasla daha yüksek bir koroner arter tutulumu insidansına sahiptir. Bu çalışmadaki amacımız, atipik KH'de yeni belirteçler belirleyerek erken tanıyı desteklemek ve gecikmiş tedaviye bağlı komplikasyonları önlemektir.

Yöntem: Hastaların demografik özellikleri, başvuru şikayetleri, klinik bulguları, ortalama trombosit hacmi (MPV), plateletkrit (PCT) ve N-terminal beyin natriüretik peptid (NT-proBNP) düzeyleri araştırıldı. Koroner arter anormallikleri iki boyutlu ekokardiyografi kullanılarak değerlendirildi.

Bulgular: Atipik KH tanısı konan 3 ile 96 ay arasında 60 çocuk dahil edildi. Kırk ardışık çocuk kontrol grubu olarak alındı. Ortalama NT-proBNP, MPV ve PCT değerleri hasta grubunda; 381,7 (±272,7) pg/mL, 5,8 (±0,93) fL ve %0,266 (±0,92) iken; kontrol grubunda ve 48,5 (±28,5) pg/mL, 8,29 (±1,12) fL ve %0,227 (±0,78), hepsi için (p<0,001) saptandı. Ayrıca, koroner arter tutulumu (KAT) olan atipik KH hastalarını, koroner arter tutulumu olmayanlar ile karşılaştırdık. Ortalama (standart deviation) NT-proBNP, KAT alt grubunda 542,9 (±226,8) pg/mL ve KAT olmayan alt grupta 171 (±161,7) pg/mL idi (p<0,001). Ortalama MPV, KAT alt grubunda 5,8 (±0,77) fL ve KAT olmayan alt grupta 6,54 (±0,95) fL idi (p<0,005). Ortalama PCT değeri KAT alt grubunda %0,264 (±0,1%) ve KAT olmayan alt grupta %0,269 (±0,08) idi.

Sonuç: MPV, PCT ve NT-proBNP'nin eş zamanlı değerlendirilmesinin atipik KH tanısında faydalı olduğunu gözlemledik. NT-proBNP ve MPV, atipik KH'de koroner arter tutulumunun belirteçleri olarak kullanılabilir.

Anahtar kelimeler: Kawasaki hastalığı, ortalama trombosit hacmi, plateletkrit, n-terminal beyin natriüretik peptid

Received: 04.06.2022
Accepted: 08.08.2022

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Cite as: Doğan E, Turan C, Yurtseven A, Eman G, Ulaş Saz E. The Use of Mean Platelet Volume, Plateletcrit, and N-terminal Brain Natriuretic Peptide as Biomarkers of Coronary Artery Involvement in Atypical Kawasaki Disease. J Dr Behcet Uz Child Hosp. 2023;13(1):30-36

INTRODUCTION

Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology that is predominantly seen in infants and children under 5 years of age[1]. KD is believed to be the most common cause of acquired heart disease in developed countries[2]. Coronary artery aneurysm and ectasia develop in approximately 15% to 25% of children with untreated KD[3,4]. Coronary artery involvement (CAI) may progress to ischemic heart disease and lead to sudden cardiac death. The incidence of these complications can be reduced to less than 5% with early diagnosis and treatments such as intravenous immunoglobulin (IVIG), steroids, biological drugs, and acetylsalicylic acid[5].

Diagnosis of KD is based on diagnostic criteria including fever, rash, cervical lymphadenopathy, and findings in the conjunctiva, lip, oral mucosa, and extremities. There is no specific test or pathognomonic clinical feature of KD. Patients who do not meet all of the diagnostic criteria are diagnosed as having atypical KD (15-20%)[3]. Atypical KD has a higher incidence of CAI compared to typical KD[6].

Additional diagnostic markers are needed for cases in which the clinical presentation is inconclusive. Markers utilized at present include the widely used C-reactive protein (CRP) elevation, high erythrocyte sedimentation rate (ESR), hypoalbuminemia, anemia, high alanine aminotransferase (ALT), leukocytosis, thrombocytosis, and pyuria[7].

N-terminal brain natriuretic peptide (NT-proBNP) is secreted into the circulation via the coronary sinus by myocytes and possibly to some degree directly by perimyocardial fibroblasts upon stimulation by ventricular stretching and various neurohormonal factors. There are reports in the literature of NT-proBNP being used as an auxiliary marker in the diagnosis of KD[8-10].

Platelets are generally known for their function in hemostasis and thrombosis, but their role in immune response and inflammation is of growing interest. Platelet indices include mean platelet volume (MPV), which reflects the average size of the platelets, and plateletcrit (PCT), which refers to the ratio of total platelet volume to blood volume. Studies in which MPV and PCT were used as inflammation markers showed that they have prognostic value in coronary artery disease[11].

We chose the KD patient group for this study due to the ongoing diagnostic challenges and the need for auxiliary markers, especially for atypical presentations. Therefore, we planned to determine the value of biochemical markers used for early diagnosis in the diagnosis and follow-up of atypical KD. In addition, we simultaneously investigated the role of MPV, PCT, and NT-proBNP in diagnosis and management as predictors of CAI. Our aim in this study was to support early diagnosis and prevent complications associated with delayed treatment by identifying new markers.

MATERIALS and METHODS

Study Design

Patients who presented to the Ege University Pediatric Emergency Department between January 2015 and December 2017 and were diagnosed with atypical KD were retrospectively included in the study. Ethics committee approval was obtained from the Ege University Faculty of Medicine Scientific Research Ethics Committee (decision no: 18-5/37, date: 08.05.2018).

Patient Selection

Children under the age of 18 years whose clinical presentation in the pediatric emergency department met the criteria for atypical KD were included. Patients with any data missing from their medical records were excluded from the study.

Definitions

Atypical KD was diagnosed according to the American Heart Association criteria[3]. Patients with fever for at least 5 days and 2 or 3 compatible clinical findings (skin rash; cervical lymphadenopathy; conjunctiva, lip, oral mucosa, and extremity findings) but not meeting the typical KD diagnostic criteria were diagnosed as having atypical KD by experienced clinicians based on evaluation of clinical and laboratory findings. Infants ≤6 months of age with KD are more likely to lack the clinical features of KD other than fever and were diagnosed as having atypical KD if echocardiogram was positive.

CAI was diagnosed if the Z-score of the left anterior descending artery or right coronary artery was ≥2.5, a coronary artery aneurysm was observed, or at least 3 other suggestive features were present, including decreased left ventricular function, mitral regurgitation, pericardial effusion, or Z-scores of 2-2.5 for the left anterior descending coronary artery or right coronary artery on transthoracic echocardiogram (TTE) performed by the same pediatric cardiologist before initiation of IVIG and acetylsalicylic acid therapy[3].

Data Collection and Patient Assessment

The patients' demographic characteristics, admitting complaints, clinical findings, MPV, PCT, red cell distribution width, leukocyte, thrombocyte, neutrophil, and lymphocyte counts, ESR, and CRP and NT-proBNP levels were investigated. Coronary artery abnormalities were evaluated using two-dimensional echocardiography (Vivid E9, GE-Vingmed Ultrasound AS, Horten, Norway). TTE evaluations were done at admission, week 1, and week 2 for all patients and was repeated intermittently thereafter in patients with CAI.

Statistical Analysis

The data were analyzed using SPSS version 22.0 for Windows (IBM Corp, Armonk, NY). Chi-squared tests were used to compare frequencies and Student's t-test was used to compare (parametric) means. The Mann-Whitney U test was used for variables with non-normal distribution. The diagnostic properties of each biomarker (NT-proBNP, MPV, and PCT) were assessed by receiver operating characteristic (ROC) curve analysis. Diagnostic reliability was evaluated based on the area under the ROC curve within 95% confidence intervals. The diagnostic markers were also analyzed in combination, with sensitivity and specificity as the main outcome measures.

RESULTS

Sixty children between the ages of 3 and 96 months who were diagnosed with atypical KD according to the American Heart Association diagnostic criteria in the Ege University Medical Faculty Pediatric Emergency Department were included in this study. In addition, 40 consecutive children of similar age and sex distribution who presented to the emergency department with non-specific chest pain and no signs of cardiac pathology were included as the control group. Children in the control group were evaluated by a pediatric emergency specialist and were found to have no infectious or rheumatological diseases. The patient group included 29 girls (48.3%) and 31 boys (51.7%). The mean age of the patients was 32.4 months (range, 3-96 months) and 15% of them were infants.

Detailed histories indicated that 68% of the patients had been diagnosed with upper respiratory tract infection, 14% with acute tonsillitis, 11% with acute gastroenteritis, and 7% with urinary tract infection before admission. Antibiotics were used by 71.7% before admission. The median duration of fever in the whole group was 7 days (interquartile range: 3). Hemogram and biochemistry parameters of the patients are shown in Table 1.

All patients underwent TTE evaluation by a pediatric cardiologist on the day of admission to the emergency

Table 1. Laboratory findings of the group diagnosed with atypical Kawasaki disease

Parameters	Mean	Minimum	Maximum	SD (±)
ESR (mm/h)	73.63	10	148	30.8
CRP (mg/dL)	8.21	0.4	25	4.9
WBC (/µL)	17,211	1,590	37,560	7,631
ANC (/µL)	11,085	895	27,690	6,011
HTC (%)	31.26	18	40	3.62
Hb (g/L)	10.3	6.5	13	1.23
MCV (fL)	77.78	62	86	4.99
AST (U/L)	53.81	18	607	79
ALT (U/L)	48.96	4	779	110.37
GGT (U/L)	39.13	4	195	47.52
Total bilirubin (mg/dL)	0.6	0.08	5.2	0.71
Direct bilirubin (mg/dL)	0.17	0.01	3	0.42
Albumin (g/dL)	3.17	1.9	4	0.41
Sodium (mEq/L)	135	122	140	2.93
Potassium (mEq/L)	4.34	2.9	5.4	0.59

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, WBC: White blood cell, ANC: Absolute neutrophil count, HTC: Hematocrit, Hb: Hemoglobin, MCV: Mean corpuscular volume, AST: Aspartate aminotransferase ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, SD: Standard deviation

department. CAI was detected in 34 patients (56.7%), Z-score of the left anterior descending coronary artery or right coronary artery was ≥2.5 in 16 patients (25%), and giant coronary aneurysm was detected in 1 patient (3.3%). Seventeen patients (28.4%) had at least 3 other suggestive features including decreased left ventricular function, mitral regurgitation, pericardial effusion, or Z-scores of 2-2.5 for the left anterior descending coronary artery or right coronary artery. The coronary arteries of 26 patients (43.3%) were evaluated as normal. CAI was significantly more frequent among patients who presented after 7 days and received IVIG treatment later (p<0.001).

The patients received standard treatment with 2 g/kg IVIG by 12-hour infusion and 80-100 mg/kg/day acetylsalicylic acid. After IVIG therapy, fever regressed in all patients. None of the patients were unresponsive to IVIG therapy.

NT-proBNP, MPV, and PCT values at admission were compared between the patients diagnosed with atypical KD and the control group. Mean [standard deviation (SD)] NT-proBNP, MPV, and PCT values were 381.7 (±272.7) pg/mL, 5.8 (±0.93) fL, and 0.266% (±0.92%) in the patient group and 48.5 (±28.5) pg/mL, 8.29 (±1.12) fL, and 0.227% (±0.78%) in the control group, respectively (p<0.001 for all; Table 2).

We also compared atypical KD patients with CAI (CAI subgroup) to those without CAI (non-CAI subgroup). These two subgroups had mean (SD) NT-proBNP values of 542.9 (±226.8) and 171 (±161.7) pg/mL (p<0.001), mean (SD) MPV values of 5.8 (±0.77) and 6.54 (±0.95) fL (p<0.005), and mean (SD) PCT values of 0.264% (±0.1%) and 0.269% (±0.08%) (p=0.82), respectively (Table 3).

A NT-ProBNP cut-off value of 267.5 pg/mL had sensitivity of 91.2% and specificity of 94% for the detection of CAI in patients diagnosed with atypical KD. When the patients were grouped by age, cut-off values for the detection of CAI in atypical KD were determined as 354.9 pg/mL for 0-12 months (sensitivity 75%, specificity 67%); 258 pg/mL for 13-24 months (sensitivity 95%, specificity 94%), 346.5 pg/mL for 25-36 months (sensitivity 86%, specificity 95%), 409 pg/mL for 27-48 months (sensitivity 66.7%, specificity 90%), and 342 pg/mL for 49-60 months (sensitivity 95%, specificity 100%).

DISCUSSION

The risk of CAI is higher in atypical KD than in classic KD[7]. A number of studies previously reported that baseline coronary artery Z-score before primary treatment, young age, prolonged fever, wider dispersion of symptoms, pyuria, and elevated serum CRP were significantly associated with the development of coronary lesions[12-15].

Table 2. Comparison of NT-proBNP, MPV, and PCT values of the patient and control groups

Parameters	Group	n	Mean	SD (±)	p-value
NT-Pro-BNP (pg/mL)	Patient	60	381.7	272.7	<0.001
	Control	40	48.5	28.5	
MPV (fL)	Patient	60	5.8	0.93	<0.001
	Control	40	8.29	1.12	
PCT (%)	Patient	60	0.266	0.92	<0.001
	Control	40	0.227	0.78	

NT-Pro-BNP: N-terminal brain natriuretic peptide, MPV: Mean platelet volume, PCT: Plateletcrit, SD: Standard deviation

Table 3. Comparison of NT-proBNP, MPV, and PCT values of patients with and without coronary artery involvement

Parameters	Group	n	Mean	SD (±)	p
NT-Pro-BNP (pg/mL)	CAI +	34	542.9	226.8	<0.001
	Normal	26	171	161.7	
MPV (fL)	CAI +	34	5.8	0.77	<0.005
	Normal	26	6.54	0.95	
PCT (%)	CAI +	34	0.264	0.1	0.82
	Normal	26	0.269	0.08	

NT-Pro-BNP: N-terminal brain natriuretic peptide, MPV: Mean platelet volume, PCT: Plateletcrit, SD: Standard deviation

Many studies have been conducted to determine NT-proBNP levels in KD. Zheng et al.[16] determined in their meta-analysis that NT-ProBNP can be used as a marker to demonstrate CAI in typical KD. In that meta-analysis, a mean cut-off value of 900 pg/mL was determined for general sensitivity and specificity in patients diagnosed with CAI in typical KD[16]. In another study, proBNP was increased in KD patients who developed CAI, and patients with serum NT-proBNP elevation >1,000 pg/mL had a ~10 times higher risk of CAI than patients with a modest increase[17]. In the present study, in the subgroup of patients with CAI, the mean (SD) NT-proBNP level was 542.9 (±226.8) pg/mL. The higher NT-proBNP level among patients with CAI suggests that this finding may predict severe disease and high risk of CAI. In our study, NT-proBNP was assessed at the time of admission and was useful in diagnosing atypical KD. A high initial NT-proBNP level supports the diagnosis of KD and indicates that CAI may develop in patients above the cut-off value. Atypical KD should be suspected in a patient presenting with fever for more than 5 days if NT-proBNP level evaluated for any reason is found to be high, even without other signs of typical KD.

Identifying age-specific cut-off values is important, especially as the diagnosis algorithms of atypical KD vary according to age. In patients under 1 year of age with fever for 7 days or longer, laboratory tests for KD are performed regardless of the presence or absence of clinical symptoms, whereas atypical KD is suspected in patients over 1 year of age who have fever for longer than 5 days and 2 or 3 clinical signs[7]. Detection of NT-proBNP levels higher than the age-specific cut-off values will inform the decision to refer patients to a pediatric cardiologist.

The cut-off value obtained in this study was lower than those reported in previous studies, which may be related to the fact that all studies evaluated in the meta-analysis were conducted in Asian populations and included patients with typical KD[16]. Our finding of a lower NT-proBNP cut-off in our population compared to children in Asian populations suggests that NT-proBNP levels may vary by race/ethnicity, and may be a first step toward determining race-specific cut-off values.

MPV and PCT are simple and easily obtained hematologic markers to evaluate platelet function. MPV increases when platelet activation and production increase, and is being utilized as a new inflammation marker[18]. MPV and PCT may be useful in addition to other conventional laboratory tests in the diagnosis of KD. Bozlu et al.[19] determined that MPV was significantly lower in incomplete KD patients compared to a control group, as in our study.

Platelets are generally recognized for their role in hemostasis and thrombosis, but their role in immune response and inflammation is also an area of interest for researchers[20]. Previous studies have shown that MPV is a predictor of increased cardiovascular risk[21]. Liu et al.[22] demonstrated in their study that low MPV predicted CAI in KD patients. In the present study, we also found that MPV was a predictor in atypical KD because patients with CAI had significantly lower values. Reduced platelet volume may be due to consumption or isolation of large platelets activated in the vascular system[23]. It has been suggested that increases in factors such as interleukin-6, granulocyte colony stimulating factor, and macrophage colony-stimulating factor during the acute phase of KD may be responsible for the decrease in platelet volume. However, the mechanism underlying the decrease in MPV in KD has not been clearly demonstrated[24].

MPV is an advantageous marker for early diagnosis because it is included in complete blood count analysis and results are obtained rapidly. In cases where other laboratory parameters that take longer to receive results or echocardiography are either not available or cannot be performed in the early period, MPV can be used to initiate treatment and monitor its effect in the early period.

PCT is the ratio of platelet volume to whole blood volume and positively correlates with platelet count and MPV. A meta-analysis showed that PCT is associated with CAI[25]. Similar to previous studies, we found that PCT was decreased in the acute phase of KD, but there was no significant difference in PCT according to the presence of CAI in this study.

In this retrospective study, we determined that NT-ProBNP and PCT values were significantly higher and MPV values were significantly lower in patients diagnosed with atypical KD compared to the control group. Patients with CAI had significantly higher NT-ProBNP level and significantly lower MPV compared to patients without CAI. However, we detected no significant difference in PCT value between patients with and without CAI. This may be attributed to the limited number of patients in our study and its retrospective design.

Study Limitations

There are several limitations to the present study. Firstly, the retrospective design of our study is the most

crucial limitation. Furthermore, the results are based on a single-center experience with a small cohort. Multicenter, prospective studies that include more patients and typical KD should be conducted.

