**ISSN:** 2146-2372 **e-ISSN:** 1309-9566

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: 12
Issue: 2
AUGUST '22

JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2022
Volume: 12
Issue: 2

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**Publisher Certificate Number:** 14521

**Online Publishing Date:** August 2022
**ISSN:** 2146-2372 **e-ISSN:** 1309-9566
International periodical journal published three times in a year.

JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2022
Volume: 12
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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.

**No fees are charged from authors for article submission, processing or publication.**

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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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**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.

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**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.

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**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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**Journal:**

Hull ML, Escareno CR, Godsland JM, Doig JR, Johnson CM, Phillips SC, Smith SK, Tavaré S, Print CG, Charnock- Jones DS: Endometrial-peritoneal interactions during endometriotic lesion establishment. Am J Pathol 2008;173:700-715. PMID: 18688027, 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;39:109-114.

**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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Care provided by nurses is especially important in the diagnosis, and prevention of malnutrition, in the decreasing hospitalization period, and hospital costs.(9) Therefore the nurses are expected to have adequate information, equipment, and skill in the field of nutrition.(3,10,11)

Duerksen et al.(14) evaluated the knowledge level, and approaches of Canadian nurses concerning nutritional problems of inpatients . In their study, they indicated that nurses failed to evaluate nutritional state of the patients adequately, and effectively which was attributed to inadequate number of auxillary personnel, time restraints, and missing documents .

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At the end of the text, references should be written double-spaced on a separate paper. Titles of the journals should be abbreviated in accordance with the citation index which includes the journal that published the article (ie: Index Medicus, Medline, Pubmed, Web of Science, TR Dizin, etc.), and if available, DOI numbers should be absolutely added. For abbreviations of the titles of the journals, please see the list of the journals published by NLM in website (http://bit.ly/2IJkey3). If title of the journal is not contained in these lists, it should be written in full. If Vancouver format is employed in the website you used for references , then copy-pasting of the reference in your reference list is recommended. References

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indicated in the text should be written in compliance with the below-mentioned sample statements:

**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.

**A chapter in a translated book:**

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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Editorial policies of the journal are conducted as stated in the rules recommended by the Council of Science Editors and reflected in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (http://www.icmje.org/). Accordingly, authors, reviewers, and editors are expected to adhere to the best practice guidelines on ethical behavior contained in this statement.

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2022
Volume: 12
Issue: 2

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JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

2022
Volume: 12
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## ORIGINAL ARTICLES

JOURNAL OF DR. BEHÇET UZ CHILDREN'S HOSPITAL

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**DOI:** 10.4274/buchd.galenos.2021.35762
J Dr Behcet Uz Child Hosp 2022;12(2):107-115

Original Article

# Epilepsy and Electroencephalographic Abnormalities in Children with Autistic Spectrum Disorder

## *Otistik Spektrum Bozukluğu olan Çocuk Olgularda Epilepsi ve Elektroensefalografi Anormallikleri*

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## ABSTRACT

**Objective:** Epilepsy and electroencephalography (EEG) abnormalities are more commonly seen in autism spectrum disorder (ASD). The aim of the present study is determine the risk factors that cause epilepsy, seizures and EEG abnormalities in cases with EEG examination who were followed with the diagnosis of ASD.

**Method:** A total of 166 cases diagnosed with ASD according to the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition in Dokuz Eylül University Faculty of Medicine Hospital Clinic of Child Psychiatry and whose neurological evaluations and examinations were performed in the pediatric neurology clinic, were included in the study. We retrospectively recorded and analyzed the cases' clinical features, the results of physical examination, imaging and EEG, comorbidities and medications they used.

**Results:** Of the cases, 74.4% were male. The mean age of cases with epilepsy diagnosis, at least one seizure and epileptic discharges on EEG was higher (p<0.001, p<0.001 and p=0.005, respectively). The history of epilepsy and having at least one seizure were more common in cases aged 11 years and older (p=0.001 and p=0.001, respectively). Abnormalities in EEG examination and epileptic discharges were detected to be more common in female ASD cases (p=0.041 and p=0.019 respectively).

**Conclusion:** In ASD cases without any underlying chronic neurological disease, female gender is a risk factor for the development of EEG abnormality and epileptic discharges. Advanced age is a risk factor for seizures and development of epilepsy in ASD cases.

**Keywords:** Autism, epilepsy, epileptic discharge, dysrhythmia

## ÖZ

**Amaç:** Otistik spektrum bozukluğunda (OSB) epilepsi ve elektroensefalografi (EEG) anormallikleri daha sık görülmektedir. Biz de kliniğimizde OSB ile izlenen ve EEG incelemesi olan olgularda epilepsi, nöbet ve EEG anormallikleri varlığına neden olan risk faktörlerini belirlemek istedik.

**Yöntem:** Dokuz Eylül Üniversitesi Tıp Fakültesi Hastanesi Çocuk Psikiyatrisi kliniğinde Mental Bozuklukların Tanısal ve Sayımsal El Kitabı 5. Baskı'ya göre OSB tanısı almış ve çocuk nöroloji Kliniği'nde nörolojik değerlendirmesi ve EEG incelemesi yapılmış olan 166 OSB olgusunu çalışmaya dahil ettik. Retrospektif olarak olguların klinik özelliklerini, fizik muayene, görüntüleme, EEG bulgularını, eşlik eden hastalıklarını ve kullandıkları ilaçları kaydettik ve analiz ettik.

**Bulgular:** Olgularımızın %74,6'sı erkekti. Epilepsi tanısı olan, en az bir epileptik nöbet geçiren ve EEG'de epileptik deşarjları olan olguların yaş ortalaması daha yüksekti (p<0,001, p<0,001 ve p=0,005, sırasıyla). On bir yaş ve daha büyük olgularda epilepsi ve en az bir nöbet geçirme öyküsü daha sıktı (p=0,001 ve p=0,001 sırasıyla). EEG'de anormallik ve epileptik deşarjlar kız OSB olgularında daha sık saptandı (p=0,041 ve p=0,019 sırasıyla).

**Sonuç:** Altta başka bir kronik nörolojik hastalığı olmayan OSB olgularında kız cinsiyet EEG'de anormallik ve epileptik deşarjların gelişimi açısından bir risk faktörüdür. OSB olgularında ileri yaş, nöbet geçirme ve epilepsi gelişimi için bir risk faktörüdür.

**Anahtar kelimeler:** Otizm, epilepsi, epileptik deşarj, disritmi

**Received:** 26.06.2021
**Accepted:** 28.12.2021

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**Cite as:** Polat İ, Hız AS, Yiş U, Ayanoğlu M, Okur D, Bayram E, Baykara HB. Epilepsy and electroencephalographic abnormalities in children with autistic spectrum disorder. J Dr Behcet Uz Child Hosp. 2022;12(2):107-115

## INTRODUCTION

Autistic spectrum disorder is a neurological dysfunction picture characterized by deficits in socialization and communication, causing neurodevelopmental delays and limited, repetitive behaviors [1]. The prevalence of autism spectrum disorder (ASD) today is reported to be 1 in 59 [2]. Although the prevalence of epilepsy remains stable in society, autistic spectrum disorders are increasingly being reported. Increased number of diagnosed cases may be due to the increased awareness of ASD among people and broader diagnostic criteria established in the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5), compared to the old diagnostic criteria [3].

Epilepsy prevalence in children with autism is reported as 2-46% [4,5]. Kanner's criteria formerly used in the diagnosis of autism identified severe and cognitively delayed cases. Therefore, only severe cases could be diagnosed according to Kanner's autism criteria, and the frequency of epilepsy among these cases was higher than ASD cohorts diagnosed according to DSM-5 [3,6]. In addition, it has been reported in the literature that the frequency of epilepsy in ASD cases is associated with intellectual delay, and the frequency of epilepsy is 21.5% in ASD cases with intellectual delay, and 8% in those without intellectual delay [3,4].

The prevalence of ASD in epilepsy cases was reported as 6.3%, much higher than the prevalence of 0.75-1.1% in the normal population [7]. Intellectual delay, male gender, being under 18 years of age, low age at first seizure and presence of symptomatic epilepsy, specific epilepsy syndrome (Dravet syndrome, infantile spasm, etc.) have been indicated as risk factors for the development of ASD in epilepsy cases [7]. The rate of ASD increases to 19.9% in infantile spasms, 41.9% in focal epilepsies and 47.4% in Dravet syndrome [7]. In addition, ASD is reported more frequently in Fragile X syndrome, Down syndrome, Angelman syndrome, Rett syndrome and neurocutaneous diseases than in the normal population [8]. Genetic studies have revealed that ASD, epilepsy and intellectual delay share common genetic features [8]. It has been observed that de novo mutations in ASD cases are a heterogeneous group, including copy number variants, single nucleotide polymorphisms and epigenetic variations [8]. Most of these variants have been found to be associated with synapse formation, neurotransmitter function, and neuronal plasticity [8]. As a result, there is mainly neuronal connectivity in ASD cases and neuronal excitability problems in epilepsy [8]. Thus, this epileptic activity may negatively affect neurodevelopment and cause ASD by disrupting both neuronal structure and neurotransmitter regulation [9]. Epileptiform discharges are associated with social-cognitive delay in Landau-Kleffner syndrome (LKS) or electrical status epilepticus in sleep (ESES) cases. However, the deficits observed in these cases do not fully meet the ASD criteria, and if epileptic discharges are eliminated by treatment, there may also be some improvement in this delay [3]. Atypical epilepsy and electroencephalography (EEG) abnormalities and epilepsy are detected more frequently in regressed ASD cases than in non-regressed ASD cases [10]. EEG abnormalities in ASD cases without seizure are reported in a wider range of 8-60% [11]. In addition, it has been shown that EEG abnormalities seen in ASD cases without seizure are associated with the delay of language, motor and cognitive skills during the first year of life. For this reason, children are in risk in terms of EEG abnormalities and seizure development in ASD [11,12].

## MATERIALS and METHODS

The study was approved by the Dokuz Eylül University Non-Interventional Research Ethics Committee (decision no: 2017/16-11 date: 15.06.2017). It was carried out in the Pediatric Neurology Clinic of Dokuz Eylül University Medical Faculty Hospital. A total of 166 ASD patients with the age range of 2 to 17 who were diagnosed according to DSM-5 diagnostic criteria and had sleep-EEG examination were included in the study. Demographic characteristics, systemic and neurological examination findings of the cases were recorded. The cases were questioned whether there was a seizure history. The seizure type and frequency of seizures were recorded. According to International League Against Epilepsy (ILAE), epilepsy is defined as a history of having at least two non-provocative seizures and/or a seizure recurrence risk of more than 60% after one seizure [13]. We collected the data from those who had at least one seizure and among the subjects who had at least one seizure, we named those who met the definition of epilepsy by ILAE as epileptic group. The antiepileptic medications used by the cases diagnosed with epilepsy were recorded. The response of the cases to antiepileptic treatment was questioned. EEG data was recorded with Nihon Kohden 9200K brand 22-channel EEG device. The international bipolar 10-20 montage system was used. EEG data was recorded at least 30 minutes during awake and sleep periods, where arousal/awake response can also be monitored. Hyperventilation and intermittent photic stimulation

were applied. EEG examinations of the cases were re-evaluated by two pediatric neurologists. The results of EEG examination were categorized into three different groups as normal, epileptic discharge (spike, sharp wave, polyspikes, generalized spike-wave complexes), and only dysrhythmia (slow wave activity) without epileptic discharge. Cranial magnetic resonance imaging (MRI) findings were recorded. The results were statistically analyzed by using the SPSS 22.0 software. The normality distribution of the dependent variables was tested by the Kolmogorov-Smirnov test. Results were expressed as mean and standard deviation or median and 25$^{th}$-75$^{th}$ percentiles where appropriate. The chi-square test was used to compare categorical variables. The p-value of less than 0.05 was regarded to be statistically significant.

### Statistical Analysis

The results were statistically analyzed by using the SPSS 22.0 software. The normality distribution of the dependent variables was tested by the Kolmogorov-Smirnov test. Results were expressed as mean and standard deviation or median and 25$^{th}$-75$^{th}$ percentiles where appropriate. The chi-square test was used to compare categorical variables. The p-value of less than 0.05 was regarded to be statistically significant.

## RESULTS

Of the cases, 74.4% (n=124) were male and 25.3% (n=42) were female. There was no age difference between the genders (p=0.313). EEG abnormality and epileptic discharges were more common in female cases than males (p=0.041 and p=0.019, respectively). However, there was no statistical difference between females and males in terms of epilepsy and history of at least one seizure (Table 1).

Abnormalities in physical examination, MR pathologies as well as comorbidities and psychiatric diseases are given in Table 2. Concomitant comorbid conditions were present in 58.3% of the cases with abnormal physical examination and 55.5% of those with abnormality in MR (p=0.003 and p=0.052, respectively). Fourteen cases (58.3%) with abnormalities on physical examination and seven cases (38.8%) with abnormalities on MR had a psychiatric comorbidity (p=0.003 and p=1.000, respectively).

EEG abnormalities were detected in 34.3% (57/166) of our cohort, while twenty-nine (17.4%) cases had a history of at least one seizure. Twenty-five (15%) of these cases had been diagnosed with epilepsy and all were receiving antiepileptic treatment. One hundred and four (95%) of patients with normal EEG had no seizure (Figure 1). It was observed that 42.1% (24/57) of the cases with abnormal EEG had a history of at least one seizure, and 17.2% (5/29) of the cases with the history of at least one seizure had a normal EEG (p<0.001) (Table 3). Among the cases with EEG abnormalities, 64.9% (n=37) had epileptic discharge and 35.1% (n=20) had a dysrhythmia/slowing down of the ground rhythm (Figure 1). It was found that seizures and epilepsy were more commonly seen in patients with epileptic discharges on EEG (p<0.001 and <0.001, respectively) (Table 3).

The mean age of the cases who had at least one seizure was higher than those without any seizures (9.1±3.8 years vs 5.5±3.2 years respectively, p<0.001). The

**Table 1. Comparision of findings of investigations in boys and girls**

| | Boys (n=124) | | Girls (n=42) | | |
|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | **p** |
| PE abnormalities (n=24) | 16 | 12.9 | 8 | 19 | 0.321 |
| MR abnormalities (n=18) | 16 | 12.9 | 2 | 4.7 | 0.129 |
| EEG abnormalities (n=57) | 37 | 29.8 | 20 | 47.6 | **0.041** |
| Epileptic discharges in EEG (n=37) | 22 | 17.7 | 15 | 35.7 | **0.019** |
| Dysrhythmia (n=20) | 15 | 12 | 5 | 11.9 | 1.000 |
| At least one seizure (n=29) | 19 | 15.3 | 10 | 23.8 | 0.241 |
| Epilepsy diagnosis (n=25) | 15 | 12 | 10 | 23.8 | 0.082 |
| Comorbidity (n=51) | 37 | 29.8 | 14 | 33.3 | 0.701 |
| Physchiatric disorder (n=51) | 40 | 32.2 | 11 | 26.1 | 0.563 |
| Mean age (years) | 6.3±3.7 | | 5.7±3 | | 0.313 |
| Mean age at seizure onset | 7.5±3.1 | | 6.9±3.6 | | 0.625 |

PE: Pulmonary embolism, MR: Magnetic resonance, EEG: Epilepsy and electroencephalography

mean age of the cases who were diagnosed as epilepsy were higher than non-epileptic cases (9.3±3.9 years vs 5.6±3.2 years respectively, p<0.001). The mean age of the cases detected to have epileptic discharge (7.6±3.4 years) was higher than those with dysrhythmia (5.2±2.8 years) (p=0.007). Of the cases, 86.7% (n=144) were under the age of 10 years. Among those aged 11 years or older, 45.4% had a history of epileptic seizure and 40.9% had a diagnosis of epilepsy (p=0.001 and p=0.001, respectively) (Table 3).

There were generalized EEG abnormalities in fourteen cases (24.6%) and focal EEG abnormalities in 43 (75.4%) cases. The most common focal EEG abnormalities was

**Table 2. Findings of neurological examination and cranial magnetic resonance imagings and comorbidities and additional physchiatric disorders and drugs**

| | n | % |
|---|---|---|
| **Abnormalities on neurological examination** | **(n=24)** | **(100%)** |
| Dysmorphic face, minor dysmorphic changes | 14 | 58.3 |
| Increased DTRs, pyramidal findings | 4 | 16.6 |
| Movement disorders, streotypic movements | 3 | 12.5 |
| Hipopigmentated/hyperpigmentated skin lesions | 3 | 12.5 |
| **Abnormalities on cranial magnetic resonance imaging studies** | **(n=18)** | **(100%)** |
| Arachnoid cyst | 4 | 22.2 |
| Periventicular leukomalasia, gliotic changes | 8 | 44.4 |
| T2 hyperintense signal changes | 4 | 22.2 |
| Dilatated ventricules, decreased white matter volume, cerebral/cerebellar atrophy | 2 | 11.1 |
| **Comorbidities** | **(n=51)** | **(100%)** |
| Preterm/premature birth | 15 | 29.4 |
| Retardation on motor milestones | 20 | 39.2 |
| Neonatal problems (low birth weight, asphyxia) | 8 | 15.6 |
| Other disorders (congenital cardiac disorders, vision problems, obesity, protein-energy malnutrition) | 8 | 15.6 |
| **Physchiatric disorders** | **(n=51)** | **(100%)** |
| Attention deficiency and hyperactivity disorder | 15 | 29.4 |
| Mental retardation | 5 | 9.8 |
| Speech delay | 31 | 60.7 |
| **Antiepileptic drugs** | **(n=25)** | **(100%)** |
| Valproic acid | 16 | 64 |
| Carbamazepine | 2 | 8 |
| Valproic acid + oxcarbazepine | 2 | 8 |
| Valproic acid + lamotrigine | 3 | 12 |
| Levetiracetam | 2 | 8 |
| **Physchiatric drugs** | **(n=68)** | **(100%)** |
| Risperidone | 38 | 55.8 |
| Aripiprazole | 13 | 19.1 |
| Risperidone + aripiprazole | 3 | 4.4 |
| Methlyphenidate | 8 | 11.7 |
| Risperidone + aripiprazole + methlyphenidate | 2 | 2.9 |
| Quetiapine | 2 | 2.9 |
| Aripiprazole + methlyphenidate | 1 | 1.4 |
| Risperidone + methlyphenidate | 1 | 1.4 |

DTR: Deep tendon reflexes

found to be the one originating from the frontocentral (37/43) (86%) region. Focal EEG abnormality and the detection of abnormality in the frontocentral region were not statistically related to gender, physical examination findings, and the presence of pathology at MR, presence of pathology in MRI, comorbid or accompanied psychiatric disease, coexistence of epileptic seizures and epilepsy and continuation of abnormality in the follow-up EEG. There was no statistically significant difference between physical examination findings or abnormality in MR and EEG abnormality, the presence of epileptic discharge, epileptic seizure history and diagnosis of epilepsy. Similarly, no statistically significant difference was detected in cases with EEG abnormality, those with epileptic discharge, those having a history of epileptic seizure, and those diagnosed with epilepsy in terms of comorbid conditions and additional psychiatric diseases. On the other hand, the rate of psychiatric medication use was higher in the cases with a history of epileptic seizure (p=0.039).

Twenty-five cases with a history of epileptic seizure were taking antiepileptic medication. Among those, antiepileptic response was partial in four (16%) cases. Five cases were using more than one antiepileptics. In the cohort, 68 (40.9%) cases were taking psychiatric medication, while nine of those were using more than one psychiatric medication. The rate of epileptic seizure history in the cases using more than one psychiatric medication (44.4%) was not significantly different than those using a single drug (22%) (p=0.212).

Five cases had a history of febrile convulsion. There was a family history of epilepsy in five cases, a family history of psychiatric disease in four cases, and a family history of both epilepsy and psychiatric disease in one case.

There were follow-up EEGs taken after at least 6 months in thirty-one cases (54.3%). In 20% of those with normal initial EEG, the control EEG was also found to be normal. On the other hand, an improvement was observed in the control EEG in 26.2% of those who had abnormality in their first EEG. While 50% of those with abnormality in the follow-up EEG were not taking any antiepileptic medications, 92.1% of those with a history of epileptic seizure were using at least one medication (p=0.020).

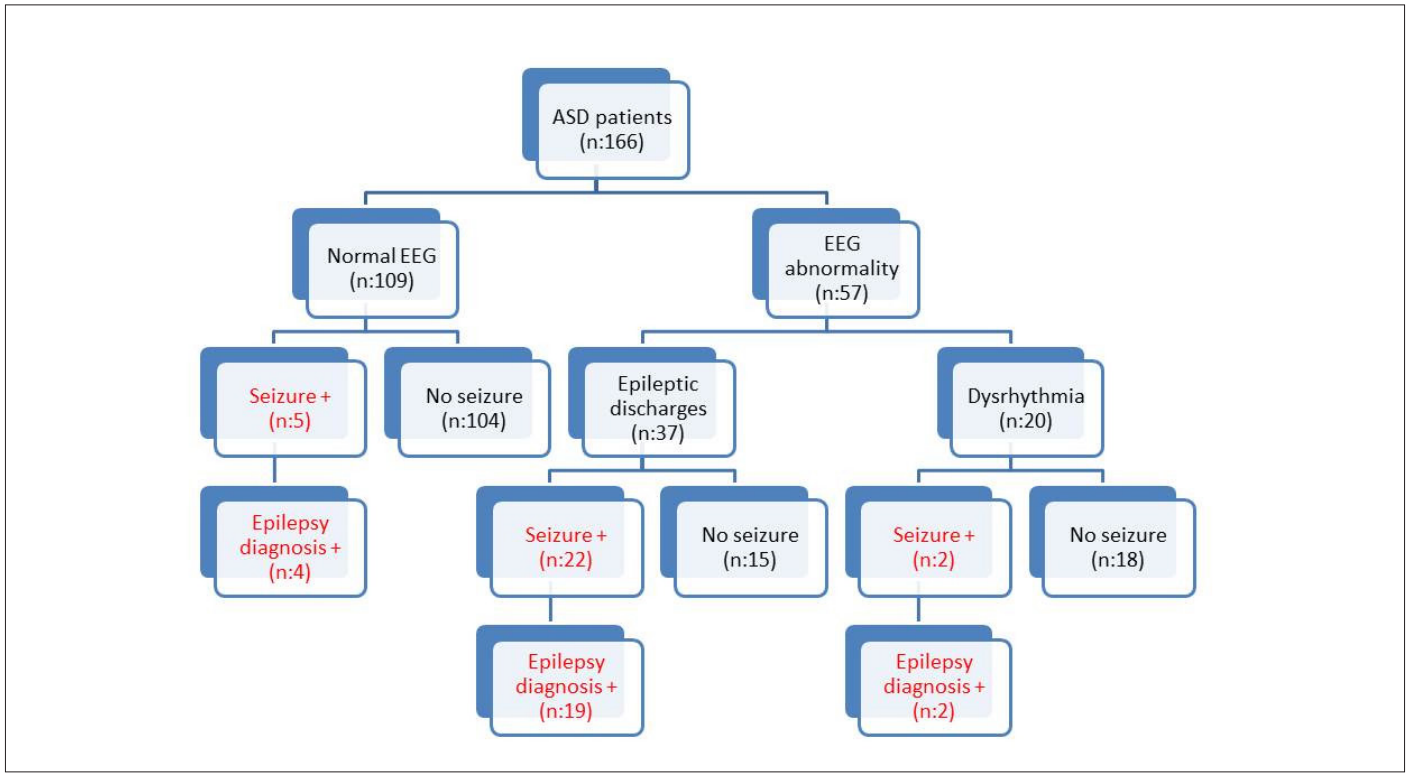


**Figure 1.** Flowchart of ASD patients with/without EEG abnormality and/or epilepsy

ASD: Autism spectrum disorder, EEG: Electroencephalography

| Table 3. Comparision of properties of patients' groups with at least one seizure and epilepsy diagnosis | | | | | | |
|---|---|---|---|---|---|---|
| | At least one seizure (n=29) | | | Epilepsy diagnosis (n=25) | | |
| | n | % | p | n | % | p |
| <10 years of age (n=144) | 19 | 13.1 | **0.001** | 16 | 11.1 | **0.001** |
| >11 years of age (n=22) | 10 | 45.4 | | 9 | 40.9 | |
| Abnormal EEG (n=57) | 24 | 43.6 | **<0.001** | 21 | 36.8 | **<0.001** |
| Normal EEG (n=109) | 5 | 4.5 | | 4 | 3.6 | |
| ED + (n=37) | 22 | 59.4 | **<0.001** | 19 | 51.3 | **<0.001** |
| No ED (n=129) | 7 | 5.4 | | 6 | 4.6 | |
| Dysrhythmia + (n=20) | 2 | 10 | 0.532 | 2 | 10 | 0.741 |
| No dysrhythmia (n=146) | 27 | 18.4 | | 23 | 15.7 | |
| EEG: Electroencephalography, ED: Epileptic discharge | | | | | | |

## DISCUSSION

In the study, we found that EEG abnormalities and especially epileptic discharges were seen more frequently in female ASD cases in our cohort. Epilepsy was detected more frequently in the cases with epileptic discharge on EEG. We observed that those diagnosed with epilepsy had a higher mean age and were followed for a longer period of time with the diagnosis of ASD. When we categorized our ASD cohort in two groups as cases under 10 years old and 11 years old or above, we detected that both groups had similar EEG abnormality rates, however, epilepsy was more frequent in the group with the age of 11 years or above. Although ASD is more frequent in males, its course is more severe in females [14]. In our cohort, 74.6% of the cases were male. In the literature, epilepsy rates in ASD cases have been reported in a wide range of 2-60%. It would be appropriate to compare our results with the studies that include cases who were diagnosed with ASD with the same diagnostic criteria, and in similar age groups. This rate differs in the range of 3.9-24.6% in studies including cases younger than 18 years of age or those defined as children [15]. However, the diagnostic criteria have not been clearly specified in all studies. While epilepsy rate has been reported to be 6.6-14.6% in studies including the cases with 0-17 age group, this rate was 24.6% in cases diagnosed with ASD in infancy and 22.5% in syndromic cases [16-20]. It was stated that the rate of epilepsy was 14.6% in the cases who were diagnosed with ASD according to the DSM-4 [16]. As can be understood from the results of the studies in the literature, epilepsy is seen much more frequently in cases diagnosed with ASD according to the diagnostic criteria applied before DSM-4 and DSM-5, in those with very early infantile onset, in those with syndromic features, and in autism cases older than 10 years of age or in adulthood [16-21]. In our study, similar to the literature, 15% of the cases were diagnosed with epilepsy, and 17.4% of the cases had seizures at least once. On the other hand, when we look at only ASD cases with the age of 11 or above in our cohort, epilepsy and experiencing at least one seizure rates increased to 45.4% and 40.9%, respectively. It was found that the mean age of the cases with epilepsy and a history of at least one seizure was also higher in our study. We included the cases who were followed up in the child psychiatry clinic with the diagnosis of ASD and applied to our child neurology outpatient clinic for EEG examination and neurological evaluation. Since our clinic is a tertiary university hospital center, we encounter more severe cases. Therefore we may have detected higher rates. Hence, the cases with ASD-like behavioral disorders who were known to have underlying severe neurological conditions such as Angelman syndrome, Rett syndrome, Dravet syndrome and West syndrome were not the subject of our study. In fact, epilepsy develops in the natural course of the disease in these cases, and the aim of our study was to detect the risk of developing epilepsy in ASD cases.

In the literature, the presence of epilepsy in ASD cases has been associated with female gender, intellectual delay, advanced age, speech problems, low socioeconomic status, and family history of ASD [3,7,21]. Although we could not find a significant difference between genders in terms of epilepsy and seizures in our study, EEG abnormalities and epileptic discharges were higher in female cases. In our cases, while concomitant attention-deficit/hyperactivity disorder and intellectual delay did not create an additional risk for epilepsy or seizures, we detected epilepsy and seizures more frequently in ASD cases with speech delay, but the difference was not statistically significant. This may be

due to the small number of cases in our cohort compared to studies and meta-analyses in which these risk factors were identified.

It has been stated in the literature that the rate of EEG abnormalities in ASD cases in the range of 8-60% [11]. On the other hand, dysrhythmic findings in EEG (asymmetry in ground rhythm, slowing, bioelectric immaturity, etc.) have been reported in various studies at different rates such as 12.5%, 13%, 21.8%, 22% and 36.8% [11,22-26]. Our cohort had EEG abnormalities in 34.3% of cases, epileptic discharges in 22.2%, and dysrhythmia in 12%. EEG abnormalities has been observed most frequently in the cases aged 5-10 years [27]. However, unlike our study, the mean age in these studies in the literature varied between 2 and 7 years. That may have been the reason we found higher mean age of the cases with epileptic discharges than those with dysrhythmia. Epilepsy and at least one seizure rates were more frequent in those who already had epileptic discharge. This situation is same with the higher mean age of those with epilepsy. EEG investigations showed abnormalities frequently on right temporal, left temporal and bitemporal regions of brain [28,29]. The right hemisphere is associated with social relationships, and the left is the region of speech. Bitemporal discharges may be related to other clinical features of ASD [29]. In another study, in which centrotemporal discharges were detected to be frequent, the population of the study was the ASD cases with seizures and regression [28]. One of the most common interictal discharges in ASD cases were those originating from the frontal regions of the brain [8]. Similarly, we also detected that the frontocentral discharges were the most frequent ones in our study. The reason for this difference from the other studies may be due to the fact that ASD cases with underlying LKS, ESES pictures or those with regression were not included in our cohort. The partial disconnectivity of the high functioning brain regions were thought to be in autism with the frontal lobe in ASD cases [30]. This may be the reason why we observed discharges in the frontocentral regions of the brain [30]. It has also been reported that epilepsy with future centrotemporal paroxysms is observed more commonly in cases with frontal EEG abnormalities [31]. Due to the small mean age of our cohort, we may have detected fewer central and temporal discharges. In a study in which 24-hour EEG recording was conducted, the rate of EEG abnormalities in ASD cases without seizures has been reported to be 60.7%. EEG abnormalities reported were spikes, sharp-wave, slow-wave, generalized spike-wave complexes, polyspikes, and paradoxical delta activity, and EEG abnormalities of all patients were shown to have occurred during sleep [28]. Detection of discharges only during sleep helps to exclude the possibility of structural defects [29]. For this reason, it is recommended to take a sleep EEG for at least 30 minutes with intermittent photic stimulation, where arousal/awake response can also be monitored [32]. Although EEG abnormalities do not cause seizure, they have adverse effects on cognition and behaviors [11]. However, in long-term follow-up, they may present with seizures, especially in adolescence period [33]. In our study, we did not find a significant difference in terms of psychiatric comorbidities in patients with EEG abnormality or epilepsy or at least one seizure compared to those without. There was a more frequent history of psychiatric medications use in those with epilepsy only.