CONCLUSION

We observed that the simultaneous evaluation of NT-proBNP, MPV, and PCT was useful for the diagnosis of atypical KD. Unlike other studies, we evaluated these parameters simultaneously and showed that NT-proBNP and MPV can be used as markers of CAI. Determination of age-specific NT-proBNP cut-off values will be useful in atypical KD, which is difficult to diagnose and can lead to serious complications. The results of this study show that NT-proBNP, MPV, and PCT can be used as early markers of atypical KD in suspected cases in which echocardiography cannot be performed. NT-proBNP and MPV can also be used as early markers of CAI and as parameters suggestive of a more severe disease course.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Ege University Faculty of Medicine Scientific Research Ethics Committee (decision no: 18-5/37, date: 08.05.2018).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: E.D., C.T., E.U.S., Concept: E.D., A.Y., Design: E.D., G.E., E.U.S., Data Collection or Processing: E.D., C.T., G.E., E.U.S., Analysis or Interpretation: E.D., C.T., G.E., E.U.S., Literature Search: E.D., C.T., E.U.S., Writing: E.D., A.Y., E.U.S.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.08076
J Dr Behcet Uz Child Hosp 2023;13(1):37-42

Original Article

Evaluation of Long-term Results and Stool Incontinence of Patients with Operated Anorectal Malformations

Opere Anorektal Malformasyonlu Hastaların Uzun Dönem Sonuçlarının ve Fekal İnkontinanslarının Değerlendirilmesi

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ABSTRACT

Objective: Anorectal malformations (ARMs) are a group of anomalies with a wide spectrum, from simple types requiring minimal surgery to complex anomalies requiring complex surgical interventions. Constipation and inability to achieve stool continence are the leading problems in the late postoperative period for patients with ARMs.

Method: This study aimed to evaluate and compare the long-term results and stool continence status of the patients with ARMs who had undergone a definitive surgery in our clinic between January 1996 and December 2009, according to anal atresia types. The patients were evaluated in terms of anomaly type, additional organ anomaly, surgeries performed, and postoperative complications.

Results: A total of 68 patients including 40 (58.82%) male, and 28 (41.18%) female cases who had undergone definitive surgery were examined. Considering all patients, the most common anomaly was anocutaneous fistula detected in 20 patients (29.4%), and the least common one was rectovesical fistula detected in 1 (1.5%) patient. When the definitive surgeries performed on patients with ARM are evaluated according to the type of ARM, most frequently anoplasty (n=20) had been performed. The most common complaint of the patients was constipation (n=20). A statistically significant relationship was found between the type of anomaly, the type of the definitive surgery performed, constipation and stool incontinence ($p<0.05$).

Conclusion: Patients should be followed up in the postoperative period for problems such as anal stenosis and constipation, and treatment should be initiated before formation of fecalith in patients with constipation.

Keywords: Anorectal malformation, constipation, incontinence

ÖZ

Amaç: Anorektal malformasyonlar (ARM) minimal cerrahi gerektiren basit tipten, kompleks cerrahi girişim gerektiren karmaşık anomalilere kadar geniş bir spektruma sahip anomali grubudur. ARM'li hastaları bekleyen postoperatif geç dönem problemlerin başında konstipasyon ve gaita kontinansının sağlanamaması gelir.

Yöntem: Bu çalışma kliniğimizde Ocak 1996-Aralık 2009 yılları arasında definitif operasyonu tamamlanmış olan ARM'li hastaların uzun dönem sonuçlarının ve gaita kontinans durumlarının anal atrezi tiplerine göre değerlendirilip, karşılaştırılması amaç edinilmiştir. Hastalar anomali tipi, ek organ anomalisi, yapılan ameliyat ve postoperatif komplikasyonlar açısından değerlendirilmiştir.

Bulgular: Definitif operasyon uygulanan 68 hasta incelendiğinde 40 hastanın erkek (%58,82) ve 28 hastanın ise kız (%41,18) olduğu görülmüştür. Tüm hastalar ele alındığında en çok karşılaşılan anomalinin 20 hastada (%29,4) tespit edilen anokutanöz fistül olup en az karşılaşılan anomali 1 hastada (%1,5) tespit edilen rektovezikal fistül olmuştur. ARM'li hastalara uygulanan definitif operasyonlar ARM tipine göre değerlendirildiğinde, en çok yapılan ameliyat (n=20) anoplasti operasyonudur. Hastalar en çok kabızlıktan şikayet etmekteydi (n=20). Anomali tipi, uygulanan definitif ameliyat ile kabızlık ve gaita kaçırma arasında istatistiksel olarak anlamlı ilişki tespit edilmiştir ($p<0,05$).

Sonuç: Hastalar postoperatif dönemde anal darlık ve kabızlık gibi problemler yönüyle takip edilmeli, kabızlık tespit edilen hastalarda dışkı taşlaşması oluşmadan tedavi başlanılmalıdır.

Anahtar kelimeler: Anorekatal malformasyon, kabızlık, inkontinans

Received: 04.07.2022
Accepted: 06.09.2022

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Cite as: Fırıncı B, Salman AB. Evaluation of Long-term Results and Stool Incontinence of Patients with Operated Anorectal Malformations.
J Dr Behcet Uz Child Hosp. 2023;13(1):37-42

INTRODUCTION

Anorectal malformations (ARMs) are seen with a frequency of 1 in 4,000-5,000 live births. A male/female ratio of 1.4-1.6 has been reported[1-3].

The majority of ARMs in boys are of the high type, while most ARMs in girls are of the low type[3].

ARMs are still mainly evaluated as low type and high type. The diagnosis of low-type ARM can be made easily by physical examination, and its treatment gives better results. In the diagnosis of high-type malformations, additional examinations are needed as well as physical examination.

ARM may be suspected by the presence of dilated colon or intraluminal calcifications, or both, during routine prenatal diagnostic ultrasonographic controls of pregnant women, especially after the 25th gestational week. Diagnosis of ARM can be done by observing the fecal matter coming from the urethra or perineum in boys and from the perineum and vestibulum in girls in the immediate postpartum period. However in some cases extrusion of fecal matter can not be detected. In this case, as previously reported by Wangensteen and Rice an invertogram film is shot or as later on proposed a "cross- table lateral view" is obtained. In addition, computed tomography, magnetic resonance imaging technique, and fistulography can also be used for diagnosis and typing of ARMs[4,5].

Patients with ARMs have attained near-normal living standards thanks to the latest developments in treatment. Dealing with the problems of these patients who are given a chance to survive after treatment, is at least as necessary as the treatment. Constipation and/or stool incontinence is one of the most critical problems and complaints awaiting patients with anal atresia who underwent definitive surgery. It is necessary to explain to the families the possibility of their children being unable to achieve stool control in the future in a realistic language without disappointing them. Likewise, families should give their children information about bowel control without causing them to have false expectations in the future.

Many additional organ anomalies may accompany high-type ARMs. Their treatment is more challenging with lower chances of continence. Posterior sagittal anorectoplasty technique (PSARP) was introduced by Peña and Devries[5] in 1982 and highly successful results started to be obtained. The treatment of ARMs aims to give the patients an opportunity to lead a near-normal life. However, it is as important as the treatment to fight the problems that the surviving patients are confronting after their definitive treatment is completed. Constipation and/or stool incontinence are the leading problems for these patients. Families should be told about the possibility of their children being unable to control their defecation in the future in a realistic language without causing disappointment. Parents should also be able to provide their children with information about control of their bowel movements to the degree that they can meet their children's expectations in cooperation This study aimed to evaluate and compare the stool continence of patients with an ARMs whose colostomies were closed.

MATERIALS and METHOD

The ethical approval was obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (number: 78, date: 17.04.2009). After obtaining ethical committee approval, the archive records of the patients with an ARM hospitalized in our clinic, between January 1996 and December 2009, according to the type of atresia, were reviewed retrospectively.

The records of patients with ARMs were accessed, and then these patients were contacted by phone and invited to the hospital for re-evaluation. Data related to gender, age, anomaly type, additional organ anomaly, operation date, operation age, postoperative complaints, postoperative treatment methods, postoperative complications, dilatation, constipation history, incontinence, psychosocial problems, and additional operations of 68 patients who agreed to participate in the study were reviewed.

Statistical Analysis

The obtained data were entered into the SPSS 20.0 program and analyzed using the chi-square test.

RESULTS

The data of 68 patients diagnosed with ARM and had definitive surgery in our hospital were examined, and only 51 of these patients were evaluated in our clinic for long-term complications. A total of 68 patients with ARM including 40 (58.82%) male, and 28 (41.18%) female cases with a median age of 7.33 years (range: 2-14 years) had undergone definitive surgery with a male/female ratio of 1.42.

The most common anomaly was anocutaneous fistula (ACF) in 20 (29.4%), and rectovestibular fistula (RVF) in 16

patients (23.5%) followed by rectourethral fistula (RUF) and rectoperineal fistula in 13 (19.1%) patients. The least common anomaly was rectovesical fistula in one (1.5%) and perineal groove in another patient (1.5%) (Table 1).

ACF, which was present in 16 patients (23.5%), was the most common type of ARM in men, followed by RUF in 13 patients (19.1%). The most common type of ARM in girls was RVF in 16 (23.5%) patients followed by rectoperineal fistula in 6 patients.

Any comorbidity was not found in 50 (73.53%) patients while additional organ anomalies were detected in 18 (26.46%) patients. Most frequently urinary system anomalies were seen (n=9) followed by cardiac (n=7), and extremity (n=2) anomalies

Urinary system anomalies were seen in 9 patients, including ureteropelvic junction stenosis (n=2), vesicourethral reflux (n=2), horseshoe kidney with accompanying hydronephrosis (n=1), hydronephrosis (n=1), nephrolithiasis (n=1), hypospadias (n=1), horseshoe kidney with accompanying vesicourethral reflux (n=1), and an isolated case of renal agenesis. Seven patients with cardiac anomalies were detected in indicated number of patients including atrial septal defect (ASD), patent ductus arteriosus (PDA), and patent foramen ovale (PFO) (n=1), ventricular septal defect (VSD), PFO (n=1), PDA, PFO (n=1), PDA, ASD (n=1), and ASD, VSD, PDA (n=1). One of the two patients with extremity anomalies had wrist and finger deformities, and the other one had bilateral club foot and duodenal atresia.

A definitive surgery was performed in a single session without opening a colostomy in 41 patients, while colostomies were created in 27 patients. The indicated number of patients had also undergone surgical interventions due to additional pathologies including ureteroneocystostomy performed with the diagnosis of vesicoureteric reflux (n=2), duodenojejunostomy due to duodenal atresia (n=1), excision of Meckel's diverticulum (n=1), anocutaneous junction revision due to prolapse of the anal mucosa (n=1), and stenosis of the sigmoid colon was dilated in one patient. In addition, two patients had an operation due to extremity anomaly (club foot and wrist deformity).

When the definitive operations performed according to the ARM type are evaluated, the most common surgical interventions was anoplasty in 20 (29.4%) patients, followed by anterior sagittal anorectoplasty (ASARP) in 15 (22.1%) and PSARP in 14 patients (20.6%)

Postoperative complications were present in 4 (5.8%) patients including recurrent RUF (n=1), anal stenosis (n=1), prolapse of the anal mucosa (n=1), and acute abdomen after colostomy repair (n=1).

Only 51 patients reached our clinic and were evaluated with face-to-face interviews. Constipation was detected in 22 (43.1%) of these 51 patients. In terms of stool continence, 36 patients (70.6%) had relevant complaints and 15 patients (29.4%) had stool incontinence. A statistically significant relationship was found between the type of anomaly and stool incontinence ($p<0.05$) (Table 2). There was no significant gender difference among children with stool incontinence (8 boys and 7 girls). No significant relationship was found between age and achievement of continence ($p>0.05$). Indicated number of ARM experienced stool control problems including cases with RUF (n=7), RVF (n=6), ACF (n=1) and rectoperineal fistula (n=1).

Colostomy was performed in 13 of 15 patients with stool continence problems, and colostomy was not performed in 2 patients. The relationship between stool incontinence and colostomy was statistically significant ($p<0.01$). Patients with ARM who had a colostomy were evaluated in terms of constipation, and any statistically significant correlation could not be found between

Table 1. Distribution of ARM by gender

			Types of ARM								Total
			ACF	RUF	PF	AEA	RPF	RVF	RVzF	Pgrv	
Gender	Boy	n	16	13	2	1	7	0	one	0	40
		%	23.5%	19.1%	2.9%	1.5%	10.3%	0.0%	1.5%	0.0%	58.8%
	Girl	n	4	0	0	1	6	16	0	1	28
		%	5.9%	0.0%	0.0%	1.5%	8.8%	23.5%	0.0%	1.5%	41.2%
Total		n	20	13	2	2	13	16	1	1	68
		%	29.4%	19.1%	2.9%	2.9%	19.1%	23.5%	1.5%	1.5%	100.0%

ARM: Anorectal malformations, ACF: Anocutaneous fistula, RUF: Rectourethral fistula, AVF: Anovestibular fistula, RPF: Rectoperineal fistula, RVF: Rectovestibular fistula, RVzF: Rectovesical fistula, Pgrv: Perineal groove, PF: Perineal fistula, AEA: Anterior ectopic anus

colostomy and constipation (p>0.05). Also, stool incontinence was detected in 11 of 24 patients with constipation, and constipation was detected in 11 of 15 patients with stool incontinence. Also a statistically significant correlation was found between stool incontinence and constipation (p<0.05).

DISCUSSION

ARMs are congenital anomalies presenting with very different clinical manifestations. They constitute the majority of pediatric gastrointestinal system anomalies. They are seen in a wide spectrum, from near-normal appearance to complex ARMs and syndromes. When all ARMs are evaluated collectively, the incidence of one or more additional anomalies varies between 25-75%[3,6]. Although many theories have been put forward related to the formation of ARM, its exact pathogenetic mechanism has not been revealed yet

The type of ARM present determines the long-term results of the patients, as well as the surgical technique and skill applied in the definitive surgery. If there is a malformation in the group with poor prognosis, these children will inevitably experience problems with stool control.

Although incontinence due to sphincter insufficiency, which is seen as the most critical problem after surgical treatment of ARM, is still a major problem, problems such as constipation, overflow incontinence due to constipation, and bowel motility disorders have come to the fore despite the advantages of surgical methods developed and applied over time[6].

Although significant advances have been made in understanding and treating ARMs, problems such as constipation, panty-soiling, and incontinence after surgery persist and can be seen at a rate of 30-70%. More than half of the patients experience bowel disorders that cause physical, psychological, and social problems[7,8].

Contrary to the literature data, in our series, most frequently low-type malformations were detected in boys, and intermediate-type RVFs in girls. Most common low-type anomaly in male patients was ACF, followed by a RUF. Among the rectourinary fistulas, rectobulbar fistula was detected. Rectoprostatic fistula was detected in only one patient. These findings are also contrary to the literature data[9,10]. In female patients, the most common anomaly type was RVF consistent with statistical data cited in the literature[11].

Accompanying additional organ anomalies are more frequently seen in cases with high-type anomalies[3,12]. Comorbidities seen in ARM patients are genitourinary (49%), musculoskeletal (43%), craniofacial (34%), cardiovascular (27%), and gastrointestinal anomalies (18%)[12,13]. Most frequently urogenital system anomalies were seen followed by cardiac anomalies[3]. Apart from these, extremity anomalies were also detected. When these findings are compared with the literature data, vertebral (especially sacral) and urinary anomalies are seen frequently, and in our country, urinary anomalies ranked on top followed by cardiac anomalies.

ASARP was performed mostly in female, and anoplasty in male patients. Compared with the literature, Peña and Hong[11] recommends opening a colostomy in all patients with high-type ARM and PSARP as definitive surgical intervention without gender discrimination. In our series, on the other hand, our colostomy rates are in line with Pena's recommendation, and the difference in our preferences for definitive surgery is remarkable.

Peña and Hong[11] advocated that all ARMs, except cloacal malformations, can be corrected using the PSARP method. However, in our clinical series, ASARP was the primary surgical technique of choice in girls

Table 2. Comparison of stool incontinence and anomaly types

			Types of ARM							Total
			ACF	RUF	PF	AEA	RPF	RVF	Pgrv	
Stool incontinence	Yes	n	one	7	0	0	one	6	0	15
		%	2.0%	13.7%	0.0%	0.0%	2.0%	11.8%	0.0%	29.4%
	No	n	13	5	2	2	7	6	1	36
		%	25.5%	9.8%	3.9%	3.9%	13.7%	11.8%	2.0%	70.6%
Total		n	14	12	2	2	8	12	1	51
		%	27.5%	23.5%	3.9%	3.9%	15.7%	23.5%	2.0%	100.0%

ARM: Anorectal malformations, ACF: Anocutaneous fistula, RUF: Rectourethral fistula, AVF: Anovestibular fistula, RPF: Rectoperineal fistula, RVF: Rectovestibular fistula, Pgrv: Perineal groove, PF: Perineal fistula, AEA: Anterior ectopic anus

with anovestibular fistula. One of the interesting results of our clinical series is that no cloacal malformation was encountered in girls. This finding is inconsistent with the literature data.

The patients were evaluated for stool control after the definitive surgery, based on the disease history, physical examination findings, and sphincter activities detected during the definitive operations. The anal tone of the patients and the presence of anal stenosis were checked with digital rectal examination. Sphincter activities were evaluated by looking at the contraction of sphincter structures on digital rectal examination. No patient with weak anal tone was detected in this examination.

The complaints of the patients and whether they received treatment for these complaints or those with these complaints faced psychosocial problems were questioned. Only 15 of the 51 evaluated patients had stained underwear and were considered in the group with stool incontinence. All but one of the 15 patients who stained underwear had a complaint of constipation, and stool incontinence was in the form of overflow incontinence. A statistically significant relationship was found between constipation and stool incontinence.

Colostomy was performed in 13 of 15 patients with stool incontinence, and these patients had high-type ARMs. Two patients were operated on in a single stage and had no colostomy history. A statistically significant correlation was detected between anomaly type, operation technique and stool incontinence, and also between colostomy opening and stool incontinence.

Constipation or staining underwear after bowel movements were not observed only in patients with high-type malformations but also such complaints were also observed in cases with low-type malformations. Almost none of the patients who had undergone definitive surgeries attended control visits at regular intervals in the long term. Patients with stool incontinence did not come for control after definitive surgery, even though they complained of staining underwear and constipation, and very few patients were treated for constipation.

The reason why the patients did not come to the control was due to the fact that they did not experience any social, psychological, or environmental problems. Half of the patients (50%) were in school, and none had social, psychological, and school-related problems. Medical treatment for constipation was started, and regular follow-ups were recommended for these patients.

In the long term, bowel training programs, enemas, or even secondary surgeries may be required in patients with ARMs, especially in patients with high-type malformations, for problems such as stool incontinence and fecal contamination[11,14].

None of the patients had enough complaints to restrict their social life. The patients did not need a second operation after the definitive surgeries performed and did not receive any bowel training program or supportive medical treatment. Indeed, they continued their lives without experiencing psychosocial problems.

The type of anomaly predicts the long-term results of the patients with ARM and the surgery performed. No matter how successful the operation is, it should be known that patients who belong to the poor prognostic group or in other words patients who have high-type ARM may have a low chance of achieving bowel control. Patients with low-type malformations are more fortunate in this regard.

In our evaluations, bowel control after definitive surgeries performed for low-type ARMs was satisfactory in more than 90% of patients, but it was not desirable in cases with high and intermediate-type ARMs.

There was no significant difference between our results and the results given in the literature, in terms of the sphincter examination performed with a nerve stimulator during the operation and digital rectal examination of the anal sphincter we performed in the postoperative period, as well as the Krickenbeck criteria used in the evaluation of the treatment results[15].

Study Limitations

This study has some limitations including its retrospective design, Besides, the patients were not evaluated using a nerve stimulator under anesthesia. Future studies can be planned prospectively to fill these deficiencies.

CONCLUSION

Since the problems detected in the patients are mainly constipation and overflow incontinence due to constipation, patients should be followed up for problems such as anal stenosis and constipation in the postoperative period, and families should be informed about getting the patients accustomed to defecation at regular intervals and at certain times. In patients

with constipation, treatment should be started before formation of fecaliths, and if necessary, the bowels should be emptied with enemas, and the patients should be fed with regular and appropriate meals to regulate their bowel movements.

Ethics

Ethics Committee Approval: The ethical approval was obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (number: 78, date: 17.04.2009).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Sugical and Medical Practices: B.F., A.B.S., Concept: B.F., A.B.S., Design: B.F., A.B.S., Data Collection or Processing: B.F., A.B.S., Analysis or Interpretation: B.F., A.B.S., Literature Search: B.F., A.B.S., Writing: B.F., A.B.S.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.48658
J Dr Behcet Uz Child Hosp 2023;13(1):43-48

Original Article

Can High Mobility Group Box 1 Protein Predict Ongoing Subclinical Inflammation in Patients With Familial Mediterranean Fever?

High Mobility Group Box 1 Proteini, Ailevi Akdeniz Ateşi Hastalarında Süregelen Subklinik Enflamasyonu Öngerebilir mi?

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ABSTRACT

Objective: Familial Mediterranean fever (FMF) is an autoinflammatory disease that commonly presents with fever, peritonitis, and pleuritis. Recent studies have reported ongoing inflammation in the attack-free period of patients with FMF. High mobility group box protein (HMGB1) is a frequently investigated marker with a strong diagnostic and prognostic role for several chronic inflammatory diseases. The objective of this study was to evaluate the role of HMGB1 in patients with FMF.

Method: This cross-sectional study included a total of 57 (25 female/32 male) consecutive patients with FMF and a control group of 60 (30 female/30 male) healthy children. Demographic and clinical data of the patients were recorded. Blood samples were obtained from participants for HMGB1 analysis.

Results: The median age of the patients was 123 months. The median follow-up time of patients was 5 years. There was no statistically significant difference between the patient and control groups in terms of age, sex, and body weight. The most frequent *MEFV* gene mutation was M694V (78%). HMGB1 was higher in the patient group than in the control group (p=0.001). The levels of HMGB1 were not different between the attack and the attack-free period (p>0.05).

Conclusion: HMGB1 is significantly higher in FMF patients independent from being in the attack period. HMGB1 may demonstrate the ongoing subclinical inflammation in patients with FMF.

Keywords: DAMPs, HMGB1, FMF, subclinical inflammation, FMF attack-free period

ÖZ

Amaç: Ailevi Akdeniz ateşi (AAA) ateş, peritonit ve plevrit ile ortaya çıkan otoenflamatuvar bir hastalıktır. Yakın zamanlı çalışmalarda AAA hastalarında atak dışı dönemde de devam eden enflamasyon gösterilmiştir. High mobility group box 1 proteini (HMGB1) güçlü bir tanısal ve prognostik belirteç olarak çok sayıda kronik enflamatuvar hastalıkta araştırılmış bir proteindir. Çalışmanın amacı AAA hastalarında HMGB1'in rolünü araştırmaktır.

Yöntem: Bu kesitsel çalışmada toplam 57 (25 kız/32 erkek) AAA hastası ile 60 (30 kız/30 erkek) sağlıklı çocuktan oluşan kontrol grubu bulunmaktadır. Hastaların demografik ve klinik verileri kaydedilmiştir. Tüm hastalardan HMGB1 analizi için kan örneği toplanmıştır.

Bulgular: Hastaların medyan yaşı 123 aydı. Medyan takip süresi 5 yıldı. Hastalar ve kontrol grubu arasında yaş, cinsiyet ve vücut ağırlığı açısından anlamlı fark yoktu. Hastalardaki en sık *MEFV* gen mutasyonu M694V (%78) idi. HMGB1 hastalarda kontrol grubuna göre anlamlı olarak yüksekti (p=0,001). HMGB1 düzeyi atak ve atak dışı dönemdeki hastalarda benzerdi (p>0,05).

Sonuç: HMGB1, AAA hastalardında atak döneminden bağımsız olarak yüksektir. AAA hastalarında süregelen subklinik enflamasyonun bir belirteci olabilir.

Anahtar kelimeler: DAMPs, HMGB1, AAA, subklinik enflamasyonu, AAA atak dışı dönem

Received: 09.08.2022
Accepted: 10.10.2022

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Cite as: Öztürk B, Baskın E, Gülleroğlu K, Avcı B, Bayraktar N, Şahin Fİ. Can High Mobility Group Box 1 Protein Predict Ongoing Subclinical Inflammation in Patients With Familial Mediterranean Fever?
J Dr Behcet Uz Child Hosp. 2023;13(1):43-48

INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive genetic disease that commonly affects the population living in the Mediterranean region[1]. The disease is characterized by repetitive self-limiting attacks of aseptic peritonitis, pleuritis, arthritis, and fever lasting for 1 to 3 days. The disease can be diagnosed with molecular genetic testing, which can identify the characteristic *MEFV* gene mutations. In the literature, many studies demonstrated that; interleukin-6 (IL-6), IL-12, IL-17, IL-18, INF-g, TNF-α, neutrophil-to-lymphocyte ratio (NLR), and red boold cell distribution width (RDW) were related to subclinical inflammation in the attack-free period[2,3]. These results support that there is ongoing inflammation in FMF patients even in the asymptomatic period of the disease[4,5]. The most devastating complication of FMF is amyloidosis, which can also develop in asymptomatic patients with subclinical inflammation[6,7]. There is no biological marker that has been reported to determine the severity of the ongoing subclinical inflammation that may predict the prognosis of the disease.

Damage-associated molecular pattern (DAMP) molecules have a role in physiological functions in the daily cycle of a cell and when they reach the extracellular environment, they have a major role in the signaling pathway of the tissue damage response[8]. High mobility group box 1 protein (HMGB1), a nuclear DNA binding protein, is one of these DAMPs[9]. HMGB1 is found in all human cells. To act like DAMPs, it should be released from the cell. It can passively be released from necrotic/apoptotic cells, and actively secreted from cells under stress such as monocytes, macrophages, and dendritic cells. HMGB1 enhances the immune response and induces the release of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 via RAGE, TLR2, and TLR4[10-12].

The inhibiting role over apoptosis-associated speck-like protein (ASC) with a caspase activation and recruitment domain (CARD) is disappeared with pyrine mutation. As a result, the release of caspase 1 leads to elevated IL-1 and NF-κB activation. The cell then proceeds to apoptosis/proptosis and triggers inflammation. HMGB1 is released actively in proptosis. This circumstance may present a different perspective on the ongoing pyrine deficiency in the attack-free period of the disease and the subclinical inflammation mechanism. The relationship between HMGB1 and autoimmune diseases such as systemic lupus, rheumatoid arthritis, and inflammatory myositis has been reported previously[11,13,14]. An elevation in HMGB1 may be seen in serum, synovial fluid, and the extracellular matrix of skin lesions in several diseases[11,13,15].

There are a few studies in the recent literature that demonstrate the subclinical inflammation in FMF patients in the attack-free period. This study aimed to determine whether the pro-inflammatory cytokine HMGB1 can be used as an indicator of inflammation in FMF patients with or without attack.

MATERIALS and METHODS

This cross-sectional study was conducted in the Pediatric Nephrology Department and was approved by the ethical board of the Başkent University (project no: KA15/350, date: 23.12.2015). The study was performed between January 2016 and November 2016. All patients and their parents signed an informed consent form before they were included in the study.

Study Population

Sixty consecutive patients with a diagnosis of FMF with or without attack were included in the study. All patients met the diagnostic criteria of Tel Hashomer[16] and Yalçinkaya[17] for FMF and all their genetic studies had been previously completed. The patients had no other concomitant diseases. Three patients were excluded due to the lack of genetic mutation analysis records, and therefore, 57 patients were analyzed in the study group. A control group was formed of 60 healthy age and sex-matched subjects selected from patients presenting for routine screening, with no known disorders, and volunteered for participation in the study with the approval of their legal guardians. The study group was aged between 5 and 18 years. Data were retrieved from the hospital records. The date of diagnosis, genetic mutation analysis, treatments of the patients, the duration of treatment, family history of the patients, and consanguineous marriage was recorded. Patients were excluded if they had any coexisting chronic disease such as amyloidosis or renal failure, or if they were not treated with colchicine.

Blood Sample Collection and Analysis

1 mL of venous blood sample was obtained for HMGB1 analysis from all participants. The blood samples were analyzed with an enzyme-linked immunosorbent assay kit for high mobility group protein 1 (HMG1) (Cloud-Clone Corp® Houston, TX, USA). This assay kit is sensitive for 12.5-800 pg/mL of HMGB1 level. The

test was performed by a laboratory assistant blinded to the groups. The blood samples of the patients were also analyzed for C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell count, fibrinogen, creatine kinase, aspartate aminotransferase, alanine aminotransferase, and blood creatinine. Urine samples were analyzed for microalbumin.

Statistical Analysis

The main outcome was the level of serum HMGB1 in patients and power analysis was used to adjust the sample size before the beginning of the study to compare the FMF patients and control group regarding recent literature. The sample size was calculated as requiring at least 49 subjects in each group for a power of 0.80 and α=0.05. Data obtained in the study were analyzed statistically using SPSS ver. 20.0 for Windows (IBM SPSS Inc., NY, USA). The Shapiro-Wilk test was used to assess the normal distribution of the data. Quantitative variables were expressed as median and minimum-maximum values and qualitative variables as frequencies. In the comparisons between groups, normally distributed variables were compared with the Student's t-test and non-normally distributed variables with the Mann-Whitney U test. Categorical variables were compared with the chi-square test. Spearman rank correlation coefficient was employed to determine the correlation between HMGB1 and other variables. A value of p<0.05 was considered statistically significant.

RESULTS

The median age of patients was 123 months (minimum: 20 - maximum: 220) and while 55 (47%) of them were boys, 62 (53%) were girls. There was no significant difference in age, sex, or body weight between the patients and the healthy control group (Table 1). The median follow-up duration was 5 years (range, 1 to 12 years) and the median age at disease onset was 5 years (range, 0 to 13 years). Five patients were in the attack period and 52 patients were in the attack-free period. Positive family history was determined in 33 (57.9%) patients with FMF, and 6 (10.5%) patients had consanguineous parents. M694V gene mutation was present in 45 (78.7%) patients with at least one allele and 19 patients had homozygous M694V mutations, one patient had homozygous M608I mutations. All patients with FMF were under colchicine treatment at a median dose of 1 mg/day (0.5-1.5 mg/day).

The median HMGB1 level was higher in patients with FMF [47.9 ng/dL (21-406)] compared to healthy control subjects [32.7 ng/dL (16-118)] (p<0.001). In FMF group, the median level of HMGB1 was 47.9 ng/dL (21-321) in girls (n=25) and 49.9 ng/dL (29-405) in boys (n=32) (p=0.303). Serum HMGB1 levels were not statistically different between patients with a ≥5 years follow-up duration of and <5 years follow-up duration [49.5 ng/dL (21-406) vs 46.7 ng/dL (23-321), respectively, p=0.533]. The levels of HMGB1 showed no difference between patients with and without a family history (p=0.566), and with and without parental consanguineous marriage (p=0.621). There was no association between serum HMGB1 levels and gene mutations. Serum HMGB1 levels were not correlated with CRP, ESR, blood cell counts, liver enzymes, fibrinogen, and urine microalbumin levels. Also, no correlation was found between the duration of follow-up and HMGB1 levels (Table 2).

However, the median hemoglobin, platelet, ESR, CRP, fibrinogen, RDW levels, and neutrophil/lymphocyte ratio were significantly different between patients with and without attack, and the levels of serum HMGB1 were not higher in patients with the attack (Table 3).

DISCUSSION

Due to the disappearance of inhibiting effect of pyrin on ASC through mutation in the MEFV gene and over-production of caspase 1, cell apoptosis/proptosis may be aggravated in patients with FMF. HMGB1 is actively released during this proptosis process[18,19]. After the release of HMGB1 from the necrotic tissues, it acts as a DAMPs molecule and induces dendritic cell maturation and migration, and controls the activation of T cells through RAGE. Thus, it acts as an important mediator of sterile inflammation as a part of native immunity[20]. These cells produce pro-inflammatory signals and cytokines

Table 1. Demographical characteristics and main laboratory findings of the groups

	FMF (n=57)	Healthy controls (n=60)	p-value
Age, months*	123 (20-220)	122.5 (22-216)	0.866
Gender, n (female/male)	25/32	30/30	0.580
Body weight, kg*	33.9 (12.5-116)	37 (14-98)	0.594
HMGB1 (ng/dL)	47.9 (21-406)	32.7 (16-118)	0.001

FMF: Familial Mediterranean fever, HMGB1: High mobility group box 1 protein. *Values given as median (minimum-maximum)

and aggravate inflammation. Recent studies related to the pro-inflammatory cytokine activity of HMGB1 have demonstrated the role of HMGB1 as a dangerous signal in autoimmune diseases such as rheumatoid arthritis, and systemic lupus erythematosus[11,21]. The most important finding of the current study was the levels of HMGB1 did not differ in attack or attack-free periods. To the best of our knowledge, no study in recent literature has evaluated HMGB1 levels in patients with FMF. The results of this study showed that HMGB1 was elevated regardless of the attack or remission period of FMF. NLR, platelet to lymphocyte ratio, MPV, and RDW have previously been reported to be elevated in patients with FMF in the attack-free period[2]. MPV, splenomegaly, and NLR have been reported as indicators of ongoing inflammation in patients with FMF[22,23]. In addition to elevated HMGB1 in the attack-free period, HMGB1 was also correlated with NLR and RDW in the current study. These parameters

Table 2. The demographic and laboratory variables in FMF patients and their correlation with HMGB1

Variable	Median	Correlation coefficient	p-value
Age (months)	126 (22-216)	-0.012	0.897
Body weight (kg)	33.9 (12.5-116)	-0.042	0.656
Creatinine (mg/dL)	0.58 (0.4-0.88)	-0.035	0.798
CRP (mg/L)	2.63 (0.2-77.8)	-0.074	0.585
ESR (mm/h)	7 (2-66)	-0.019	0.891
White blood cell (k/µL)	7660 (3970-13930)	0.059	0.664
Fibrinogene (mg/dL)	306 (192-602)	-0.144	0.287
Colchicine dose (mg/day)	1 (0-1.5)	0.175	0.192
Follow-up time, years	5 (1-12)	0.020	0.882
Urine microalbumine (mg/gr/cre)	9.2 (3-34.9)	-0.022	0.871
MPV (fL)	7.33 (5.3-10.7)	0.112	0.447
Neutrophyl/lymphocite	1.22 (0.4-4.3)	R=0.350	0.02
RDW (%)	13.35 (10.6-17.4)	R=0.285	0.04

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, MPV: mean platelets volume, RDW: Red blood cell distribution width, FMF: Familial Mediterranean fever, HMGB1: High mobility group box 1 protein

Table 3. The laboratory findings in patients with and without FMF attack

	Patients in attack period (n=5)	Patients in attack-free period (n=52)	p-value
HMGB1 (ng/dL)	42.2 (35-100)	48.3 (21-406)	0.774
CRP (mg/L)	51 (36.5-77.8)	1.8 (0.2-65)	<0.001
Fibrinogene (mg/dL)	452 (412-551)	295 (192-602)	<0.001
Erythrocyte sedimentation rate (mm/h)	30 (9-66)	6.5 (2-31)	0.001
Hemoglobine (g/dL)	12.3 (10.6-13)	13.1 (10.2-16)	0.035
White blood cells (k/µL)	8290 (4670-11600)	7485 (3970-13930)	0.356
Platelets (k/µL)	387000 (214000-495000)	295000 (157000-478000)	0.048
Creatinin (mg/dL)	0.55 (0.51-0.61)	0.59 (0.4-0.88)	0.206
AST (U/L)	20 (16-29)	22 (13-35)	0.651
ALT (U/L)	15 (7-23)	17 (9-49)	0.250
CK (U/L)	92 (37-127)	100 (32-280)	0.519
Urine microalbumine (mg/gr/cre)	7.2 (4.2-9.9)	9.2 (3-34.9)	0.262
Neutrophyl/lymphocite	1.85 (0.7-4.1)	1.18 (0.4-4.3)	0.04
RDW (%)	15.5 (14.4-16.2)	13 (10.6-17.4)	0.04
MPV (fL)	7.2 (5.7-8.4)	7.33 (5.3-10.7)	0.482

HMGB1: High mobility group box 1 protein, CRP: C-reactive protein, AST: Alanin aminotransferaz, ALT: Aspartate aminotransferaz, RDW: Red blood cell distribution width, MPV: Mean platelets volume , FMF: Familial Mediterranean fever, CK: Creatine kinase

may indicate subclinical inflammation in patients with FMF.

As an indicator of ongoing inflammation, the elevated HMGB1 in the attack-free period in patients under colchicine treatment may indicate insufficient control of the subclinical inflammation. Although colchicine treatment is known to reduce attack frequency and serious complications such as amyloidosis, to date, the clinical outcomes of the subclinical inflammation despite colchicine treatment are not known[24]. Gunes et al.[25] reported that kidney damage in patients with FMF is due to ongoing minimal inflammation and that urinary microalbumin can be used as a marker of kidney damage. The urine microalbumin and HMGB1 were not found to be correlated in the current study. This may be related to the short disease duration time of the patients[26]. Therefore, considering the limited exposure of patients to ongoing subclinical inflammation and the absence of pathologic microalbuminuria may have concealed the predictive value of HMGB1 for amyloidosis in this study. Long-term outcomes of these patients may give an idea about the predictive value of HMGB1 for amyloidosis.