### Study Limitations

It is a limitation that we included complex cases in the study, since the study was carried out in a tertiary institution.

## CONCLUSION

In summary, although ASD is more common in males, epilepsy and EEG abnormalities accompany ASD more often in females. Although EEG abnormalities are detected in ASD cases in the first decade of life, early age EEG abnormalities have a negative effect on cognitive development, and epilepsy in ASD cases begins to be seen more frequently after the age of 10. While EEG abnormalities are most frequently seen in the frontocentral, in ASD cases with epilepsy, epileptic discharges occur more commonly in bitemporal and central regions. Many genetic and environmental factors are considered among the common causes of the coexistence of ASD and epilepsy/EEG abnormalities. The detection rates of epilepsy and/or EEG abnormality in ASD cases vary greatly. It is also not yet clear how much EEG abnormality detected without seizures affects the development of epilepsy in which age group. Risk factors can be more clearly determined in studies conducted with more limited groups with well-defined and similar characteristics (gender, age group, underlying disease/genetic variant, age of onset of epilepsy, age of onset of ASD findings, etc.).

### Ethics

**Ethics Committee Approval:** The study was approved by the Dokuz Eylül University Non-Interventional Research Ethics Committee (desicion no: 2017/16-11 date: 15.06.2017).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: İ.P., A.S.H., U.Y., M.A., D.O., E.B., H.B.B., Concept: İ.P., A.S.H., U.Y., M.A., D.O., E.B., H.B.B., Design: İ.P., A.S.H., U.Y., M.A., D.O., E.B., H.B.B., Data Collection and/or Processing: İ.P., A.S.H., U.Y., M.A., D.O., E.B., H.B.B., Analysis and/or Interpretation: İ.P., A.S.H., U.Y., M.A., D.O., E.B., H.B.B., Literature Search: İ.P., A.S.H., U.Y., M.A., D.O., E.B., H.B.B., Writing: İ.P.

**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.2022.84704
J Dr Behcet Uz Child Hosp 2022;12(2):116-119

Original Article

# How Does Respiratory Rate Affect Alveolar Ventilation in Pediatric Patients?

*Pediatrik Hastalarda Solunum Hızı Alveolar Ventilasyonu Nasıl Etkiler?*

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## ABSTRACT

**Objective:** Minute ventilation is a combination of alveolar ventilation (V'alv) and dead space ventilation which is also a result of multiplication of respiratory rate (RR) by tidal volume. V'alv is the volume of the air which reaches the alveoli per minute by definition. The aim of this study was to examine the effect of RR on V'alv in a pediatric physiologic bench setting.

**Method:** In our study, respiratory parameters of a male child, approximately 1 year old, 78 cm in length and ideal body weight of 10 kg, were simulated. This model was ventilated in two different RR settings and with two different breathing circuits which has different dead space (DS) values. However amount of $CO_2$ was kept same during whole bench. Static compliance, static resistance, end-tidal-carbondioxide, positive end expiratory pressure, peak inspiratory pressure, inspiratory time, expiratory-time values of each breathing circuit were taken as the mean and standard deviation of repeated measurements.

**Results:** V'Alv decreased from 1.84±0.3 L/m to 1.63±0.5 L/m (p<0.001) in the pediatric circuit and decreased from 1.95±0.3 L/m to 1.83±0.5 L/m (p<0.001) in neonatal circuit group.

**Conclusion:** Younger patients should be ventilated with higher RR because of their physiology. Additionaly, regarding the current guidelines in pediatric mechanic ventilation, higher RR should be selected in restrictive lung disease condition. Therefore, clinicians should be more alert particularly in the younger and/or in the restrictive lung disease group regarding both the increased RR and the increased percentage of instrumental DS which results in a decreased V'alv.

**Keywords:** Alveolar ventilation, dead space, respiratory rate, minute ventilation, mecahnical ventilation, pediatric intensive care unit

## ÖZ

**Amaç:** Dakika ventilasyonu, alveolar ventilasyon (V'alv) ve ölü boşluk (DS) ventilasyonunun bir kombinasyonudur ve tanım olarak solunum hızının (RR) alveollere dakikada ulaşan tidal hacim ile çarpılmasının bir sonucudur. Bu çalışmanın amacı, pediatrik bir fizyolojik model ortamında RR'nin V'alv üzerindeki etkisini incelemekti.

**Yöntem:** Çalışmamızda yaklaşık 1 yaşında, 78 cm boyunda ve ideal vücut ağırlığı 10 kg olan bir erkek çocuğun solunum parametreleri simüle edildi. Bu model iki farklı RR ayarında ve farklı DS değerlerine sahip iki farklı solunum devresi ile ventile edilmiştir. Ancak tüm fizyolojik modelleme boyunca $CO_2$ miktarı aynı tutulmuştur. Statik komplians, statik direnç, end-tidal-karbondioksit, ekspirium sonu pozitif basınç, tepe inspiratuvar basınç, inspirium süresi, ekspirium süresi değerleri her solunum devresinin tekrarlanan ölçümlerin ortalaması ve standart sapması olarak alınmıştır.

**Bulgular:** Pediatrik devrede V'Alv 1,84±0,3 L/m'den 1,63±0,5 L/m'ye (p<0,001), yenidoğan devresi kullanıldığında ise 1,95±0,3 L/m'den 1,83±0,5 L/m'ye (p<0,001) gerilemiştir.

**Sonuç:** Daha genç hastalar fizyolojileri nedeniyle daha yüksek RR ile ventile edilmelidir. Ayrıca pediatrik mekanik ventilasyonda güncel kılavuzlara göre restriktif akciğer hastalığı durumunda daha yüksek RR seçilmelidir. Bu nedenle klinisyenler özellikle yaşı küçük hastalarda ve/veya restriktif akciğer hastalığı grubunda hem artmış RR hem de V'alv'de azalma ile sonuçlanan artmış instrumental DS yüzdesi nedeni ile daha uyanık olmalıdırlar.

**Anahtar kelimeler:** Alveolar ventilasyon, ölü boşluk, solunum hızı, dakika ventilasyonu, mekanik ventilasyon, pediatrik yoğun bakım ünitesi

**Received:** 08.09.2021
**Accepted:** 03.01.2022

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**Cite as:** Ceylan G, Atakul G, Topal S, Çolak M, Soydan E, Sarı F, Seven P, Saraç Sandal Ö, Ağın H. How Does Respiratory Rate Affect Alveolar Ventilation in Pediatric Patients? J Dr Behcet Uz Child Hosp. 2022;12(2):116-119

## INTRODUCTION

The purpose of mechanical ventilation is to partially or fully support the patient's respiratory workload using a mechanical ventilator. Minute ventilation is a combination of minute alveolar ventilation (V'alv) and minute dead space ventilation, which is also a result of multiplying the respiratory rate (RR) by the tidal volume (VT). V'alv is by definition the volume of air which reaches the alveoli per minute. Dead space (DS) is a combination of physiological dead space (DSp) and instrumental dead space (DSi). DSp is a term used to describe the region where no gas exchange occurs in the lung [1]. It is unavoidable that DSi is added to DSp during mechanical ventilation. The lower the VT used, the more DS affects ventilation and decreases V'alv. In addition, positive end-expiratory pressure (PEEP) also affects DS ventilation in physiological conditions [2]. The aim of this study was to examine the effect of RR on V'alv in pedatric patients.

## MATERIALS and METHODS

The study was performed during the period from September 1-30, 2020 using B&B test lungs. In order to mimic the physiology and respiratory parameters of a healthy, male, 1-year old child, the height was chosen as 78 cm and the corresponding ideal body weight (IBW) was selected as 10 kg. Firstly, the static compliance (Cs) and static resistance (Rs) values were reached in the test lung according to the model [3]. Then the system was titrated with an amount of $CO_2$ appropriate to the patient's IBW in the amounts regulated by the Sierra Mass Flow Controller (with accuracy of ±2.0 of full scale for 100 mL from 201-300 slpm, Sierra Instruments, California, USA) via the T-tube and $CO_2$ diffuser. The required minute $CO_2$ flow ($V'CO_2$) for the selected physiological model was calculated as 63 mL/m using the Brody equation [$VCO_2$=5.56 x ($PBW^{1.05}$)] [4]. To achieve better $CO_2$ diffusion in the physiological lung, different $CO_2$ models were tested. For the closest comparison between the natural flow-volume curve and the standard volumetric capnograph graphics loop, we selected a ring-shaped diffuser as the most appropriate. After stabilizing both the lung mechanics and end-tidal $CO_2$ ($EtCO_2$) values on the patient model, the model was ventilated using two different breathing circuits (HAMILTON-BC8022 for pediatrics DSi and HAMILTON-BC8010 for neonates, Hamilton Medical AG, Bonaduz, Switzerland). To be able to compare the effect of RR, we reached the same minute ventilation during ventilation by using a volume-targeted, pressure-regulated mechanical ventilation mode (APV-CMV). The study design was crossover. Sequencing from opaque envelopes prepared before each measurement was used for randomization of the measurements. When the baseline RR was lower and RR increased after crossing over, the expired VT target was decreased accordingly. Measurements were repeated five times in accordance with the crossover study design. Values for Cs, Rs, $EtCO_2$, PEEP, peak inspiratory pressure (PIP), inspiratory time (Ti), and expiratory time (Te), and pressure support (PS) for each breathing circuit were taken as the mean and standard deviation (SD) of repeated measurements. For the measurement of $EtCO_2$ values, we used two different methods [5,6] and took the mean of these two measurement methods. After completing this first group of tests with different breathing circuits, the circuit with greater dead space was selected for the further testing. In the second group of tests, none of the parameters of the phsyiological lung model were changed, however RR was increased by 30% from the initial group. During all study phases, ventilation variables were measured breath-by-breath and downloaded from the ventilator's RS32 communication port using a memory card. The data was in binary form; therefore, it was converted to text files by the clinician and then transferred to a statistical analysis program. As this is a bench study, approval from the ethics committee was waived and only institutional permission was obtained.

### Statistical Analysis

The data acquired during the crossover phases were assessed for the distribution; therefore, the continuous data were expressed either in mean and SD, or median and interquartile range. The Wilcoxon test was used to analyze the data. A p-value of less than 0.05 was considered statistically significant for all comparisons.

## RESULTS

Among the respiratory circuits tested in first model, Dsi1 was measured as 22.5 mL and Dsi2 as 17.4 mL. Both Dsi differences (Dsi1-Dsi2=ΔDSi)=14 mL were calculated. There was no statistically significant difference between the Cs values reached in both cases (Cs1=1.07 mL/cm$H_2O$/kg, Cs2=1.1 mL/cm $H_2O$/kg; p=0.78). However, the resistance of the smaller breathing circuit was higher than the largerone (Rs1=18.4 cm $H_2O$/L/s, Rs2=21.9 cm $H_2O$/L/s; p=0.012). Conversely, the V'alv value measured by the smaller respiratory set was higher compared to the value measured with the larger set (V'alv1=1.95±0.2 L/m, V'alv2=1.84±0.3 L/m). As the other respiratory parameters (RR, Ti, Te, PS, PEEP, PIP) were not changed, there was no statistically significant difference between them.

In the second group of tests, RR was increased by 30% from 30 b/min to 39 b/min. This resulted in a decrease in Ti and Te. In addition, VTe was decreased to 6.4 mL in order to compare the DS effect. By decreasing Vte to lower values, we were able to keep the minute ventilation equal in both phases of the second group of tests. V'alv value measured with the higher DSi (V'alv3) was 1.63±0.5 L/m, whereas V'alv4 was measured as 1.84±0.3 L/m while using the other circuit with smaller DSi.

## DISCUSSION

In our study, we demonstrated that the increase in RR resulted in a decrease in V'alv. This is because the RR is a multiplier for V'alv, which must be subtracted from the total minute ventilation. Considering the normal RR for younger children is higher than for older ones or adults, this effect will be more prominent in younger patients [7]. During ventilation with the pediatric circuit, V'alv decreased from 1.84±0.3 L/m to 1.63±0.5 L/m when we increased the rate from 30 to 39. Similarly when RR was increased with the same percentage during ventilation with the neonatal circuit, V'alv decreased from 1.95±0.3 L/m to 1.83±0.5 L/m. This is clear evidence of the fact that for both circuits, an increase in RR caused a decrease in V'alv. Another reason for the decrease in V'alv is the other multiplier of the DS ventilation, which is DSi itself. The increase in DSi values in the breathing circuit also caused a decrease in the V'alv value in the model during our study. Simply by changing the DSi by keeping RR at 30, V'alv decreased from 1.95±0.2 L/m to V'alv2=1.84±0.3 L/m ($p<0.001$) in the neonatal circuit. When RR was kept at 39 b/m, V'alv decreased from 1.84±0.3 L/m to 1.63±0.5 L/m ($p<0.001$). To achieve the same V'alv in the model, it is necessary to increase either RR or VT, similar as the clinician would do [5,8]. However, particularly the increases in RR cannot affect V'alv values linearly [9]. For this reason, clinicians should either use breathing sets with a low DSi value or avoid using fittings and heat and moisture exchanger filters that are likely to increase DSi when using a low DSi is not possible. DSi will be higher in neonatal and infant patients than in adult or adolescent populations, and the increase will be greater the smaller the patient is. In addition, younger patients should be ventilated with a higher RR because of their physiology. According to the current guidelines in pediatric mechanical ventilation, a higher RR should be selected in restrictive lung disease conditions [10,11].

### Study Limitations

The one main limitation of this study is that rather than providing clinical data, we tested the bench settings optmized to mimic the physiology and respiratory parameters of a healthy, male, 1-year old child. Nevertheless, these settings may help us to understand the mechanisms of real physiology with reproducibilty of the specific, predefined test conditions.

## CONCLUSION

Our results demonstrated that a higher RR resulted with lower V'alv. Particularly in the smaller and/or the restrictive lung disease group, clinicians should therefore be more alert in terms of both the increased RR and the increased percentage of DSi that results with a decrease in V'alv.

### Ethics

**Ethics Committee Approval:** As this is a bench study, approval from the ethics committee was waived and only institutional permission was obtained.

**Informed Consent:** Informed consent is not required.

**Peer-review:** Internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: G.C., Concept: G.C., S.T., E.S., P.S., H.A., Design: G.C., M.Ç., E.S., P.S., H.A., Data Collection and/or Processing: G.C., G.A., S.T., F.S., Ö.S.S., Analysis and/or Interpretation: G.C., S.T., M.Ç., E.S., P.S., Ö.S.S., H.A., Literature Search: G.C., G.A., S.T., M.Ç., E.S., F.S., P.S., Ö.S.S., H.A., Writing: G.C., G.A., S.T., M.Ç., P.S., Ö.S.S., H.A.

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

**Financial Disclosure:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Gokhan Ceylan is the only author with a conflict of interest; he is employed by Hamilton Medical AG in the Department of Medical Research. None of the other authors have a conflict of interest. The manuscript has not been previously published elsewhere and is not currently under consideration for publication elsewhere, including online.

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**DOI:** 10.4274/buchd.galenos.2022.87533
J Dr Behcet Uz Child Hosp 2022;12(2):120-127

Original Article

# Predictive Factors of Organ Involvement in Childhood Henoch-Schonlein Purpura

## *Henoch-Schönlein Purpuralı Çocuklarda Organ Tutulumunu Belirleyen Faktörler*

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## ABSTRACT

**Objective:** Henoch-Schonlein purpura (HSP) is the most common vasculitis of childhood, presenting with immunoglobulin A-dominant immune deposits. Unless there is an organ involvement, the prognosis of HSP is excellent. In this study, we aimed to evaluate clinical and laboratory risk factors for organ involvement in patients with HSP.

**Method:** Our study sample consisted of 95 children with HSP and 75 healthy controls. Clinical and laboratory parameters recorded at the first admission to the hospital were retrospectively evaluated. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated based on the complete blood counts.

**Results:** Leucocyte, neutrophil and lymphocyte counts, C-reactive protein, PLR, platelet distribution width, and NLR were significantly higher in the HSP group than in the control group ($p=0.008$, $p<0.001$, $p=0.003$, $p<0.001$, $p=0.002$, $p<0.001$, $p=0.002$, and $p<0.001$, respectively). In the HSP group, NLR, PLR and lymphocyte were significantly higher among the patients with renal involvement and those with gastrointestinal involvement. Neutrophil levels were correlated with renal involvement. Additionally, the older age onset of the disease and elevated antistreptolysin O (ASO) levels were associated with renal involvement.

**Conclusion:** NLR, PLR and lymphocyte counts may be used as inflammatory indicators of renal and gastrointestinal involvement in HSP. In addition, the neutrophil count is associated with renal involvement. The older age onset of HSP and elevated ASO levels are risk factors for renal involvement in HSP.

**Keywords:** Child, gastrointestinal involvement, Henoch-Schonlein purpura, renal involvement

## ÖZ

**Amaç:** Henoch-Schönlein purpurası (HSP) çocukluk çağının en sık görülen vaskülitidir ve immünoglobulin A'nın baskın olduğu immün birikintilerle kendini gösterir. Organ tutulumu olmadıkça HSP'nin prognozu mükemmeldir. Bu çalışmada HSP'li hastalarda organ tutulumu için klinik ve laboratuvar risk faktörlerini değerlendirmeyi amaçladık.

**Yöntem:** Çalışma örneklemi HSP'li 95 çocuk ve 75 sağlıklı kontrolden oluştu. Hastaneye ilk başvuruda kaydedilen klinik ve laboratuvar parametreleri geriye dönük olarak değerlendirildi. Nötrofil-lenfosit oranı (NLR) ve trombosit-lenfosit oranı (PLR), tam kan sayımlarına göre hesaplandı.

**Bulgular:** Lökosit, nötrofil ve lenfosit sayıları, C-reaktif protein, PLR, trombosit dağılım genişliği ve NLR, HSP grubunda kontrol grubuna göre anlamlı olarak daha yüksekti ($p=0,008$, $p<0,001$, $p=0,003$, $p<0,001$, $p=0,002$, $p<0,001$, $p=0,002$ ve $p<0,001$, sırasıyla). HSP grubunda böbrek ve gastrointestinal tutulumu olan hastalarda NLR, PLR ve lenfosit anlamlı olarak daha yüksekti. Nötrofil seviyeleri böbrek tutulumu ile korele idi. Ek olarak, hastalığın ileri yaşta başlaması ve yüksek antistreptolizin O (ASO) seviyeleri böbrek tutulumu ile ilişkiliydi.

**Sonuç:** NLR, PLR ve lenfosit sayıları, HSP'de renal ve gastrointestinal tutulumun enflamatuvar göstergeleri olarak kullanılabilir. Ek olarak, nötrofil sayısı böbrek tutulumu ile ilişkilidir. HSP'nin daha ileri yaşta başlaması ve yüksek ASO seviyeleri, HSP'de böbrek tutulumu için risk faktörleridir.

**Anahtar kelimeler:** Çocuk, gastrointestinal tutulum, Henoch-Schonlein purpurası, böbrek tutulumu

**Received:** 16.12.2021
**Accepted:** 31.01.2022

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**Cite as:** Akyol Önder EN, Ertan P. Predictive Factors of Organ Involvement in Childhood Henoch-Schonlein Purpura. J Dr Behcet Uz Child Hosp. 2022;12(2):120-127

**The study had been presented in a scientific meeting (11th International Pediatric Nephrology E-Congress, 4-5 September 2021) as a poster.**

## INTRODUCTION

Henoch-Schonlein purpura (HSP) is the most frequently seen pediatric leukocytoclastic vasculitis characterised by the deposits of immunoglobulin A (IgA) in the small vessel walls of the skin, joint, gastrointestinal (GI) system and kidney [1]. Its incidence is 3-26.7 cases per 100,000 people [2,3]. Palpable purpura is reported in all patients, while joint involvement (JI) is observed in 60-80%, GI involvement in >50%, and renal involvement in 40-50% of patients [4,5]. Although HSP is usually self-limited, GI bleeding and HSP nephritis can be life-threatening conditions during the course of the disease [5]. It is important to assess the risk factors of organ involvement in HSP for the prediction of the disease prognosis.

HSP-GI involvement with severe GI bleeding and intestinal perforation represents the major risk in the acute period of HSP [6]. The manifestation of renal involvement in HSP varies from microscopic haematuria and intermittent proteinuria to severe nephrotic syndrome, and long-term outcome of HSP depends on the presence of renal involvement [7,8]. Approximately, 1-2% of the patients with renal involvement may progress to renal failure [8]. Therefore, it is crucial to predict organ involvement early for effective management and follow-up [9]. Thus, there is a need for reliable prognostic markers to predict organ involvement in HSP. Additionally, the predictive parameters of organ involvement in HSP should be identified.

We aimed both to determine the best prognostic markers in predicting organ involvement in children with HSP, and investigate the predictive factors for GI and renal involvement in HSP.

## MATERIALS and METHODS

After obtaining the ethical approval from Manisa Celal Bayar University Ethics Committee (approval number: 20.478.486/1044, date: 01.12.2021), the study was conducted in our nephrology division with children diagnosed with HSP between December 2010 and December 2020 according to the EULAR/PRINTO/PRES diagnostic criteria [10]. The control group was consisted of healthy patients admitted to our unit without any inflammatory symptoms. Children with any inflammatory, immunologic or other chronic disorder and those taking steroids or other medication(s) were excluded.

GI involvement was defined as the presence of occult blood in stool, melena, or haematochezia. Renal involvement was identified based on the existence of one of the conditions: haematuria (>5 red cells per microscopic field under 40X magnification), proteinuria (a spot urine protein/creatinine ratios of >0.5 mg/mg and >0.2 mg/mg in children aged <2 , and ≥2 years of age, respectively), nephrotic syndrome (hypoalbuminemia, hyperlipidaemia, nephrotic range proteinuria with a protein/creatinine ratio of >2 mg/mg), and/or nephritic syndrome (haematuria, hypertension, nephritic range proteinuria with a protein/creatinine ratio of 0.2-2 mg/mg) [3].

The patients' medical history, demographic characteristics, and initial laboratory data were retrospectively obtained from patient files and the hospital records. Haemoglobin levels, leucocyte, lymphocyte, neutrophil, monocyte and platelet counts, platelet indices [mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW)], and red cell distribution width (RDW), C-reactive protein (CRP) and anti-streptolysin-O (ASO) levels were recorded at the time of the patients' first admission to the hospital. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are low-cost and useful biomarkers for predicting severity and outcome of many inflammatory diseases including HSP [11-18]. NLR and PLR are calculated using the complete blood counts. Immunoglobulin levels and complement components C3 and C4 were noted. Urinalysis and faecal occult blood tests were also recorded. Renal biopsies were performed in 23 patients with nephrotic- range proteinuria or prolonged nephritis.

### Statistical Analysis

Frequencies and percentages were computed for qualitative parameters. Continuous data were demonstrated as mean ± standard deviation. The distributions of data were assessed with histogram, and the Shapiro-Wilk or Kolmogorov-Smirnov test. The chi-square test was performed for testing relationships on qualitative parameters. Mann-Whitney U test or Student's t-test were utilized to measure the difference in continuous data. The Pearson and Spearman analyses were carried out to measure the correlation between the variables. The association of laboratory parameters with renal and GI involvements in HSP was measured by the receiver operating characteristic (ROC) curve analysis. Differences were determined as significant at a p-value of <0.05 in all analyses. The data was conducted with SPSS 22.0.

## RESULTS

This study involved ninety-five patients with HSP and 75 healthy children. The demographic factors and laboratory variables are shown in Table 1. The HSP group contained 39 female and 56 male children with a mean age of 14.9±4.1 years. The control group comprised 40 female and 35 male children with a mean age of 13.6±2.5 years. No significant difference was detected among the groups in terms of age and gender (p=0.07 and p=0.11, respectively). The leucocyte, lymphocyte and neutrophil counts and the CRP, PLR, PDW and NLR levels were significantly higher in the HSP group (p=0.008, p=0.003, p<0.001, p<0.001, p=0.002, p<0.001, and p<0.001, respectively). No significant difference among the groups were found in terms of haemoglobin MPV, PCT and RDW values, monocyte, and platelet counts, (Table 2). Upper respiratory tract infection (URTI) was present in 39 (41%) children.

In the HSP group, there were 57 (60%) patients with renal involvement. In five (8.8%) of these patients, renal involvement was identified at the time of diagnosis. Among the remaining patients, renal involvement was found within the first month in 43 (75.4%), first to third months in five (8.8%), fourth to sixth months in three (5.3%), and the first year in one (1.8%) case. Patients presented with isolated haematuria (n=15), isolated proteinuria (n=10), nephritic syndrome (n=10), and nephrotic syndrome (n=23). Table 2 presents the comparison of the demographic characteristics and laboratory data between the patients with and without renal involvement in the patients with HSP. The mean age at the onset of the disease, neutrophil and lymphocyte counts, and PDW, PLR, NLR and ASO levels were significantly higher among the patients with renal involvement compared to those without (p=0.001, p=0.011, p=0.003, p=0.028, p=0.015, p<0.001, and p=0.036, respectively). However, no statistically significant difference was detected between these two subgroups in terms of gender and, leucocyte, monocyte, platelet counts; CRP, haemoglobin, MPV, PCT and RDW levels (p=0.15, p=0.27, p=0.43, p=0.64, p=0.6, p=0.86, p=0.93, p=0.54, and p=0.36, respectively). There was also no significant difference among the patients with and without renal involvement in terms of presenting signs, such as abdominal pain, joint, and GI or scrotal involvement (p=0.49, p=0.25, p=0.7, and p=0.2, respectively). We determined positive correlations between the presence of renal involvement and neutrophil, lymphocyte counts, PDW, PLR, NLR and C3 values (r=0.24, p=0.017; r=0.32, p=0.02; r=0.22, p=0.03; r=0.25, p=0.015; r=0.36, p<0.001; and r=0.23, p=0.025, respectively). Leucocyte counts was negatively associated with renal involvement (r=-0.084 and p=0.4).

Twenty-two (23%) patients with HSP had GI involvement. The data of the children with and without

**Table 1. Demographic characteristics and laboratory parameters of the HSP and control groups**

| Characteristics | HSP group (n=95) | Control group (n=75) | p-value |
|---|---|---|---|
| Age at onset (years) (mean ± SD) | 14.9±4.1 | 13.6±2.5 | 0.07 |
| Gender (F/M) | 39/56 | 40/35 | 0.11 |
| CRP (mg/dL) | 2.3±6.3 | 0.24±0.2 | **<0.001** |
| Haemoglobin (g/dL) (mean ± SD) | 12.3±1.4 | 12.6±1.4 | 0.35 |
| Leukocytes ($10^3$/μL) (mean ± SD) | 11.5±10 | 8.9±3.9 | **0.008** |
| Neutrophils ($10^3$/μL) (mean ± SD) | 6.7±3.1 | 4.4±2.4 | **<0.001** |
| Lymphocytes ($10^3$/μL) (mean ± SD) | 2.9±1.2 | 3.7±2.2 | **0.003** |
| Monocytes ($10^3$/μL) (mean ± SD) | 0.6±0.3 | 0.6±0.2 | 0.57 |
| Platelets ($10^3$/μL) (mean ± SD) | 355±110 | 340±80 | 0.3 |
| MPV (fL) (mean ± SD) | 8.3±1.1 | 8.6±0.95 | 0.09 |
| PCT (mean ± SD) | 0.29±0.1 | 0.29±0.06 | 0.9 |
| PDW (mean ± SD) | 15.9±0.7 | 15.6±0.43 | **<0.001** |
| PLR (mean ± SD) | 139±65 | 110±47 | **0.002** |
| NLR (mean ± SD) | 2.8±2.4 | 1.56±1 | **<0.001** |
| RDW (%) (mean ± SD) | 13.6±1.4 | 15.1±1.3 | 0.18 |

HSP: Henoch-Schonlein purpura, SD: Standard deviation, F: Female, M: Male, CRP: C-reactive protein, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, RDW: Red cell width distribution

GI involvement are presented in Table 3. The PLR and NLR values were found to be higher in the HSP subgroup of patients with GI involvement (p=0.003 and p=0.036, respectively). No significant difference was detected between the patients with and without GI involvement in terms of age, gender, and leukocyte, lymphocyte, neutrophil, monocyte, and platelet counts; CRP, haemoglobin, MPV, PCT, PWD and RDW values (p=0.5, p=0.6, p=0.42, p=0.41, p=0.88, p=0.92, p=0.72, p=0.63, p=0.86, p=0.14, p=0.82, p=0.2, and p=0.12, respectively). The presence of GI involvement was found positively associated with PLR and NLR (r=0.310, p=0.002 and r=0.217, p=0.035, respectively) and negatively associated with leucocyte counts (r=-0.229, p=0.007).