The most common mutations in FMF patients are M694V, V726A, M680I, and M694I[27,28]. The mutations in the current study were compared with the literature and 78% of patients had M694V mutation in at least one allele. Prasad et al.[29] reported that patients with M694V mutation had earlier disease onset, had less response to colchicine, and more frequently developed amyloidosis. However, no significant relationship between M694V mutation and poor prognosis of the disease has been demonstrated[30]. Similarly, no significant HMGB1 difference was observed between the patients with and without M694V mutation in this study. As the relationship between the type of mutation and the prognosis is considered, it may be concluded that HMGB1 may not predict prognosis in these patients. There is a need for further studies with more patients and longer follow-ups to clarify the relationship between HMGB1 and the prognosis of the disease and the risk for amyloidosis.

As expected, the acute inflammation markers were higher in patients in the FMF attack period but HMGB1 was similar to that of patients in the attack-free period in the current study. HMGB1 has been reported to be elevated in later periods of inflammation[31]. However, HMGB1 was elevated in all patients in the current study, whether in the attack or attack-free period, so it can be concluded that HMGB1 is elevated in patients with FMF regardless of whether they are in attack or attack-free period. It can also be speculated that HMGB1 in patients with FMF probably projects the long-term inflammation of the patients which may indicate the effective control of the disease with colchicine treatment.

Study Limitations

However, the limited number of patients in the attack period was a major limitation of this study, and studies with a higher number of patients in the attack period of the disease are needed to draw definitive conclusions about the differences in HMGB1. In addition, the lack of control groups including various types of autoinflammatory diseases limits the study to rule out the specificity of HMGB1 in patients with FMF.

CONCLUSION

The results of this study demonstrated that serum HMGB1, which indicates cellular stress and ongoing subclinical inflammation, is elevated in patients with FMF when compared to healthy controls. In addition, the elevated HMGB1 in patients with FMF is not associated with the attack period of the disease.

Ethics

Ethics Committee Approval: This cross-sectional study was conducted in the Pediatric Nephrology Department and was approved by the ethical board of the Başkent University (project no: KA15/350, date: 23.12.2015).

Informed Consent: All patients and their parents signed an informed consent form before they were included in the study.

Authorship Contributions

Surgical and Medical Practices: B.Ö., Concept: K.G., B.A., N.B., F.İ.Ş., Design: B.Ö., E.B., Data Collection or Processing: B.Ö., N.B., F.İ.Ş., Analysis or Interpretation: N.B., Literature Search: B.Ö., Writing: B.Ö., F.İ.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: Başkent University Research Fund (23/12/2015-15/109) KA15/350.

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DOI: 10.4274/buchd.galenos.2022.90922
J Dr Behcet Uz Child Hosp 2023;13(1):49-53

Original Article

Food Protein-induced Enterocolitis Syndrome: A Single-center Experience

Besin Proteini İlişkili Enterokolit Sendromu: Tek Merkez Deneyimi

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ABSTRACT

Objective: Food protein-induced enterocolitis syndrome (FPIES) is an uncommon, non-IgE (immunoglobulin E) -mediated food allergy that mainly affects infants and young children. Our study aims to evaluate the etiology, clinical phenotypes, and tolerance status of our cases with FPIES.

Method: The file records of the patients who were followed up with the diagnosis of FPIES in the Departments of Pediatric Allergy and Gastroenterology of our hospital, between September 2016 and June 2022 were examined, and families who could not attend follow-up visits regularly were contacted by phone.

Results: Twelve (66.66%) of 18 cases with a mean age of admission of 33.0±27.5 (2-108) months were boy. The triggers of FPIES were fish in 66.66%, cow's milk in 16.66%, eggs in 5.55%, eggs and milk in 5.55%, and potato in 5.55% of the patients. While 94.44% of the cases had acute FPIES, and 44.4% of them had early-onset (<9 months) FPIES. The most common symptoms were vomiting (100%), diarrhea (38.88%), pallor (27.77%), lethargy (22.22%). Food-specific IgE sensitization was found in 5.55% of the patients, while tolerance developed in 33.33% of the cases during the follow-up. The mean age of tolerance development was 63±42 (19-112) months.

Conclusion: It is important to have knowledge about the symptoms of FPIES for accurate and early diagnosis. While cow's milk is the most prevalent triggers of FPIES in the literature, fish was at the forefront in our series. Despite the limited number of cases, our results are important in terms of giving us an idea about the triggers of FPIES in the western regions of Turkey.

Keywords: Food protein-induced enterocolitis syndrome, food allergy, fish allergy, children, oral food challenge

ÖZ

Amaç: Besin proteini ilişkili enterokolit sendromu, çoğunlukla bebekleri ve küçük çocukları etkileyen, immünoglobulin E (IgE) aracılı olmayan, nadir görülen bir besin alerjisidir. Çalışmamızda besin proteini ilişkili enterokolit sendromlu hastalarımızın etiyoloji, klinik fenotipleri ve tolerans durumlarının değerlendirilmesi amaçlanmıştır.

Yöntem: Hastanemiz Çocuk Alerji ve Çocuk Gastroenteroloji Bölümleri'nde Eylül 2016-Haziran 2022 tarihleri arasında besin proteini ilişkili enterokolit sendromu tanısı ile izlenen olguların dosya kayıtları incelendi ve düzenli takibe gelemeyen ailelere telefonla ulaşıldı.

Bulgular: Ortalama başvuru yaşı 33,0±27,5 (2-108) ay olan 18 olgunun 12'si (%66,66) erkek idi. Olguların %66,66'sında balık, %16,66'sında inek sütü, %5,55'inde yumurta, %5,55'inde yumurta ve süt, %5,55'inde patates tetikleyici idi. Olguların %94,44'ü akut, %44,4'ü erken başlangıçlı (<9 ay) besin proteini ilişkili enterokolit sendromu idi. En sık semptom kusma (%100), diyare (%38,88), solukluk (%27,77), letarji (%22,22) idi. Olguların %5,55'inde şüpheli gıdaya ait IgE duyarlılığı saptandı. İzlemde hastaların %33,33'ünde tolerans gelişti. Ortalama tolerans gelişme yaşı 63±42 (19-112) ay idi.

Sonuç: Semptomlar hakkında bilgi sahibi olmak doğru ve erken tanı için önemlidir. Literatürde en yaygın tetikleyiciler inek sütü ve soya iken, bizim serimizde balık ön plandadır. Olgu sayımız sınırlı olmasına rağmen sonuçlarımız Türkiye'nin batı bölgelerindeki tetikleyiciler hakkında bize fikir vermesi açısından önemlidir.

Anahtar kelimeler: Besin proteini ilişkili enterokolit sendromu, besin alerjisi, balık alerjisi, çocuk, besin yükleme testi

Received: 28.06.2022
Accepted: 25.11.2022

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Cite as: Bahçeci S, Kuyum Töz P, Ayar M. Food Protein-induced Enterocolitis Syndrome: A Single-center Experience. J Dr Behcet Uz Child Hosp. 2023;13(1):49-53

INTRODUCTION

Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE (immunoglobulin E)-mediated food allergy that presents with gastrointestinal symptoms[1]. FPIES affects the entire gastrointestinal system and presents with recurrent episodes of vomiting, diarrhea, lethargy, pallor, hypothermia, and hypovolemic shock in severe cases. In chronic FPIES, the major symptoms are intermittent vomiting, diarrhea (sometimes contaminated with mucus and/or blood), and growth retardation[2].

The prevalence and incidence of FPIES are still unknown[2]. Its cumulative incidence in infants is estimated to be 0.015-0.7%. Its prevalence in infants in the United States (US) was reported to be 0.51%[3]. While studies from Israel, Spain, Australia reported its incidence as 0.34%, 0.35%, and 0.015%, respectively[4]. As has been indicated, boys are slightly more frequently affected than girls[1].

Its pathogenesis is still unclear, and any relevant diagnostic biomarker has not been reported up to now. Although the diagnosis of FPIES is based on clinical history, oral food challenge (OFC) test is still the gold standard for the confirmation of the diagnosis[2]. It is important to exclude other causes, and metabolic disorders while establishing the diagnosis[3]. The fact that the resulting symptoms are not specific and typical to allergy, the prolonged period of time between food intake and the onset of symptoms (1-4 hours), low levels of awareness among clinicians, and the lack of diagnostic biomarkers lead to difficulties in diagnosis. Allergy tests are usually negative. For these reasons, FPIES can usually be misdiagnosed, and/or its diagnosis may be delayed[2,3].

Cow's milk is reported to be the most prevalent culprit food. However, triggers may differ according to geographical regions, the eating habits of a specific society, the place of a certain food in the diet, time to the onset of complementary feeding, and genetic predispositions[5].

Although the prevalence of FPIES is gradually increasing with the use of currently available diagnostic criteria and the enhanced level of awareness, our knowledge of this subject is still limited. The data from Turkey are similarly limited. Our study aimed to evaluate the etiology, clinical phenotypes, and tolerance status of our cases with FPIES.

MATERIALS and METHODS

Subjects

Eighteen cases who were followed up with the diagnosis of FPIES in the Departments of Pediatric Allergy and Pediatric Gastroenterology of our hospital, between September 2016 and June 2022 were included in the study. The clinical and laboratory findings were recorded from the patients' medical files, and families who could not come to follow-up regularly were contacted by phone.

Methods

The diagnosis of the FPIES was made according to the criteria specified in the guidelines[3]. Cases with suspected metabolic disease were not included in the study.

Serum-specific IgE was studied with UniCAP 100 system fluorescence enzyme immunoassay (Phadia, Uppsala, Sweden). A result above 0.35 kUA/L was considered as positive.

The skin prick tests (SPT) were performed by physicians using a commercial food extracts (ALK Abelló, Horsholm, Denmark). Histamine was used as positive control and saline as negative control. The mean diameter of the wheal was measured after 15 minutes, with a mean diameter greater than 3 mm considered as positive.

The OFC test was used for diagnostic purposes in the presence of a single episode, in cases with a suspected clinical history or unknown food trigger in the past. In the OFC test, the responsible food was administered at a dose of 0.06-0.6 g/kg.bw (in a way not to exceed a total of 3 g of protein or a total of 10 g of food) in 3 equal doses at 30-minute intervals, and the cases were observed for 4-6 hours. OFC test was considered positive if vomiting started within 1-4 hours after food intake (usually prolonged recurrent vomiting) and in the presence of at least two minor criteria[3].

At least 1 year after the last allergic reaction, the cases were re-evaluated for developing tolerance by the OFC test under hospital conditions.

The study was approved by the İzmir Bakırçay University Non-Invasive Clinical Research Ethics Committee (decision number: 636, date: 22.06.2022).

Statistical Analysis

IBM SPSS version 22.0 (Armonk, New York, United States) was used for all statistical analyses. Numbers

and percentages were presented for discrete variables, while means and standard deviations were presented for continuous variables.

RESULTS

Twelve (66.66%) of 18 cases with a mean age of admission of 33.0±27.5 (2-108) months were boy, and the mean age at symptom onset was 12.3±12.8 (1-60) months. The triggers of FPIES were fish in 66.66% (n=12), cow's milk in 16.66% (n=3), eggs in 5.55% (n=1), eggs and milk in 5.55% (n=1), and potato in 5.55% (n=1) of the cases. While 94.44% (n=17) of the cases had acute FPIES, and 44.4% (n=8) of them had early-onset (<9 months) FPIES. The most common symptoms were vomiting (100%), diarrhea (38.88%), pallor (27.77%), and lethargy (22.22%). During diagnostic procedure, food-specific IgE value and SPT were positive in only one case. In the case with IgE sensitization, the culprit food was cow's milk. Atopic dermatitis was present in 11.11% of the cases, and IgE-mediated food allergy in 5.55%. Tolerance developed in 33.33% of the patients during the follow-up. The mean age of tolerance development was 63±42 (19-112) months. Baked-milk tolerance emerged in two patients who developed cow's milk-induced FPIES at the age of 9 and 12 months, respectively (Table 1, 2). The tolerance developed earlier in the patients with milk and egg induced FPIES [13.3±5.1 (9-19) months] compared to the patients with fish-induced FPIES [78.6±36.2 (43-115) months] (p=0.037).

DISCUSSION

As far as we know, our study is the first survey investigating the incidence of FPIES in the Aegean region of Turkey. In our study, the most common trigger of FPIES was fish, and a single food was responsible for FPIES in 94.44% of our cases. IgE sensitization to the trigger food was rarely found, and tolerance developed earlier in cases with milk and egg-induced FPIES.

In some countries, fish has been reported as the second most common (12-33%) food after cow's milk[6]. In Greece, fish (54%) was reported to be the most prevalent food[7].

Cow's milk is considered to be most frequent culprit food in infancy, while solid food FPIES tends to present after infancy as the most important triggers in the etiology of FPIES. Although cow's milk is the most frequent culprit food in the literature, fish was at the forefront in our series.

In terms of solid foods, in the literature the most frequently reported trigger foods were grain, egg and fish; but fish is at the forefront of our patients, similar to the Greece cohorts.

Table 1. Patient characteristics

Number of patients, n	18
Male patients, n (%)	12 (66.66)
Family history of atopy*, n (%)	6 (33.33)
Atopic disease, n (%) Eczema n (%) IgE- mediated food allergy, n (%)	3 (16.66) 2 (11.11) 1 (5.55)
No. of episodes in total, n	56
No. of episodes at diagnosis, (mean ± SD)	3.3±0.9
Skin prick test positive at diagnosis, n (%)	1 (5.55)
Specific IgE positive at diagnosis, n (%)	1 (5.55)
Diagnosis based on clinical history, n (%)	15 (83.33)
Diagnosis based on positive OFC, n (%)	3 (16.66)

OFC: Oral food challenge, IgE: Immunoglobulin E, SD: Standard deviation. *Defined as asthma, eczema, IgE- Mediated food allergy, or allergic rhinitis reported in parent or sibling

Table 2. Clinical characteristics of patients and trigger foods

		n (%)
Admission symptoms	Vomiting	18 (100)
	Diarrhea	7 (38.8)
	Pallor	5 (27.77)
	Lethargy	4 (22.22)
Symptom duration	Acute	17 (94.44)
	Chronic	1 (5.55)
Hospitalization for the acute FPIES episode	Yes	12 (66.66)
	No	6 (33.34)
Age of onset	Early <9 months	8 (44.4)
	Late ≥9 months	10 (55.56)
Severity	Mild-moderate	8 (44.44)
	Severe	10 (55.55)
Trigger foods	Fish	12 (66.66)
	Cow's milk	3 (16.66)
	Egg	1 (5.55)
	Cow's milk and egg	1 (5.55)
	Potato	1 (5.55)
Tolerance	With tolerance development	6 (33.34)
	Without tolerance development	12 (66.66)

FPIES: Food protein-induced enterocolitis syndrome

In the studies from Turkey, while Arik Yilmaz et al.[8] reported cow's milk as the most prevalent trigger in the Central Anatolia Region, Ocak et al.[9] reported that the most prevalent trigger in the same region was egg, followed by fish. In a multicenter study by Metbulut et al.[10], milk, egg white, and fish were indicated as the most common trigger foods. Trigger foods may differ according to geographical regions, the eating habits of society, the place of the trigger food in the diet, time to the onset of complementary feeding, and genetic predispositions[5]. Differences in breastfeeding rate and duration, the formulas used, and the order and time of giving complementary foods are among the reasons for geographical differences. Other possible reasons for geographical variations are differences in gut microbiota and genetics[6].

Allergic reactions to more than one food are rare. It appears that patients with cereal-induced FPIES are at higher risk of allergic reactions, especially against other types of cereals. This association was observed in the studies from Europe, Australia, and the US[6,11-13]. US studies reported allergic reactions to both cow's milk and soy in 16-29% of patients. However, this association was not common in European studies[12,14]. Metbulut et al.[10] reported the incidence of FPIES occurring with multiple foods was 21.9%. Multiple food allergies were present in only 5.55% of our cases.

In our study, the frequency of atypical FPIES was significantly lower (Table 1). IgE sensitization to trigger foods was not found in 94.45% of the patients. This incidence rate was comparable with those reported in the studies performed in the US, Australia, and Italy (94-97%)[6,11,14]. However, the rate of IgE sensitization is higher in European countries (13-16%)[6,7,15]. In the multicenter study conducted by Metbulut et al.[10] in Turkey, the rate of atypical FPIES was 20%. In another study examining children with FPIES in Turkey, specific IgE positivity was found in 26% of the cases[8]. Caubet et al.[12] reported that cases with IgE sensitization to cow's milk had a more persistent course than those who did not. In our study, the only case with IgE sensitization was the case with cow's milk-induced FPIES. In this case, baked milk tolerance developed at the age of 12 months.

FPIES is mainly diagnosed based on history, typical complaints and clinical findings, exclusion of other possible diagnoses, and challenge tests. According to the diagnostic criteria for acute FPIES, vomiting within 1-4 hours suspected food intake and absence of cutaneous or respiratory symptoms and at least three of the nine minor criteria should be present in combination. In chronic FPIES, the presence of intermittent, and not otherwise explained vomiting and/or diarrhea (with or without blood), improvement of symptoms within 3-10 days after elimination of the suspected trigger food, and the emergence of acute FPIES symptoms after re-exposure are required diagnostic criteria[3,16]. Vomiting, diarrhea, pallor were the most frequent symptoms in our cases and 66.66% of the cases were admitted to the emergency department. In a study of 462 cases, severe symptoms (such as hypotension) were reported in 5% of patients[14]. Likewise, one of our cases with metabolic acidosis and cow's milk as the culprit food was hospitalized two times and followed up.

In our study, the tolerance developed at a later stage in fish-related FPIES cases, when compared to milk and egg-induced FPIES. This finding is in line with existing literature as well[1].

Study Limitations

The retrospective design, the small number of subjects, and the inability to evaluate some cases in time for developing tolerance due to the pandemic can be listed among the limitations of our study.

CONCLUSION

FPIES is an uncommon, non-IgE-mediated food allergy. It is important to have knowledge about its symptoms for accurate and early diagnosis. Thus, serious reactions can be prevented by eliminating the offending food. Despite the limited number of cases, our results are important in terms of giving us an idea about the trigger foods prevalent in the western regions of Turkey.

Ethics

Ethics Committee Approval: The study was approved by the İzmir Bakırçay University Non-Invasive Clinical Research Ethics Committee (decision number: 636, date: 22.06.2022).

Informed Consent: Retrospective study.

Peer-review: Internally peer reviewed.

Author Contributions

Surgical and Medical Practices: S.B., P.K.T., Concept: S.B., P.K.T., Design: S.B., P.K.T., Data Collection or Processing: S.B., P.K.T., M.A., Analysis or Interpretation: S.B., P.K.T., M.A., Literature Search: S.B., Writing: S.B.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.39129
J Dr Behcet Uz Child Hosp 2023;13(1):54-60

Original Article

The Association of Screen Time with Isometric Back and Leg Muscle Strength in School-aged Children

Okul Çağı Çocuklarda Ekran Süresinin İzometrik Sırt ve Bacak Kas Gücü ile İlişkisi

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ABSTRACT

Objective: Long recreational screen time affects many aspects of children's health. We aimed to assess the association of screen time with isometric back and leg muscle strength in school-aged children.

Method: Healthy children aged between 7-11 years were enrolled in this study. Gender of the children, their weight, and height at enrollment were recorded. A research assistant surveyed the parents of each child with face-to-face interview technique in order to analyse child's screen-viewing behavior. Children participating in the study were divided into 2 groups according to their average daily screen time (group 1: ≤ 2 hours, group 2: >2 hours). The children in Groups 1 and 2 were also divided into subgroups according to their body mass index (BMI) percentiles. The back and leg muscle strength were measured by using isometric back-leg strength dynamometer. The demographic characteristics, BMI Z-scores and the back and leg muscle strength of the children in groups were compared statistically.

Results: A total of 307 children including 103 boys (33.6%) and 204 girls (66.4%) were enrolled in the study. There were 204 (66.4%) and 103 (33.6%) children in groups 1 and 2, respectively. There was no statistically significant difference between the two groups in terms of back and leg muscle strength. The BMI Z-score of children positively correlated with their back and leg muscle strength, respectively.

Conclusion: This study could not find an association between screen time and both back and leg muscle strength of children. There is a need for further studies to analyse the effects of other confounding factors such as physical activity, sedentary behaviors, sleep duration and quality, sociodemographic factors and seasonal influence in associaton with screen time in muscle strength outcome measures in children.

Keywords: Child, dynamometer, muscle strength, screen time

ÖZ

Amaç: Sağlıklı okul çağındaki çocuklarda ekran süresinin izometrik sırt ve bacak kas gücü ile ilişkisini değerlendirmeyi amaçladık.

Yöntem: Çocukların ekran izleme sürelerini analiz etmek için bir araştırma görevlisi her çocuğun ebeveyniyle yüz yüze görüşme tekniğiyle anket yaptı. Araştırmaya katılan çocuklar ortalama günlük ekran sürelerine göre 2 gruba ayrıldı (grup 1: ≤2 saat/gün, grup 2: >2 saat/gün). Grup 1 ve grup 2'deki çocuklar da vücut kitle indeksi (VKİ) persentillerine göre alt gruplara ayrıldı. Sırt ve bacak kas kuvvetleri izometrik sırt-bacak kuvveti dinamometresi kullanılarak ölçüldü. Gruplardaki çocukların demografik özellikleri, VKİ Z-skorları ve izometrik sırt ve bacak kas güçleri istatistiksel olarak karşılaştırıldı.

Bulgular: Çalışmaya toplam 307 çocuk alındı. Grup 1 ve grup 2'de sırasıyla 204 (%66,4) ve 103 (%33,6) çocuk vardı. Sırasıyla izometrik sırt ve bacak kas kuvvetleri açısından iki grup arasında istatistiksel olarak anlamlı bir fark yoktu. Çocukların VKİ Z-skoru, sırasıyla izometrik sırt ve bacak kas güçleri ile pozitif korelasyon gösterdi.

Sonuç: Bu çalışmada sırasıyla çocukların ekran başında geçirilen süre ile izometrik sırt ve bacak kas güçleri arasında bir ilişki bulunamamıştır. Fiziksel aktivite, sedanter davranışlar, uyku süresi ve kalitesi, sosyodemografik faktörler ve ekran süresi ile ilişkili mevsimsel etki gibi diğer karıştırıcı faktörlerin çocuklarda kas gücü sonuçları üzerindeki etkilerini analiz etmek için daha ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Çocuk, dinamometre, kas gücü, ekran süresi

Received: 28.09.2022
Accepted: 12.12.2022

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Cite as: Orak SA, Çelebi Tayfur A, Zinnuroğlu I, Yücel A, Yılmaz D, Günbey S. The Association of Screen Time with Isometric Back and Leg Muscle Strength in School-aged Children. J Dr Behcet Uz Child Hosp. 2023;13(1):54-60

INTRODUCTION

Increased levels of physical activity and fitness during childhood and adolescence are associated with reduced risk of many diseases in adulthood such as cardiovascular disease, diabetes, selected cancers, musculoskeletal conditions and depression[1]. Physical exercise strongly modifies metabolic potential, morphology, and physiology of skeletal muscles. Regular physical activity leads to mitochondrial biogenesis, fast-to-slow fiber transformation, expansion of the muscle capillary bed, changes in substrate metabolism, an increase in the size of muscle fibers and muscle strength[2].

Long recretional time spent in front of TV and computer screen is a reason of failure to comply with recommendations on effective time spent with daily physical activities, thus muscle strength development[3]. The American Academy of Pediatrics (AAP) recommends limiting children's screen time in order to promote developmental, psychosocial and physical health[4]. There are few studies in the literature examining the effects of screen time on muscle strength in children[5,6]. In this study, the relationship between screen time and muscle strength in healthy primary school-aged children was evaluated.

MATERIALS and METHODS

Healthy children aged between 7-11 years were enrolled in this study. This study was approved by the Ankara Keçiören Training and Research Hospital Clinical Research Ethics Committee (decision no: 1284, date: 11.01.2017), and all participants and at least one parent gave his/her informed consent. Children who had malignant, infectious, inflammatory, endocrinological, neurological and/or muscular disease and/or growth retardation, and/or attended any sport program in the last 6 months were excluded. The sociodemographic characteristics of the children were recorded. A research assistant surveyed the parents of each child with face-to-face interview technique in order to analyse child's screen-viewing behavior. Children participating in the study were divided into 2 groups according to their average daily screen time (group 1: ≤2 hours, and group 2: >2 hours). The children in groups 1 and 2 were also divided into subgroups according to their body mass index (BMI) percentiles (A: normal, BMI=5p-<85p; B: overweight, BMI=85p-<95p and obese, BMI≥95p BMI=5p-<85p; B: overweight, BMI=85p-<95p and obese, BMI≥95p) [7]. BMI was calculated using the formula: body weight (kg)/ height^2 (m^2)[8]. The AAP recommends creation and implementation of a family media use plan. The 2-hour limit for daily screen time was determined according to AAP recommendations for children aged ≥6 years[4].

The measurements of muscular strength was carried out after each child rested for 30 minutes after feeding. The back and leg muscle (extensors of the knees and back) strengths of the children were measured using an isometric back-leg strength dynamometer (Baseline© back-leg-chest dynamometer Fabrication Enterprises, NY, USA). In order to measure back muscle strength, after a period of warm-up time the child pulled the dynamometer bar vertically upwards exerting his/her maximum force in the position of standing, knees tense, placing his/her feet on the dynamometer stand, arms tense, straight back and body slightly bent forward[9]. For leg muscle strength, after a period of warm-up time, the child pulled the dynamometer bar vertically upwards exerting his/her maximum force in the position of standing, knees twisted, placing his feet on the dynamometer stand, arms tense, straight back and body slightly bent forward[9]. After repeating this set of exercises for 3 times, the best values for back and leg muscle strength obtained were recorded. Back and leg muscle strength were measured in pounds, and pounds were converted to kilograms by multiplying the value by a coefficient of 0.45.

Statistical Analysis

In the study descriptive statistics for categorical variables were reported as numbers, percentages, and pie charts. Statistics for continuous variables were reported with mean, standard deviation, median, minimum and maximum values. Chi-square test was performed to compare categorical variables. The relationship between categorical and continuous variables was examined by box plot and line plot graphs. In accordance with the distribution assumptions, the differences between the groups were examined with the Mann-Whitney U test. The effect of one or more continuous variables on another continuous variable was examined with simple linear regression and multiple linear regression methods. The effect of a continuous variable on a categorical variable was analyzed using the Logistic Regression method. IBM SPSS Statistics 22 was used for statistical analysis. A value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 307 children including 103 boys (33.6%) and 204 girls (66.4%), (age range: 6 yr + 8 mos - 8 yr + 8 mos)

were included in the study. The mean ages of the male and female children enrolled in the study were 9 yr + 6 mos and 1 yr + 5 mos, respectively. According to CDC 2000 growth reference chart[7] respective number of patients were underweight (n=11; 3.58%), healthy weight (n=179; 58.3%), overweight (n=52; 16.9%) and obese (n=65; 21.1%). Daily screen time was more than 2 hours in group 1 (n=204; 66.4%) and ≤2 hours in group 2 (n=103; 33.6%) (Table 1). There were 28 (27.1%) boys and 75 (72.8%) girls in group 1 and 75 (36.7%) boys and 129 (63.2%) girls in group 2. The mean ages in groups 1, and 2 were 9 yr + 7 mos, and 9 yr +6 mos, respectively. Eight (2.6%) children did not spend time in front of the screen, while average daily screen time was 1-2 hours/day in 142 (46.3%), 3-4 hours in 125 (40.7%), 5-6 hours in 28 (9.1%) and >6 hours/in 4 (1.3%) children, in weekdays. On weekends, 3 (1%) children did not spend time in front of the screen, while average daily screen time was 1-2 hours in 117 (37.1%), 3-4 hours in 144 (46.9%), 5-6 hours in 39 (12.7%) and >6 hours in 4 (1.3%) children.

The average daily screen time of boys (3.1±1.4 hours; range: 0.7-7 hours) and girls (2.7±1.2 hours; range: 0-7 hours) were recorded. A statistically significant difference was found between boys and girls in terms of daily screen time (p=0.018).

Back muscle strength (n=307), both back, and leg muscle strength (n=114) were measured in indicated number of children.

The mean back muscle strength of 307 children was 33.29±13.23 kg (range: 6.75-72 kg). It was 35.82 kg ±14.99 kg (range: 6.75-72 kg) in group 1 (n=103) and 34.1 kg ±13.78 kg (range: 9-72 kg) in group 2 (n=204). There was no statistically significant difference between groups in terms of back muscle strength (p=0.273) (Table 1).

The mean back muscle strength was 31.02±11.41 kg (range: 9-54 kg) in girls and 37.81±15.33 kg (range: 6.75-72 kg) in boys. A statistically significant difference was found between girls and boys in terms of back muscle strength (p<0.001) (Table 2).

The mean leg muscle strength of 114 children was 34.91 kg ±14.84 kg (range: 9-72 kg). It was 34.84 kg ±14.64 kg (range: 9-72 kg) in group 1 (n=47) and 34.26 kg ±15.06 kg (range: 9-72 kg) in group 2 (n=67). There was no statistically significant difference between groups in terms of leg muscle strength (p=0.577) (Table 1).

The mean leg muscle strength of girls (n=76) and boys (n=38) were 31.13±12.33 kg (range: 9-72) and 42.48±16.6 kg (range: 9-72), respectively. A statistically significant difference was found between girls and boys in terms of leg muscle strength (p<0.001) (Table 2).

Statistically significant positive correlations were found between the back and leg muscle strength of children, their ages and BMI Z-scores (group 1-age: p<0.001, r=0.56; group 2-age: p<0.001 r=0.52 and group 1-BMI: p<0.001 r=0.41; Group 2-BMI: p<0.001, r=0.46) (Graphic 1, 2). There was no correlation between children's back and leg muscle strength and maternal age, paternal age, maternal educational level, paternal educational

Table 1. Back and leg muscle strength of children grouped according to their average daily screen time (group 1: ≤2 hours, group 2: >2 hours)

Groups	N	Mean back muscle strength (kg)	Range (kg)	p-value
Group 1	103	35.82 kg ±14.99	6.75-72	0.273
Group 2	204	34.1 kg ±13.78	9-72	
		Mean leg muscle strength (kg)	**Range (kg)**	**p-value**
Group 1	47	34.84 kg ±14.64	9-72	0.577
Group 2	67	34.26 kg ±15.06	9-72	

N: number

Table 2. Back muscle strength and leg muscle strength of girls and boys

Gender	N	Mean back muscle strength (kg)	Range (kg)	p-value
Girls	204	31.02±11.41	9-54	<0.001
Boys	103	37.81±15.33	6.75-72	
		Mean leg muscle strength (kg)	**Range (kg)**	**p-value**
Girls	76	31.13±12.33	9-72	<0.001
Boys	38	42.48±16.64	9-72	

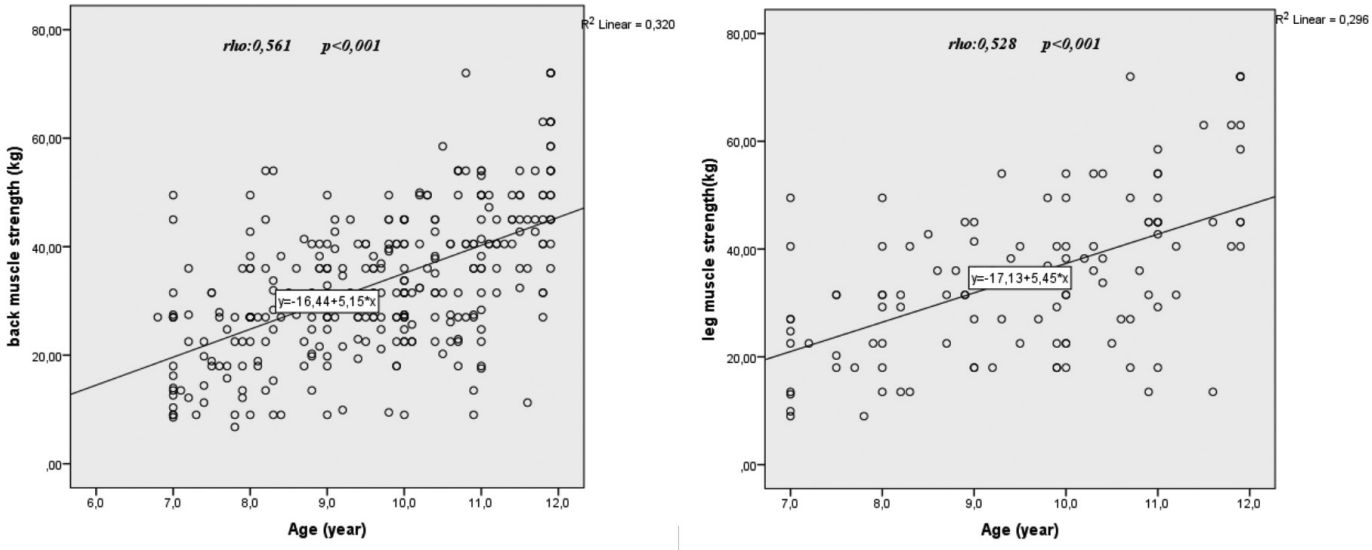
N: number

level, monthly income, maternal employment status and presence of a sibling. One year increase in age caused an increase of 4,539 kg in back, and 9,426 kg in leg muscle strength. Also, one unit increase in BMI caused an increase of 0.829 kg in back and of 3,205 kg in leg muscle strength.

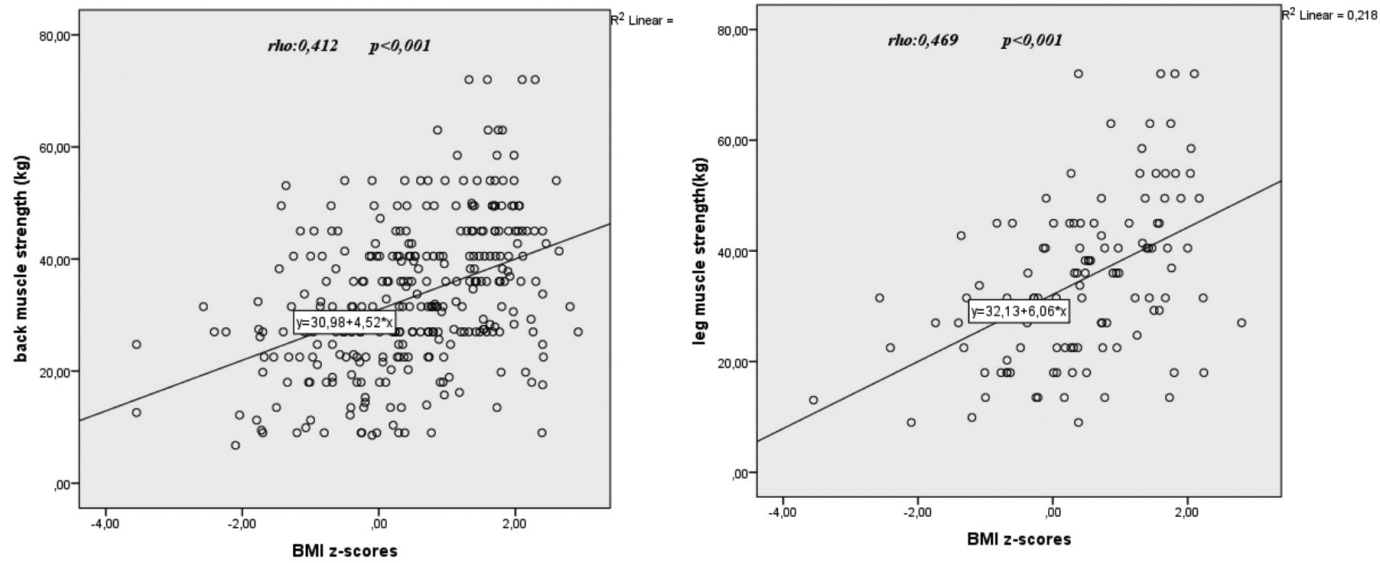
When back and leg muscle strengths were adjusted according to BMI Z-scores, neither back nor leg muscle strength significantly differed among patients in groups 1 and 2.

DISCUSSION

Adequate muscle mass and muscle strength are considered as reliable measures of overall health[10]. Development of muscular strength in children depends on age, height, gender, sexual maturation, genetic background, neuromuscular integrity, physical activity level, BMI and limb dominance[11]. Long recreational time spent in front of TV and computer screens often while sitting or lying down limit the time spent for daily physical activity and may lead a decrease in muscle



Graphic 1. Correlation between age and back and leg muscle strength of children



Graphic 2. Correlation between BMI Z-scores and back and leg muscle strength of children

BMI: Body mass index

mass, isometric muscle strength and bone density[12]. However, there are few studies investigating the effect of screen time on muscle strength of children and adolescents[6,12,13]. Ours is one of few studies analysing association between school-age children's muscle strength and screen time.