JI was detected in 34 (36%) of patients with HSP mostly involving ankles. Twenty-three (68%) patients with JI had renal, 9 (26%) of them had GI involvement. When the patients with and without JI evaluated, no statistically significant difference was determined between presenting symptoms of HSP, such as abdominal pain, renal, GI or scrotal involvement (p=0.07, p=0.26, p=0.6, p=0.3, respectively). Also, no statistically significant difference was assessed between these two subgroups in terms of age, gender and leucocyte, neutrophil, lymphocyte, monocyte, platelet counts, and CRP, haemoglobin, MPV, PCT, PWD, PLR, NLR and RDW levels (p=0.66, p=0.68, p=0.68, p=0.25, p=0.78, p=0.47, p=0.67, p=0.56, p=0.16, p=0.67, p=0.66, p=0.16, p=0.17, p=0.29, and p=0.66, respectively).

Figure 1 and Table 4 present the area under the curve (AUC) values acquired from the ROC analyses of the patients with RI in the HSP group. According to this analysis, NLR, lymphocyte count, PLR, and neutrophil counts had AUC values of 0.725, 0.679, 0.658, and 0.634, respectively (p<0.001, p=0.003, p=0.009 and p=0.028, respectively).

Figure 2 and Table 4 show the AUC values obtained from the ROC analysis of the cases with GI involvement in the HSP group. The results revealed that PLR, lymphocyte count, and NLR had AUC values of 0.709,

**Table 2. Demographic characteristics and laboratory parameters of the patients with and without RI in the HSP group**

| Characteristics | RI subgroup (n=57) | Non-RI subgroup (n=38) | p-value |
|---|---|---|---|
| Age at onset (years) (mean ± SD) | 10.3±3.4 | 7.8±3.5 | **0.001** |
| Gender (F/M) | 20/37 | 19/19 | 0.15 |
| CRP (mg/dL) (mean ± SD) | 2±2.4 | 3.3±9.6 | 0.6 |
| Haemoglobin (g/dL) (mean ± SD) | 12.4±1.5 | 12.3±1.3 | 0.86 |
| Leukocytes ($10^3$/μL) (mean ± SD) | 12.4±13 | 10±3.4 | 0.27 |
| Neutrophils ($10^3$/μL) mean ± SD) | 7.3±3.4 | 5.7±2.3 | **0.011** |
| Lymphocytes ($10^3$/μL) mean ± SD) | 2.6±0.9 | 3.3±1.3 | **0.003** |
| Monocytes ($10^3$/μL) (mean ± SD) | 0.63±0.3 | 0.67±0.3 | 0.43 |
| Platelet counts ($10^3$/μL) (mean ± SD) | 350±109 | 361±113 | 0.64 |
| MPV (fL) (mean ± SD) | 8.3±1.16 | 8.3±0.96 | 0.93 |
| PCT (mean ± SD) | 0.28±0.1 | 0.29±0.08 | 0.54 |
| PDW (mean ± SD) | 16.1±0.6 | 15.8±0.7 | **0.028** |
| PLR (mean ± SD) | 151±72 | 120±49 | **0.015** |
| NLR (mean ± SD) | 3.4±2.9 | 1.9±0.9 | **<0.001** |
| RDW (%) (mean ± SD) | 13.6±1.6 | 13.7±1.1 | 0.36 |
| Elevated IgA | 8/49 | 2/36 | 0.2 |
| Decreased C3 | 6/54 | 5/33 | 0.7 |
| Elevated ASO | 22/36 | 7/31 | 0.036 |
| Abdominal pain | 20/37 | 16/22 | 0.49 |
| Joint involvement | 23/34 | 11/27 | 0.25 |
| GI involvement | 14/43 | 8/30 | 0.7 |
| Scrotal involvement | 5/52 | 3/38 | 0.2 |

RI: Renal involvement, HSP: Henoch-Schonlein purpura, SD: Standard deviation, F: Female, M: Male, CRP: C-reactive protein, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, RDW: Red cell width distribution, IgA: Immunoglobulin A, ASO: Anti-streptolysin O, GI: Gastrointestinal involvement

**Table 3. Demographic characteristics and laboratory parameters of the HSP-GI and HSP-non-GI groups**

| Characteristics | HSP-GI patients (n=22) | HSP-non-GI (n=73) | p-value |
|---|---|---|---|
| Age at onset (years) | 9.7±3.5 | 9.2±3.7 | 0.5 |
| Gender (F/M) | 8/14 | 31/42 | 0.6 |
| CRP (mg/dL) (mean ± SD) | 2.3±3.4 | 2.3±7 | 0.63 |
| Hemoglobin (g/dL) (mean ± SD) | 12.5±1.8 | 12.3±1.3 | 0.86 |
| Leukocytes ($10^3$/μL) (mean ± SD) | 9±3.8 | 12±11 | 0.42 |
| Neutrophils ($10^3$/μL) (mean ± SD) | 6.6±3 | 6.7±3.1 | 0.88 |
| Lymphocytes ($10^3$/μL) (mean ± SD) | 2.4±1.3 | 3±1.1 | 0.41 |
| Monocytes (mean ± SD) ($10^3$/μL) | 0.64±0.4 | 0.65±0.31 | 0.92 |
| Platelets ($10^3$/μL) (mean ± SD) | 363±139 | 352±100 | 0.72 |
| MPV (fL) (mean ± SD) | 8.6±1.3 | 8.2±1 | 0.14 |
| PCT (mean ± SD) | 0.29±0.1 | 0.29±0.07 | 0.82 |
| PWD (mean ± SD) | 15.8±0.8 | 16±0.6 | 0.2 |
| PLR (mean ± SD) | 172±65 | 129±62 | **0.003** |
| NLR (mean ± SD) | 3.2±1.8 | 2.65±2.6 | **0.036** |
| RDW (%) (mean ± SD) | 13.3±0.9 | 13.7±1.5 | 0.12 |
| Elevated IgA | 2/20 | 10/63 | 0.08 |
| Decreased C3 | 4/18 | 9/66 | 0.7 |
| Elevated ASO | 8/14 | 21/52 | 0.5 |
| Abdominal pain | 14/8 | 22/51 | **0.049** |
| Joint involvement | 9/13 | 25/48 | 0.57 |
| Renal involvement | 14/8 | 43/30 | 0.7 |
| Scrotal involvement | 2/20 | 6/67 | 0.3 |

HSP-GI: Henoch-Schonlein purpura-gastrointestinal involvement, HSP-non-GI: Henoch-Schonlein purpura without gastrointestinal involvement, SD: Standard deviation, F: Female, M: Male, CRP: C-reactive protein, MPV: Mean platelet volume, PCT: Platecrit, PWD: Platelet width distribution, PLR: Platelet/lymphocyte ratio, NLR: Neutrophil/lymphocyte ratio, RDW: Red cell width distribution, IgA: Immunoglobulin A, ASO: Anti-streptolysin O

**Table 4. Receiver operating characteristic analysis for HSP-RI and HSP-GI**

| Test result variable (s) | Area under the curve | p-value | 95% Confidence interval | |
|---|---|---|---|---|
| | | | Lower bound | Upper bound |
| HSP-RI | | | | |
| NLR | 0.725 | **<0.001** | 0.624 | 0.827 |
| Lymphocyte | 0.679 | **0.003** | 0.575 | 0.771 |
| PLR | 0.658 | **0.009** | 0.545 | 0.771 |
| Neutrophil | 0.634 | **0.028** | 0.522 | 0.746 |
| PWD | 0.605 | 0.086 | 0.489 | 0.721 |
| HSP-GI | | | | |
| PLR | 0.709 | **0.003** | 0.562 | 0.856 |
| Lymphocyte | 0.691 | **0.004** | 0.587 | 0.781 |
| NLR | 0.644 | **0.042** | 0.512 | 0.776 |

HSP-RI: Henoch-Schonlein Purpura-Renal Involvement, HSP-GI: Henoch-Schonlein Purpura-Gastrointestinal Involvement, PWD: Platelet width distribution, PLR: Platelet/lymphocyte ratio, NLR: Neutrophil/lymphocyte ratio

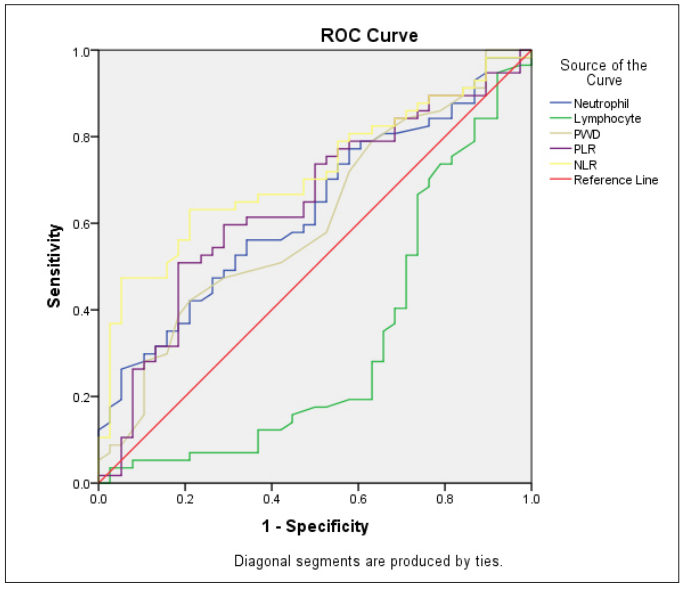


**Figure 1.** Area under the curve values of the ROC analysis of the patients with renal involvement in the HSP group

ROC: Receiver operating characteristic, HSP: Henoch-Schonlein purpura, PWD: Platelet distribution width, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio

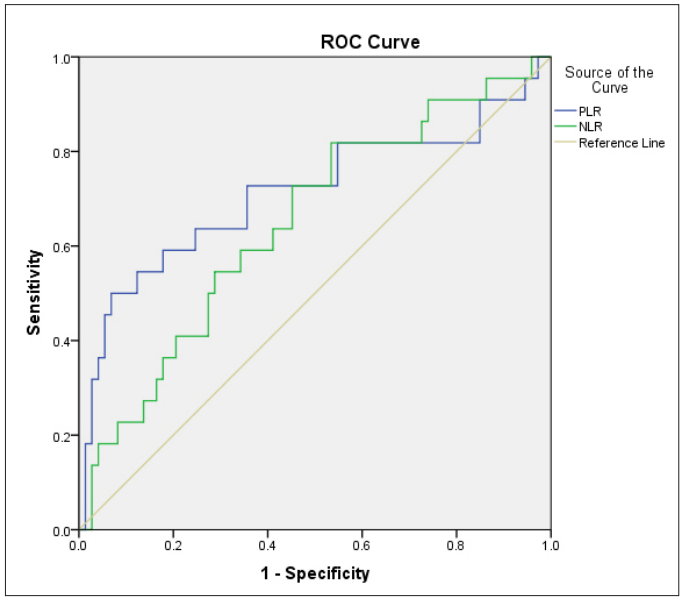


**Figure 2.** Area under the curve values of the ROC analysis of the patients with gastrointestinal involvement in the HSP group

ROC: Receiver operating characteristic, HSP: Henoch-Schonlein purpura, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio

0.691, and 0.644, respectively (p=0.003, p=0.004, and p=0.042, respectively).

## DISCUSSION

HSP is the commonest pediatric vasculitis of presenting with the accumulation of IgA within the walls of small vessels [1,2]. GI, and renal involvement indicate acute and chronic course of the disease, respectively [4,19]. The etiopathogenesis of HSP has not yet been well defined; however, the abnormal expression of inflammatory cytokines is the most associated factor [11]. Neutrophils, lymphocytes and platelets are key mediators in the inflammatory pathways of HSP [11,12]. PLR and NLR are low-cost and simple methods used to estimate the severity and prognosis of several inflammatory diseases [13-15] and chronic diseases, such as prostate, breast, colon and renal cancers [16]. NLR and PLR have also been described to reflect the activity of rheumatoid arthritis and lupus erythematosus [13]. In addition, NLR is considered as a useful marker to evaluate Behçet's disease activity [14]. Platelets are likely to contribute to inflammation, and therefore platelet indices are used in various diseases, such as cardiovascular disease, Crohn disease, diabetic nephropathy, rheumatoid arthritis, and ankylosing spondylitis [17,18]. Recently, neutrophil-, lymphocyte- and platelet-associated markers; e.g., NLR, PLR, MPV and PDW have been increasingly reported to serve as the predictors of GI and renal involvement in HSP [9,12,20-23]. In the current study, we planned to determine the predictive factors for organ involvement in HSP and define possible biomarkers correlated with the presence of GI, and renal involvement.

Renal involvement is reported in a wide range (20-80%) of patients with HSP [3]. Consistent with the literature, renal involvement was detected in 57 (60%) of the patients with HSP in this study. It has been suggested that renal involvement is associated with the more severe form of HSP and more frequently seen among older children [20,21]. We also found a significantly higher age at the time of diagnosis among the children in the HSP group with renal involvement compared to those without. Kim et al. [20] and Karadağ et al. [24] reported NLR to be higher in patients with renal involvement. Ekinci et al.[25] reported elevated neutrophil and NLR in biopsy-proven nephritis. Yakut et al. [26] observed significantly elevated MPV in HSP cases presenting with renal involvement. We have shown that lymphocyte, and neutrophil counts, NLR, and PLR were significantly higher in the HSP subgroup with renal involvement. In the ROC analysis, the AUC values indicated that NLR was the parameter that was

most related to renal involvement. ASO, an indicator of streptococcal infection, is a specific streptolysin antibody. Most patients with HSP are reported to have a history of URTI, especially streptococcal infection with high levels of ASO [27]. Chan et al. [21] determined that relatively higher ASO levels were related with renal involvement in HSP. We also reported elevated ASO levels in children with renal involvement in our HSP group. However, the mechanism causing the relationship between streptococcal infection and renal involvement in HSP is still debated [28].

GI involvement is the most severe acute complication of HSP. In this study, 22 (23%) patients with GI involvement had HSP. Ekinci et al. [25], Yakut et al. [26], and Makay et al. [29] evaluated that NLR was associated with GI bleeding. Karadağ et al. [24] observed that NLR, PLR, neutrophil and platelet levels were elevated in HSP cases with GI involvement. Gayret et al. [30] found that both PLR and NLR were significantly higher in children with HSP presenting with GI involvement. We also observed that PLR and NLR were with GI involvement in our HSP group.

JI was detected in 34 (36%) patients with HSP. This ratio was lower than those reported in other studies [4,19]. NLR and PLR were not found to be associated with JI in this research.

### Study Limitations

The limitations of the current study are that its single-centre and retrospective nature and small sample size.

## CONCLUSION

In conclusion, NLR, PLR and lymphocyte count may be useful parameters in predicting renal and GI involvement in HSP. In addition, neutrophil counts are related to renal involvement. Its onset at a relatively older age and elevated ASO levels are risk factors for renal involvement. When we evaluated platelet indices, we found that PDW was significantly higher in the HSP group compared to the control group and in the subgroup with renal involvement compared to the patients without. However, PDW was not correlated with GI involvement. We also determined that MPV was not related to organ involvement in HSP. Further prospective, large-scale and multicentre studies are needed to corroborate our findings.

### Ethics

**Ethics Committee Approval:** The study was approved by the Manisa Celal Bayar University Ethics Committee (approval number: 20.478.486/1044, date: 01.12.2021).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: E.N.A.Ö., P.E., Concept: E.N.A.Ö., P.E., Design: E.N.A.Ö., P.E., Data Collection and/or Processing: E.N.A.Ö., Analysis and/or Interpretation: E.N.A.Ö., P.E., Literature Search: E.N.A.Ö., P.E., Writing: E.N.A.Ö.

**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.2022.36459
J Dr Behcet Uz Child Hosp 2022;12(2):128-135

Original Article

# Nutritional Status of Pediatric Intensive Care Patients with Chronic Disease

## *Kronik Hastalığı Olan Çocuk Yoğun Bakım Hastalarının Beslenme Durumları*

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## ABSTRACT

**Objective:** This study aims to evaluate the relationship between enteral nutrition and mortality in children with chronic diseases who need to be hospitalized in the pediatric intensive care unit (PICU).

**Method:** The data of the patients who were admitted to intensive care between January 2014 and December 2019 were retrospectively supplied from the hospital database. Demographic data, the presence of underlying diseases, the diagnoses during intensive care admission, the history of previous hospitalization, homecare requirement, the presence of gastrostomy, feeding type during homecare before hospitalization, the amount and type of nutrition were recorded.

**Results:** A total of 186 patients hospitalized in the PICU due to an acute critical illness and with an underlying chronic disease were included in the study. The median age was 49 (17.75-104.5) months, it was observed that 53.8% of the patients were required homecare. The most common chronic disease was central nervous system pathologies accompanied by neurological impairment in swallowing functions (37.3%), while the most common cause of the acute disease was lower respiratory tract infections (48.9%). The overall mortality was 9.7%, and it was found that the majority of the patients who were died were the patients requiring homecare and fed with nasogastric tube at home (p=0.002, p=0.014).

**Conclusion:** It is observed that patients with percutaneous endoscopic gastrostomy are relatively low, hence feeding tube is frequently preferred in children with dysphagia during homecare. However, it is demonstrated that the feeding tube is an independent risk factor for mortality.

**Keywords:** Nutrition, home care, pediatric intensive care unit, mortality

## ÖZ

**Amaç:** Çocuk yoğun bakım yatış (ÇYBÜ) ihtiyacı gösteren, kronik hastalığa sahip çocuklarda, nutrisyonel denge ve mortalite arasındaki ilişkinin araştırılması planlamıştır.

**Yöntem:** Ocak 2014-Aralık 2019 tarihleri arasında yoğun bakımda yatan ve kronik hastalığı olan tüm hastaların verileri geriye dönük olarak tarandı. Yaş, cinsiyet, kilo, boy, büyüme çizelgeleri, vücut kitle indeksleri, altta yatan hastalıkların varlığı, yoğun bakıma yatış sırasındaki tanılar, önceki yatış öyküsü, evde bakım gereksinimi, gastrostomi varlığı, yatış öncesi evde bakım sırasında beslenme şekli, beslenme miktarı ve türü kaydedildi.

**Bulgular:** Çalışmaya altta yatan hastalığı olan ve akut ciddi hastalık nedeniyle yoğun bakıma yatırılan 186 hasta dahil edildi. Yaş ortancasının 49 (17,75-104,5) ay olduğu çalışmamızda %53,8 hastanın evde bakıma muhtaç olduğu görüldü. Alt solunum yolu enfeksiyonları yatışların en sık akut sebebi iken; santral sinir sitemi patolojilerine eşlik eden yutma disfonksiyonuyla giden nörolojik hastalıklar en sık altta yatan kronik hastalıklar olarak göze çarptı (%48,9, %37,3). Mortalitenin %9,7 olduğu çalışmada, kaybedilenlerin evde bakıma muhtaç, nazogastrik beslenmenin yapıldığı çocuklardan oluştuğu gözlendi (p=0,002, p=0,014).

**Sonuç:** Kronik hastalığa sahip çocuklarda, günlük nutrisyonel dengenin yetersiz kalması mortalite ile ilişkilidir. Tek tipte sulu gıdalarla beslenme mortalite için bağımsız risk faktörüdür. Multidisipliner yaklaşımla nutrisyon ekiplerinin kurulması ve dengeli nutrisyonun sağlanması sağ kalımı artıracaktır.

**Anahtar kelimeler:** Beslenme, evde bakım, çocuk yoğun bakım ünitesi, mortalite

**Received:** 30.05.2021
**Accepted:** 31.01.2022

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**Cite as:** Saraç Sandal Ö, Ongun EA, Ceylan G. Nutritional Status of Pediatric Intensive Care Patients with Chronic Disease. J Dr Behcet Uz Child Hosp. 2022;12(2):128-135

## INTRODUCTION

Chronic disease is defined as a condition that deviates from normal status or shows permanent disability, irreversible, as a consequence of pathological changes, special training for rehabilitation, nursing, observation and supervision for a long time [1]. Chronic disease prevalence is ranged from 1 to 2% and affects daily activities [2]. Diseases such as congenital anomalies, congenital heart diseases, chronic pulmonary diseases, hypoxic-ischemic encephalopathy, cerebral palsy, metabolic diseases, trauma-related long-term organ injuries (extremity paralysis, traumatic brain injury, etc.), chronic renal failure affecting various organs are considered as a part of chronic diseases. However, the medical care requirement of the patients might increase after the presence of a neuromotor disease resulted in bed ridden [1,2].

Nutritional problems observed in children with chronic diseases hold an important place in the medical care and treatment of these children as well. Studies have shown that the frequency of feeding problems in children with cerebral palsy is between 30 and 90% and the malnutrition rate is 90% [3]. It is known that malnutrition negatively affects morbidity and mortality by causing growth restriction. The most common feeding problems can be listed as gastroesophageal reflux (GER), oral-motor dysfunction, swallowing disorders and food refusal behaviors. Complications that may develop due to these problems are other negative factors affecting the quality of life [4]. These complications include inadequate food intake, development of malnutrition, immune system suppression secondary to malnutrition leading to infections, and aspiration (oral-motor dysfunction or GER). Life-threatening recurrent respiratory tract infections due to aspiration and rarely apnea requiring resuscitation can be observed. Protein and calorie requirements of children should be regulated depending on the age and activity of the children where growth and development must be followed closely in children with nutritional problems and the presence of chronic disease [5].

It is known that patients with neuromotor retardation frequently require pediatric intensive care unit (PICU) admission due to complications caused by the underlying chronic diseases that disrupt vital functions, severe infections and nutritional disorders. International guidelines recommended that oral or enteral nutrition without the presence of any contraindications should be initiated as soon as possible in critical children [4,5]. It has been determined that early trophic feeding improves the immune system through the lymphoid tissue of the intestinal mucosa and reduces mortality and morbidity. Guidelines suggest that the dietary intake of such children at home should be arranged according to the age, gender and daily activity of the patient after discharge from intensive care unit [6].

As the only tertiary pediatric care center within Sivas provincial borders, medical care is provided to all critically ill children requiring surgical and internal intensive care. Similarly with the literature, our clinical observations indicate that many critical patients whose caloric intake is limited during hospitalization are at risk for entering into a catabolic state and subsequently developing malnutrition that ultimately results in increased morbidity and mortality [7-9]. The primary goal of this study is to evaluate the dietary intake of children with chronic diseases who require intensive care unit admission and of those with growth retardation; providing effective and correct nutrition and taking preventive measures against malnutrition after discharge. The secondary aim of the study is to investigate whether the nutrition is adequate or not during intensive care unit stay and to establish our protocol for the nutrition of critically ill children within the department of our university.

## MATERIALS and METHODS

After obtaining Local Ethics Committee approval for the study from the Cumhuriyet University Clinical Research Ethics Committee (decision number: 2019-04/11, date: 17.04.2019), the data of the patients who were admitted to intensive care between January 2014 and December 2019 were retrospectively supplied from the hospital database. The authors anounced 'acute onset of disease' as the acute sickness that resulted in the PICU admission. Both acute onset of disease and chronic underlying conditions were collected from the hospital database. Demographic characteristics including age, gender, weight, height, previous hospitalizations, children in need of home-care or those with technology dependence (enteral nutrition via gastrostomy and home ventilation via tracheostomy) were assessed for analysis.

Antropometric evaluation consisted of the measurements of height-for-age Z score (HAZ), weight-for-age Z score (WAZ), weight-for-height Z-scores (WHZ) to assess the degree of nutritional status in the child [10]. Z score of body mass index (BMI): (calculation of weight in kilograms divided by height in meters squared) was also obtained for children older than five years

of age. Z score classification defined by World Health Organization (WHO) was categorized as normal nutrition: >-1 standart deviation score (SDS), mild undernultrition: SDS between -1 and -2, moderate undernutrition: SDS between -2 and -3, severe undernutrition: <-3 [11]. The group categorization was based on survival.

We got the height of the patients with supine length measurement. The patient was made to lie down supine. Using a flexible measuring tape the length between the vertex of the head and the heel was measured. The measurement was taken up to one decimal point. Although this measure is undereasy to do, it may be unreliable in patients with joint contractures. In those patient who had contractures we got four point method of measurement.

Nutrition data consisted of variables such as the route of nutrition (via nasogastric tubing or gastrostomy), the volume and type of daily nutrition (breast milk, enteral nutrition, aqueusfood, solid food prior to hospitalization. Nutritional status and basal caloric demand at home-care for each gender was calculated by the recommendations of The Food and Agriculture Organization/WHO/United Nations University Expert Consultation for Human Energy Requirements and The Society of Turkish Pediatric Gastroenterology, Hepatology and Nutrition [12]. The children whose daily caloric intake at home-care were below the target dietary caloric requirement were identified.

Due to technical difficulties to monitore resting energy expenditure (REE) using indirect calorimetry at PICU, the investigators used the Schofield-WH method for calculation REE and total enery expenditure (TEE) in the hospitalization period [13-15]. Basal caloric demand during the PICU-stay admssion was calculated from TEE which was considered as REE plus 5% of activity factor and 15% for day-to-day variability [16,17]. Thus the duration to achieve target caloric intake at PICU was extracted from the patient data.

### Statistical Analysis

Analysis were performed using Statistical Package for Social Sciences (SPSS Inc., Chi, IL) for Windows version 23 program. The distribution of the data were analyzed by the Kolmogorov-Smirnov test. Student t-test was used for normally distributed data where Mann-Whitney U test for abnormal. Multivariate regression analysis was used to evaluate independent risk factors to predict mortality. Descriptive statistics were used as numbers and percentages for categorical variables, mean ± SD for normally distributed continuous variables, and median (distance between quartiles 25-75%) for abnormally distributed continuous variables.

## RESULTS

A total of 186 patients who suffer an underlying chronic disease and hospitalized due to an acute onset of critical illness were included in the study. The median age was 49 (17.75-104.5) months. Of the study group, 53.8% of the children were in need of homecare assistance. The most common chronic disease was central nervous system pathologies accompanied by neurological impairment in swallowing functions (37.3%), while the most common cause of acute onset of disease was lower respiratory tract infections (48.9%; Table 1). The growth stature of the population can be observed at Table 2. The Z score of WHZ was lower than -3 in 14.2% of the children under five years and in 5% of for the ones older than five years. Moreover, an underlying chronic disease resulted in short stature (HAZ <-3) in 42.5% of the patients under five and 25% of the study group who were older than five years (Table 2).

### Homecare

The route of nutrition at home-care via nasogastric tubing and gastrostomy was 20.4% and 18.8% respectively. The daily routine of nutrition revealed that the daily caloric intake were below target dietary caloric requirement in 24.7% of the children (Table 3). Nutrition by solid foods remained at 46.2% in the study population.

### Nutrition in Pediatric Intensive Care Unit

Due to acute onset of a life-threatining sickness, enteral feeding was initiated to critically-ill child at median 2 days (interquartile range 25-75%: 1-2 days) once the cardiorespiratory balance was achieved. A median duration of five days was recorded to achieve the target caloric intake at PICU stay. However enteral nutrition could not reach the target calories in deceased children due to either gastrointestinal intolerance or high doses of inotropic support (Table 3).

### Mortality

The overall mortality was 9.7%. Older children with male gender, lower Z scores of WHZ and BMI were more likely to die due to the acute life-threatining conditions

(p=0.015, p=0.015, p=0.023, p=0.001; Table 4). Moreover, these children required home-care assistance and nutrition with nasogastric feeding more often (p=0.002, p=0.014). Sepsis was significantly associated with mortality (p=0.024).

A multiple regression model was created for mortality risk prediction including four independent variables of Pediatric Risk of Mortality 3 (PRISM 3) score, sepsis, lower daily caloric intake than target caloric requirement at home-care and nutrition by liquid foods. The model predicted the highest odds of mortality for children fed by liquid foods at home-care (OR: 9.149, p=0.002, 95% CI: 1.182 - 16.158; Table 5).

| **Table 1. Demographic characteristics** | |
|---|---|
| **Variables** | **(IQR %)** |
| Age (months)* | 49 (17.75-104.5) |
| Weight (kg)* | 13 (7.9-28) |
| Height (cm)* | 92 (70-120) |
| Variables | Patients total n=186 (n%) |
| Gender ratio (M/F %) | 92/94 (49.5%) |
| Previous hospitalization | 158/186 (84.9%) |
| In need of homecare | 100/186 (53.8%) |
| Tracheostomized | 27/186 (14.5%) |
| Acute diseases causing pediatric intensive care unit admission | Patients total n=186 (n%) |
| Seizure | 15 (8.1%) |
| Sepsis | 26 (14%) |
| LRTI | 91 (48.9%) |
| Cardiopulmonary arrest | 4 (2.2%) |
| Dehydration/shock | 6 (3.2%) |
| Bronchiolitis/asthma | 28 (15.1%) |
| Heart failure | 4 (2.2%) |
| Trauma-bleeding | 4 (2.2%) |
| Underlying chronic disease | Patients total n=186 (n%) |
| Hematologic | 8 (4.3%) |
| Gastrointestinal | 4 (2.2%) |
| Endocrine | 18 (9.7%) |
| Nephrologic | 12 (6.5%) |
| Muscle disorder | 23 (12.4%) |
| Respiratory | 11 (5.9%) |
| Neurologic | 69 (37.1%) |
| Genetic anomaly | 25 (13.4%) |
| Cardiovascular | 14 (7.5%) |
| Immunologic | 9 (4.8%) |

*Median (IQR: Interquartile range 25-75%), LRTI: Lower respiratory tract infection, n: Number, M: Male, F: Female

| **Table 2. Growth status of the population** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | **<5 years** | | | | | **>5 years** | | | | |
| **WHO Z scores** | **>-1** | **-1/-2** | **-2/-3** | **>-3** | **SDS median (25-75%)** | **>-1** | **-1/-2** | **-2/-3** | **>-3** | **SDS median (25-75%)** |
| **Weight-for-age** | | | | | | | | | | |
| **n (%)** | 29 (27.4%) | 17 (16%) | 27 (25.5%) | 33 (31.1%) | -2.18 (-4.46)-(-0.61) | 45 (56.3%) | 12 (15%) | 14 (17.5%) | 9 (11.3%) | -0.80 (-2.16)-(0.29) |
| **Weight-for-height** | | | | | | | | | | |
| **n (%)** | 68 (64.2%) | 15 (14.2%) | 8 (7.5%) | 15 (14.2%) | -0.47 (-1.43)-(0.77) | 55 (68.8%) | 10 (12.5%) | 11 (13.8%) | 4 (5%) | 0.30 (-1.62)-(1.23) |
| **Body mass index** | | | | | | | | | | |
| **n (%)** | 67 (63.2%) | 14 (13.2%) | 10 (9.4%) | 15 (14.2%) | -0.3 (-1.51)-(0.80) | 57 (71.3%) | 10 (12.5%) | 9 (11.3%) | 4 (5%) | 0.19 (-1.20)-(1.53) |
| **Height-for-age** | | | | | | | | | | |
| **n (%)** | 30 (28.3%) | 22 (20.8%) | 9 (8.5%) | 45 (42.5%) | -2.14 (-5.46)-(-0.73) | 32 (40%) | 13 (16.3%) | 15 (18.8%) | 20 (25%) | -1.67 (-3.25)-(-0.26) |

WHO: World Health Organization, SDS: Standard deviation score

| Table 3. The nutritional status of the study population | | | |
|---|---|---|---|
| **Homecare** | **Patients n (%)** | **Nutrition at PICU** | **Patients n (%)** |
| Nutrition via gastrostomy | 35/186 (18.8%) | Parenteral nutrition | 15 (8.1%) |
| Nutrition via nasogastric tube | 38/186 (20.4%) | Initiation of enteral feeding (days) | 2 (1-2) |
| Enteral feeding product | 48 (25.8%) | Duration to achieve target caloric intake (days) | 5 (3-5) |
| Liquid food | 53 (28.5%) | PICU stay (days) | 9 (9-10) |
| Solid food | 86 (46.2%) | Hospital stay (days) | 15 (14-16) |
| Daily caloric intake below target dietary caloric requirement | 46 (24.7%) | Exitus | 18 (9.7%) |
| PICU: Pediatric intensive care unit, n: Number | | | |

## DISCUSSION

The present study revealed that nutritional support was inadequate in critically ill children during homecare where approximately one-quarter of the children were taken below ideal daily calorie intake. The overall mortality rate was 9.7% which tends to be higher in children getting homecare compared to PICU. Sepsis was the major cause of death. The age of the deaths was two-fold older than the survivors, where most of the deaths were female. In addition, most of the deaths have been fed on aqueous foods via nasogastric tubes.

The critical illness causes evident endocrine and metabolic alterations associated with autonomic and immune changes. These changes include insulin resistance and catabolism [18,19]. Besides these alterations, critically ill children often encounter feeding problems caused by feeding intolerance, thus leading to malnutrition during the PICU stay period [20]. The rate of malnutrition at the time of admission to PICU is ranged from 15 to 25% even in developing countries. Malnourishment and macronutrient deficits in critically ill children have been related to increased complications including infections, weakness, prolonged mechanical ventilation and delayed recovery.

In contrast, optimal nutrition is essential for the treatment of critically ill children [21]. Various studies have reported that severe malnourishment in children leads to a higher risk of mortality compared to healthy ones in developing countries than in developed countries [22-24]. Similar with the previous studies, sepsis was the major cause of death in the present study [25]. There is a lack of data available where children with malnutrition have poor outcomes than in children with optimal nutritional status. Previous studies demonstrated that the presence of malnourishment elevates the mortality rate of children admitted to PICU [8,9,26]. Moreover Thukral et al. [27] showed that critically ill children with malnutrition and an elevated PRISM score have a trend towards a higher mortality rate.

Furthermore, the present study revealed that patients who died in PICU had a previous history of homecare those already had a higher PRISM score compared to others. Interestingly, it is also showed that mainly aqueous feeding is an independent risk factor for mortality. This finding suggested that aqueous foods may increase the aspiration risk causing aspiration pneumonia, thus requiring PICU admission and leading to severe infection/sepsis with an elevated risk of mortality as well.

However, mortality could not merely be affected by PICU stay but also related to several factors including demographic and clinical features of the population, infrastructure, paramedical conditions, and nutritional status of the patient [26,27]. Elucidating the aspects of malnutrition as an independent factor for the cause of death is of crucial importance, in order to interpret the anthropometric data and prioritization of applications and main strategies. Evaluation of malnutrition is usually used to define the risk of morbidity and mortality.

### Study Limitations

This study has some limitations. First, age-specific clinical assessments could not be completed associated with the limited number of patients that would directly affect the nutritional status of the children. Second, the number of patients with severe malnutrition was low to predict a contributing factor for nutrition. Third, samples were collected from a single center which restricts the generalizability of the results of this study.

| Table 4. The clinical characteristics of the patients | | | |
|---|---|---|---|
| | **Mortality (n=18)** | **Survival (n=168)** | **p** |
| **Growth charts and demographics** | | | |
| Age (month)* | 90 (65.75-151.75) | 46 (17-100.75) | **0.015** |
| Weight (kg)* | 17 (8-33.75) | 12.25 (7.8-26) | 0.334 |
| SDS-WAZ | -2.28 (-5.34)-(-1.83) | -1.39 (-2.47-0.80) | **0.012** |
| Height (cm) | 111 (86-133.75) | 88 (69-120) | **0.045** |
| SDS-BMI | -2.19 (-4.12)-(-0.43) | -0.11 (-1.27-1.23) | **0.001** |
| SDS-WHZ | -2.26 (-2.71)-(0.72) | -0.05 (-1.27-1.09) | **0.023** |
| SDS-HAZ | -2.18 (-6.17)-(-0.54) | -1.67 (-4.55)-(-0.41) | 0.971 |
| Gender (Female) | 14 (77.8%) | 80 (47.6%) | **0.015** |
| Previous hospitalization | 14 (77.8%) | 144 (85.7%) | 0.276 |
| In need of homecare | 16 (88.9%) | 84 (50.0%) | **0.002** |
| Tracheostomy | - | 27 (16.1%) | - |
| PRISM-3 score* | 35 (16-38) | 5 (2-6%) | **<0.001** |
| **Acute onset of disease** | | | |
| Impaired consciousness | - | 19 (11.3%) | - |
| Seizure | - | 15 (8.9%) | - |
| Sepsis | 6 (33.3%) | 20 (11.9%) | **0.024** |
| Cardiac arrest | 2 (11.1%) | 2 (1.2%) | **0.047** |
| Dispnea | 6 (33.3%) | 22 (13.1%) | 0.022 |
| Heart Failure | - | 4 (2.4%) | - |
| Dehydrationyon/shock | 2 (11.1%) | 4 (2.4%) | 0.105 |
| Trauma/bleeding | - | 4 (2.4%) | - |
| Underlying chronic conditions | | | |
| Hematologic | 2 (11.1%) | 6 (3.6%) | 0.175 |
| Gastrointestinal | - | 4 (2.4%) | - |
| Endocrine | - | 18 (10.7%) | - |
| Nephrologic | - | 12 (7.1%) | - |
| Muscle disorder | 2 (11.1%) | 21 (12.5%) | 0.611 |
| Chronic pulmonary disease | - | 11 (6.5%) | - |
| Genetic | 5 (27.8%) | 20 (11.9%) | 0.073 |
| Cardiac | - | 14 (8.3%) | - |
| Immune defficiency | 2 (11.1%) | 7 (4.2%) | 0.212 |
| Neurologic | 8 (44.4%) | 61 (36.3%) | 0.497 |
| **Nutrition at homecare** | | | |
| Enteral feeding products | - | 48 (28.6%) | - |
| Liquid food | 13 (72.2%) | 40 (23.8%) | **<0.001** |
| Solid food | 6 (33.3%) | 80 (47.6) | 0.248 |
| Nutrition via nasogastric tube | 8 (44.4%) | 30 (17.9%) | **0.014** |
| Nutrition via gastrostomy | 4 (22.7%) | 31 (18.5%) | 0.450 |
| Daily caloric intake below target dietary caloric requirement | 14 (77.8%) | 32 (19.0%) | **<0.001** |
| **Nutrition in PICU** | | | |
| Initiation of enteral feeding (days)* | 2 (2-4) | 2 (1-2) | 0.371 |
| Duration to achieve target caloric intake (days)* | - | 5 (4-5) | - |
| PICU stay (days)* | 5 (3.75-7) | 10 (9-10) | **<0.001** |
| Hospital stay (days)* | 5 (3.75-7) | 17 (16-17) | **<0.001** |

Chi-square or Fischer's Exact test (if necessary) was performed. *Median (interquartile range 25-75%);. SDS: Standard deviation score, WAZ: Weight-for-age Z score, BMI: Body mass index, WHZ: Weight for height Z score, HAZ: Height-for-age Z score, PICU: Pediatric intensive care unit, PRISM-3: Pediatric Risk of Mortality 3

| Table 5. Multivariate regression analysis for mortality | | | | |
|---|---|---|---|---|
| | Odds ratio | %95 Confidence interval | | |
| | | Lower bound | Upper bound | p |
| Daily caloric intake below target dietary caloric requirement | 0.100 | 0.009 | 1,138 | 0.063 |
| Sepsis | 0.127 | 0.010 | 1,692 | 0.118 |
| PRISM-3 score | 0.599 | 0.432 | 0,830 | **0.022*** |
| Liquid feeding at home | 9,149 | 1,182 | 16,158 | **0.002*** |
| *p<0.05; PRISM-3: Pediatric Risk of Mortality 3 | | | | |

## CONCLUSION

In conclusion, this study showed that mortality is closely associated with the lack of recommended daily calorie intake in critically ill children. Supplementing daily calorie intake with a balanced diet in children requiring homecare is of great importance. Since the number of patients with PEG is seen to be relatively low, feeding tube is often preferred during home care in children with swallowing difficulties. However, it is demonstrated that the feeding tube is an independent risk factor for mortality. The establishment of nutrition team consisted of a dietitian, a pediatric gastroenterologist, a pediatrician and family physicians might decrease the mortality rate in in critically ill children requiring medical care. In addition, we think that we will contribute to the quality of home healthcare services by sharing the findings of this study with the provincial health directorate.

### Ethics

**Ethics Committee Approval:** Cumhuriyet University Clinical Research Ethics Committee approval was obtained (decision number: 2019-04/11, date: 17.04.2019).

**Informed Consent:** Since the study design was retrospective and data were collected anonymously, informed consent was waived.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: Ö.S.S., E.A.O., Concept: Ö.S.S., E.A.O., Design: Ö.S.S., E.A.O., G.C., Data Collection and/or Processing: Ö.S.S., E.A.O., G.C., Analysis and/or Interpretation: Ö.S.S., E.A.O., G.C., Literature Search: Ö.S.S., E.A.O., G.C., Writing: Ö.S.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.2022.26097
J Dr Behcet Uz Child Hosp 2022;12(2):136-141

Original Article

# The Importance of Upper Pouch Contrast X-ray Radiography in Esophageal Atresia: A Retrospective Analysis

## *Özofagus Atrezisinde Üst Kese Kontrastlı X-ışını Radyografisinin Önemi: Retrospektif Analiz*

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## ABSTRACT

**Objective:** To investigate the effectiveness of preoperative upper pouch radiography in determining the treatment method in neonates with esophageal atresia (EA) and also to analyze the contribution of radiographic assessment of upper esophageal level to the prediction of anastomotic tension.

**Method:** Neonates who underwent primary esophagus repair due to EA with distal fistula between 2014 and 2020 were analyzed retrospectively. Upper esophageal levels assessed on preoperative upper pouch radiographs and during surgery were compared.

**Results:** A total of 36 cases were included in the study. Contrast-enhanced upper pouch radiograms were obtained using a thin catheter in 18 (50%) patients and using a thick catheter in 18 (50%) patients. The upper esophageal levels of preoperative pouch radiographs and surgery were consistent in 80.5% (n=29) and not consistent in 19.5%. The rate of compliance between radiographic and intraoperative levels was found to be significantly higher in the use of thick catheters compared to thin catheters (p=0.03). Six patients (16.6%) whose upper pouch level was in the 1st or 2nd thoracic vertebra had tense anastomosis.

**Conclusion:** Withdrawing the upper pouch radiography by advancing a thick catheter is effective for determining the actual level of the upper esophagus. If the upper esophageal pouch is at the 1st or 2nd thoracic level on the radiographs obtained with a thick catheter, it can be predicted that the anastomosis will be tense.

**Keywords:** Esophageal atresia, diagnosis, newborn, surgery, upper pouch graphy

## ÖZ

**Amaç:** Özefagus atrezili (ÖA) yenidoğanlarda preoperatif üst poş radyografisinin tedavi yönteminin belirlenmesindeki etkinliğini araştırmak ve ayrıca üst özefagus seviyesinin radyografik değerlendirmesinin anastomoz gerilimi tahminine katkısını analiz etmektir.

**Yöntem:** 2014-2020 yılları arasında distal fistüllü ÖA nedeniyle primer özefagus onarımı yapılan yenidoğanlar retrospektif olarak incelendi. Preoperatif üst poş grafilerinde ve ameliyat sırasında değerlendirilen üst özefagus seviyeleri karşılaştırıldı.

**Bulgular:** Çalışmaya toplam 36 olgu dahil edildi. On sekiz (%50) hastada ince kateter, 18 (%50) hastada kalın kateter kullanılarak kontrastlı üst poş grafileri çekildi. Preoperatif poş radyografileri ve intraoperatif üst özefagus seviyeleri %80,5'te (n=29) tutarlıydı; %19,5'te tutarlı değildi. Kalın kateter kullanımında radyografik ve intraoperatif düzeyler arasındaki uyum oranı ince kateterlere göre anlamlı olarak yüksek bulundu (p=0,03). Üst poş seviyesi 1. veya 2. torasik vertebrada olan altı hastada (%16.6) anastomoz gergin olarak yapılabildi.

**Sonuç:** Kalın kateter ilerletilerek üst poş radyografisinin çekilmesi, üst özefagusun gerçek seviyesinin belirlenmesinde etkilidir. Kalın kateter ile çekilen grafilerde üst özefagus poşu 1. veya 2. torasik seviyede ise anastomozun gergin olacağı tahmin edilebilir.

**Anahtar kelimeler:** Özefagus atrezisi, tanı, yenidoğan, cerrahi, üst poş grafisi

**Received:** 19.09.2021
**Accepted:** 04.02.2022

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**Cite as:** Sağ S, Orbay TM, Gül C, Celayir A. The Importance of Upper Pouch Contrast X-ray Radiography in Esophageal Atresia: A Retrospective Analysis. J Dr Behcet Uz Child Hosp. 2022;12(2):136-141

## INTRODUCTION

Tracheoesophageal fistula (TEF) and esophageal atresia (EA) are congenital anomalies characterized by an abnormal communication between the trachea and the proximal and/or distal esophagus segments. The incidence of EA is 2.99 cases per 10,000 births [1].

EA with TEF is a congenital malformation requiring surgery soon after birth [2]. Accurate preoperative determination of the distance between esophageal pouches is mandatory in order to establish an ideal surgical plan [2]. Anteroposterior chest radiography can provide a rough idea of the distance between pouches, whereas it mostly cannot assess this distance precisely. Contrast radiography is widely used for imaging the dilated upper pouch and also for assessing fistulas and interpouch distance [2,3]. Some studies suggested that contrast radiography may be misleading for estimating exact location of pouch levels in the preoperative period when an appropriate-sized catheter and technique are not employed [4,5]. Nevertheless, studies conducted on this subject are very limited and most of them include a limited number of cases [5].

The aim of this study was to investigate the effectiveness of upper pouch radiography in the assessment of upper pouch level in cases with EA and to analyze the contribution of radiographic assessment of upper esophageal level to the precise prediction of anastomotic tension.

## MATERIALS and METHODS

The study included 36 neonates with EA and TEF (Type C) who were operated on in our clinic between January 2014 and April 2020. The diagnosis of EA was confirmed radiologically by thoracoabdominal radiographs with the use of a rubber catheter/an infant feeding tube in situ and upper pouch radiograms were obtained in all cases. Intraoperatively upper esophageal pouch level, fistula level, anastomotic tension findings, catheter size, demographic findings (gender, birth weight) and gestational age were recorded. Patients whose radiographs could be retrieved from the hospital database were included in the study. The first pouch radiograms of some patients who were referred to our department from external centers for surgery were not available in the hospital database and these patients were excluded from the study. The study was approved by the local ethics committee (decision number: 142, date: 08.07.2020).

### Statistical Analysis

Data were analyzed using SPSS for Windows version 18.0 (Chicago, IL, USA). Descriptive statistics were expressed as frequencies (n) and percentages (%) for categorical variables and as mean ± standard deviation and median (minimum-maximum) values for continuous variables. Categorical variables were compared using chi-square test or Fisher's Exact tests. A p value of $<0.05$ was considered to be statistically significant.

## RESULTS

The study population consisted of 36 neonates including 20 (55.5%) girls and 16 (45.5%) boys. Median gestational age was 34 weeks (range; 28-41 weeks). Median birth weight was 2137 g (range; 1100-3480 g). Associated congenital heart disease was present in 28 (77.7%), anorectal malformation in two (5.5%), and duodenal atresia in one (2.7%) patient.

Thoracoabdominal radiography confirmed the presence of EA in all patients. Contrast-enhanced [iohexol (Omnipol; Polifarma, İstanbul, Turkey)] upper pouch radiograms were obtained using a thin catheter [<10 F (Bıçakcılar, İstanbul, Turkey)] in 18 (50%) and a large-caliber catheter [10-12 Fr rigid rubber (Bıçakcılar, İstanbul, Turkey)] in 18 (50%) patients. Primary repair was performed via thoracotomy through the 3$^{rd}$ or 4$^{th}$ intercostal space. During the surgery, assessment of the upper pouch was performed with the help of a catheter which was inserted with sufficient pressure to allow the catheter to curl up at the lowest point of the upper pouch. Radiographically detected levels of upper esophageal pouch complied with intraoperative findings in 29 (80.5%), but they were more proximal in six (16.7%), and more distal in one (2.8%) patient. Radiographs obtained with insertion of a thin catheter demonstrated that the upper pouch level were consistent with the intraoperative pouch level in 12 (66.7%), and more proximally located in six (33.3%) out of 18 patients. Radiographs of the remaining 18 patients were obtained using a larger-caliber catheter and the level of the pouch detected on the radiographs was consistent with the intraoperative pouch level in 17 (94.4%) patients. In one patient where a large-caliber catheter was used, the pouch level was more distal on radiographs compared to its intraoperative level.

Figure 1 shows a radiograph obtained using a thin catheter (radiographically and intraoperatively detected levels were incompatible, and the upper esophageal pouch was revealed at the 3$^{rd}$ thoracic vertebral level during surgery).

Figure 2 shows a radiograph obtained with a larger caliber catheter (radiographic and intraoperative levels were compatible).

The rate of compatibility between radiographically and intraoperatively detected levels was found to be significantly higher in the group catheterized with a larger-caliber catheter compared to the group in whom a thin catheter was used (p=0.03). Table 1 presents the effectiveness of catheter size on compatibility between radiographically and intraoperatively detected levels of upper esophageal pouches.

Primary repair was performed via thoracotomy through the 3rd intercostal space in patients in whom the upper pouch was detected at the level of the 1st and 2nd thoracic vertebra on radiography and it was performed through thoracotomy incision into the 4th intercostal space for the other patients. Primary anastomosis was performed successfully in all cases without further need for elongation techniques.

TEF communicated with the trachea at the carina in 32 (88.9%), and at the level of the 3rd thoracic vertebra in the remaining four (11.1%) patients. Six patients (16.6%) whose upper pouch level was at the level of the 1st or 2nd thoracic vertebra had tense anastomosis. In these patients, pouch radiographs were obtained using a large-caliber catheter in four and a thin catheter in two patients. In the same patients, radiographically

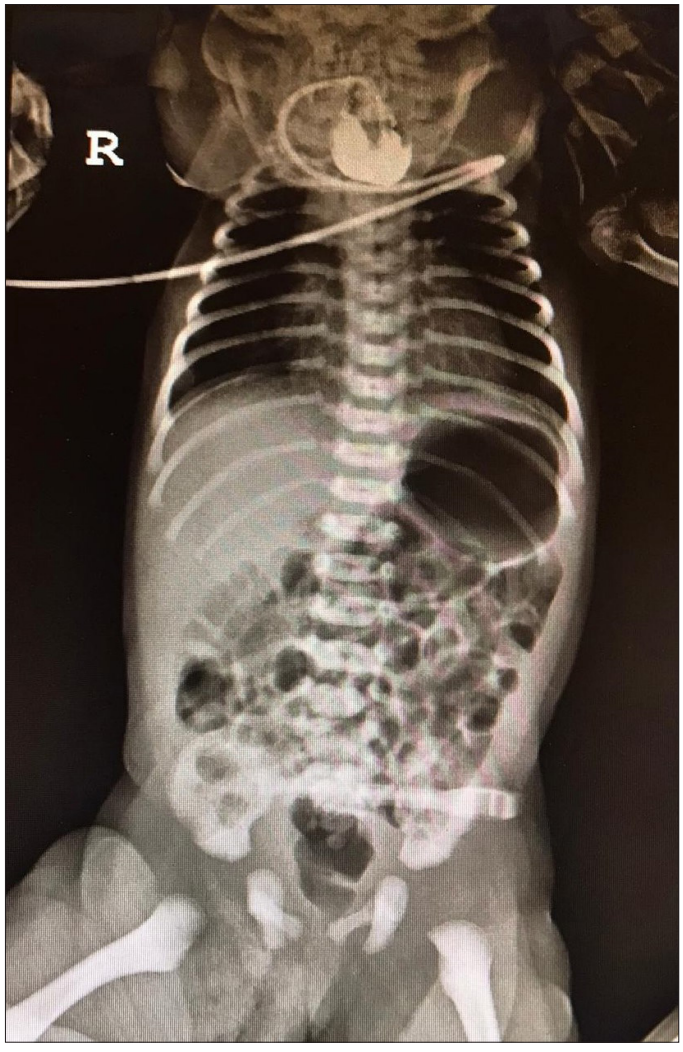


**Figure 1.** Radiograph obtained with a thin catheter in a patient (radiographic and intraoperative levels were not compatible and the upper esophageal pouch was detected at the 3rd thoracic vertebral level during surgery)

**Table 1. Effectiveness of catheter size on the compliance between radiographic and intraoperative upper pouch levels**

| | Catheter size | |
|---|---|---|
| | **Thin** | **Thick** |
| **Consistent** | 12[a] (66.7%) | 17[b] (94.4%) |
| **Inconsistent** | 6 (33.3%) | 1 (5.6%) |
| $p^{a-b}$=0.03 | | |

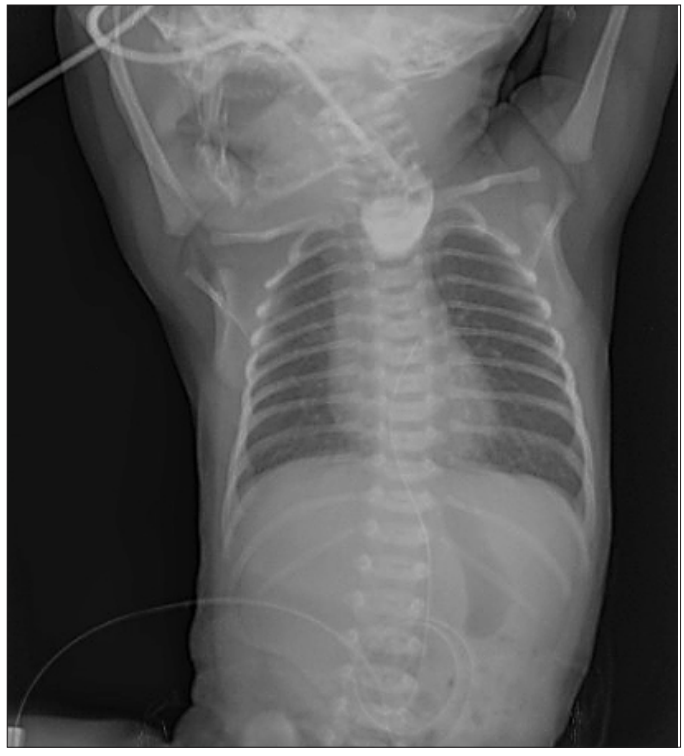

**Figure 2.** Radiograph obtained with a thick catheter in a patient (radiographic and intraoperative levels were compatible) (2nd thoracic vertebra level)

and intraoperatively detected levels were compliant in five patients, whereas in the remaining one patient, a larger caliber catheter was used and the pouch was detected more distally on radiograms compared to its intraoperatively detected level of the pouch. There were two vertebral gaps between the proximal and distal esophagus in all cases with tense anastomosis. In these patients, the anastomosis was tense despite the use of extra muscle relaxants. Moreover, the knots of some sutures could not fit properly or the sutures were cutting through the tissue. Minor anastomotic leakage was observed in two patients with tense anastomosis. No serious postoperative complications occurred in other cases. Table 2 presents data on gender distribution,

**Table 2. Gender distribution, radiographic and intraoperative upper pouch levels, tendency for anastomosis, and catheter sizes**

| Sex | Level of upper pouch on X-ray (thoracic vertebra) | Level of upper pouch intraoperation (thoracic vertebra) | Level of fistula (thoracic vertebra) | Anastomosis tendency | Catheter size |
|---|---|---|---|---|---|
| **M** | **1** | **1** | **3** | **Tense** | **Thin** |
| **F** | **2** | **2** | **3** | **Tense** | **Thin** |
| **M** | **3** | **1** | **4** | **Tense** | **Thick** |
| F | 2 | 3 | 3 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thin |
| F | 3 | 4 | 4 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thin |
| F | 4 | 4 | 4 | Comfortable | Thin |
| F | 4 | 4 | 4 | Comfortable | Thin |
| M | 3 | 3 | 4 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thin |
| M | 5 | 5 | 5 | Comfortable | Thin |
| **F** | **1** | **1** | **4** | **Tense** | **Thick** |
| **M** | **2** | **2** | **4** | **Tense** | **Thick** |
| **F** | **1** | **1** | **4** | **Tense** | **Thick** |
| F | 4 | 4 | 4 | Comfortable | Thick |
| M | 3 | 3 | 4 | Comfortable | Thick |
| F | 4 | 4 | 4 | Comfortable | Thick |
| M | 2 | 3 | 4 | Comfortable | Thin |
| M | 2 | 2 | 3 | Comfortable | Thick |
| M | 2 | 2 | 4 | Comfortable | Thick |
| M | 3 | 3 | 4 | Comfortable | Thick |
| M | 1 | 3 | 4 | Comfortable | Thin |
| F | 3 | 3 | 4 | Comfortable | Thick |
| F | 4 | 4 | 4 | Comfortable | Thick |
| F | 4 | 4 | 4 | Comfortable | Thick |
| F | 5 | 5 | 5 | Comfortable | Thick |
| M | 2 | 4 | 4 | Comfortable | Thin |
| M | 3 | 3 | 4 | Comfortable | Thick |
| F | 3 | 3 | 4 | Comfortable | Thick |
| M | 4 | 4 | 4 | Comfortable | Thick |
| M | 4 | 4 | 4 | Comfortable | Thick |
| M | 2 | 3 | 4 | Comfortable | Thin |

M: Male, F: Female

radiographically and intraoperatively detected upper pouch levels, tendency for anastomosis, and catheter sizes.

## DISCUSSION

EA and/or TEF is one of the most challenging congenital anomalies for pediatric surgeons. It is very important to determine the exact location of the upper pouch and fistula as well as inter- pouch gap for surgery plan [2-5]. Magnetic resonance imaging (MRI) and computed tomography (CT) have been used preoperatively to define, and evaluate the tracheoesophageal anatomy. Although MRI seems advantageous for imaging the pouch and fistula line and for prenatal detection of accompanying anomalies, debates continue about its sensitivity, cost and effective use [1-3]. Preoperative CT scan of chest has many advantages, but it involves significant exposure to ionizing radiation [5]. Nevertheless, experience with these imaging methods in newborns in the preoperative period is quite limited. Transport to the radiology center, radiation risk, sedation or general anesthesia seem to be its major disadvantages [2]. Recent studies concluded that there is no role for CT scan or MRI in the routine preoperative assessment of EA [2,5]. The authors reported that performing CT or MRI in preoperative period provide limited information that may contribute to modifying the surgical plan [2,3,5].

Contrast esophagram is still used in many clinics for the preoperative evaluation of the esophagus since it can detect the location of the dilated upper esophageal pouch in relation to the thoracic inlet and also reveal a proximal TEF [2,6]. However, the downsides of this technique include aspiration risk and though rarely, inability to show fistula due to mucus plug and radiation [2,7]. Nonetheless, the risk of aspiration is minimal when the technique is performed meticulously, and the technique can provide highly useful information regarding the preoperative period when used with an appropriate-sized catheter and a proper extraction technique [4,8,9].