The AAP recommends imposing restrictions on types of media platforms used, and hours of media used per day for children aged 6 years or older to ensure that screen time does not displace sleeping, playing, conversation and physical activities[4]. Studies have shown that children and adolescents are more likely to spend more time in sedentary activities such as watching television and playing computer games[14]. Many studies found a significant negative association between screen time and physical activity in children and adolescents[15-17]. In a cross-sectional study including 606 adolescents, screen time was found to be inversely associated with isometric trunk muscle strength independent of lifestyle, sociodemographic factors, cardiorespiratory fitness and waist circumference[18]. In our study, no statistically significant difference was found between children spending >2 or ≤2 hours a day in front of the screen in terms of back and leg muscle strength. This finding might be due to engagement of children in physical activity during screen was on, that was not reported by the parents during face-to-face interviews or on the contrary due to lack of physical activity during the daytime among most of children enrolled in the study. Its effect on muscle strength might have been revealed when more children with excessive screen time had been involved in the study, but limited number of children with a daily screen time of >6 hours were enrolled in this study. The average daily screen time of boys was significantly longer than girls. This finding is consistent with previous studies reporting that boys are spending substantially more time in front of TVs, and computer monitors than girls both during the weekdays and at the weekend[19].

Strength of the back and leg muscles of girls was significantly lower than boys. Nevertheless, when strength of the back, and leg muscles were adjusted for BMI-Z-score, the gender difference in muscle strength was not statistically significant. Muscle strength appears to increase in both boys and girls until about the age of 14 years where it begins to plateau in girls and a spurt in muscular strength is evident in boys[20]. Hormonal differences during puberty are responsible for a gradual increase in the strenght development of boys which is maintained at approximately the same rate in the strength development of girls seen during their preadolescent years[21]. The lack of difference between girls and boys in terms of muscle strength can be explained by the fact that the majority of children included in the study were in the prepubertal stage, where the muscle strength of girls and boys is essentially equal.

Muscle size and muscle strength increase throughout preadolescence and adolescence due to changes in muscle mass and muscle fiber size. Gender, individual body size, growth, maturity and motor competence and level of physical activity effect muscular strength[22,23]. As expected, back, and leg muscle strength positively correlated with age ($p<0.001$, rho:0.561 and $p<0.001$ rho:0.528, respectively) in children enrolled in this study and in fact, gender effect on back and leg muscle strength was not remarkable in children with normal BMI.

The relationship between media exposure and obesity has been widely studied[24,25]. A meta-analysis reviewing the results from 16 studies examining the relationship between screen time and overweight/obesity in children has shown that daily screen time of ≥2 hours was likely to be associated with greater risk of overweight/obesity than daily screen time of <2 hours[25]. The possible mechanisms to explain the effects of screen media exposure on obesity include displacing physical activity, increasing energy intake and reducing sleep[24]. The previous studies have shown that children with high BMI values have greater absolute measures of grip strength and leg extension power, but have lower core and upper body strength when compared to children with lower BMI[26]. Obese individuals have reduced maximum muscle strength relative to body mass in their anti-gravity muscles compared to non-obese persons. High levels of adiposity may impair agonist muscle activation in the adolescents[27]. In our study, a positive correlation was found between BMI values and both back, and leg muscle strength. Nevertheless, in our study, when back and leg muscle strength were adjusted according to BMI Z-scores, longer screen time apparently had not any significant negative effect on muscle strength.

Study Limitations

The relatively scarce number of children enrolled in this study may be considered as one limitation of our study. Besides, the information on screen time, screen-based sedentary behaviors and daily physical activity were gathered from the self-reports of the study participants which could lead a bias in interpretation of

the results. Furthermore we did not query school-related screen (video game/computer/TV) use which could likely underestimate the total amount of screen time of children. In fact, we did not analyse jump performance signifying the action of passive and active components of lower limb muscles and the motor performance tasks like throwing which are used as indicators of specific aspects of muscular strength in children[28].

CONCLUSION

This study could not find an association between screen time and back and leg muscle strength measurements of children, respectively. We suggest that only the screen time per se may not have substantial effect on muscle strength outcomes. There is a need for further studies to analyse the effects of other confounding factors such as physical activity, sedentary behaviors, sleep duration and quality, sociodemographic factors and seasonal influence in association with screen time on outcome measures of muscle strength.

Acknowledgement: This article was created from the master's thesis titled "Evaluation of the effect of screen time on muscle strength in school age children" completed by the first author.

Ethics

Ethics Committee Approval: This study was approved by the Ankara Keçiören Training and Research Hospital Clinical Research Ethics Committee (decision no: 1284, date: 11.01.2017).

Informed Consent: All participants and at least one parent gave his/her informed consent.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Concept: S.A.O., A.Ç.T., D.Y., Design: S.A.O., I.Z., Data Collection or Processing: S.A.O., Analysis or Interpretation: S.A.O., A.Ç.T., A.Y., D.Y., S.G., Literature Search: S.A.O., A.Ç.T., Writing: S.A.O., A.Ç.T.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.75688
J Dr Behcet Uz Child Hosp 2023;13(1):61-69

Original Article

The Most Common Causes of Morbidity and Mortality in Late Preterm Infants: 8-year Single-center Experience

Geç Prematüre İnfantlarda En Yaygın Morbidite ve Mortalite Nedenleri: 8 Yıllık Tek Merkez Deneyimi

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ABSTRACT

Objective: Late preterm infants are immature regarding respiratory, metabolic, neurological, and immunological features and have a high risk for morbidity and mortality. Therefore, it is aimed to draw attention to the problems that may develop in newborn care by scanning all hospitalization and mortality rates of late preterm infants.

Method: In this retrospective study, late preterm infants hospitalized in a tertiary university hospital between January 1, 2007, and December 31, 2014, having the study admission criteria, were enrolled.

Results: A total of 1,088 late preterm infants were included in the eight-year study period. According to their gestational weeks, the infants were divided into three main groups; 31.4% (n=342) were in group 1 (340/7-346/7), 30.6% (n=333) were in group 2 (350/7-356/7), 38% (n=413) were in group 3 (360/7-366/7). The most common causes for admission to the neonatal intensive care unit were hyperbilirubinemia, suspected sepsis and infections, respiratory morbidities, poor feeding, and hypoglycemia; reasons for rehospitalization included jaundice, infections, suspicion of sepsis, and poor feeding. In addition, neonatal transient tachypnea, apnea, hypoglycemia, early-onset sepsis, healthcare-associated infection were most common in those born at 34-346/7 gestational weeks; respiratory distress syndrome, pneumonia, late-onset sepsis were most frequently in those born at 35-356/7 gestational weeks.

Conclusion: As a result, late preterm infants are at risk for respiratory disorders, sepsis, jaundice, and metabolic problems; the need for intervention increases as gestational age decreases. The delivery timing should be planned with these problems in mind, and the follow-up and treatment of late preterm infants should be carried out accordingly.

Keywords: Preterm birth, gestational age, late premature, morbidity, mortality

ÖZ

Amaç: Geç preterm bebekler solunum, metabolik, nörolojik ve immünolojik açıdan olgunlaşmamış olup morbidite ve mortalite açısından yüksek risk taşırlar.

Yöntem: Bu retrospektif çalışmaya, üçüncü basamak bir çocuk hastanesi yenidoğan yoğunbakım ünitesinde, 1 Ocak 2007 ile 31 Aralık 2014 tarihleri arasında yatırılan ve çalışma kriterlerine uygun geç prematüre bebekler dahil edildi.

Bulgular: Sekiz yıllık süreyi kapsayan çalışmaya toplam 1,088 geç prematüre bebek dahil edildi. Bebekler gebelik haftalarına göre üç ana gruba ayrıldı. Grup 1, 342 (%31,4) geç pretermden oluşup 34-346/7gebelik haftasında doğan, grup 2, 333 (%30,6) geç pretermden oluşup 35-356/7 gebelik haftasında doğan, ve grup 3, 413 (%38) geç pretermden oluşup 36-366/7 gebelik haftasında doğanlar idi. Yenidoğan yoğun bakım ünitesine en sık başvuru nedenleri hiperbilirubinemi, şüpheli sepsis ve enfeksiyonlar, solunumsal morbiditeler, yetersiz beslenme ve hipoglisemi olup; yeniden hastaneye yatış nedenleri arasında sarılık, enfeksiyonlar, şüpheli sepsis ve kötü beslenme vardı. Yenidoğan geçici takipnesi, apne, hipoglisemi, erken başlangıçlı sepsis, sağlık hizmeti ilişkili enfeksiyon 34-346/7 gebelik haftasında doğanlarda yaygın olup; respiratuvar distress sendromu, pnömoni, geç başlangıçlı sepsis en sık 35-356/7 gebelik haftasında doğanlarda idi.

Sonuç: Sonuç olarak geç prematüreler solunumsal rahatsızlıklar, sepsis, sarılık ve metabolik problemler açısından risk altında olup; gebelik yaşı azaldıkça müdahale ihtiyacı artar. Doğum zamanı bu sorunlar göz önünde bulundurularak planlanmalı ve geç prematüre bebeklerin takip ve tedavisi buna göre yapılmalıdır.

Anahtar kelimeler: Erken doğum, gebelik haftası, geç prematürite, morbidite, mortalite

Received: 02.09.2022
Accepted: 21.12.2022

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Cite as: Cem E, Örs R. The Most Common Causes of Morbidity and Mortality in Late Preterm Infants: 8-year Single-center Experience. J Dr Behcet Uz Child Hosp. 2023;13(1):61-69

INTRODUCTION

Late preterm infants have attracted attention for the last 20 years since they comprise approximately 80% of all births and have a higher risk of short- and long-term morbidity and mortality than term infants[1]. Late preterm birth rates in the United States increased from 6.82% to 7.09% between 2014 and 2016, accounting for approximately 72% of all preterm births[2].

Preterm labor, premature rupture of membranes, the increasing occurrence of multiple births due to assisted reproductive technologies, advanced maternal age, maternal obesity, and maternal and physician concern about complications such as hypoxic-ischemic encephalopathy and birth traumas in advanced gestational weeks are all possible contributors to the rising incidence of late preterm birth[3-5].

Although late preterm infants have a better prognosis and survival rate than premature infants, they are more likely to develop respiratory complications, infections, feeding issues, hyperbilirubinemia, hypothermia, and hypoglycemia[6]. In addition, the duration of hospitalization and readmission rates after discharge are two to three times higher in late preterm infants than in term infants, resulting in increased healthcare costs and, ultimately, a public health problem[7].

The aim of this retrospective study, which was planned considering these problems of late premature, was to evaluate the clinical and laboratory findings, the leading indications of hospitalization, the cause and rate of mortality in all infants at $34^{0/7}$-$36^{6/7}$ gestational weeks in our neonatal intensive care unit (NICU). In addition, it was planned to compare our results with national and international studies, explain the reasons for their differences, and emphasize all the problems that may develop in late preterm babies to attract the attention of pediatricians and obstetricians.

MATERIALS and METHODS

This study was conducted in a tertiary university hospital. The study included 1,088 late preterm infants born between $34^{0/7}$ and $36^{6/7}$ weeks admitted to the NICU between January 1, 2007, and December 31, 2014.

The patients were divided into three main groups: infants born at $34^{0/7}$-$34^{6/7}$ were classified as group 1, infants born at $35^{0/7}$-$35^{6/7}$ were classified as group 2, and infants born at $36^{0/7}$-$36^{6/7}$ were classified as group 3.

Definitions

Late premature: According to World Health Organization, the American Academy of Pediatrics (AAP), and the American College of Obstetrics and Gynecology (ACOG), infants born between $34^{0/6}$ weeks of gestation and $36^{6/7}$ weeks from the mother's last menstrual cycle were considered late preterm [8-10].

Respiratory disorders: Tachypnea (respiratory rate >60/min), suprasternal-intercostal-subcostal retractions, groaning, cyanosis, apnea, or oxygen requirement were all considered signs of postpartum respiratory distress. Respiratory distress syndrome (RDS) was defined as cyanosis, tachypnea, intercostal retractions, persistent or progressive groaning for 48-96 hours of life, and diffuse reticulonodular appearance and air bronchogram on chest X-ray[11]. Pneumonia was defined as respiratory distress with cough, fever, malnutrition, pathological clinical and respiratory findings, evidence of infiltration-consolidation, reticulonodular appearance with air bronchograms, and pleural fluid seen in X-ray[12]. Transient tachypnea (TTN) of the newborn was defined as pulmonary vascular prominence, increased lung aeration, and fluid in the fissures on the chest X-ray accompanied by tachypnea and moderate respiratory distress that started shortly after birth and generally resolved within 3-5 days[13]. Apnea was defined as a 20-second or more prolonged decrease in oxygen saturation and respiratory arrests accompanied by bradycardia [14].

Hypoglycemia: According to AAP guidelines, the lower limit for low blood sugar in an asymptomatic late preterm infant was accepted as 25 mg/dL in the first four hours of life and 35 mg/dL between 4-24 hours. The lower limit of blood glucose was accepted after 24 hours as 47 mg/dL[15].

Hypocalcemia: Serum calcium value <7 mg/dL was accepted as hypocalcemia[16].

Hyperbilirubinemia: According to the Bhutani nomogram, late preterm infants who required phototherapy were included [17].

Neonatal sepsis: The presence of at least three of the following clinical findings, such as bradycardia or tachycardia, hypotension, hypotonia, seizure, apnea, tachypnea, cyanosis, respiratory distress, deterioration of skin color and perfusion, malnutrition, irritability, lethargy, and the presence of positive blood or other cultures, was defined as neonatal sepsis. Sepsis detected in the first three days of life was considered early neonatal

sepsis and noticed after the 4th day of life was considered late-onset neonatal sepsis. Nosocomial sepsis was defined as a late preterm infant with signs of infection 48-72 hours after hospitalization or rehospitalization within the previous ten days [18].

Infants referred to another center after birth whose file information was lacking from the study were excluded.

Patients' data were collected from medical records, including prenatal problems, mothers' age, type of delivery, gestational week, birth weight, gender, indications of hospitalization, all physical examination-clinical-laboratory-radiological findings, causes, and rates and reasons for rehospitalization and mortality.

Statistical Analysis

The late preterm infants were divided into three groups based on their gestational weeks, and the statistical analyses were performed on these groups' axis. The Kruskal-Wallis scale was used to compare numerical values according to gestational week groups. Statistical analysis was performed using SPSS Statistical Software (version 20; SPSS, Chicago, IL, USA). Categorical variables were presented with frequency and percentage, while numerical variables were presented using tables as (mean ± SD) or (median) range (minimum, maximum). The Spearman's Rho correlation test was used to determine the correlations between numerical variables, and p values corresponding to the correlation values were obtained. A multivariate logistic regression model was designed to investigate the effect of the variables on the gestational week. In addition, a chi-square test and post-hoc analysis were used to investigate the association level among variables, with significance set to $p<0.05$.

This study was approved by the Necmettin Erbakan University, Meram Faculty of Medicine Ethics Committee for Non-pharmaceutical and Medical Device Research (decision no: 2015-282, date: 16.09.2015).

RESULT

Comparison of Demographic Data and Clinical Features of Three Late Preterm Groups

During these eight years, 1,088 late preterm infants hospitalized in the NICU were included in the study. According to their gestational weeks, the infants were divided into three main groups; 31.4% (n=342) were in group 1 ($34^{0/7}$-$34^{6/7}$), 30.6% (n=333) were in group 2 ($35^{0/7}$-$35^{6/7}$), 38% (n=413) were in group 3 ($36^{0/7}$-$36^{6/7}$). Of the 1,088 infants, 53.7% (n=584) were male, 45.9% (n=499) were female, and the remaining 5 (0.4%) infants had ambiguous genitalia. The male gender was more dominant at all gestational weeks (%55 in group 1; %50.7 in group 2, %55 in group 3). Among all late premature, the rate of cesarean section (C/S) was as high as 85.9% (n=934). When each gestational week was evaluated separately, C/S deliveries were statistically higher than spontaneous vaginal deliveries (SVD) (p=0.001).

While 84.6% (n=921) of all pregnancies were spontaneous, 15.4% (n=167) were accomplished via assisted reproductive techniques (ART). In addition, the majority of ART pregnancies were born at $34^{0/7}$-$34^{6/7}$ gestational weeks, whereas spontaneous pregnancies were born at $36^{0/7}$-$36^{6/7}$ gestational weeks. The distinction was statistically significant (p=0.003).

The multiple pregnancy rate among all groups was 20.6% (n=224). Of these, 17% (n=184) were twin pregnancies, and 3.6% (n=40) were triplet pregnancies. While the majority of triplet pregnancies were born at $34^{0/7}$-$34^{6/7}$ gestational weeks, there were no triplet births at $36^{0/7}$-$36^{6/7}$ gestational weeks. Regarding multiple pregnancies, the difference between gestational week groups was significant (p=0.012).

Post-hoc analysis revealed a statistically significant difference between groups 1 and group 3 regarding C/S delivery, while a statistically significant difference between group 1- group 3 and group 2- group 3 for ART and multiple pregnancies. The epidemiological characteristics of all three groups are summarized in Table 1.

Comparison of Three Late Preterm Groups' Reasons for Admission to the NICU

The most common reasons for admission to the NICU for all late preterm infants were jaundice, suspected or proven sepsis or another infectious disease, respiratory disorders, malnutrition, and hypoglycemia (detailed in Table 2).

Among the respiratory diseases, TTN and apnea were more common in group 1 than in those group 2 and group 3 (8.4%, n=29; 5%, n=17, respectively), while RDS and pneumonia were more common in group 2 than in those group 1 and group 3 (6.3%, 21; 12.6%, n=42, respectively). There was a statistically significant difference between the groups regarding apnea and RDS (p=0.032, p=0.009, respectively).