The caliber and rigidity of the catheter used in radiographic examination is of paramount importance, and studies emphasize the importance of using a rigid rubber catheter instead of a soft feeding tube for the diagnosis of EA [6,9]. Many authors recommend a rigid 10-F nasogastric tube for radiography [10]. Using a thinner tube may cause the catheter curl up in the posterior pharynx or upper pouch [10-12]. In our series, the radiographically and intraoperatively detected upper pouch levels showed a high rate of compliance (80.5%). Moreover, this rate was lower in the patients where a thin catheter was used compared to those in whom a larger-caliber catheter was employed (66.7% vs. 94.4%). These findings implicate that the thin catheter is less effective in determining the lowest level of the upper pouch, mainly because it is curled up inside the pouch due to its more flexible nature.

Preoperative assessment of the level of the upper pouch and interpouch distance is important for determining the location of the incision [2]. In our study, we planned the thoracotomy site based on the location of the upper pouch and interpouch distance, whereby the 3rd intercostal space was selected in cases with upper pouches and the 4th intercostal space in the presence of lower pouches. In doing so, better surgical field dominance was achieved and primary repair was easily performed in all patients. In some cases, however, radiographs were misleading and thus led to difficulties during surgery. Although we believe that a large-caliber catheter should be used when performing pouch radiography, pushing a this catheter with excessive force during the radiographic examination may produce misleading results about the pouch level in the preoperative period, as was seen in one of our patients.

Literature indicates that an appropriate technique is also important besides the caliber and type of the catheter for determining the level of the upper pouch on radiography [4,13]. Lyall et al. [13] found that the level of the upper pouch detected on radiography was seriously affected by the position of the head in porcine animal model. The authors also noted that a full flexion of the neck was associated with a 9% reduction in the length of the esophagus [13]. In addition, the size and position of the upper pouch are influenced by swallowing and breathing as well [4]. Accordingly, we consider that in our study these factors may also be effective in the errors experienced in the assessment of the level of the upper pouch on radiography.

In our study, in six patients, the upper pouch was determined at the 1st and 2nd vertebral level in contrast-enhanced radiography. Thus, we preoperatively predicted that the anastomosis could be tense. Indeed, intraoperatively, tense anastomoses were detected in these six patients. Although the number of our cases in our study was limited, our findings indicate that contrast-enhanced radiography can help predict possible anastomotic tension. There is no detailed study about effectiveness of preoperatively obtained upper

pouch radiography in neonates with EA in the literature. Further studies are required to compare our findings.

In recent years, preoperatively, endoscopy and bronchoscopy have been used for determining the location of TEF and the interpouch distance and also for evaluating vocal cords and anomalies such as tracheomalacia and tracheal cleft [2]. In our clinic, bronchoscopy and esophagoscopy are not routinely performed for every patient, and are only performed in patients with suspected proximal fistula. In patients with suspected proximal fistula, bronchoscopy and esophagoscopy are performed in consideration of the patient's weight. However, they cannot be performed in our study anyway, because the caliber of our endoscope is not suitable for infants weighing less than 2000 grams.

### Study Limitations

The limitation of the study was that it was a retrospective study and the data were obtained from medical records of hospital. Non-standardized evaluation is also a serious limitation of such retrospective studies.

## CONCLUSION

As a summary, the assessment of the upper pouch level is highly important for determining the surgical technique and incision site and also for predicting anastomotic tension in cases with EA. Performing the upper pouch radiography by inserting a large- caliber catheter is effective for determining the actual level of the upper esophagus. If the upper esophageal pouch is at the 1st or 2nd thoracic level on the radiographs obtained with a large-caliber catheter, the anastomosis is likely to be tense.

### Ethics

**Ethics Committee Approval:** The study was approved by the Zeynep Kamil Woman and Children Diseases Training and Research Hospital Ethics Committee (decision number: 142, date: 08.07.2020).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: T.M.O., C.G., A.C., Concept: S.S., A.C., Design: S.S., A.C., Data Collection and/or Processing: T.M.O., C.G., A.C., Analysis and/or Interpretation: S.S., A.C., Literature Search: S.S., Writing: S.S., T.M.O., C.G., A.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.2022.79027
J Dr Behcet Uz Child Hosp 2022;12(2):142-150

Original Article

# Iron, Vitamin D and B12 Levels of Young Children with Autism Spectrum Disorder at Diagnosis

*Otizmli Küçük Çocuklarda Tanı Anında Demir, Vitamin D ve B12 Seviyeleri*

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## ABSTRACT

**Objective:** The etiology of autism spectrum disorder (ASD) remains unclear. The study aims to evaluate the relationship between ASD and preventable nutritional risk factors as iron, vitamin D, and B12 deficiency.

**Method:** Medical records of ASD-diagnosed children, ages 1.5-4 years old, were retrospectively reviewed. Hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV), red cell distribution width (RDW), ferritin, 25-hydroxyvitamin D, and vitamin B12 levels at diagnosis were compared with age and sex-matched typically developing control group.

**Results:** Eighty-five ASD-diagnosed and 63 typically developing children were included. Mean Hb, Htc, B12, and median MCV, RDW, ferritin and 25-hydroxyvitamin D levels were similar between the groups (p>0.05). Iron deficiency (ID) was in 39.5% and 35.5% of the children in the study and control groups, respectively (p>0.05). Iron deficiency anemia (IDA) prevalence in the study and control groups were 6.1% and 4.8% (p>0.05). Vitamin D insufficiency and deficiency were 15.1% and 16.4% in the study group, and 23.4% and 10.6% in the control group, respectively (p>0.05). B12 deficiency was detected in 20.2% and in 8.9% of the children in the study and control groups respectively, without significance (p>0.05). No relationship was observed between the severity of ASD symptoms and Hb, ferritin, B12, and 25-hydroxyvitamin D levels (p>0.05).

**Conclusion:** Young children with ASD did not at greater risk for ID, IDA, vitamin D and vitamin B12 deficiency than typically developing controls. But, our results support the necessity of evaluating children with ASD in terms of iron parameters, vitamin D and B12 levels.

**Keywords:** Autism spectrum disorder, iron, vitamin D, vitamin B12, children

## ÖZ

**Amaç:** Otizm spektrum bozukluğunun (OSB) etiyolojisi halen belirsizliğini korumaktadır. Bu çalışmanın amacı OSB ile demir, D vitamini ve B12 eksikliği gibi beslenme ile ilişkili önlenebilir risk faktörleri arasındaki ilişkiyi değerlendirmektir.

**Yöntem:** Yaşları 1,5-4 arasında değişen OSB tanısı almış çocukların tıbbi kayıtları geriye dönük olarak incelendi. Tanı anındaki hemoglobin (Hb), hematokrit (Htc), ortalama eritrosit hacmi (MCV), eritrosit dağılım genişliği (RDW), ferritin, 25-hidroksivitamin D ve vitamin B12 düzeyleri benzer yaş ve cinsiyetteki sağlıklı gelişen kontrol grubu ile karşılaştırıldı.

**Bulgular:** OSB tanılı 85, sağlıklı gelişen 63 çocuk dahil edildi. Ortalama Hb, Htc, B12 ve ortanca MCV, RDW, ferritin ve 25-hidroksivitamin D düzeyleri gruplar arasında benzerdi (p>0,05). Demir eksikliği (DE) çalışma ve kontrol grubundaki çocukların sırasıyla %39,5'inde ve %35,5'inde saptandı (p>0,05). Çalışma ve kontrol gruplarında demir eksikliği anemisi (DEA) prevalansı %6,1 ve %4,8 idi (p>0,05). D vitamini yetersizliği ve eksikliği çalışma grubunda sırasıyla %15,1 ve %16,4, kontrol grubunda ise sırasıyla %23,4 ve %10,6 bulundu (p>0,05). B12 eksikliği, çalışma ve kontrol grubundaki çocukların sırasıyla %20,2'sinde ve %8,9'unda saptanırken, fark anlamlı değildi (p>0,05). OSB belirtilerinin şiddeti ile Hb, ferritin, B12 ve 25-hidroksivitamin D düzeyleri arasında ilişki gözlenmedi (p>0,05).

**Sonuç:** OSB'li küçük çocuklar, sağlıklı olarak gelişen kontrollere göre ID, DEA, vitamin D ve vitamin B12 eksikliği açısından artmış riske sahip değildir. Ancak sonuçlarımız OSB'li çocukların demir parametreleri, D vitamini ve B12 düzeyleri açısından değerlendirilmesi gerekliliğini desteklemektedir.

**Anahtar kelimeler:** Otizm spektrum bozukluğu, demir, D vitamini, B12 vitamini, çocuklar

**Received:** 11.09.2021
**Accepted:** 08.03.2022

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**Cite as:** Çelik P, Ayrancı Sucaklı İ, Yakut Hİ. Iron, Vitamin D and B12 Levels of Young Children with Autism Spectrum Disorder at Diagnosis. J Dr Behcet Uz Child Hosp. 2022;12(2):142-150

## INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder typically seen in early childhood, characterized by deficits in social interaction, restricted and repetitive behaviors [1]. It is estimated that one in 54 children has been identified with ASD with a global increase in the prevalence of ASD [2]. This increasing prevalence has encouraged researchers to question the relationship between ASD and preventable and changeable environmental risk factors for central nervous system dysfunction such as iron, vitamin D, and B12 deficiencies. Children with ASD are at risk for nutritional deficiencies because of frequently accompanying food selectivity and refusal of foods other than with certain colors and nutrient contents [3].

Vitamin D deficiency is the most frequently investigated environmental risk factor in infants and children with the diagnosis of ASD. Vitamin D plays an essential role in neuronal differentiation, proliferation, apoptosis, synaptic plasticity, immunomodulation, gene expression, and reduction of oxidative stress [4]. The findings of former studies on the relationship between serum vitamin D levels and ASD are controversial. Some studies suggested that vitamin D plays a role in the etiology of autism and low vitamin D levels are associated with the frequency and severity of autism, while others could not report any statistically significant interrelationship [5-9].

Another environmental risk factor investigated regarding the relationship with ASD is iron deficiency (ID). There is substantial evidence concerning the critical role of iron on learning, attention, memory, and psychomotor functions. Decreased iron concentration in the brain may affect serotonergic and dopaminergic systems, cortical networks, and myelin production [10-12]. Since children with ID often consume similar diets with their mothers, expectedly their mothers may also have ID [13]. Maternal ID can cause maternal depression, apathy, and low cognitive functioning. Therefore, ID can cause adverse developmental outcomes directly through its effect on brain functioning and indirectly through the non-responsive caregiver-child interaction [13]. Also, it has been shown that children with ID are less curious, less happy, and have less social interaction with the environment than healthy children [13]. Therefore, it can be considered that ID may increase the severity of ASD symptoms. Some studies have reported a high prevalence of ID and anemia in children with ASD [14-16], while others have reported that children with ASD are not at greater risk for ID than the general population [3,17].

Some studies have also investigated the relationship between ASD and vitamin B12, and reported that children with ASD had lower B12 and higher homocysteine levels compared with healthy controls [18-20], and indicated that treatment with B12 may alleviate ASD symptoms by reducing oxidative stress [21,22].

The studies exploring the relationship between vitamin D levels, iron parameters, and ASD have yielded conflicting results. There are limited data in the literature investigating the relationship between B12 and autism. Our research questions were: 1) Are the vitamin B12, vitamin D, and iron levels lower in children with newly diagnosed with ASD compared to their healthy peers? 2) Is there any relationship between severity of ASD symptoms and these micronutrients? Our study aimed to investigate the iron parameters, 25-hydroxyvitamin D, and vitamin B12 levels among children with ASD at the time of diagnosis and determine the relationship between severity of ASD symptoms and levels of these micronutrients.

## MATERIALS and METHODS

This retrospective cohort study was conducted at the University of Health Sciences Turkey, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital, Developmental Behavioural Pediatrics (DBP) Outpatient Clinic. The study was approved by the Ethics Committee of the same hospital (decision no: 2019-144, date: 05/28/2019). Children aged 1.5-4 years and diagnosed with ASD between January 1, 2015, and May 1, 2019 with available hemogram, ferritin, 25-hydroxyvitamin D, and vitamin B12 test results at the time of diagnosis were included in the study.

The control group included children without any detected developmental delay admitted to the DBP Outpatient Clinic within the study period and underwent the tests required for the study group. Children in both groups used vitamin D regularly within the first year of life. Patients with a history of chronic neurologic, genetic and metabolic diseases, gestational age less than 32 weeks, birth weight less than 1500 grams, history of using iron and vitamin preparations within the last three months, and those using diuretics or antiepileptics were not included in the study. An additional exclusion criteria for the control group was malnutrition and eating disorders.

The developmental evaluation was conducted by developmental and behavioral pediatricians based on Bronfenbrenner and Ceci's [23] bioecological theory, International Classification of Functioning, Disability, and Health framework [24], family-centered care [25], and Guide for Monitoring Child Development [26]. Bronfenbrenner and Ceci's [23] bioecological theory postulates that early childhood development holds through dynamic interactions between the child's biological, psychological, and social functioning and his/her immediate environment consisting of parents and other family members, and the distant environment including social opportunities that support this basic structure. This theory offers a helpful perspective for understanding and supporting child development. The child's health status was evaluated as an integral part of the body functions and structures, the participation and limitations in his/her activities, and the environmental and personal factors [24]. Health and education services were planned, delivered, and evaluated by a mutual collaboration with families who had a unique role in the child's life [25]. The Guide for Monitoring Child Development [26] is a validated method based on an open-ended interview with the primary caregiver for the developmental monitoring and early detection of developmental difficulties in low and middle-income countries [26]. Children whose development was compatible with their age in terms of expressive, and receptive language, relationship, fine and gross motor domain according to developmental evaluation were included in the control group. The ASD was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition criteria for ASD [1]. The severity of ASD symptoms was evaluated by the Childhood Autism Rating Scale (CARS) [27-29]. The CARS is a behavioral rating scale used to diagnose and evaluate the severity of ASD [28]. The Turkish validity and reliability of the scale was performed [27]. Sum scores can range from 15 to 60, and scores more than 30 indicate that the child is in the autistic range. Scores between 30-36 and 37-60 are categorized as mild, and severe autism, respectively [28].

Serum ferritin, 25-hydroxyvitamin D, and vitamin B12 levels were measured by the radioimmunoassay method on Beckman Coulter DXI800 autoanalyzer. Hemogram was studied on Beckman Coulter HmX hematology analyzer.

Hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV), red cell distribution width (RDW), and ferritin levels were used to evaluate serum iron parameters. ID was defined as ferritin <12 ng/mL; and iron deficiency anemia (IDA) as Hb <11 gr/dL, Htc <34%, MCV <73 fL, and ferritin <12 ng/mL [30,31]. American Academy of Pediatrics considers serum 25-hydroxyvitamin D levels ≤15 ng/mL as vitamin D deficiency and 25-hydroxyvitamin D levels between >15-20 ng/mL as vitamin D insufficiency [32]. We categorized vitamin D levels measured between 21 March, and 23 September as summer, and between 23 September-21 March as winter 25-hydroxyvitamin D levels. A serum vitamin B12 level below 200 pg/mL was considered as vitamin B12 deficiency [33].

### Statistical Analysis

The data obtained were analyzed using the SPSS 20 package program for MAC. Chi-square test were used for comparing the percentages between groups. Statistical differences of the mean values between groups were analyzed using the t-test for the variables with normal and Mann-Whitney U test without normal distribution. Paired t-test was used for the time comparison in the group for the normally distributed data and the Wilcoxon test for not normally distributed data. Descriptive statistics were expressed as numbers and percentages for categorical variables and as mean ± standard deviation or median (minimum-maximum) for the continuous variables. Spearman tests were used for evaluating the correlation between different variables. For all tests, statistical significance was defined as $p<0.05$.

## RESULTS

We followed 192 children with ASD between January 2015 and May 2019. The laboratory results were available at the time of diagnosis in 121 of them. We excluded 36 patients with chronic conditions such as neurologic (n=23), genetic (n=7), surgical (n=4), allergic (n=7) diseases and history of very low birth weight (n=5). We finally assessed a total of 148 children including 85 with an ASD diagnosis, and 63 controls. Demographic characteristics of the cases are shown in Table 1. The mean age of the children was 30.3±8.6 months. The age, gender, birth weight, gestational age, and fathers' educational level were similar between the groups ($p>0.05$). The percentage of mothers who were educated more than 8 years was higher in the control group ($p=0.007$).

Hemogram, ferritin, vitamin B12, and vitamin D levels were available in 144, 143, 140, and 120 children, respectively. In the study group, 60 of 85 children's CARS scores were available. The comparison of Hb, Htc, MCV, RDW, ferritin, vitamin D and vitamin B12 levels between both groups is shown in Table 2.

The mean Hb, Htc, vitamin B12, and median MCV, RDW, and ferritin levels were similar between the groups (p>0.05). The median vitamin D levels in summer and winter were similar between the study and the control groups (p=0.535 and p=0.569). In the study group 39.5% and in the control group 35.5% of the children had ID (p=0.623). We determined that 6.1% of the study, and 4.8% of the control group had IDA without astatistically significant intergroup difference (p=0.744). Vitamin D insufficiency and deficiency were observed in 15.1% and 16.4% of the study (p=0.249), and 23.4% and 10.6% of the control group, (p=0.374) respectively. We found vitamin B12 deficiency in 20.7% and 8.9% of the study and the control groups without a statistically significant difference (p=0.072) (Table 3).

**Table 1. Demographic characteristics of the groups**

| | Study group n=85 | Control group n=63 | Total n=148 | p |
|---|---|---|---|---|
| **Gender*** | | | | |
| Male | 72 (84.7) | 48 (76.2) | 120 (81.1) | 0.191 |
| Female | 13 (15.3) | 15 (23.8) | 28 (18.9) | |
| Age, month† | 31.12±8.32 | 29.27±8.91 | 30.33±8.60 | 0.197 |
| Mothers' age‡ | 28 (18-42) | 27 (19-42) | 28 (18-42) | 0.197 |
| Fathers' age‡ | 31 (20-55) | 31 (22-48) | 31 (20-55) | 0.704 |
| **Education levels of mothers*** | | | | |
| ≤8 years | 36 (42.9) | 11 (20.4) | 47 (34.1) | 0.007 |
| >8 years | 48 (57.1) | 43 (79.6) | 91 (65.9) | |
| **Education levels of fathers*** | | | | |
| ≤8 years | 26 (31) | 15 (27.8) | 41 (29.7) | 0.690 |
| >8 years | 58 (69) | 39 (72.2) | 97 (70.3) | |
| Birth weight, gram† | 3236±518 | 3263±413 | 3246±480 | 0.757 |
| Gestational age, week‡ | 40 (35-42) | 40 (37-42) | 40 (35-42) | 0.903 |

*n (%), †mean ± Standard deviation, ‡median (minimum-maximum). Missing data of ten parents educational information are excluded

**Table 2. Comparison of Hb, Htc, MCV, RDW, ferritin, vitamin D, and vitamin B12 levels in the groups**

| | Study group n=85 | Control group n=63 | Total n=148 | p |
|---|---|---|---|---|
| Hb* | 12.36±1.04 | 12.21±0.84 | 12.30±0.96 | 0.357 |
| Htc* | 36.53±2.84 | 36.06±2.48 | 36.33±2.69 | 0.302 |
| MCV† | 75.8 (16.5-88.3) | 76 (60.1-83.3) | 75.9 (16.5-88.3) | 0.482 |
| RDW† | 14.8 (12.2-26.4) | 14.6 (12.6-19.9) | 14.7 (12.2-26.4) | 0.368 |
| Ferritin† | 15.9 (1.9-63.3) | 14.7 (2-53) | 14.8 (1.9-63.3) | 0.717 |
| Vitamin D† | 24.3 (10-70.3) | 24 (7.5-52.9) | 24 (7.5-70.3) | 0.851 |
| Vitamin D, winter† | 24.1 (10-61) | 24.8 (12.3-52.9) | 24.3 (10-61) | 0.569 |
| Vitamin D, summer† | 25.2 (10.4-70.3) | 23.8 (7.5-43.6) | 23.9 (7.5-70.3) | 0.535 |
| Vitamin B12* | 375.0±186.7 | 364.1±179.4 | 370.6±183.2 | 0.729 |

*mean ± Standard deviation, , †median (minimum-maximum). Hb: Hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, RDW: Red cell distribution width

**Table 3. Comparison of iron, vitamin D, vitamin B12 deficiency, and iron deficiency anemia between the groups**

| | Study group n=85 | Control group n=63 | Total n=148 | p |
|---|---|---|---|---|
| Iron deficiency | 32 (39.5) | 22 (35.5) | 54 (37.8) | 0.623 |
| Iron deficiency anemia | 5 (6.1) | 3 (4.8) | 8 (5.6) | 0.744 |
| Vitamin D insufficiency | 11 (15.1) | 11 (23.4) | 22 (18.3) | 0.249 |
| Vitamin D deficiency | 12 (16.4) | 5 (10.6) | 17 (14.2) | 0.374 |
| B12 deficiency | 17 (20.2) | 5 (8.9) | 22 (15.7) | 0.072 |

Available micronutrient results (study/control group): Ferritin (81/62); hemogram (82/62); vitamin D (73/47); vitamin B12 (84/56)

Mild and severe autism symptoms were found in 8 and 52 children according to CARS scores, respectively. There was no correlation between CARS scores and Hb, serum ferritin, 25-hydroxyvitamin D, and vitamin B12 levels (Table 4). There was no significant relationship between the severity of autism symptoms and 25-hydroxyvitamin D, Hb, ferritin, and vitamin B12 levels (p>0.05).

## DISCUSSION

Our data have shown that children with ASD aged between 1.5 and 4 years were not at a greater risk for ID, IDA, vitamin D, and vitamin B12 deficiency compared with age and gender-matched healthy controls.

There are many studies investigating the relationship between vitamin D levels and ASD. A variety of case-control studies from different countries and races reported that children and adolescents with ASD had lower vitamin D levels [20,34-47], however, some studies in the literature have indicated the opposite of these results [48-54]. Recently, in a meta-analysis of 24 case-control studies, Wang et al. [55] showed that children and adolescents with ASD had significantly lower vitamin D levels than the control group. Quantitative integration of 10 case-control studies which reported odds ratio among these studies revealed that low vitamin D levels were associated with increased risk of ASD (odds ratio: 5.23, 95% confidence interval: 3.13; 8.73, p<0.0001, I2=78.2%). Surprisingly we did not observe any statistically significant difference in vitamin D levels between children with ASD diagnosis and healthy controls. Only 6 of the 24 case-control studies in the meta-analyses by Wang et al. [55] included children below five years of age. Our results may be associated with some relevant factors. Firstly, newly diagnosed children below four years of age were included in our study. Secondly, parents had not yet restricted dietary milk protein or casein. In addition, children's daily routines and exposure to sunlight were probably similar to the control group, as they were newly diagnosed and have not started to receive early intervention services yet.

Geographic location and latitude are also factors affecting vitamin D levels [55]. Studies from African and Asian countries showed that ASD diagnosed children had lower vitamin D levels than the control group. However, studies from Europe reported higher but non-significant vitamin D levels in the ASD diagnosed children than the control group. Studies from America reported no difference in vitamin D levels between the children with and without ASD. The mean vitamin D level of children living in the low-latitude areas was higher than children living in high and middle latitudes. Studies from Turkey also showed different results. While some studies reported that vitamin D concentration in children with ASD was lower than the controls, others reported no difference in vitamin D concentration [20,42,50,56]. Guler et al. [50] reported that mean vitamin D concentrations in children with ASD were 25.58±10.31, and 25.35±9.92 in the control group (p>0.05). We observed similar vitamin D levels in our study with children of younger age group. Also, approximately one-third of children in both the study and the control groups had vitamin D insufficiency or deficiency (≤20 ng/mL) in our study. This high rate may be related to the characteristics of the city where this study was conducted. Ankara has high latitude and receives short-term sunlight exposure. Unexpectedly we could not find a seasonal variation in vitamin D levels. These results may be related to the small sample size and the study was not designed to detect the difference in seasonal variations.

Increasing data from preclinical studies and case-control studies about the relationship between vitamin D levels and ASD have prompted randomized controlled trials (RCTs) investigating the effect of vitamin D supplementation on symptoms of ASD. However, the results of RCTs have been inconsistent, and a recent meta-analysis of RCTs showed that vitamin D supplementation in children with ASD provides a small improvement in hyperactivity scores but there was no statistically significant effect on the severity of core symptoms of ASD such as disrupted social interaction,

**Table 4. Correlations between the Childhood Autism Rating Scale scores and ferritin, vitamin D, vitamin B12, and hemoglobin levels**

| | CARS | Ferritin | Vitamin D | Vitamin B12 | Hb |
|---|---|---|---|---|---|
| CARS | 1.000 | - | - | - | - |
| Ferritin | 0.013 | 1.000 | - | - | - |
| Vitamin D | 0.193 | 0.103 | 1.000 | - | - |
| Vitamin B12 | -0.068 | 0.107 | 0.133 | 1.000 | - |
| Hb | -0.082 | 0.272** | 0.125 | 0.044 | 1.000 |

**Correlation is significant at the 0.01 level (2-tailed). Hb: Hemoglobin, CARS: Childhood autism rating scale

communication, repetitive and restricted behaviors and interests [57]. Contrary to studies that found a significant negative correlation between serum 25-hydroxyvitamin D levels and CARS, in our study no relationship was found between severity of ASD and serum vitamin D levels [45,47]. This result may be related to our sampling method which included mostly the children with severe ASD symptoms, and we could not reach the CARS score of the entire sample because of the retrospective design of the study.

Although there is some biological evidence showing the possible potential relationship between vitamin D levels and ASD, the fundamental mechanism of this relationship is not well understood. While interpreting the vitamin D levels of children diagnosed as ASD, it is important to consider some variables such as methodologic differences in the studies (e.g diagnostic methods/criteria, method of measuring vitamin D levels), duration of the sunlight exposure, diet, geography, age, ethnic and genetic factors.

In a case-control study, Bener et al. [40] showed that in a case group with a mean of 5.39±1.66 years, mean serum iron, Hb, ferritin, Htc were significantly lower than the control group. Gunes et al. [58] similarly found significantly lower mean serum iron level, Hb, ferritin, Htc in children diagnosed as ASD with a mean age of 9.73±4.20 years than the healthy control group, with a significant correlation between ID parameters and autism severity. In contrast to these case-control studies, a recent meta-analysis reported that the available evidence was inconsistent about whether children with ASD had lower iron levels or not [17]. In our study, we did not detect any significant differences between the groups in terms of Hb, Htc, MCV, RDW, and ferritin levels. We observed that frequency rates of IDA and ID were strikingly high in the whole sample (5.6%, and 37.8%, respectively). Compared to the control group, the frequency of IDA and ID were higher in the study group, without any statistically significant difference. The role of iron in brain development is noteworthy, and as in the study of Pivina et al., [59] our study also emphasizes that we should follow iron parameters of the children both with an ASD diagnosis and healthy individuals. In contrast to the study of Gunes et al., [58] we did not find any relationship between Hb, Htc, ferritin levels and severity of ASD.

Methyl B12 is an essential cofactor in the antioxidant system and has a role in the transmethylation pathway by providing methyl groups for the methionine-homocysteine cycle. Methyl B12 administration was reported to improve cellular methylation capacity, decrease oxidative stress, and alleviate ASD symptoms in a subgroup of children with the diagnosis of ASD [21,22]. Additionally, this responder subgroup exhibited significant improvement in blood plasma levels of glutathione, a potential biomarker of response, and a mechanism of improvement, which may include increased antioxidant capacity and reduced oxidative stress. These preliminary results indicated a strong trend toward improvement following methyl B12 administration in a subgroup of children with autism, warranting further research into the efficacy of methyl B12 and potential biomarkers in the evaluation of response to this treatment. Lower serum vitamin B12 levels, and significantly higher homocysteine levels were detected in children diagnosed with ASD [19,20,60-62]. In contrast to the literature, our study revealed lack of any significant difference between children diagnosed as ASD and healthy controls in terms of mean vitamin B12 levels which may be related to the younger age of the study sample compared with the studies in the literature. Although vitamin B12 deficiency was more frequent in the study group compared to the control group (20% and 8,9%, respectively), this intergroup difference was not statistically significant. However, our findings support the need for monitoring vitamin B12 levels of children diagnosed as ASD.

### Study Limitations

Our findings can not be generalized due to the results of a single center. One of the limitations of our study is the variables affecting the level of the micronutrients, such as nutrition-related factors, longevity of sunlight exposure, selective nutrition, sensory hypersensitivity were not evaluated because of the retrospective design of the study. Another limitation is that serum iron and iron-binding capacity were not evaluated. However, the inclusion of a gender-, and age-matched control group and the evaluation of the data of youngest aged ASD children at the time of diagnosis are the strengths of our study.

## CONCLUSION

In conclusion, although children diagnosed with ASD are not at a greater risk for ID, IDA, vitamin D, and vitamin B12 deficiency, our preliminary findings support the necessity of monitoring these preventable risk factors. Parents of children with ASD should be guided about providing a balanced diet for their children,

appropriate exposure to sunlight, iron and vitamin D supplementation during infancy, and not following non-evidence-based diets. Further studies are necessary to determine the potential relationship between ASD and iron, vitamin D and B12 deficiency, and their underlying mechanisms.

### Ethics

**Ethics Committee Approval:** The study was approved by the Ethics Committee of the University of Health Sciences Turkey, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital (decision no: 2019-144, date: 05/28/2019).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: P.Ç., İ.A.S., Concept: P.Ç., H.İ.Y., Design: P.Ç., İ.A.S., H.İ.Y., Data Collection and/or Processing: P.Ç., İ.A.S., Analysis and/or Interpretation: P.Ç., Literature Search: P.Ç., Writing: P.Ç., İ.A.S., H.İ.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.2022.88942
J Dr Behcet Uz Child Hosp 2022;12(2):151-158

**Original Article**

# Respiratory Viruses in Pediatric Patients with Suspected COVID-19 at the Early Stages of the Pandemic: A Single-center Experience

## *COVİD-19 Şüpheli Pediatrik Hastalarda, Pandeminin Erken Döneminde Diğer Solunum Yolu Virüs Enfeksiyonları: Tek Merkez Deneyimi*

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## ABSTRACT

**Objective:** This study aimed to report the respiratory tract viruses we detected in the respiratory polymerase chain reaction (PCR) samples taken from patients admitted to the Pediatric Emergency Service with suspicion of coronavirus disease-2019 (COVID-19) in the early stages of the pandemic, in addition to the clinical course, and laboratory features of the disease caused by these identified respiratory tract viruses.

**Method:** All upper respiratory tract PCR samples were taken simultaneously from patients suspected of having COVID-19 disease. All pediatric patients who came to the Pediatric Emergency Department with suspicion of COVID-19 disease between March and June 2020 were included in the study. We retrospectively compared the laboratory findings, clinical manifestations, and primary outcomes of the children aged between 1 month and 18 years infected with respiratory viruses (RVs) and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus.

**Results:** Fifty-eight pediatric patients were tested. SARS-CoV-2 virus was detected in 27 (46.6%) patients and other RVs in 31 (53.4%) patients. The detection rate of SARS-CoV-2 was significantly higher in the older age group of children ($p<0.01$). We didn't detect co-infections with SARS-CoV-2 and other RVs in these patients. Compared to the children with COVID-19, those infected with other RVs required markedly higher rates of oxygen supplementation ($p<0.01$). There was no need for hospitalization in the COVID-19 patient group, and 23 of 31 critically ill children infected with other RVs were followed up in the pediatric intensive care unit.

**Conclusion:** RVs are common causes of childhood infections and may cause critical illness. Infections caused by other RVs progressed with more severe clinical findings than those of COVID-19 disease in pediatric patients. During the COVID-19 pandemic, other RVs that cause mortality and morbidity in children should be also kept in mind.

**Keywords:** Respiratory viruses, COVID-19, pediatric intensive care unit, pediatric emergency care

## ÖZ

**Amaç:** Bu çalışmada, pandeminin erken döneminde koronavirüs hastalığı-2019 (COVİD-19) şüpheli hastalarda, diğer solunum yolu virüsleri açısından nazal sürüntü polimeraz zincir reaksiyonu (PCR) örnekleri çalışılarak; şiddetli akut solunum yolu sendromu koronavirüsü 2 (SARS-CoV-2) enfeksiyonu ve diğer solunum yolu virüsleriyle enfekte hastaların klinik ve laboratuvar özelliklerinin karşılaştırılması ve çocukluk yaş grubunda diğer virüslerin de patojen olarak akılda tutulması gerektiğini vurgulamak amaçlanmıştır.

**Yöntem:** Çalışmaya 11 Mart 2020 ve 30 Haziran 2020 tarihleri arasında Çocuk Acil Servise başvuran ve SARS-CoV-2 enfeksiyonu şüphesi olan 1 ay-18 yaş arası tüm hastalar dahil edilmiştir. COVİD-19 şüpheli tüm hastalardan diğer solunum yolu virüsleri açısından nazal sürüntü PCR örnekleri ve COVİD-19 PCR örnekleri eş zamanlı olarak alınmıştır. Solunum yolu virüsleri ile enfekte olan hastaların laboratuvar bulguları, klinikleri ve izlemdeki sonuçları SARS-CoV-2 enfeksiyonu olan hastalarla karşılaştırılmıştır.

**Bulgular:** Çalışmaya 58 hasta dahil edilmiştir. Bu hastaların 27'sinde (%46,6) SARS-CoV-2, 31'inde (%53,4) diğer solunum virüsleri saptanmıştır. Bu hasta grubunda COVİD-19 ve diğer solunum yolu viral etkenleriyle koenfeksiyon saptanmamıştır. COVİD-19 enfeksiyonu olan hastalarla karşılaştırıldığında diğer solunum yolu virüs enfeksiyonu olan daha küçük yaşlardaki hastalarda oksijen desteği alma oranı daha yüksektir ($p<0,01$). COVİD-19 ile enfekte olan hastalarda hastane yatışı gerekmemiştir. Solunum yolu virüsleriyle enfekte olan 31 hastanın 23'ü solunum sıkıntısı-solunum yetmezliği nedeniyle çocuk yoğun bakım ünitesinde izlenmiştir.

**Received:** 30.09.2021
**Accepted:** 08.03.2022

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**Cite as:** Zengin N, Bal A, Atik S, Şen Bayturan S, Akçalı S. Respiratory Viruses in Pediatric Patients with Suspected COVID-19 at the Early Stages of the Pandemic: A Single-center Experience. J Dr Behcet Uz Child Hosp. 2022;12(2):151-158

**Sonuç:** Solunum yolu virüsleri çocuklarda sık görülen enfeksiyon etkenlerindendir ve kritik hastalığa neden olabilirler. Özellikle daha küçük yaş grubundaki çocuklarda diğer solunum yolu virüslerinin neden olduğu enfeksiyonlar, COVİD-19'a göre daha şiddetli klinik bulgularla seyretmektedir. Çocuklarda önemli mortalite ve morbidite nedeni olan diğer solunum yolu patojenleri, pandemi sürecinde de izolasyon önlemleri ve tedavi-izlem açısından önemli olup akılda tutulmalıdır.

**Anahtar kelimeler:** Solunum yolu virüsleri, COVİD-19, çocuk yoğun bakım, çocuk acil servis

## INTRODUCTION

In December 2019, cases of pneumonia of unknown etiology were reported in Wuhan city of Hubei Province, China, which spread rapidly from Wuhan city to other provinces in China and abroad [1,2]. The name of the infection and the virus was determined as coronavirus disease-2019 (COVID-19) and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), respectively [3]. World Health Organization reported that COVID-19 had reached a pandemic on March 11, 2020 [4]. The clinical spectrum of COVID-19 disease can range from asymptomatic to critical illness. Milder symptoms are observed in children compared to adults, and the most common clinical findings in children, and adults are fever and cough [5,6]. Compared to adults, children have a better prognosis. Recovery in pediatric patients is possible within 1-2 weeks, and hospitalization is less often necessary in pediatric patients than adults [7].

The infection is transmitted human-to-human with respiratory droplets. Mainly, transmission occurs when infected person sneezes or coughs during close contact with people [8,9]. Laboratory findings, SARS-CoV-2 nucleic acid tests, serological tests, and radiological findings aid in the diagnosis [10]. Laboratory findings as lymphopenia, thrombocytopenia, and leukopenia, elevated erythrocyte sedimentation rate, C-reactive protein (CRP), and lactate dehydrogenase are more commonly seen [11]. Lung X-ray and computed tomography (CT) scanning can be used for diagnosis and assessment of disease progression. Lung X-ray is not recommended as the first choice diagnostic tool. In severe cases, a white lung pattern can be seen in plain chest X-rays [10]. Chest CT is more valuable than a plain chest X-ray. Ground-glass opacities and consolidations are the most common chest CT findings [5,12]. Nucleic acid amplification tests are the most commonly used method to confirm the diagnosis. SARS-CoV-2 was detected in nasopharyngeal and oropharyngeal samples obtained using the reverse-transcription polymerase chain reaction (RT-PCR) method [13].

Pneumonia caused by the other viral agents has also been seen in this period. Respiratory syncytial virus (RSV), influenza, parainfluenza viruses (PIV), human rhinovirus, adenovirus (ADV), and human metapneumovirus (hMPV) are the most common viral pneumonia agents in the child population [14]. Viral pathogens are responsible for pediatric emergencies and intensive care admissions because they cause acute respiratory infections, respiratory distress, and sepsis-like clinical presentation, especially in young children [15-18]. Pneumonia and bronchiolitis account for 20-50% of hospital admissions and lead to non-elective pediatric intensive care unit (PICU) admissions [19-20]. In children with comorbidities, the risk of mortality and morbidity significantly increases [21,22]. Molecular microbiological examination techniques enable the detection of many viruses that commonly cause acute respiratory infections in children, including influenza viruses RSV, ADV, PIV, and hMPV [23]. The recognition of SARS-CoV-2 infection is vital for appropriate infection control measures and potentially promising antiviral therapy. On the other hand, clinicians should also consider SARS-CoV-2 co-infection. SARS-CoV-2 co-infection rates with other respiratory viruses have been reported in the range of 0-20% [24]. COVID-19 protocols allowed us to test for the respiratory virus in all pediatric patients with suspected COVID-19 that attended our Pediatric Emergency Department in the early stages of the pandemic. In this study, we aimed to ascertain the respiratory virus (including SARS-CoV-2) present in respiratory samples of pediatric patients with suspected COVID-19 disease who attended to the Pediatric Emergency Department at the early stages of the pandemic. Our analysis compared diseases caused by SARS-CoV-2 and other viral agents detected in the pediatric population in terms of clinical features, and laboratory findings.

## MATERIALS and METHODS

Patients who applied to the pediatric emergency department with respiratory system symptoms at the beginning of the pandemic were included in the study. Respiratory molecular PCR and SARS-CoV-2 PCR samples from these patients were taken in the pediatric emergency isolation room. The clinical manifestations of COVID-19 in children were not known clearly in the

early stages of the pandemic, so we performed necessary laboratory tests in all patients. The records of 58 patients aged between 1 month and 18 years taken to the pediatric emergency clinic in a tertiary care hospital between March 2020 - June 2020 were examined. All necessary data of the patients were obtained retrospectively from the electronic database records of the hospital. SARS-CoV-2-positive and other respiratory virus- positive patients were compared in two groups. The laboratory diagnosis of COVID-19 was based on a positive result obtained in the hospital laboratory via real-time (RT)-PCR testing of SARS-CoV-2 in nasopharyngeal swabs. The lung radiograms were evaluated in patients with respiratory distress who required hospitalization. The diagnosis of upper respiratory tract infection (URTI) and lower respiratory tract infections (LRTI) such as bronchopneumonia, pneumonia, and acute bronchiolitis was made by evaluating relevant disease symptoms, physical examination findings, and radiological findings in association. Mild symptomatic patients were followed up with isolation measures and discharged. The follow-up of the discharged patients was made via phone calls by the pediatric emergency physicians. Moderate to severe symptomatic patients were monitored in isolation rooms until the SARS-CoV-2 PCR examinations were concluded. Necessary permissions were taken from The Republic of Turkey Ministry of Health. Ethical approval was received from the Ethics Committee of Manisa Celal Bayar University Medical Faculty of Health Sciences (decision no: 465, date: 22.07.2020). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

### Patient Management

The Turkish Public Health Directive Guidelines prepared according to the recommendations of the COVID-19 advisory board were used as the main indications for PICU admission. Mild symptomatic patients were followed up with isolation measures and discharged from Emergency Department (ED). According to isolation measures, patients requiring respiratory and organ support were transferred to PICU until SARS-CoV-2 PCR results were obtained. Chest radiography was performed in patients who only needed respiratory support. None of the patients required CT. The standard definitions were used to evaluate chest radiographs at admissions, such as normal lung X-ray, bronchopneumonia, atelectasis, and air leaks (pneumothorax, pneumomediastinum, etc.). The respiratory conditions of the patients were evaluated clinically. Low- or high-flow oxygen delivery methods were chosen according to the $SpO_2$ levels of the patients. High-flow nasal cannula (HFNC) oxygen therapy was administered in patients with hypoxemic respiratory failure by wearing appropriate personal protective equipment due to the risk of aerosolization. These patients were closely monitored as for the progression of clinical deterioration. The most experienced physician intubated four patients with respiratory failure with a rapid sequence intubation protocol. Routine isolation measures in PICU were maintained until the SARS-CoV-2 PCR results of the patients were obtained. Fluid/electrolyte balance, antibiotics, nutrition, other supportive treatments, and weaning from invasive/noninvasive mechanical ventilation were arranged according to the PICU protocol.

### Statistical Analysis

Study databases were evaluated using SPSS 20.0 (SPSS Inc, Chicago, Ill). Descriptive data were expressed as median (25-75 percentiles) for continuous variables. As appropriate, categorical variables were compared using the $\chi 2$ or Fisher Exact tests. Mann-Whitney U test was used for comparing nonparametric variables. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

The records of 58 pediatric patients who were taken to our pediatric emergency clinic between March 2020 and June 2020 were examined retrospectively: SARS-CoV-2 was detected in 27 (46.6%) and other respiratory viruses in 31 (53.4%) patients. Twenty-eight (48.2%) male, and 30 (51.7%) female patients were included in the study. The median ages of patients with COVID-19, and those infected with other respiratory viruses were 108 (48-168), and 9 months (3-24), respectively. Co-infection with SARS-CoV-2 virus and other respiratory viruses was observed in any patient. The detection rate of SARS-CoV-2 was significantly higher in older children ($p<0.001$). In 27 pediatric patients, the way of transmission was identified as close contact with diseased family members or exposure to people infected with COVID-19. Twenty-seven children had symptoms of mild URTI such as sore throat, fever, and positive SARS-CoV-2 RT-PCR test results. There were no severe or critically ill patients. On admission, frequent symptoms were cough, fever, and myalgia in 14 (51.8%) of 27 SARS-CoV-2 positive patients. COVID-19 patient group had no severe clinical symptoms. Mild symptomatic patients were discharged home and followed up according to the protocols of our health ministry.

The clinical severity of patients infected with other respiratory viruses ranged from mild to severe respiratory distress. Twenty (64.5%) patients presented with moderate to severe respiratory distress due to acute LRTI were managed with HFNC oxygen therapy. Two patients who developed respiratory failure during the follow-up with HFNC and two patients admitted to the ED with respiratory failure were intubated and followed up on mechanical ventilation. Twenty-four (77.4%) patients were followed up in the intensive care unit. Inpatients were younger than outpatients (p=0.005). The patients with an underlying disease required more frequent hospitalizations (p=0.01). In terms of laboratory values, leukocyte, neutrophil and lymphocyte counts, CRP, and PCT values at admission were markedly higher in the other respiratory virus-infected inpatients. Moreover, eosinophil counts of the patients who required hospitalization were dramatically lower than those of outpatients (p=0.035) (Table 1).

The median white blood cell (WBC) count was 7670/μL (4300-10300) in COVID-19 patients, while it was 9750/μL, (7920-12640) in patients infected with other respiratory viruses, (p=0.013)., Median absolute neutrophil count/absolute lymphocyte (ALC) values were 2715/μL (1770-6710)/ 2005/μL (1331.5-3522.5); In COVID-19 patients, while they were 5280/μL (3250-8440)/2825/μL (2090-4270) in patients infected with other respiratory viruses (p=0.059/p=0.015). Serum aminotransferase (AST), serum alanine aminotransferase (ALT), CRP, procalcitonin (PCT), D-dimer values were statistically higher in the group infected with other viruses. The median fibrinogen level was determined as 260 (249.75-393.75) in COVID-19 patients and as 246 (171.5-368) in the group infected with other respiratory viruses (p=0.208). The median age of patients infected with respiratory tract pathogens was nine months (3-24) with a male dominancy (male:16; 51.6%) / female:15; 48.3%). Respiratory viruses identified in nasopharyngeal swab samples in order of decreasing frequency were as follows: RSV (13-22.4%), metapneumovirus (3-5.3%), rhinovirus (3-5.3%), influenza A (1-1.7%), ADV (1-1.7%), human parechovirus (1-1.7%), rhinovirus + ADV (2-3.4%), RSV + bocavirus (2-3.4%), RSV + ADV (1-1.7%), RSV + rhinovirus (1-1.7%), rhinovirus + enterovirus (1-1.7%), bocavirus + ADV (1-1.7%), parainfluenza + metapneumovirus (1-1.7%). LRTI and URTI were diagnosed according to clinical signs and symptoms. No mortality was observed in both groups (Table 2).

## DISCUSSION

In this study, respiratory tract viruses and SARS-CoV-2 in children aged one month to 18 years attended to our pediatric emergency clinic between March 2020 and June 2020 with signs of respiratory tract infection and followed up as out- or in-patients were examined. Most studies indicate that children infected with COVID-19 have mild clinical manifestations characterized by relatively nonspecific symptoms such as cough, sore throat, nasal congestion, and fever. COVID-19 leads a much milder course than that seen in adults, and understanding the reasons for this may enable us to develop potential methods for treatment. The rates of hospital -including intensive care unit- admissions in adults have been reported as 10-33%, while the reported

**Table 1. Demographic and laboratory variables**

| Variables | SARS-CoV-2 (+) (n=27) | Other RV (n=31) | p |
|---|---|---|---|
| Age (Months) (Median, 25-75 p) | 108 (48-168) | 9 (3-24) | <0.001 |
| Sex (F/M) | 15/12 | 15/16 | 0.586 |
| WBC (Median, 25-75 p); | 7670 (4300-10300) | 9750 (7920-12640) | 0.013 |
| ANC | 2715 (1770-6710) | 5280 (3250-8440) | 0.059 |
| ALC | 2005 (1331.5-3522.5) | 2825 (2090-4270) | 0.159 |
| Eosinophil | 40 (40-127.5) | 5 (0-250) | 0.035 |
| AST (Median, 25-75 p) | 22.5 (21-35.5) | 38.5 (31.5-49.5) | <0.001 |
| ALT (Median, 25-75 p) | 20 (11-19.5) | 14.5 (15.5-35) | 0.006 |
| CRP (Median, 25-75 p) | 0 (0-0) | 3.65 (2-17.9) | <0.001 |
| Procalsitonin (Median, 25-75 p) | 0.03 (0-0.05) | 0.16 (0.1-1) | 0.002 |
| D-dimer (Median, 25-75 p) | 125 (96.5-386.5) | 719 (251.5-1358) | 0.001 |
| Fibrinogen (Median, 25-75 p) | 260 (249.75-393.75) | 246 (171.5-368) | 0.208 |

SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2, RV: Respiratory virüse, F: Female, M: Male, WBC: White blood cell, ANC: Absolute neutrophil, ALC: Absolute lymphocyte, AST: Aminotransferase, ALT: Alanine aminotransferase, CRP: C-reactive protein

rates of hospital, and PICU admissions in children have ranged between 5.7-20%, and 0.58-2%, respectively [25-28]. Among the pediatric patient population with COVID-19, the highest rate of hospitalization occurs in infants [29]. Our data have shown that the other respiratory tract infections might be more severe compared to COVID-19. In this study, the laboratory findings, including WBC, eosinophil counts, AST, ALT, CRP, PCT, D-dimer values, were different between these two groups. There was no need for mechanical ventilation support in SARS-CoV-2 -positive patients. While most of the other respiratory virus-positive patients required supplemental oxygen, HFNC, and mechanical ventilation (noninvasive/invasive) support. Laboratory findings are variable for laboratory - confirmed cases of COVID-19 in pediatric patients. In a systematic review of laboratory - confirmed COVID-19 cases in children, mostly normal complete blood counts, but lower WBC counts in 17%, and neutropenia or lymphocytopenia in 13% of the patients were indicated [30,31]. Also, eosinopenia (29.5%) was observed in another study [32]. Approximately 30% of the patients had elevated CRP (CRP was reported as >5 mg/L in most studies) or PCT (PCT was reported as >0.5 ng/mL). On the other hand, elevated inflammatory markers and lymphocytopenia may indicate multisystem inflammatory syndrome in children. AST values were elevated in 12% of the patients. In our study, WBC counts, AST, ALT, CRP, PCT, and D-dimer were statistically significantly higher in the group infected with other viruses. There is insufficient data on coagulation test results in children with COVID-19. A study of adult patients with COVID-19 showed increased D-dimer levels and prothrombin time in intensive care patients [33]. In the pediatric patients, an elevated D-dimer level was more frequent in infants than in the other age groups which may suggest that infants might become more seriously infected than older children during COVID-19 pandemic. Studies have shown that children with RSV have significantly lower lymphocyte counts and that severe stress reduces lymphocyte counts, especially CD4-positive T cells and CD8-positive T cells in septic patients requiring pediatric intensive care and ALC counts of patients decrease as well [34,35]. Although the most common viral agent in our study was RSV, lymphocyte counts were not statistically significant in the group infected with other viruses due to the limited number of patients and the presence of other viruses. Eosinophils make up only a small percentage of circulating leukocytes (1-3%) and potent pro-inflammatory cells [36,37]. In addition, eosinophils are involved in protective immunity, including antiviral responses. The pathophysiology of eosinopenia in COVID-19 is multifactorial, including direct eosinophil apoptosis caused by inhibition of eosinophil outflow from the bone marrow or by type 1 interferons released during acute infection [38]. Eosinopenia occurs in response to acute inflammation and sepsis. Studies have shown that lower eosinophil levels are associated with poor outcomes in critically ill patients [39]. In our study,

**Table 2. Isolated respiratory viruses and oxygen delivery methods**

| RV type (n,%) | Simple $O_2$ mask (n) | Nonrebreathing mask (n) | HFNC oxygen therapy (n) | MV (n) |
|---|---|---|---|---|
| Adenovirus (1, 1.7%) | - | - | 1 | - |
| RSV (13, 22.4%) | 2 | 1 | 9 | 1 |
| Metapneumovirus (3, 5.3%) | - | - | 1 | 2 |
| Rhinovirus (3, 5.3%) | 2 | - | 1 | - |
| Influenza A (1, 1.7%) | - | 1 | - | - |
| Human parechovirus (1, 1.7%) | - | - | 1 | - |
| RSV + Adenovirus (1, 1.7%) | - | - | - | - |
| RSV + Bocavirus (2, 3.4%) | - | - | 2 | - |
| RSV + Rhinovirus (1, 1.7%) | - | - | - | 1 |
| Rhinovirus + Enterovirus (1, 1.7%) | - | - | 1 | - |
| Rhinovirus + Adenovirus (2, 3.4%) | - | - | 2 | - |
| Adenovirus + Bocavirus (1, 1.7%) | - | - | 1 | - |
| Parainfluenza + Metapneumovirus (1, 1.7%) | - | - | 1 | - |
| SARS-CoV-2 (27, 46.6%) | - | - | - | - |

RV: Respiratory viruses, HFNC: High-flow nasal cannula, MV: Mechanical ventilation, RSV: Respiratory syncytial virus, SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2

eosinopenia was associated with severe disease in the group infected with other viruses. The chest X-ray appearance of LRTI due to other viruses was not specific. Chest X-ray was not performed in the COVID-19 patient group due to signs of URTI. In this study, almost all of the COVID-19- positive pediatric patients had mild upper airway infection symptoms, and they weren't hospitalized with COVID-19 infection. On the other hand, the COVID-19 receptor uses an angiotensin-converting enzyme (ACE). For input host cells, and angiotensin-converting enzyme-2 decreases with age. Pulmonary ACE concentration is still low in the children compared to that measured in adults. Children have a strong innate immune response due to trained immunity (secondary to frequent viral infections and live vaccines), possibly leading to early control of infection at the site of entry. However, young infants and children with underlying diseases may constitute high-risk groups and may need careful monitoring [40]. We think that respiratory failure may be more severe in infections caused by other respiratory tract viruses. Especially the youngest children (0-6 months) had higher RSV-related hospitalization rates than older children. A meta-analysis by Lansbury et al. [41] involving adults and children found that 3% of the patients hospitalized with COVID-19 were co-infected with another respiratory virus. This meta-analysis mainly detected RSV and influenza virus [41]. We attributed the absence of co-infection in our patients to the fact that the seasonal characteristics of influenza and other respiratory viruses were not observed during our study period and that isolation measures were implemented beginning from the early stages of the pandemic.

### Study Limitations

This study has been performed on a limited number of patients due to a decrease in hospital admissions with the curfews in the early stages of the pandemic.

## CONCLUSION

Respiratory viral infections are common in childhood and constitute important reasons for admission to the pediatric ED and hospitalization in pediatric intensive care, especially during the winter months. Interventions such as staying at home, complying with social distance, closing schools, travel restrictions; measures not specific to SARS-CoV-2 prevent transmission of other respiratory viruses. Detection of viral infections also plays an important role in the isolation and treatment of critically ill patients during the pandemic. Infections caused by other respiratory viruses in pediatric patients progressed with more severe clinical findings than COVID-19.

### Ethics

**Ethics Committee Approval:** Ethical approval was received from the Ethics Committee of Manisa Celal Bayar University Medical Faculty of Health Sciences (decision no: 465, date: 22.07.2020).

**Informed Consent:** Since this study had a retrospective design, informed consent was not sought.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Concept: N.Z., A.B., S.A., S.Ş.B., S.Ak., Design: N.Z., A.B., Data Collection and/or Processing: N.Z., S.A., Analysis and/or Interpretation: N.Z., A.B., S.Ş.B., Literature Search: N.Z., A.B., Writing: N.Z.

**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.2022.23590
J Dr Behcet Uz Child Hosp 2022;12(2):159-163

Original Article

# Could Hematologic Parameters Have a Predictive Role in Pediatric Hashimoto Thyroiditis?

## *Pediatrik Hashimoto Tiroiditinde Hematolojik Parametrelerin Prediktif Rolü Olabilir mi?*

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## ABSTRACT

**Objective:** Hashimoto thyroiditis (HT) is an autoimmune thyroid disease evolving as a result of lymphocyte infiltration and chronic inflammation. Although adult studies have shown that hematologic parameters, such as platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII) can be used as biomarkers of HT, knowledge is limited concerning the pediatric age group. The aim of our study is to investigate the potential of hematologic biomarkers in predicting HT in children.

**Method:** Children with HT (n=165) who were followed in the Pediatric Endocrinology Department of our hospital between July 2020 and July 2021, were enrolled in the present retrospective cross-sectional study. Hemogram values were compared with those of age-matched control group (n=122).

**Results:** The average leukocyte (p>0.05), platelet (p>0.05), and absolute neutrophil (p>0.05) counts, NLR (p>0.05) and SII (p>0.05) in the children with HT were not statistically different from those of the control group. Although PLR values were significantly higher in the HT group than the control group (p<0.05), in receiver operating characteristic curve analysis, PLR values had low specificity and sensitivity, in predicting HT.

**Conclusion:** Our study has shown that NLR and SII are not useful indicators in predicting HT in children. Although there is a statistically significant difference in PLR values, we think that PLR is not a useful marker due to its low specificity and sensitivity.

**Keywords:** Hashimoto thyroiditis, pediatric, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, systemic immune-inflammation index

## ÖZ

**Amaç:** Hashimoto tiroiditi (HT), lenfosit infiltrasyonu ve kronik enflamasyon sonucu gelişen otoimmün bir tiroid hastalığıdır. Erişkin hastalarda yapılan çalışmalarda trombosit-lenfosit oranı (PLR) ve nötrofil-lenfosit oranı (NLR) ve sistemik immün-enflamasyon indeksi (SII) gibi hematolojik parametrelerin HT'nin biyobelirteçleri olarak kullanılabileceği gösterilmiş olsada, pediatrik yaş grubunda bilgi sınırlıdır. Çalışmamızın amacı, çocuklarda HT'yi öngörmede hematolojik biyobelirteçlerin potansiyelini araştırmaktır.

**Yöntem:** Bu retrospektif kesitsel çalışmaya hastanemiz çocuk endokrinoloji bölümünde Temmuz 2020 ile Temmuz 2021 tarihleri arasında takip edilen HT'li çocuklar (n=165) ile aynı yaş ve cinsiyetteki kontrol grubu (n=122) karşılaştırıldı.

**Bulgular:** HT'li çocuklar ve kontrol grubu karşılaştırıldığında ortalama lökosit sayısı (p>0.05), trombosit sayısı (p>0,05), mutlak nötrofil sayısı (p>0,05), NLR (p>0,05) ve SII (p>0,05) istatistik olarak fark saptanmadı. HT grubunda PLR değerleri kontrol grubuna göre anlamlı olarak daha yüksek olmasına rağmen (p<0,05), alıcı işlem karakteristikleri (receiver operating characteristic curves) analizinde, PLR değerleri HT'yi öngörmede düşük özgüllük ve duyarlılığa sahipti.

**Sonuç:** Çalışmamız, NLR ve SII'nin çocuklarda HT'yi öngörmede yararlı göstergeler olmadığını göstermektedir. PLR değerinde istatistiksel olarak anlamlı bir fark olmasına rağmen, özgüllüğü ve duyarlılığının düşük olması nedeniyle yararlı bir belirteç olmadığını saptanmıştır.

**Anahtar kelimeler:** Hashimoto tiroiditi, pediatrik, nötrofil-lenfosit oranı, trombosit-lenfosit oranı, sistemik bağışıklık-enflamasyon indeksi

**Received:** 30.10.2021
**Accepted:** 25.03.2022

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**Cite as:** Kırkgöz T, Özkan B. Could Hematologic Parameters have a Predictive Role in Pediatric Hashimoto Thyroiditis?. J Dr Behcet Uz Child Hosp. 2022;12(2):159-163

## INTRODUCTION

Hashimoto thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is the most common cause of acquired thyroid diseases in children with or without goiter [1]. It is a thyroid gland specific autoimmune disease, characterized by autoimmune-mediated destruction [2]. The prevalence of HT in the pediatric population peaks at puberty. HT is more common in females and the presentation of the disease is rare before the age of 3 years. Strong female preponderance and also high prevalence in patients with Down and Turner syndrome have been reported. Clinical manifestations of HT are observed in the pediatric population in a spectrum ranging from completely normal, to severe thyroid dysfunction [3]. The pathology of HT is characterized with diffuse lymphocytic infiltration of thyroid gland with T-cells, fibrosis, parenchymal atrophy, evidence of goiter or thyroid glandular atrophy, elevated serum anti-thyroid antibodies and dysfunction to varying degrees [4].