Table 1. The comparison of demographic data and clinical features of three groups				
	Group 1 ($34^{0/7}$-$34^{6/7}$)	**Group 2 ($35^{0/7}$-$35^{6/7}$)**	**Group 3 ($36^{0/7}$-$36^{6/7}$)**	**p-value**
Demographic data				
Number, n(%)	342 (31.4%)	333 (30.6%)	413 (38%)	
Gender, n (%)				
Male	188 (55%)	169 (50.7%)	227 (55%)	
Female	154 (45%)	164 (49.3%)	186 (45%)	
Type of delivery				
C/S ratio, n(%)	308 (90%)	289 (86.8%)	338 (81,8%)	0.001[b]
SVD ratio, n(%)	34 (10%)	44 (13.2%)	75 (18.2%)	
The mean duration of hospitalization days (mean ± SD)	11.49±13.6	10.39±15.2	9.21±14.3	>0.05
Method for achieving pregnancy, n(%)				
Spontaneous	278 (81,3%)	276 (82,9%)	367 (88.9%)	0.003[b,c]
ART	64 (18.7%)	57 (17.1%)	46 (11.1%)	
Multiple pregnancy ratio, n (%)	78 (23%)	72 (21.6%)	72 (17.6%)	0.012[b,c]

n: Number, C/S: Cesarean section, SVD: Spontaneous vaginal delivery, SD: Standard deviation, ART: Assisted reproductive techniques

[b]statistically significant difference between group 1-3 (p=0.001 for C/S; p=0.002 for ART; p<0.000 for multiple pregnancy)

[c]statistically significant difference between group 2-3 (p=0.01 for ART; p=0.002 for multiple pregnancy

Table 2. Reasons for NICU admission in late preterm infants	
Indications of NICU admission	**n (%)**
Jaundice	578 (53.1%)
Suspected sepsis	573 (52.6%)
Hypoglycemia	319 (29.3%)
Pneumonia	116 (10.7%)
RDS	42 (3.9%)
TTN	84 (7.7%)
Apnea	34 (3.1%)
Hypocalcemia	344 (31.6%)

NICU: Neonatal intensive care unit, RDS: Respiratory distress syndrome, TTN: Transient tachypnea of the newborn

In total, 53.4% (n=581) of infants had sepsis. Early-onset sepsis was observed in 69.9% (n=406) of the cases, nosocomial sepsis was observed in 23.2% (n=135), and late-onset sepsis was observed in 6.9% (n=40) cases. The risk of early-onset and nosocomial sepsis was inversely proportional to gestational week. Late-onset neonatal sepsis was detected in group 2, with the highest percentage. However, a statistically significant difference was found in early-onset sepsis between gestational weeks (p<0.01). Blood cultures were positive in 18.2% (n=106) of late preterm infants with sepsis, and other (urine- bronchoalveolar lavage- cerebrospinal fluid-wound) cultures were positive in 7.7% (n=45). Antibiotics were administered to 81.7% (n=475) of the infants. The most common agents detected in patients with early-onset sepsis were coagulase-negative *Staphylococcus* CoNS (n=24), *Klebsiella pneumonia* (n=10), and *Staphylococcus aureus* (n=5). The most common agents noticed in patients with late-onset sepsis were CoNS (n=34), S*taphylococcus aureus* (n=10), and *Klebsiella pneumonia* (n=5). Furthermore, *Staphylococcus*, *Klebsiella*, and *Pseudomonas* species were the most common pathogens of nosocomial infections.

Hypoglycemia and hypocalcemia were seen at the highest percentage in group 1 (35.7%, n=122; 35.4%, n=121, respectively). Hypocalcemia was observed, particularly on the second or third postnatal days, with no systemic findings. There was a statistically significant difference between the weeks of gestation for both conditions (p<0.001, p=0.003, respectively).

Jaundice requiring phototherapy was observed in 53.1% (n=578) of all infants. The highest rate of jaundice was observed in group 1 and was statistically significant (p=0.043). Jaundice was reported in the first seven days of life, particularly in the 2-5. days. Twenty-one babies required exchange transfusions.

There was a statistically significant difference between groups 1-3 in terms of RDS, apnea, hypoglycemia, hypocalcemia, early-onset sepsis, and jaundice in the post-hoc analysis. In addition, while there was a statistically significant difference in hyperbilirubinemia

between groups 1 and 2, there was a significant difference between groups 2 and 3 in RDS and hypocalcemia.

Sixty-nine (6.3%) late preterm infants were readmitted to the hospital after discharge. Although there was no statistically significant difference in gestational weeks, group 1 had the highest readmission rate (p>0.05). Jaundice and infectious disease were the leading causes of rehospitalization.

In total, seventy-seven (7.07%) late preterm infants died. Group 2 had the highest mortality percentage for the 34th gestational week; however, there was no statistically significant difference among the three groups. The most significant reasons for mortality were congenital anomalies and sepsis (respectively %44.1, n=34; %22, n=17). Other causes of death included hydrops fetalis, asphyxia, and complete atrioventricular block (AV block). The reasons and rates of hospitalization according to gestational weeks are summarized in Table 3.

DISCUSSION

Extensive clinical studies evaluating the differences among late preterm infants according to their gestational week were limited. Therefore, in this descriptive study, we compared 1,088 late preterm infants. Certain differences were found in these study as; while TTN, apnea, hypoglycemia, early-onset sepsis, and nosocomial sepsis were most common in those born at 34-34$^{6/7}$ weeks of gestation, RDS, pneumonia, and late-onset sepsis were most common in those born at 35-35$^{6/7}$ gestational weeks. Compared to other groups, those born at 36 weeks of gestation had lower rates of these morbidities.

Late preterm infants constituted 21.6% of all infants needing NICU and 51.8% of the premature group in our 8-year retrospective study. While the rate of late preterm birth was 9.6% in a recent 3-year survey[19], this rate was 21.6% in our study. It is thought that the rates vary according to the region where the high-risk pregnancy centers are located, as well as the differences in patients.

As stated in most studies in the literature, it was observed that C/S proportions were high in our research, and C/S percentages increased as the gestational week decreased[19-21]. In addition, studies have stated that increased multiple pregnancy rates with ART contribute to preterm birth[22,23]. Refuerzo et al.[24] observed that the mean week of birth was 35.3 weeks in twins and 32.2 weeks in triplets, and the rate of late prematurity in twins was 50%. Similar to the reports, multiple births were observed to occur at earlier gestational weeks in our study. In addition, triplet pregnancy was at the highest rate at 34th gestational weeks, supporting that

Table 3. Comparison of the reasons for admission to NICU between the three groups

Indications of NICU admission	Group 1 n=342 (34$^{0/7}$-34$^{6/7}$)	Group 2 n=333 (35$^{0/7}$-35$^{6/7}$)	Group 3 n=413 (36$^{0/7}$-36$^{6/7}$)	p-value
RDS	16 (4.7%)	21 (6.3%)	5 (1.2%)	**0.009**[b,c]
TTN	29 (8.4%)	24 (7.2%)	31 (7.5%)	>0.05
Apnea	17 (5%)	8 (2.4%)	9 (2.2%)	**0.032**[b]
Pneumonia	34 (10%)	42 (12.6%)	40 (9.6%)	>0.05
Hypoglycemia	122 (35.7%)	97 (29.1%)	100 (24.1%)	**<0.001**[b]
Hypocalcemia	121 (35.4%)	115 (34.5%)	108 (26.1%)	**0.003**[b,c]
Early-onset sepsis	140 (41%)	131 (39.3%)	135 (32.7%)	**0.012**[b]
Late-onset sepsis	8 (2.3%)	16 (4.8%)	16 (3.9%)	>0.05
Nosocomial sepsis	49 (14.3%)	39 (11.7%)	47 (11.4%)	>0.05
Jaundice	203 (59.4%)	159 (47.8%)	216 (52.3)	**0.043**[a,b]
Rehospitalization	26 (7.6%)	19 (5.7%)	24 (5.8%)	>0.05
Mortality	24 (7.01%)	24 (7.2%)	29 (7.02)	>0.05

NICU: Neonatal intensive care unit, RDS: Respiratory distress syndrome, TTN: Transient tachypnea of the newborn

[a]statistically significant difference between group 1-2 (p=0.003)

[b]statistically significant difference between group 1-3 (p=0.004 for RDS; p=0.036 for apnea; p<0.000 for hypoglycemia; p=0.004 for hypocalcemia; p=0.02 for early-onset sepsis; p=0.031 for jaundice)

[c]statistically significant difference between group 2-3 (p<0.000 for RDS; p=0.005 for hypocalcemia)

the number of fetuses was significantly associated with prematurity.

Late preterm babies are more prone to complications such as heat instability, hypoglycemia, respiratory distress, apnea, jaundice, and feeding difficulties than term babies[25]. According to a study by Hakan et al.[19], the most common problems of late preterm infants were respiratory distress (49%) and indirect hyperbilirubinemia (21%). In our study, however, jaundice and sepsis were the leading causes of hospitalization, followed by respiratory diseases.

Late preterm babies are more likely than term babies to develop apnea, TTN, RDS, pneumonia, pulmonary hypertension, and respiratory failure due to being born with lung and ventilation center immaturity[26,27]. In our study, pneumonia, TTN, and RDS were the most common causes of respiratory morbidity, as reported in the literature. The occurrence and severity of respiratory distress decreased as gestational age increased, with the highest at 34 weeks and the lowest at 39 weeks[5].

In the literature, antenatal corticosteroid administration decreased the incidence of RDS in extremely premature infants, but the same result was not observed in late preterm infants[28]. However, the rate of RDS was lower in our study than in the literature; 50% of these infants were born at the 35th gestational age. This was attributed to infants born at the 35th gestational week receiving fewer antenatal corticosteroids than infants born at the 34th gestational week. Although the rates of RDS were significantly lower in those born at 36 weeks of gestation compared to 34 and 35 weeks of gestation, the findings indicated that prophylaxis in late preterm infants remains necessary.

TTN and other respiratory problems are caused by prematurity and performing a cesarean delivery before labor begins. TTN rates ranged between 3.9% and 9.9%, decreasing as the gestational week increased[5]. TTN rates were reported to be low in our report, which was attributed to the late prematurity of all infants included in the study, as well as the fact that respiratory distress persisted for more than 24 hours in the majority of infants, necessitating the early initiation of empirical antibiotic therapy with the diagnoses of suspected sepsis/pneumonia. In addition, TTN was highest in infants born at 34 weeks of gestation and lowest in infants born at 35 weeks, contrary to the literature. The possible reason was that infants born at the 35th gestational week had more severe respiratory diseases than TTN.

In our research, pneumonia rates were reported to be greater than the general literature and comparable to data from our country[19,29-31]. These rates were related to the monitoring of complicated infants. Apnea is caused by an immature neurological system, physiological immaturity of the respiratory system, and a lack of coordination between feeding, swallowing, and breathing[29]. According to a meta-analysis, the incidence of apnea in late preterm infants was 0.9% and 0.05% in term infants and decreased as gestational age increased[32]. In this study, ten percent of late preterm infants have significant prematurity apnea. Half of them were born at 34 weeks, statistically higher than those at 36 weeks. This rate was consistent with the literature and was attributed to the apparent physiological immaturity as the gestational week declines.

The risk of hypoglycemia was increased in late preterms because of insufficient glycogen stores, particularly in challenging conditions such as hypothermia, sepsis, and feeding difficulties. The incidence of hypoglycemia is known to enhance inversely with gestational age[33]. The incidence of hypoglycemia in late preterm infants is approximately three times higher than in term infants, according to studies similar to ours.[34-36]. In our study, the rate of hypoglycemia was significantly higher at the 34th gestational week, particularly compared with the 36th gestational week; as the gestational week increased, the rate of hypoglycemia decreased. These findings were generally consistent with previous research.

Similar to Picone et al.[37], hypocalcemia was observed mainly on the second or third postnatal days, with no systemic findings, in our study. Furthermore, hypocalcemia decreased as the gestational week increased, with the lowest incidence in those born at the 36th gestational week. This situation was similar to the literature and was attributed to the insufficiency of parathormone secretion due to incomplete parathyroid development.

Likewise to our findings, the rates of jaundice in late preterm infants were significantly higher in the literature [5,38]. Similar to the literature, jaundice was most common between the second and fifth days of life. Those born at the 34th gestational week had the highest rate, compared with the 35th and 36th gestational weeks. However, contrary to popular opinions in this study, those born at the 35th gestational week had the lowest percentage of jaundice.

Early-onset neonatal and nosocomial sepsis are frequent in late preterm infants and are significant

causes of morbidity and mortality. The probability of culture positivity increases as gestational age decreases. The rate of clinical sepsis was 10% in the study by Hakan et al.[19], and a quarter of patients had positivity in the blood culture. Our study's blood culture positivity rate was 18.2%, higher than the literature. The rate of sepsis increased 33 times in the premature birth group in McIntire and Leveno's[39] studies, where sepsis screening was performed at 34 and 39 weeks of gestation. In our study, the incidence of sepsis was reported to be high in late preterm infants, which was consistent with the literature. Our study's high rates of sepsis were associated with being a developing country, the patients' poor financial and living conditions, intensive follow-up of high-risk pregnancies and infants, and accepting many referrals from various centers. Similar to the literature, it was observed that the probability of early-onset sepsis and nosocomial sepsis increased as the gestational week decreased. On the other hand, late-onset sepsis was highest in those born at the 35th gestational week and lowest in those born at the 34th gestational week. This situation was related to the predominance of early-onset and nosocomial sepsis in the types of sepsis seen at 34 weeks of gestation.

There have been a few studies on the agents that cause sepsis in late preterm infants[19,40]. While group B *Streptococcus* and *Escherichia coli* are the most common pathogens in early-onset sepsis in developed countries[41], *Escherichia coli* is uncommon as a causative agent in our study, and group B *Streptococcus* was not observed.

In this report, the most common agent in early-onset sepsis was CoNS, followed by *Klebsiella pneumonia* and *Staphylococcus aureus*. The most common agent in late-onset sepsis was CoNS, followed by *Staphylococcus aureus* and *Klebsiella pneumonia*. *Staphylococci, Klebsiella, Pseudomonas, Enterococci,* and *Candida* species were causative agents in nosocomial sepsis. Bacteria that caused death were Gram-negative agents in early and late-onset sepsis, consistent with neonatal sepsis cases in the literature[42].

The length of hospital stay is inversely proportional to gestational week (e.g., 6-11 days, 4-6 days, and 3-4 days in infants born at 34th, 35th, and 36th weeks, respectively) [43]. In a study conducted in Spain, the mean length of hospital stay for late preterm infants were six days and 2.8 days for term infants, with a statistically significant difference[44]. Similarly, prematurity was reported to affect our study's length of hospital stay substantially. Furthermore, it was noticed that following complicated infants, receiving more referrals, and avoiding early infant discharge as much as possible increased hospitalization rates.

Despite the high morbidity risks, late preterm infants are typically cared for in the healthy infant neonatal unit after birth and discharged from the hospital at 2-3 days of age, which increases hospital readmission rates. As a result, understanding the causes and risks of morbidity in late preterm infants is critical for post-discharge follow-up and preventing complications. According to the literature and our findings, the hospital readmission rate in late preterm infants is 1.5-3 times higher than in term newborns[7,44]. The lower hospital readmission rates in our study compared to other studies in the literature were attributed to the avoidance of early discharge in late preterm babies in our unit. Similar to the literature, in our study, the highest readmission rate was observed at 34 weeks of gestation, and as the week of gestation increased, the readmission rate decreased. Jaundice was the most common reason for hospital readmission, followed by infections, suspicion of sepsis, and malnutrition[26,45].

In the study of Karnati et al.,[5] late preterm infants were approximately four times more likely than term infants to die from congenital malformations, neonatal bacterial sepsis, and placental, cord, and membrane complications in infancy. A recent study conducted in Spain showed that 1-year mortality rates were higher in late preterm infants than in term infants[44]. Our study's mortality rate among all late preterm infants was 7%. Although the rates are very similar during the gestational weeks, infants born at the 35th gestational week have the highest mortality rate; infants born at the 34th gestational week have the lowest rate. According to the literature, the death rate decreases as the gestational week increases, but the rates were nearly equal in our study. This rate was attributed to the fact that most patients who died had congenital malformations rather than late prematurity. And these infants were mainly born at 35 and 36 weeks of gestation.

Study Limitations

Several considerations should be noted when interpreting the results. First, this was a single-center retrospective study with inherent limitations compared to randomized clinical trials. Due to the retrospective review of medical records, we had limited access to perinatal data. However, we believe it is a good sample size as it is an 8-year study with 1,088 cases. Furthermore,

while late preterm and term babies have been compared in the literature, late preterm infants were compared within themselves in our research, and similar studies are still scarce.

CONCLUSION

Numerous short-term complications have been observed in late preterm infants, including respiratory distress, hyperbilirubinemia, feeding intolerance, hypoglycemia, and sepsis. Since of morbidities and a high readmission rate after discharge, these infants have a prolonged hospital stay. As a result, discharge planning and follow-up are crucial to reducing re-admission rates of late preterm infants, who are a greater risk group, and promoting healthy growth and development. Furthermore, new approaches must be developed to reduce the off-label C/S ratio. Premature births are thought to be preventable in this manner.

Ethics

Ethics Committee Approval: This study was approved by the Necmettin Erbakan University, Meram Faculty of Medicine Ethics Committee for Non-pharmaceutical and Medical Device Research (decision no: 2015-282, date: 16.09.2015).

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Author Contributions

Surgical and Medical Practices: E.C., Concept: R.Ö., Design: R.Ö., Data Collection or Processing: E.C., Analysis or Interpretation: R.Ö., Literature Search: E.C., Writing: E.C.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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DOI: 10.4274/buchd.galenos.2022.18942
J Dr Behcet Uz Child Hosp 2023;13(1):70-75

Case Report

A Newborn with Arhinia: Suspected BAM Syndrome

Burunsuz Bir Yenidoğan: BAM Sendromu

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ABSTRACT

Bosma arhinia microphthalmia (BAM) syndrome is a rare condition, characterized with eye defects, complete absence of nose, and hypogonadotropic hypogonadism. The symptoms and severity of disorder can alter from one patient to another. The etiology of the majority of the reported cases has remained unknown. The case report of a female baby, who was born through vaginal delivery with characteristic features of midface hypoplasia, nasal aplasia, hypertelorism and other anomalies related to BAM syndrome and challenges during follow-up period are shared in this article.

Keywords: Craniofacial dysmorphology, arrhinia, eye defects, hypogonadotropic hypogonadism, BOSMA, BAM

ÖZ

Bosma arhinia mikroftalmi (BAM) sendromu, burnun tamamen yokluğu, göz anomalileri ve hipogonadotropik hipogonadizmin ile karakterize nadir bir durumdur. Semptomlar ve şiddeti olgular arasında değişiklik göstermektedir. Bildirilen olguların çoğunluğunun etiyolojisi bilinmemektedir. Bu yazıda, 35. gebelik haftasında vajinal yolla doğan, orta yüz hipoplazisi, nazal aplazi, hipertelorizm ve BAM sendromuna bağlı diğer anomalileri olan bir kız bebek ve takip sürecindeki tecrübeler paylaşılmıştır.