Neutrophils (N) and platelets (P) play an active role in inflammation and have regulatory roles in the immune system. Recent studies have shown that the rates of various parameters such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in the hemogram have been used as effective predictive markers for the prognosis, survival and morbidity in a wide variety of diseases including autoimmune diseases such as psoriasis, ulcerative colitis, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, Takayasu's arteritis, Behçet's disease and also various malignancies [5]. Another marker is systemic immune-inflammation index (SII) formulated by (N×P)/L where N, P and lymphocyte (L) represent N, P and lymphocyte counts, respectively. SII is associated with various diseases especially with malignancies, cardiovascular and infectious diseases [6].

In this study, we hypothesized that hemogram parameters may have a predictive value in pediatric patients with HT. To this end, NLR, PLR and SII levels were examined in 165 pediatric patients with HT and 122 patients in the control group.As far as we know, this is the first study that examines the predictive role of hematologic parameters (PLR, NLR ans SII) in pediatric patients with HT.

## MATERIALS and METHODS

A total of 287 children (165 HT patients, and 122 healthy controls) were enrolled in this retrospective study. Patients data obtained from a retrospective scan of files of the patients who were admitted to our outpatient clinic between Janunary 2021 and December 2021. This study was approved by the University of Health Sciences Turkey İzmir Dr. Behçet Uz Pediatric Diseases and Surgery training and research Hospital Clinical Research Ethics Committee. (decision number: 2021/15-04, date: 07.10.2021)

Patients with other autoimmune diseases such as diabetes mellitus, chronic inflammatory disease, and patients with conditions that directly affect hematologic parameters such as, hematologic diseases, patients on anticoagulation therapy, hepatic or renal disorders, heart failure, myeloproliferative disorders, and acute or chronic infection were excluded from the study.

Hemogram parameters were determined using Sysmex NX1000 Automated Hematology System. All blood samples were obtained after 12 hours of fasting and analyzed within one hour after venipuncture. NLR and PLR values were calculated by dividing the N and P counts by absolute L counts, respectively. SII is calculated by (N×P)/L formula. Thyroid-stimulating hormone (TSH) and free thyroxine ($fT_4$) parameters were studied using Abbott Architect I 2000 SR® immunoassay analyzer.

Laboratory data were uploaded from computerized patient database. Demographic characteristics of the patients, and laboratory findings were reviewed from the files of the participants. Patients did not undergo any investigations other than those routinely requested. Hemoglobin (Hgb), hematocrit (Hct), leukocyte, N, L, P counts, C-reactive protein (CRP), serum TSH, $fT_4$, free triiodothyronine, and anti-thyroglobulin (anti-TG), anti-thyroid peroxidase (anti-TPO) values were recorded.

### Statistical Analysis

Data obtained from this study were analyzed using GraphPad Prism (statistical software, version 8.0.0). Values are expressed as mean ± standard deviation. The statistical comparisons for mean values were performed using paired t-test for parametric, and Mann-Whitney test for nonparametric variables. Chi-square test was used to compare differences between categorical variables, and receiver operating characteristic (ROC) curve analysis was conducted to find out the cut-off values for PLR parameters. In the analyses, p=0.05 was accepted as the level of statistical significance.

## RESULTS

One hundred and sixty- five HT patients [138 females (F) and 27 males (M)] and one hundred and twenty-two healthy controls (98 F and 24 M) were enrolled in this study. The mean ages of the patient, and the control

groups were was 13.5±3.3, and 12.6±2.8 years, respectively. F/M ratios of the HT patient and control groups were 5.1 and 4.0, respectively. NLR and SII were a little bit, but not statistically significantly higher in the HT group compared to the control group (1.65±1.09 and 1.42±0.49; p>0.05 for NLR, 512±373 and 450±275; p>0.05 for SII, respectively). However, PLR was significantly higher in HT patients to the control group (126.7±83.7, and 111.3±33.5; p=0.02, respectively). White blood cell (WBC), P, N, counts and CRP were not significantly different between HT patients and the control group. Whereas, L counts, levels of Hgb and Htc were statistically significantly lower in HT patients compared to the control group (2.74±0.91 and 2.99±0.92; p<0.05 for L, 12.8±0.99 and 13.3±1.2; p<0.05 for Hgb, 38.5±3 and 39.5±3.3; p<0.05 for hct, respectively). Laboratory characteristics of the two groups are presented in Table 1.

ROC curve analysis was performed for PLR parameter. The descriptive cut-off value of PLR was 112.8 with 61.7% sensitivity and 62.1% specificity. The area under curve for PLR was 0.596±0.04, and p=0.018) (Figure 1). In the correlation analysis, NLR and PLR values were not significantly correlated with anti-TPO and anti- TG values (p=0.33 r=0.07 for PLR-anti-TPO, p=0.15, r=0.11 for PLR-anti-TG, p=0.63 r=-0.03 for NLR-anti-TPO and p=0.33 r=0.07 for NLR-anti-TG).

## DISCUSSION

HT is the most common cause of acquired hypothyroidism in childhood and adolescence.

Although the etiology of HT is still not fully understood, its pathogenesis reflects the combination of immunologic, genetic, and environmental factors [7,8]. As for the pathophysiology of disease, HT develops due to especially increased sensitized t-cell activation and cytokine levels. Ns and Ls are involved in the production of these cytokines. Hematologic parameters can be easily calculated from hemograms. Relationship with hematologic markers and HT disease have been reported in several studies [9-12].

Although WBC, N, and P counts were not statistically different between HT patients and the control group, L counts were lower in the HT group. Similarly, Bilge et al. [11] showed that L counts were lower in HT patients, while Cengiz et al. [13] showed that the number of Ls decreases in acute inflammatory phase of subacute granulomatous thyroiditis which can be explained with accumulation of Ls in the thyroid gland. In addition, hematocrit and hemoglobulin levels were lower in HT patients in comparison with control subjects. This fact may be accounted for the crucial effect of thyroid hormones on erythropoiesis via erythropoietin production enhancement and also proliferation of erythroid progenitors. However, iron-deficient anemia negatively affects thyroid hormone status [14].

Inflammatory conditions temporarly change N and L counts and abnormalities in the activation of Ns and Ls and defective apoptosis may lead to development of autoimmune disorders. NLR has been recently described as a simple and novel inflammatory marker in malignancy,

**Table 1. Laboratory characteristics of the patient and control groups**

| | Patients group | Control group | p |
|---|---|---|---|
| Age (mean ± SD) | 13.5±3.3 | 12.6±2.8 | >0.05 |
| TSH (mean ± SD) | 10.4±19.5 | 2.1±1.0 | <0.05 |
| $fT_4$ (mean ± SD) | 0.97±0.23 | 1.0±0.12 | >0.05 |
| Hb (mean ± SD) | 12.8±0.99 | 13.3±1.2 | <0.05 |
| Hct (mean ± SD) | 38.5±3 | 39.5±3.3 | <0.05 |
| Leukocyte ($10^3/mm^3$) (mean ± SD) | 7.63±2.2 | 7.7±2.0 | >0.05 |
| Neutrophil ($10^3/mm^3$) (mean ± SD) | 4.09±1.84 | 3.92±1.6 | >0.05 |
| Lymphocyte ($10^3/mm^3$) (mean ± SD) | 2.74±0.91 | 2.99±0.92 | <0.05 |
| Platelet ($10^3/mm^3$) (mean ± SD) | 306±75 | 315±81 | >0.05 |
| PLR (mean ± SD) | 126.7±83.7 | 111.3±33.5 | <0.05 |
| NLR (mean ± SD) | 1.65±1.09 | 1.42±0.49 | >0.05 |
| SII (mean ± SD) | 512±373 | 450±275 | >0.05 |
| CRP (mg/dL) (mean ± SD) | 0.3±0.2 | 0.3±0.2 | >0.05 |

TSH : Thyroid stimulating hormone, $fT_4$ : Free thyroxine, Hb: Hemoglobulin, Hct: Hematocrit, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, SII: Systemic immune-inflammation index, CRP: C-reactive protein

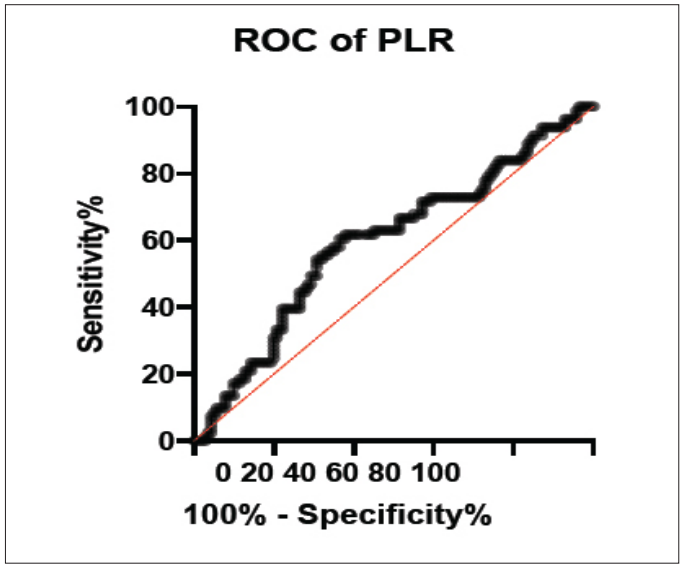


**Figure 1.** ROC curves for the PLR. For PLR, AUC was 0.596, with 61.7% sensitivity and 62.1% specificity

ROC: Receiver operating characteristic, PLR: Platelet-to-lymphocyte ratio, AUC: Area under curve

autoimmune inflammatory and cardiovascular diseases. In adult studies, NLR is statistically significantly higher in the HT group than the control group and higher NLR values reported might be a reliable predictive marker of the clinical course of the Hashimoto throiditis [9,11,12]. Moreover, Bilge et al. [11] showed that levothyroxine ($LT_4$) treatment decreases NLR and PLR. In the literature, some studies showed that $LT_4$ replacement therapy decreases inflammation and oxidative stress. In our study, although NLR was a little bit , but not significantly higher in the HT group which may be explained with changes in hemogram parameters during childhood. This study has demonstrated that NLR is not correlated with either thyroid antibodies or disease prognosis. Thus, we think that NLR is a nonspecific marker for all autoimmune diseases and is not a useful tool for the prediction of diagnosis and prognosis of HT.

Some literature stuides have shown that, PLR can provide valuable information about autoimmune and rheumatologic diseases and some malignancies. PLR values reflected shifts in P, L, N, or monocyte counts. Some authors have suggested that interpretation of PLR together with other hematologic markers have more accurate diagnostic value in inflammatory rheumatic diseases and also predicts related comorbidities [13]. Some literature studies have demonstrated the relationship between PLR values and diagnosis and disease activity in adult HT patients [9,11,12]. Ps interact with leukocytes in autoimmune diseases and are regarded as central players in the pathophysiology of especially vascular inflammation. In our study PLR was significantly higher in the HT group but ROC analysis revealed that sensitivity and specificity is not strong enough to use PLR values in predicting HT (Figure 1). In addition, PLR values were not correlated either with thyroid antibodies or with disease prognosis, as were NLR values.

The last marker, we compared between the two groups, was SII. It correlates positively with N and P counts, and negatively with L counts, and clinical significance of SII have been reported in inflammatory diseases and malignancies. In our study there were not significant differences between the HT and control groups in terms of SII values.

### Study Limitations

This study was a retrospective basis and represented a single-center experience. The limited number of patients and, short follow-up period are major limitations of the study. Higher number of patients should be followed up for longer periods to achieve a higher statistical significance.

## CONCLUSION

Our study suggested that NLR and SII are not useful indicators in predicting the course of HT. In addition, although there was a statistically significant difference between the HT, and the control groups in terms of PLR values we think that it is not a useful marker due to its low specificity and sensitivity.

**Acknowledgments:** We would like to thank to the parents and the patients reported in this study.

### Ethics

**Ethics Committee Approval:** This study was approved by the University of Health Sciences Turkey, İzmir Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Clinical Research Ethics Committee. (decision number: 2021/15-04, date: 07.10.2021)

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: T.K., Concept: T.K., B.Ö., Design: T.K., B.Ö., Data Collection and/or Processing: T.K., Analysis and/or Interpretation: T.K., B.Ö., Literature Search: T.K., B.Ö., Writing: T.K., B.Ö.

**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.2022.37539
J Dr Behcet Uz Child Hosp 2022;12(2):164-168

Original Article

# Endocrine Surgery and Pediatic Surgery Partnership Reduces Complication Rate of Pediatric Thyroidectomy

## *Endokrin Cerrahisi ile Çocuk Cerrahisi Ortaklığı Pediatrik Tiroidektomide Komplikasyon Oranını Azaltır*

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## ABSTRACT

**Objective:** Thyroidectomy is not a common procedure performed in childhood. The aim of this study is to evaluate the clinical data and postoperative results of pediatric patients who underwent thyroidectomy in our center performed by a pediatric and an endocrine surgeon working in collaboration.

**Method:** Patients under the age of 18 who underwent thyroidectomy between 2008-2020 were included in this study. Demographic data, clinical data, preoperative evaluation results and postoperative complications were reviewed retrospectively.

**Results:** A total of 21 patients were included in the study. Postoperative bleeding, surgical site infection, transient and permanent recurrent nerve palsy, and permanent hypocalcemia were not detected in any patient. Transient hypocalcemia developed in only 2 patients (9.5%) in the postoperative period.

**Conclusion:** Small sample size and avoiding central neck dissection may also be effective in our low postoperative complication rate. However, it was thought that pediatric thyroidectomy procedure performed by a general surgeon and a pediatric surgeon experienced in endocrine surgery in collaboration may change the postoperative complication rate.

**Keywords:** Adolescent, fine needle aspiration biopsy, pediatric thyroid surgery, thyroid, thyroidectomy, thyroid nodule

## ÖZ

**Amaç:** Tiroidektomi çocukluk çağında sık uygulanan bir işlem değildir. Bu çalışmanın amacı bir çocuk cerrahı ve bir endokrin cerrahın birlikte çalıştığı merkezimizde tiroidektomi yapılan çocuk hastaların klinik verilerini ve ameliyat sonrası sonuçlarını değerlendirmektir.

**Yöntem:** Çalışmaya 2008-2020 yılları arasında tiroidektomi yapılan 18 yaş altı hastalar dahil edildi. Demografik veriler, klinik veriler, ameliyat öncesi değerlendirme sonuçları ve ameliyat sonrası komplikasyonlar geriye dönük olarak incelendi.

**Bulgular:** Çalışmaya 21 hasta dahil edildi. Hiçbir hastada postoperatif kanama, cerrahi alan enfeksiyonu, geçici veya kalıcı rekürren sinir felci ve kalıcı hipokalsemi saptanmadı. Postoperatif dönemde sadece 2 hastada (%9,5) geçici hipokalsemi gelişti.

**Sonuç:** Postoperatif komplikasyon oranının düşük olmasında örneklem büyüklüğünün küçük olması ve santral boyun diseksiyonunun olmaması da etkili olabilir. Ancak pediatrik tiroidektomi prosedürünün endokrin cerrahisi konusunda deneyimli bir genel cerrah ve çocuk cerrahı tarafından birlikte uygulanmasının postoperatif komplikasyon oranını değiştirebileceği kanısına varılmıştır.

**Anahtar kelimeler:** Adölesan, ince iğne biyopsisi, pediatrik tiroid cerrahisi, tiroid, tiroidektomi, tiroid nodülü

**Received:** 15.02.2022
**Accepted:** 07.04.2022

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**Cite as:** Sayan A, Üstün M, Mert M, Karaali C, Köylüoğlu G. Endocrine Surgery and Pediatic Surgery Partnership Reduces Complication Rate of Pediatric Thyroidectomy. J Dr Behcet Uz Child Hosp. 2022;12(2):164-168

## INTRODUCTION

Since the indications for thyroidectomy in children are more limited than in adults, thyroidectomy is not a frequently performed procedure in childhood [1]. Therefore, data in the literature on the pediatric thyroidectomy procedure are limited. When the literature is reviewed, the most common indication for thyroidectomy in children and adolescents is the presence of thyroid nodules [1,2]. The incidence of thyroid nodules in children and adolescents is lower than in adults, and the risk of malignancy in thyroid nodules in childhood is higher [3]. Although it is rarely seen in children, childhood thyroid nodules should be evaluated well because of the high risk of malignancy.

The 2016 guideline of the American Thyroid Association (ATA) recommends that more than 25 thyroid surgeries should be performed annually in order to be experienced in thyroid surgery [4]. It is difficult for a pediatric surgeon to reach these numbers and be experienced in thyroid surgery due to the small number of patients. Endocrine surgeons experienced in thyroidectomy have limited experience with pediatric patients.

The aim of this study is to evaluate the clinical findings and postoperative results of pediatric patients who underwent thyroidectomy performed by pediatric surgeon and an endocrine surgeon in collaboration in a tertiary center.

## MATERIALS and METHODS

### Ethics Commitee Approval

The study was conducted after obtaining approval from University of Health Sciences Turkey, İzmir Tepecik Education and Research Hospital Non-Interventional Research Ethics Committee (decision no: 2021/12-15, date: 15.12.2021).

### Study Design

Patients under the age of 18 who underwent thyroidectomy in our center between 2008 and 2020 were included in the study. Demographic data, ultrasonographic (USG) nodule size (mm), fine needle aspiration biopsy (FNAB) results, surgical procedure (hemi/total/completion thyroidectomy), pathology results, operation time (minutes), postoperative hospitalization time (days), presence of postoperative bleeding, transient or permanent hypocalcemia and/or recurrent laryngeal nerve *(N. laryngeus recurrens)* (RLN) paralysis, or a syndrome that constitutes prophylactic thyroidectomy indication were retrospectively analyzed. FNAB results were evaluated according to The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) criteria neuromonitoring is not performed in our center except for high-risk patients. Since the aim of this study was to evaluate the complication rates, the cases that underwent neuromonitorization were excluded from this study.

Postoperative serum calcium level below 8 mg/dL was defined as hypocalcemia. Hypocalcemia lasting less than six months was defined as transient, and hypocalcemia lasting longer as permanent hypocalcemia. Direct laryngoscopy was performed on the patient with dysphonia, dyspnea and swallowing disorder, and RLN function was evaluated. RLN palsy lasting more than 6 months and proven by laryngoscopy was considered permanent paralysis.

### Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA).

## RESULTS

Seven (33.3%) male, and 14 (66.6%) female patients were enrolled in the study. The median interquartile range (IQR) age of the patients was 14 (11.5-16) (range 5-17) years. The median IQR thyroid nodule size was 30 (14-38) (range 0-46) mm. Total thyroidectomy was performed in 17 (81%), completion thyroidectomy in 2 (9.5%) and hemi-thyroidectomy in 2 (9.5%) patients. Two (9.5%) patients whose hemithyroidectomy specimens obtained were reported as having malignant histopathology, underwent completion thyroidectomy. Mean operation time was 116.7 [standard deviation (SD): ±21.4, range: 70-140] minutes. The mean duration of hospitalization in the postoperative period was 2 (SD: ±0.6, range: 1-4) days. Pathology specimen results and FNAB results are given in Table 1. The pathology result of one patient who underwent prophylactic total thyroidectomy due to the diagnosis of multiple endocrine neoplasia 2 (MEN 2) was reported as medullary microcarcinoma, and the pathology result of the other patient who was operated with the same indication was reported as C-cell hyperplasia. In addition, the pathology result of a patient who had no additional syndromic diagnosis and was operated for suspected malignancy was reported as medullary microcarcinoma. Demographic and clinical data of all patients are given in Table 2. Distant metastasis

**Table 1. Pathology specimen results and fine needle aspiration biopsy results**

| FNAB results (acording to TBSRTC) | Pathology specimen results | |
|---|---|---|
| | Benign | Malignant |
| 1 | 1 | 0 |
| 2 | 6 | 2 |
| 3 | 0 | 1 |
| 4 | 3 | 2 |
| 5 | 1 | 2 |
| 6 | 0 | 1 |

FNAB: Fine needle aspiration biopsy, TBSRTC: The Bethesda System for Reporting Thyroid Cytopathology

or cervical lymph node metastasis was not detected in any patient who received histopathologic diagnosis of malignancy in the preoperative and peroperative period.

Postoperative complications as postoperative bleeding, surgical site infection, transient or permanent RLN paralysis, and permanent hypocalcemia were not detected in any of the patients. In total, 2 (9.5%) patients developed transient hypocalcemia in the postoperative period. None of the patients required parathyroid autotransplantation.

**Table 2. Demographic and clinical data of all patients**

| | Number of patients | | |
|---|---|---|---|
| Sex | | | |
| Male | 7 (33.3%) | | |
| Female | 14 (66.7%) | | |
| Age (mean ± SD) | 13.3±3.5 | min-max: 5-17 | median (IQR): 14 (11.5-16) |
| Ultrasonographic nodule size (mm) (mean ± SD) | 26.2±14.2 | min-max: 0-46 | median (IQR): 30 (14-38) |
| FNAB results (acording to TBSRTC) | | | |
| Bethesda 1 | 1 (4.8%) | | |
| Bethesda 2 | 8 (38.1%) | | |
| Bethesda 3 | 1 (4.8%) | | |
| Bethesda 4 | 5 (23.8%) | | |
| Bethesda 5 | 3 (14.3%) | | |
| Bethesda 6 | 1 (4.8%) | | |
| Type of operation | | | |
| Hemithyroidectomy | 2 (9.5%) | | |
| Completion thyroidectomy | 2 (9.5%) | | |
| Total thyroidectomy | 17 (81%) | | |
| Operation time (min.) (mean ± SD) | 116.7±21.4 | min-max: 70-140 | median (IQR): 125 (115-130) |
| Pathology results | | | |
| Bening | 11 (52.4%) | | |
| Papillary carsinoma clasical variant | 3 (14.3%) | | |
| Medullary microcarcinoma | 2 (9.5%) | | |
| Papillary microcarcinoma | 1 (4.8%) | | |
| Papillary carsinoma follicular variant | 2 (9.5%) | | |
| Papillary carsinoma oncocytic variant | 1 (4.8%) | | |
| C cell hyperplasia | 1 (4.8%) | | |
| Complications | | | |
| Transient hypoparathyroidism | 2 (9.5%) | | |
| Transient RLN paralysis | 0 | | |
| Permanent hypoparathyroidism | 0 | | |
| Permanent RLN paralysis | 0 | | |
| Hospitalization day (mean ± SD) | 2±0.6 | min-max: 1-4 | median (IQR): 2 (2-2) |
| | n=21 (100%) | | |

SD: Standard deviation, FNAB: Fine needle aspiration biopsy, TBSRTC: The Bethesda System for Reporting Thyroid Cytopathology, RLN: Recurrent laryngeal nerve *(N. laryngeus recurrence)*, IQR: Interquartile range, min: Minumum, max: Maximum

## DISCUSSION

Although most thyroid nodules are benign, the thyroid gland is more susceptible to radiation and carcinogenesis in children. Therefore, although thyroid nodules are rarely seen in childhood, the risk of malignancy is higher [5,6]. Thyroid nodules are the most common indication for thyroidectomy. Literature data on peroperative evaluations and postoperative complications are limited, as thyroidectomy is not a frequently performed procedure due to the rarity of thyroid nodules in childhood and adolescence.

The most common endocrine complication after thyroidectomy, both in adults and children, is hypocalcemia. Hypocalcemia develops secondary to hypoparathyroidism that occurs due to trauma to the parathyroid glands during surgery or devascularization of the parathyroid gland [7,8]. de Jong et al. [9] investigated a case series consisting of 106 patients, and reported that although the incidence of postoperative hypocalcemia and hypoparathyroidism in children who underwent total thyroidectomy was higher compared to adults, it was not associated with age, indication for surgery, and extent of surgery. Radakrishnan et al. [10] reviewed 15 studies including 1552 cases, and reported that hypocalcemia developing after thyroidectomy in the pediatric population is particularly common in high-risk groups. Wu et al. [11] evaluated 184 patients whose FNAB results were classified in categories IV-V-VI according to TBSRTC criteria or who underwent early thyroidectomy for rearranged during transfection (RET) germline mutation (MEN2A, MEN2B, or familial medullary thyroidcarcinom), and reported that extrathyroidal involvement and central neck dissection are two independent risk factors for postoperative hypoparathyroidism. In addition, it was emphasized that surgeons operating in these patients should be aware of the relatively high risk of postoperative hypoparathyroidism, that they should master special intraoperative techniques, including liberal use of parathyroid autotransplantation for devascularized parathyroid glands, and exercise due care during surgery to preserve parathyroid functions [11]. In our study, as a postoperative complication transient hypocalcemia was observed in only 2 patients. These patients were operated for differentiated thyroid cancer and RET germline mutation (MEN2A) in line with the literature. Presumably, our lower postoperative complication rates were related to the small sample size, refraining from performing central neck dissection in any of the patients, and intraoperative collaboration of a pediatric surgeon who is familiar with pediatric neck anatomy and a general surgeon (>30 thyroid surgeries per year) experienced in endocrine surgery.

Wood et al. [12] reported that optimal surgical results would be achieved in pediatric thyroidectomy with the cooperation of pediatric endocrinologists as well as high-volume endocrine surgeons and pediatric surgeons. Scheumann et al. [13] reported that high-volume endocrine surgeons had achieved better outcomes, shorter postoperative hospitalization time, and lower costs after thyroidectomy and parathyroidectomy in children compared to pediatric surgeons, general surgeons, or otolaryngologists. Considering the data in our study, it was thought that a collaborative approach between pediatric surgeons and endocrine surgeons would yield better results.

While evaluating pediatric thyroid nodules, decision to perform FNAB according to USG findings and clinical features is debatable. ATA recommends USG-guided FNAB for nodules larger than 1 cm. In addition, USG-guided FNAB is also recommended in case of ultrasonographically detected hypoechogenicity, irregular margin, increased intranodular blood flow, presence of microcalcifications, abnormal cervical lymph nodes (except for hyperfunctional nodules requiring direct surgery) that are smaller than 1 cm. FNAB results are evaluated according to TBSRTC [14,15] criteria. In the article reported by Partyka et al., [16] 186 FNAB results from 154 patients were evaluated and FNAB was found as a sensitive and highly specific modality for evaluating thyroid nodules in the pediatric patient group. In our study, FNAB results were similarly consistent with the pathology reports.

### Study Limitations

The limitations of the study are that it is based on retrospective data analysis and there is no control group.

## CONCLUSION

Thyroidectomy is a less common surgical procedure performed in childhood compared to adults. Thyroid nodules are the most common indication for pediatric thyroidectomies and FNAB is a safe method in the evaluation of thyroid nodules. Therefore, we believe that thyroidectomy performed by a general surgeon experienced in endocrine surgery and a pediatric surgeon in collaboration will reduce the possible postoperative complication rates.

### Ethics

**Ethics Committee Approval:** The study was conducted after obtaining approval from Health Sciences Turkey, İzmir Tepecik Education and Research Hospital Non-Interventional Research Ethics Committee (decision no: 2021/12-15, date: 15.12.2021).

**Informed Consent:** Since the study design was retrospective and data were collected anonymously, informed consent was waived.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: A.S., M.Ü., M.M., C.K., Concept: A.S., M.Ü., M.M., C.K., G.K., Design: A.S., M.Ü., M.M., C.K., Data Collection and/or Processing: A.S., M.M., Analysis and/or Interpretation: A.S., M.Ü., M.M., C.K., G.K., Literature Search: M.M., Writing: M.Ü., M.M.

**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.2022.92678
J Dr Behcet Uz Child Hosp 2022;12(2):169-175

**Original Article**

# Serum 25-Hydroxyvitamin D Levels in Preterm Infants Born at Gestational Age of ≤32 Weeks and Prematurity-related Morbidities and Complications

## *Gebelik Yaşı ≤32 Hafta Olan Preterm İnfantlarda Serum 25-Hidroksivitamin D Düzeyleri ve Prematürite İlişkili Morbidite ve Komplikasyonlar*

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## ABSTRACT

**Objective:** To investigate the association between vitamin D levels and prematurity-related morbidities and complications in preterm infants born at ≤32 gestational weeks

**Method:** Newborns having a gestational age of ≤32 weeks were included in the study. Lower 25-hydroxyvitamin D (25-OHD) levels (≤15 ng/mL) were detected in the study, and normal 25-OHD levels (>15 ng/mL) were in the control group. Neonatal and maternal demographic features, laboratory findigs, clinical outcomes, prematurity-related morbidities and complications were investigated for two groups.

**Results:** A total of 122 preterm infants were evaluated in the study. The study group consisted of 73 (60%) and the control group comprised 49 (40%) infants. The study group more frequently used antenatal steroid (p=0.046). First and fifth minute - Apgar scores were lower in the study group (p=0.001 and p=0.003, respectively). Duration of invasive mechanical ventilation was longer in the study group (p=0.02). Late-onset sepsis (LOS) was more often detected in the study group (p=0.0001). The incidence of hemodynamically significant patent ductus arteriosus (hsPDA) and metabolic bone disease of prematurity (MBD) was higher in the study group (p=0.001 and p=0.04, respectively).

**Conclusion:** Significant relationships were found between low vitamin D levels and LOS, hsPDA and MBD. Vitamin D usage during pregnancy is important to avoid maternal and neonatal vitamin D deficiency and its consequences.

**Keywords:** Preterm infant, morbidity, 25-hydroxyvitamin D level

## ÖZ

**Amaç:** Bu çalışmanın amacı, ≤32 gebelik haftasında doğan prematüre bebeklerde vitamin D düzeyleri ile prematüriteye bağlı morbidite ve komplikasyonlar arasındaki ilişkiyi değerlendirmektir.