Anahtar kelimeler: Kraniyofasiyal dismorfoloji, arini, göz anomalisi, hipogonadotropik hipogonadizm, BOSMA, BAM

Received: 08.11.2022
Accepted: 29.11.2022

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Cite as: Armağan C, Üçüncü Egeli T, Akyıldız C, Erdoğan KM, Erdoğan F, Duman N, Özkan H. A Newborn with Arhinia: Suspected BAM Syndrome. J Dr Behcet Uz Child Hosp. 2023;13(1):70-75

INTRODUCTION

Bosma arhinia microphthalmia (BAM) syndrome is a rare condition, characterized with eye defects, complete absence of nose, and hypogonadotropic hypogonadism. In 1981, Bosma et al.[1] described two males with severe hypoplasia of the nose, hypoplasia of the eyes, sensory abnormalities of taste and smell, and hypogonadism. Around the same time, Ruprecht and Majewski[2] described two German siblings with similar defects (Ruprecht-Majewski Syndrome). Since that time, BAM syndrome has been reported in fewer than 100 patients worldwide. Although SMCHD1[3,4], FSHD2[5] and other gene mutations were detected in a few cases, its etiology remained mostly unknown[6]. The symptoms and severity of the disorder can alter from one patient to another. Neonates, and obligatory nasal breathers with the absence of external nose, are at risk of airway collapse. There are no clear guidelines about management in the delivery room. We report a late preterm neonate with arhinia and other congenital malformations consistent with BAM syndrome.

CASE REPORT

A female baby who was born via vaginal delivery at 35th week of gestational age due to late deceleration of fetal heart rate detected during the non-stress test in a 25-year-old healthy mother during routine controls, was intubated and referred to our hospital because of dysmorphic features and lack of respiratory effort in the delivery room. Patient's family stated that regular follow-ups were realized throughout the pregnancy and there was no abnormality in the prenatal history. In addition, there was no consanguinity between the parents and they had two healthy boys aged seven and nine years. The patient's birth weight (2,180 g: 29th pctl), birth length (45 cm: 37th pctl), and head circumference (30.5 cm: 17th pctl) were as indicated[7]. In the physical examination; midface hypoplasia, nasal aplasia, hypertelorism, low-

placed left eyeball, downslanting palpebral fissures, low ear, several skin tags in front of right ear, severely malformed left external auditory canal, high-arched palate, large gap between both big toes and second toes were detected (Figure 1).

Abdominal ultrasonographic and echocardiographic examinations did not reveal any abnormal findings other than patent foramen ovale. In the ccomputed tomography examination of the skull bones; no aeration was observed in the left tympanic bone, and total osseous atresia was noted in the left external ear canal. Aeration was not observed in both mastoid bones. The external auditory canal opening to the right side was very thin. Quite hypoplastic nasal bone, and absence of the nasal cavity were noted. Bilateral Cochleas and vestibules were normal. However, semicircular canals could not be evaluated (Figure 2). In the cranial magnetic resonance imaging (MRI) of the patient whose electroencephalography examination was normal for his age, lipoma in the supracerebellar region, frontoethmoidal encephalocele, hypoplasia in the cerebellar vermis and left external auditory canal atresia were detected. No abnormal findings were found in the blood tests (Table 1). Moderate conductive hearing loss on the left and severe conductive hearing loss on the right were detected by hearing test.

Karyotyping and microarray analyzes were performed subsequently. Karyotype analysis revealed the presence of 46,XX. In microarray analysis, an 85 Kbp deletion of unknown clinical significance characterized by the presence of an *Online Mendelian Inheritance in Man* (OMIM) gene [CATION CHANNEL AMILORIDE-SENSITIVE, NEURINAL, 1; *ACCN1* (601789)] in the 17q12 region was detected. The data obtained from microarray analysis was searched in the Database of Genomic Variants, DECIPHER, OMIM and other related databases with the methods recommended in the literature. Thus, this small deletion was thought to be not related to the patient's clinical features.

The patient was followed up as intubated, and breastfeeding was started at the postnatal 48th hour. Tracheostomy was opened for airway patency on the seventh day. The patient was followed up on mechanical ventilator for fifteen days and received antibiotherapy with the diagnosis of late neonatal sepsis during this period. Due to difficulties in oral intake, the patient, whose follow-up was continued with a heat and moisture exchanger, was fed via an orogastric tube. Oral feeding was provided with the development of sucking/swallowing reflex and after being monitored with her mother for seven days, the patient was discharged on the postnatal 60th day (Figure 3). On evaluation performed after two weeks, the patient's body weight (3.185 g: 11th pctl), height (50 cm: 12th pctl), and head circumference (35 cm: 22th pctl) were masured as indicated[8]. Blood tests performed during mini-pubertal period and routine evaluations are given in Table 1.

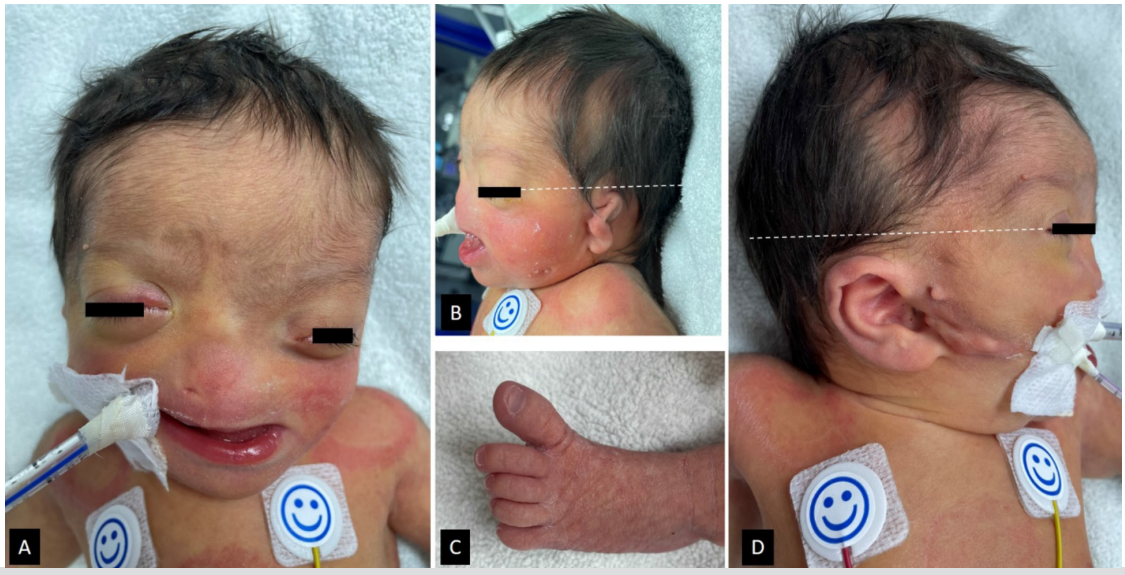

Figure 1. A) Midface hypoplasia, nasal aplasia, hypertelorism, low placed left eyeball, down slanting palpebral fissure, low ear, B) severely malformed left external auditory canal, C) large gap between both big toes and second toes, D) several skin tags in front of right ear

DISCUSSION

The frontonasal processes appear around the fourth gestational week of age and give rise to the majority of the skeletal elements of the face during the processes of proliferation, differentiation and apoptosis[9]. Malformations that may occur during this process are manifested by various craniofacial anomalies such as Apert syndrome, BAM syndrome, Crouzon syndrome and Treacher Collins syndrome etc. BAM syndrome, firstly defined in 1981, has three decisive components: arhinia, ocular malformations and hypogonadotropic hypogonadism[10]. Some of the patients were shown to have mutations in *SMCHD1*, *FSHD2* and other genes[3-6]. However, genetic mutations have not been identified in most cases or medical centers have failed to perform relevant genetic analyzes. However, any known pathogenic mutations have not been identified in most of the cases or medical centers have failed to perform further genetic analyzes Several candidate genes including *ALX4, PAX6, FGF, RAX, SOX2* and *CHX10* have been evaluated, and yet no significant associations have been found[11]. According to OMIM (603457) database, BAMS related only to *SMCHD1* gene locating on chromosome 18p11. *SMCHD1*, codes a protein regulating gene activity by altering the structure of DNA, and plays an essential role in the inactivation of X chromosome[12].

Table 1: Patient's laboratory results at specified times

	Postnatal 3rd day	2nd month
Hb (gr/dL)	16.8	9.8
MCV (fL)	118	89.9
Blood Glucose (mg/dL)	85	98
BUN (mg/dL)	5.4	7.7
Cre (mg/dL)	0.67	0.22
Albumin (g/dL)	2.9	4.09
AST/ALT (U/L)	64/17	31/18
TSH [m (IU)/mL]	6.5	3.4
fT_4 (ng/dL)	1.57	1.1
ACTH (pg/mL)	-	58
Cortisol (µg/dL)	-	32.3
FSH [m (IU)/mL]	-	<0.001
LH [m (IU)/mL]	-	<0.00
Estradiol (pg/mL)	-	<11.80

Hb: Hemoglobin, MCV: Mean corpuscular volume, BUN: Blood urea nitrogen, Cre: Plasma creatinine, AST: Aspartate aminotransferase, ALT: Alanine transaminase, TSH: Thyroid stimulating hormone, fT_4: Free thyroxine, ACTH: Adrenocorticotropic hormone, FSH: Follicle stimulating hormone, LH: Luteinizing hormone

Arhinia leads to severe airway impairment and poor feeding in neonates. Considering that neonates are requisite nasal breathers, it is very difficult, but crucial for the maintenance of airway patency in the delivery room. Mouth breathing is a learned reflex which is acquired around sixth months. Hence, an obstructed airway can drive to respiratory distress[13]. In our case, antenatal diagnosis was unknown, the patient was intubated in the delivery room, and a tracheostomy was performed on the seventh day. In the reported cases, all patients, with very few exceptions, were intubated soon after birth[10,14-16]. Detection of arhinia during the antenatal period would generally be possible by using qualified ultrasound devices and adroit ultrasonographers. There are five cases diagnosed with isolated arhinia antenatally. Two of these cases did not survive, one of them was intubated on the third day, and one of them was discharged without intubation[14,16,17]. Knowing what to expect in the delivery room has an important place

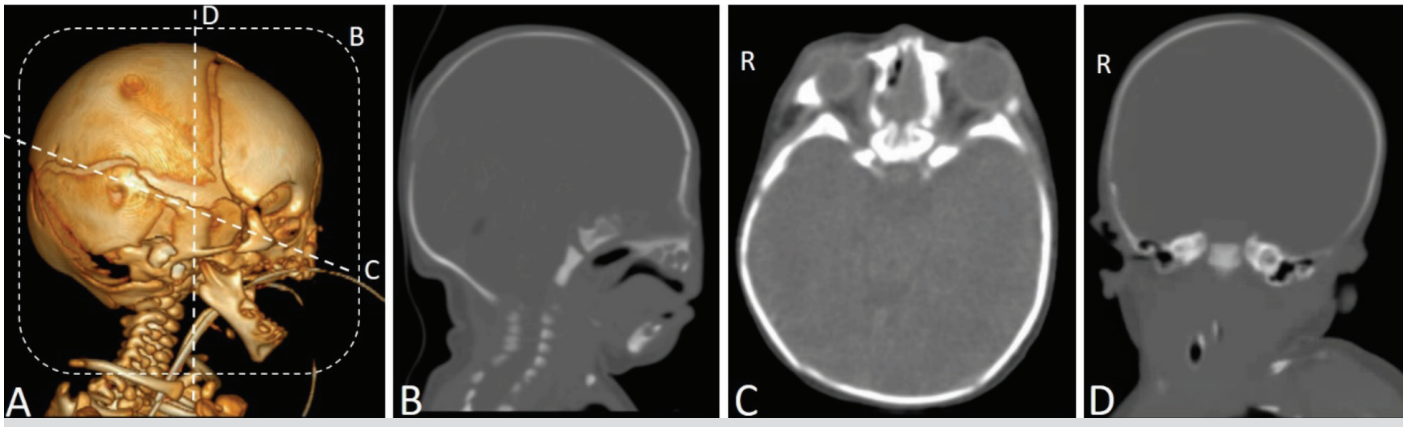

Figure 2. A) 3D model of skull bones, B, C) Sagittal and transverse section The nasal bone was observed to be quite hypoplastic, and the nasal cavity was not observed. D) Aeration was not observed in bilateral mastoids. The external auditory canal opening on the right was very thin. Bilateral cochlea and vestibule are normal

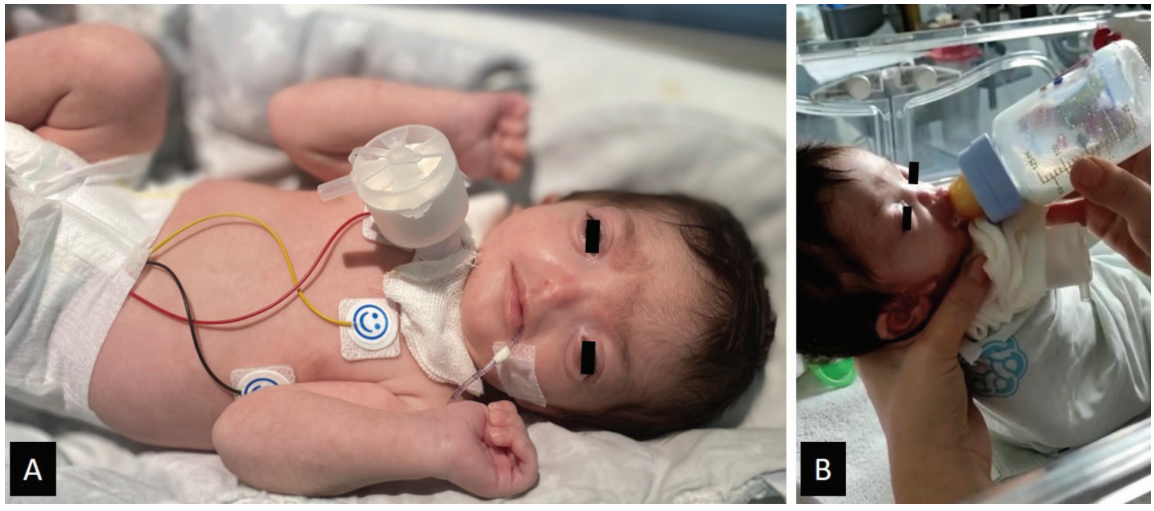

Figure 3. A) Spontan breathing, with a heat and moisture exchanger on 42nd day, B) Oral feeding started on day 51

in the future interventions to be performed. In addition, in children with nasal agenesis, disturbances in the oral food intake are expected, since simultaneous breathing is not possible when sucking. Oral food intake can be ensured with an orogastric tube taking into account the risk of cyanosis.

High-arched palate and absence of nasolacrimal ducts are the most common accompanying midfacial anomalies[10,17]. Our patient had high-arched palate and ear abnormalities described above. Ear anomalies are not well described in children with BAM syndrome. Only a few cases had hypoplasia of the auditory canals, mild conductive or sensorineural hearing loss, preauricular pits, small over-folded ears and helical crus anomalies[6,10,18,19].

Ocular malformations including coloboma, microphthalmia, anophthalmia, hypertelorism, downslanting palpebral fissures are well described[10,16]. Hypertelorism, low-placed left eyeball and downslanting palpebral fissure were observed in our patient. In addition to the described findings tigroid retinal pattern together with ectropion was observed but coloboma wasn't noted during ophthalmologic examination. The patient could make eye contact and recognize caregivers around two months of age.

Facial malformations are generally seen with central nervous (CNS) system anomalies. In the case reports and reviews, most associated CNS anomalies are absence of olfactory tracts/bulbs, frontal or frontonasal encephalocele and thin corpus callosum[10,16]. MRI scan findings in our patient have been described above. Excepting three cases (one had abnormal aortic root, one had patent ductus arteriosus, questionable aortic coarctation and pulmonary and tricuspid valve insufficiency, and one had patent ductus arteriosus), no cardiac anomalies were described in the literature[10,20,21]. In our patient's echocardiography, a patent foramen ovale was observed.

Hypogonadotropic hypogonadism, cryptorchidism, umbilical/inguinal hernia and hypospadias were also described in these patients[10,16]. Generally, case reports on exclusively neonatal cases have been published, whereas some cases have dealt with older patients[1,10,21-23]. Though cases of isolated arhinia and delayed puberty in newborns and infants have been reported[24-26], these cases had not undergone hormonal examination at the time of mini puberty. Mini puberty describes the ephemeral activation of the hypothalamic-pituitary-gonadal axis during early months of childhood. Between the 2nd and the 10th weeks of age levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) increase, make a peak, then decrease and stay at prepubertal levels until puberty[27]. In our patient around nine weeks of age, FSH, LH and estradiol levels were compatible with hypogonadotropic hypogonadism (Table 1). No shock, hypoglycemia or electrolyte abnormality were observed during the patient's follow-up period in the neonatal intensive care unit, suggesting the presence of hypothalamic-pituitary axis insufficiency.

There is no consensus about the type and timing of the reconstructive surgery to be performed. Some

authors perform reconstructive surgery in the neonatal period, while others delay it until adolesence[28]. In our case, the patient was followed up monthly with in situ tracheostomy by related departments of pediatrics, ophthalmology and ENT. Surgical correction was planned by departments of ENT and esthetic, plastic and reconstructive surgery after childhood.

Although a patient with BAM syndrome may require intensive medical support early in life due to difficulty in breathing and feeding, they usually become healthy and reproductive later on. Antenatal diagnosis is important to determine the necessary intervention to be performed in the delivery room. Owing to the rarity of BAM syndrome, there are no standardized relevant treatment protocols or guidelines. Affected individuals should be monitored by medical care team including ENT, esthetic, plastic and reconstructive surgeons, ophthalmologists, and pediatric endocrinologists.

Acknowledgments: We would like to express our gratitude to the patient's parents for their understanding and cooperation in this study.

Informed Consent: Informed consent was obtained from the parents of the case included in this report.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: C.A., T.Ü.E., C.Ak., F.E., N.D., H.Ö., Concept: T.Ü.E., N.D., Design: C.A., C.Ak., F.E., Data Collection or Processing: C.A., T.Ü.E., F.E., Analysis or Interpretation: K.M.E., N.D., H.Ö., Literature Search: C.A., T.Ü.E., Writing: C.A., C.Ak., K.M.E.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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