**Yöntem:** Gebelik yaşı ≤32 hafta olan yenidoğanlar çalışmaya dahil edildi. Düşük 25-hidroksivitamin D düzeyi (≤15 ng/mL) çalışma grubu, normal 25- hidroksivitamin (>15 ng/mL) kontrol grubu olarak tanımlandı. Her iki grup için neonatal ve maternal demografik özellikler, laboratuvar bulguları, klinik sonuçlar, prematürite morbiditeleri ve komplikasyonları değerlendirildi.

**Bulgular:** Toplam 122 preterm infant çalışmada değerlendirildi. Çalışma grubunu 73 (%60) infant, kontrol grubunu ise 49 (%40) infant oluşturdu. Çalışma grubunda antenatal steroid kullanımı daha sıktı (p=0,046). Birinci ve beşinci dakika Apgar skorları çalışma grubunda daha düşüktü (sırasıyla; p=0,001 ve p=0,003). Çalışma grubunda invaziv mekanik ventilasyon süresi daha uzundu (p=0,02). Geç başlangıçlı sepsis çalışma grubunda daha sık saptandı (p=0,0001). Hemodinamik anlamlı patent duktus arteriyozus ve prematürenin metabolik kemik hastalığı insidansı çalışma grubunda daha yüksekti (sırasıyla; p=0,001 ve p=0,04).

**Sonuç:** Düşük vitamin D seviyesi ile geç başlangıçlı sepsis, hemodinamik anlamlı patent duktus arteriyozus ve prematürenin metabolik kemik hastalığı arasında anlamlı ilişki saptandı. Gebelik sırasında vitamin D kullanımı, maternal ve neonatal vitamin D eksikliğini ve sonuçlarını önlemek için önemlidir.

**Anahtar kelimeler:** Preterm infant, morbidite, 25-hidroksivitamin D seviyesi

**Received:** 16.02.2022
**Accepted:** 08.04.2022

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**Cite as:** Baldan E, Yarci E. Serum 25-Hydroxyvitamin D Levels in Preterm Infants Born at Gestational Age of ≤32 Weeks and Prematurity-related Morbidities and Complications. J Dr Behcet Uz Child Hosp. 2022;12(2):169-175

## INTRODUCTION

Vitamin D has functional roles in many systems, such as bone metabolism and fetal growth [1,2]. Vitamin D receptors (VDRs), which are found in osteoblasts, bronchial epithelial cells, alveolar type II cells, intestinal and skin epithelial cells, kidney tubules, parathyroid gland epithelium, pancreatic beta cells and immune system cells, are responsible for its effects [3]. It is best known for its effects on phosphorus (P) and calcium (Ca) homeostasis; but it also has considerable functions in fetal lung development, supporting the functions of the innate and adaptive immune system, intestinal cell proliferation, and differentiation, induction of apoptosis, stabilization of vascular smooth muscle and endothelial cells [4-9].

Deliveries occurring before the 37th gestational weeks is defined as preterm birth. There are approximately 15 million preterm births in the world every year, and approximately one million of these newborns die due to various complications [10,11]. Patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH), respiratory distress syndrome (RDS), early-onset sepsis (EOS), late-onset sepsis (LOS), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), periventricular leukomalacia, metabolic bone disease of prematurity (MBD) are the major morbidities [12,13].

This study aimed to investigate the association between vitamin D levels and prematurity-related morbidities and complications in preterm infants born at gestational age (GA) of ≤32 weeks.

## MATERIALS and METHODS

This single center retrospective study was carried out between April 2019 and April 2021 in Dörtçelik Children's Hospital, neonatal intensive care unit (NICU) in Bursa, Turkey. Ethical committee approval was received from Uludağ University Faculty of Medicine Clinical Research Ethics Committee (decision no: 2021-11/17, date: 11.08.2021).

Newborns with a GA of ≤32 weeks were included in the study. The 25-hydroxyvitamin D (25-OHD) levels measured at postnatal six hours in the NICU, were used for the categorization of preterm infants. The infants with low (≤15 ng/mL), and normal (>15 ng/mL) 25-OHD levels were allocated into the study, and the control groups, respectively [8]. Maternal demographic data related to maternal age, presence of concomitant disease and multiple pregnancies were used. The characteristics of the newborns such as birth weight (BW), GA, sex, GA-adjusted BW, mode of delivery, Apgar scores, antenatal steroid use, duration of invasive and noninvasive mechanical ventilation (MV), oxygen support, and hospitalization, total parenteral nutrition (TPN) and body weight at discharge and were recorded.

Blood samples for the measurement of alkaline phosphatase (ALP), parathyroid hormone (PTH), P, magnesium (Mg) and Ca were obtained from all participants at postnatal six hours in the NICU. Serum PTH and 25-OHD levels were measured by chemiluminescent immunoassay analyzer (Abbott i2000, Abbott Laboratories, USA). The photometry method on the Beckman Coulter AU680 analyzer (Danaher Corporation, Brea, CA, USA) was used to measure Mg, P, Ca and ALP levels.

Prematurity - related morbidities and complications such as RDS, pulmonary hemorrhage, EOS, LOS, hemodynamically significant patent ductus arteriosus (hsPDA), MBD, NEC, ROP, IVH, BPD and mortality were recorded.

### Statistical Analysis

SPSS version 20.0 software (SPSS Inc. Chicago, IL, USA) was used for statistical analysis. Categorical values were summarized as numbers and percentages. Continuous data with nonnormal distribution were presented as median [interquartile range (IQR)]. Mann-Whitney U test was used for non-normal distributions. The Fisher's Exact and chi-squared tests were used for categorical variables. A p value of <0.05 was accepted for statistical significance.

## RESULTS

Between April 2019 and April 2021, 122 preterm infants were evaluated including 73 (60%) infants in the study and 49 (40%) in the control group. The study group used antenatal steroids more frequently (p=0.046). The study group had lower 1 and 5- minute Apgar scores compared to the control group (p=0.001 and p=0.003, respectively). Other demographic characteristics were similar between the groups. All demographic characteristics are given in Table 1.

All laboratory findings were similar between the groups. When the groups were evaluated for the clinical outcomes, only duration of invasive MV was longer in the study group (p=0.02). The other clinical outcomes were similar between the groups (Table 2).

While the frequency of EOS was similar in both groups, the frequency of LOS was higher in the study group (p=0.0001). Also, hsPDA and MBD were more frequently detected in the study group (p=0.001 and p= 0.04, respectively). Other morbidities and complications were similar between the groups (Table 3).

## DISCUSSION

Effects of vitamin D on mineral and bone metabolism have been known for a long time. Recent studies have shown that VDRs are found in many tissues and vitamin D affects many systems [14,15]. Maternal vitamin D, which shows transplacental transmission throughout pregnancy, is the most important determinant of fetal and neonatal vitamin D levels [16,17]. In the literature, low vitamin D levels in the neonatal period have been linked to an increased risk of wheezing and asthma in later childhood [18].

Neonatal sepsis causes seriuos morbidity and mortality in the neonatal period. While EOS is associated with maternal transmission of microorganisms, LOS is encountered as a complication of prolonged NICU stay, especially in preterm infants [19,20]. Vitamin D affects cells of the immune system [21]. It has been reported that vitamin D deficiency in cord blood increases the risk of neonatal sepsis in preterm infants, but vitamin D deficiency was not found to be an independent risk factor for sepsis [8]. Contrary to that finding, low maternal and neonatal vitamin D levels were found to be associated with EOS in term infants [19]. In an another study, vitamin

**Table 1. Neonatal and maternal demographic features of the groups**

| Variables | Study group n=73 | Control group n=49 | p |
|---|---|---|---|
| GA at birth, weeks, median (IQR) | 31 (30-32) | 30 (30-31) | 0.88[a] |
| Birth weight, g, median (IQR) | 1400 (1148-1733) | 1570 (1200-1700) | 0.91[a] |
| Sex, male, n (%) | 39 (53) | 21 (43) | 0.25[b] |
| Type of delivery, C/S, n (%) | 66 (90) | 42 (86) | 0.42[b] |
| SGA, n (%) | 21 (29) | 11 (22) | 0.53[b] |
| Antenatal steroid usage, n (%) | 34 (47) | 14 (29) | 0.046[b] |
| 1- minute Apgar score, median (IQR) | 7 (6-8) | 8 (7-9) | 0.001[a] |
| 5- minute Apgar score, median (IQR) | 8 (8-9) | 9 (9-10) | 0.003[a] |
| Multiple pregnancies, n (%) | 21 (29) | 7 (14) | 0.06[b] |
| Maternal age, year, median (IQR) | 30 (23-33) | 27 (23-35) | 0.97[a] |
| Concomitant diseases, n (%) | 37 (51) | 30 (61) | 0.25[b] |

[a]Mann-Whitney U test, [b]Chi-square test, GA: Gestational age, IQR: Interquartile range, g: gram, C/S: Cesarean section, SGA: Small for gestational age

**Table 2. Laboratory findings and clinical outcomes of the groups**

| Variables | Study group n=73 | Control group n=49 | p |
|---|---|---|---|
| Ca, mg/dL, median (IQR) | 8.3 (7.7-9.1) | 8.5 (8.2-9.4) | 0.08[a] |
| P, mg/dL, median (IQR) | 5.5 (4.7-6.3) | 5.6 (4.7-6.1) | 0.66[a] |
| Mg, mg/dL, median (IQR) | 2 (1.8-2.6) | 2 (1.8-2.7) | 0.74[a] |
| ALP, IU/L, median (IQR) | 191 (147-244) | 192 (155-210) | 0.26[a] |
| PTH, pg/mL, median (IQR) | 49 (31-160) | 45 (26-110) | 0.11[a] |
| TPN, day, median (IQR) | 24 (14-44) | 23 (14-30) | 0.36[a] |
| Noninvasive MV, day, median (IQR) | 2 (0-7) | 1 (0-4) | 0.18[a] |
| Invasive MV, day, median (IQR) | 0 (0-6) | 0 (0-4) | 0.02[a] |
| $O_2$ support, day, median (IQR) | 2 (1-6) | 2 (1-3) | 0.43[a] |
| Body weight*, g, median (IQR) | 2345 (2073-2645) | 2400 (2050-2505) | 0.64[a] |
| Hospitalization**, day, median (IQR) | 41 (29-60) | 36 (28-52) | 0.4[a] |

[a]Mann-Whitney U test, *Body weight at discharge, **Duration of hospitalization, Ca: Calcium, IQR: Interquartile range, P: Phosphorus, Mg: Magnesium, ALP: Alkaline phosphatase, PTH: Parathyroid hormone, TPN: Total parenteral nutrition, MV: Mechanical ventilation, $O_2$: Oxygen

| Table 3. Prematurity - related morbidities and complications of the groups | | | |
|---|---|---|---|
| **Variables** | **Study group n=73** | **Control group n=49** | **p** |
| RDS, n (%) | 38 (52) | 18 (36) | 0.09[a] |
| EOS, n (%) | 12 (16) | 5 (10) | 0.33[a] |
| LOS, n (%) | 50 (68) | 8 (16) | 0.000[a] |
| Pulmonary hemorrhage, n (%) | 5 (7) | 0 (0) | 0.08[a] |
| hsPDA, n (%) | 22 (30) | 3 (6) | 0.00[a] |
| MBD, n (%) | 16 (22) | 4 (8) | 0.04[a] |
| NEC, n (%) | 13 (18) | 13 (26) | 0.25[a] |
| ROP, n (%) | 16 (22) | 12 (24) | 0.74[a] |
| IVH, n (%) | 16 (22) | 18 (36) | 0.07[a] |
| BPD, n (%) | 4 (5) | 0 (0) | 0.15[a] |
| Mortality, n (%) | 4 (5) | 0 (0) | 0.15[a] |

[a]Chi-square test, RDS: Respiratory distress syndrome, EOS: Early - onset sepsis, LOS: Late - onset sepsis, hsPDA: Hemodynamically significant patent ductus arteriosus, MBD: Metabolic bone disease, NEC: Necrotizing enterocolitis, ROP: Retinopathy of prematurity, IVH: Intraventricular hemorrhage, BPD: Bronchopulmonary dysplasia

D levels was lower in preterm infants diagnosed with LOS and the risk of LOS increased in case of vitamin D levels of ≤9.5 ng/mL [5]. Dhandai et al. [22] demonstrated that low vitamin D levels increase the risk of LOS in the late preterm and term infants. In the present study, while there was no relationship between vitamin D levels and EOS, vitamin D levels were lower in preterm infants who developed LOS. In studies evaluating the effects of vitamin D on the immune system and its association with neonatal sepsis, low vitamin D levels were found to increase the risk of both EOS and LOS. Similar to the literature, our study has found that preterm infants with low vitamin D levels had a higher risk of LOS. However, no relationship was identified between vitamin D levels and EOS.

BPD and RDS are major respiratory complications of preterm infants [23]. Surfactant is synthesized in alveolar type II cells and decreases alveolar surface tension. RDS is a neonatal respiratory system disorder caused by impared or decreased secretion of surfactants [24]. BPD is a disease characterized by long - term respiratory failure and its etiology and mechanism are not fully understood [25]. Vitamin D effects surfactant synthesis and secretion through VDRs in alveolar type II cells [26,27]. Low vitamin D levels were reported to be an independent risk factor for RDS [28]. Similar to that finding, maternal and neonatal vitamin D levels were lower in patients with BPD [29]. In another study evaluating RDS and BPD, vitamin D levels were lower in preterm infants with RDS (≤30 GA) and BPD (<34 GA) [23]. Contrary to that, Matejek et al. [17] found no association between vitamin D levels and RDS and BPD. We found that low vitamin D levels were not risk factors for these diseases.

Requirement for respiratory support in newborns is inversely proportional to GA [30]. Two studies reported that duration of invasive MV did not differ significantly according to vitamin D levels [17,31]. In addition, Matejek et al. [17] stated that the duration of noninvasive MV did not differ between vitamin D groups. Contrary to these studies, two studies reported that the duration of both invasive and noninvasive MV was longer in groups with low vitamin D levels [18,23]. Another study showed that the duration of invasive MV was longer in the lower vitamin D group [28]. Some studies in the literature have shown that the duration of oxygen support is longer in preterm infants with low vitamin D levels [18,23]. In our study, duration of invasive MV is longer in the study group, but without any intergroup difference in terms of duration of noninvasive MV and oxygen support. Prolonged exposure to high oxygen concentrations causes lung damage which plays a role in the development of BPD [25]. The similarity in the duration of oxygen support between the groups may explain the lack of difference in BPD.

MBD is mainly caused by abnormalities in Ca and P metabolism due to nutritional, environmental and biomechanical factors [32,33]. In MBD, abnormal bone mineralization, cortical and trabecular damage to the bones are observed. In severe cases, it can cause osteopenia and pathological fractures [32,34]. The usage of fortified breast milk, preterm formulas and advances in neonatal care has reduced the incidence of MBD in recent

years [34,35]. Ca and P are transmitted from the mother to the fetus most frequently in the third trimester [34]. Vitamin D deficiency and prolonged parenteral nutrition are other important risk factors for MBD [32]. In our study, although neonatal cholestasis was similar in both groups, the frequency of MBD was higher in the study group. Although there are studies for determining the optimal vitamin D supplementation to prevent MBD in preterm infants, there are no studies about relationship between vitamin D levels in first day after birth and MBD.

NEC is a multifactorial disease whose pathophysiology is not fully understood [36,37]. Genetic predisposition, inadequate intestinal function, excessive inflammatory response and alterations in microbiota play a role in the pathophysiology [36]. In a recent study, the incidence of NEC was 7% in very low birth weight infants followed in the NICU [38]. Vitamin D exerts its function in intestinal tissue by induction of cell proliferation, differentiation and apoptosis through VDRs [6]. In a study, maternal and neonatal vitamin D levels were found to be lower in the NEC group but only maternal vitamin D level was detected to be a significant predictor for NEC [39]. In another study, no association was found between vitamin D levels and NEC [17]. In our study any significant difference was not observed between the groups in terms of NEC.

PDA is an important congenital heart disease in preterm infants. While the ductus arteriosus is functionally closed within postnatal 72 hours in term infants, this closure may be delayed in preterm infants [40]. GA and BW are inversely related to the risk of PDA [41]. After birth, increased partial pressure of oxygen in the arterial blood, decreased prostaglandin E2 and prostacyclin 2 levels negatively effect ductal closure [42,43]. An animal study, showed that Ca flow through Ca channels and increased Ca sensitivity play a role in ductal closure. In the same study, it was observed that closure was delayed with Ca channel blockers [44]. Cakir et al. [41] measured ionized Ca (iCa) at the 1st and 48th postnatal hours in the hsPDA and non-hsPDA groups, and found that iCa levels was lower in the group with hsPDA. A study examining the association between vitamin D levels and PDA, showed that low vitamin D does not increase the risk of PDA [18]. In our study, although GA, BW and Ca levels were similar between the groups, hsPDA was higher in the study group.

ROP is another morbidity of preterm infants closely related to low BW and low GA. RDS, BPD, prolonged high oxygen delivery are other important risk factors [45,46]. In a study, it was stated that vitamin d deficiency may be effective in the development of ROP [45]. Similarly, Kim et al. [18] found that ROP was more common in patients with severe vitamin D deficiency. On the other hand, in our study, no relationship was found between vitamin D levels and ROP which may be related to similarities between our groups in terms of the most important ROP risk factors such as GA, BW, RDS, BPD and duration of oxygen support.

The risk of IVH increases with prematurity and low BW. Immature germinal matrix, hypoxic and ischemic brain damage, fluctuations in cerebral blood pressure, hemostatic abnormalities play a role in the pathogenesis [4]. Vitamin D exerts its effect on vascular smooth muscle cells by reducing angiogenesis, inflammation, proliferation and providing vascular endothelium stability [4,47]. Boskabadi et al. [4] found that preterm infants with IVH had lower serum vitamin D levels. In our study, the development of IVH was similar between the groups.

The present study focused on the relationships between neonatal 25-OHD levels and prematurity-related morbidities and complications.

### Study Limitations

This study has several limitations. Firstly, the maternal 25-OHD levels at the time of delivery were not evaluated. Secondly, pregnant women were given vitamin D supplementation beginning from the 12th weeks of gestation. The usage patterns of vitamin D (no usage, irregular use, regular use) were not examined in the study. As exposure to sunlight is the most important factor for vitamin D synthesis and use of sun - protective clothing is a major factor in this process which were not examined in the study. Another limitation was the small sample size of the study population.

## CONCLUSION

In this study, significant relationships were found between low vitamin D levels and LOS, hsPDA and MBD. Our study shows similar results as well as differences when compared with the studies in the literature, Further studies with larger sample size are required to achieve precise results. The advances in prenatal and neonatal care increase the chance of survival of newborns. This condition also causes an increase in morbidity due to prematurity. The main aim should be to prevent these morbidities before they occur. Therefore, vitamin D

usage during pregnancy is important to avoid vitamin D deficiency and its consequences.

### Ethics

**Ethics Committee Approval:** Ethical committee approval was received from Uludağ University Faculty of Medicine Clinical Research Ethics Committee (decision no: 2021-11/17, date: 11.08.2021).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: E.B., E.Y., Concept: E.B., E.Y., Design: E.B., E.Y., Data Collection and/or Processing: E.B., E.Y., Analysis and/or Interpretation: E.B., E.Y., Literature Search: E.B., E.Y., Writing: E.B., E.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.2022.73454
J Dr Behcet Uz Child Hosp 2022;12(2):176-183

Original Article

# Association Between Testicular Microlithiasis and Ultrasound-based Testicular Volume in Pediatric Population

## *Pediatrik Popülasyonda Testiküler Mikrolitiazis ve Ultrason ile Ölçülen Testiküler Volüm Arasındaki İlişki*

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## ABSTRACT

**Objective:** A potential relationship between testicular microlithiasis (TM) and testicular atrophy in childhood might increase the risk of testicular malignancy and infertility in adulthood. The present study aimed to determine the effect of the presence of TM on testicular volume by comparing the ultrasound-based testicular volumes between boys with TM and controls.

**Method:** A total of 140 boys (mean ± standard deviation, 9.86 years ± 5.44 years; age range, 0-18 years) with two descended testes were classified into a study group of 70 patients with TM (TM group) and an age-matched control group of 70 boys without TM (non-TM group). The TM group was subdivided based on the number of microliths in one transducer field as 'mild TM' (5-20 microliths) and 'severe TM' (>20 microliths). The ultrasound-based testicular volume (mL) was estimated by the Lambert equation as 0.71× length × width × depth.

**Results:** The average testicular volume in the TM group was 1.44 (0.70-4.68) mL and 3.09 (0.84-14.65) mL in the non-TM group. A lower testicular volume was observed in patients with TM, however, this difference was not significant (p=0.096). The average testicular volumes in patients with 'severe TM' and 'mild TM' were not significantly different (p=0.106). A lower testicular volume was found in older boys (≥15 years) with 'severe TM'.

**Conclusion:** We found no significant association in the testicular volume between boys with TM and controls, however, a lower testicular volume was observed in boys with TM. Thus, a close clinical follow-up might be considered in these patients.

**Keywords:** Testicular microlithiasis, testicular volume, ultrasonography

## ÖZ

**Amaç:** Çocukluk çağında testiküler mikrolitiazis (TM) ile testiküler atrofi arasındaki potansiyel bir ilişki, yetişkin döneminde testis malignitesi ve infertilite riskini artırabilir. Bu çalışma, TM'li erkek çocuklar ve kontroller arasında ultrason ile ölçülen testiküler volümlerini karşılaştırarak TM varlığının testis hacmi üzerindeki etkisini belirlemeyi amaçladı.

**Yöntem:** Çalışmamıza bilateral skrotal yerleşimli testisi olan toplam 140 erkek çocuk (ortalama ± standart sapma, 9,86 yıl ± 5,44 yıl; yaş aralığı, 0-18 yıl) dahil edildi. Çocuklar TM'li 70 hastadan oluşan bir çalışma grubu (TM grubu) ve aynı yaştaki TM'si olmayan 70 erkek çocuktan oluşan bir kontrol grubu (TM olmayan grup) olarak sınıflandırıldı. TM grubu, bir prob alanındaki mikrolit sayısına göre 'hafif TM' (5-20 mikrolit) ve 'şiddetli TM' (>20 mikrolit) olarak iki subgruplara ayrıldı. Testiküler volümü (mL), Lambert denklemi ile (0,71x uzunluk x genişlik x derinlik) hesaplandı.

**Bulgular:** Ortalama testis hacmi TM grubunda 1,44 (0,70-4,68) ml ve TM olmayan grubunda 3,09 (0,84-14,65) mL idi. TM'li hastalarda daha düşük testis hacmi gözlendi, ancak bu fark anlamlı değildi (p=0,096). 'Şiddetli TM' ve 'hafif TM' hastalarında ortalama testis hacimleri anlamlı olarak farklı değildi (p=0,106). 'Şiddetli TM' olan büyük erkek çocuklarda (≥15 yaş) daha düşük testis hacmi bulundu.

**Sonuç:** TM'li erkek çocuklar ve kontroller arasında testis hacminde anlamlı bir ilişki bulamadık, ancak TM'li erkek çocuklarda testis hacmi daha düşüktü. Bu nedenle bu hastaların yakın klinik takibi gerekebilir.

**Anahtar kelimeler:** Testiküler mikrolitiyazis, testis hacmi, ultrasonografi

**Received:** 02.02.2022
**Accepted:** 12.04.2022

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**Cite as:** Çolak E, Özkan B. Association Between Testicular Microlithiasis and Ultrasound-based Testicular Volume in Pediatric Population. J Dr Behcet Uz Child Hosp. 2022;12(2):176-183

## INTRODUCTION

Testicular microlithiasis (TM) is characterized by the presence of five or more microcalcifications in a single view of a testicular ultrasound [1]. TM is asymptomatic and commonly found incidentally on imaging [1,2]. The prevalence of TM is reported in the literature to range from 1.6% to 5.3% with a higher prevalence among children with testicular atrophy, undescended testis, genetic diseases (Down syndrome, Klinefelter syndrome), history of orchiopexy, and personal or family history of testicular germ cell tumor [3-5]. Several studies have also reported an association between TM and primary testicular neoplasia in the pediatric population [3]. According to the pediatric urology guideline of the European Association of Urology, self-examination of the testis is recommended on a monthly basis in boys with associated risk factors from puberty onwards. However, close clinical follow-up could be considered, if TM is still existing during the transition to adulthood [1]. In a 5-year follow-up study of 63 adults with TM by DeCastro et al. [6], only one patient had developed a testicular mixed germ cell tumor. As a result, a self-examination of the testicles was purposed for asymptomatic individuals with TM [6].

Testicular volume has been examined in children with undescended testis, varicocele, hydrocele, and Down syndrome [7-12], however, to date, the testicular volume in pediatric TM was reported in only one study with a small sample size (n=23) [13]. Our study reports the effect of the presence of TM on testicle volume in subjects from the newborn period up to 18 years of age.

Since a potential relationship between testicular atrophy and TM in childhood might increase the risk of testicular malignancy and infertility in adulthood, here we aimed to compare the ultrasound-based testicular volume between controls and boys with TM.

## MATERIALS and METHODS

### Ethical Statement

This comparative and cross-sectional study conformed to the Declaration of Helsinki and was approved by the University of Health Sciences Turkey, İzmir Dr Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Clinical Research Ethics Committee (approval number: 2021/16-06, date: 21.10.2021).

### Study Design

Between November 2018 and June 2021, a total of 232 patients underwent ultrasound examination of testes in the Department of Radiology. Patients with testicular torsion (n=4); orchitis (n=16); testicular trauma (n=3); testicular mass (n=1); cryptorchidism (n=46); previous scrotal surgery (n=12); chromosomal anomalies (n=3); congenital adrenal hyperplasia (n=0); and age over 18 years (n=0) were excuded from the analysis.

The diagnosis of classic TM was established on the following ultrasonographic criteria: Five or more non-shadowing hyperechogenic foci in one testis or in one transducer field, microlith size between 1 and 3 mm, uniform distribution, and preserved testicular shape. Boys with less than 5 microliths in a single view (n=7) [14], unilateral TM (n=0), and abnormal biomarker levels ($\alpha$-fetoprotein and $\beta$-human chorionic gonadotrophin) (n=0) were excluded from the study. Finally, a study group of 70 patients with two descended testes with TM (TM group) and an age-matched control group of 70 boys with two descended testes without TM (non-TM group) were enrolled. The TM group was divided into two subgroups based on the number of microliths in one transducer field: 'mild TM' (5-20 microliths) and 'severe TM' (>20 microliths) (Figure 1). The patients with

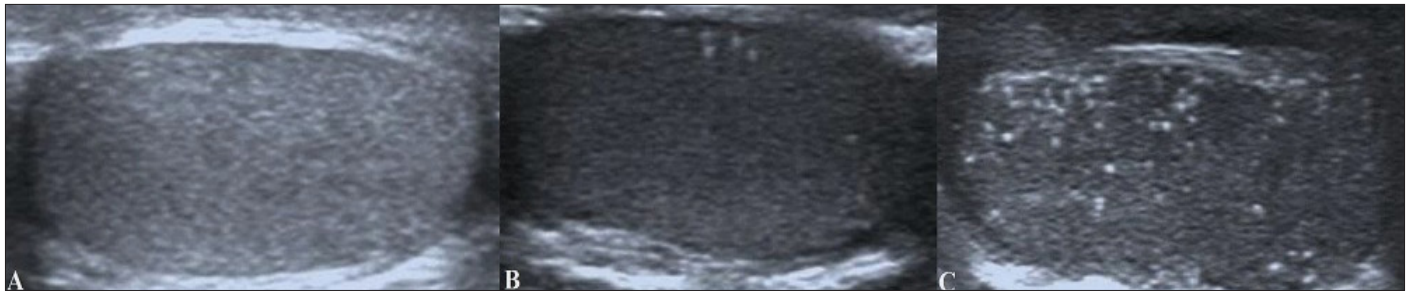


**Figure 1.** Sagittal ultrasound images of the testis (A) without testicular microlithiasis (non-TM group); (B) with 5-20 microliths in one transducer field (mild TM group); and (C) >20 microliths in one transducer field (severe TM group)

TM: Testicular microlithiasis

TM had normal levels of serum α-fetoprotein (α-FP) and β-human chorionic gonadotrophin (β-hCG).

The scrotal ultrasound scans were carried out by a single board-certified radiologist on an ultrasound machine (Aplio 500, Toshiba Medical System, Otawara, Japan) with a 5-12 MHz linear transducer. The ultrasound images were examined in two planes (transverse and sagittal). The maximum length (L) and width (W) of each testis were measured on a sagittal view and the maximum depth (D) was obtained from the transverse plane. The testicular volume (mL) was estimated by the Lambert equation as 0.71×L×W×D [15]. The testicular atrophy index (TAI) was calculated to evaluate the effect of the presence of TM on testicle volume by the following formula: TAI = (non-TM average testicle volume-TM average testicle volume)/non-TM average testicle volume x100 and expressed as a percentage [16].

Testicular atrophy was defined as a more than 50% reduction in the testicle volume compared to the normal testis (TAI >50%).

### Statistical Analysis

The distribution of the numeric variables was skewed based on a Kolmogorov-Smirnov test ($p<0.05$). Qualitative variables were reported as percentages and numeric variables were presented as median [interquartile range (IQR) 25-75]. Non-normally distributed data were log-transformed before regression analysis or the Mann-Whitney U test was used to compare the differences between groups. Spearman's correlation coefficients were used for correlations.

Analyses were conducted using SPSS statistical software (version 20, SPSS Inc., Chicago, IL, USA). Statistical significance was considered as a p-value of <0.05.

## RESULTS

### Study Population

The mean age (±SD) of the boys was 9.86 years (±5.44) and ranged from 0 to 18 years. The study and age-matched control groups were classified into five age groups: group 1 (0-2 years; n=7); group 2 (3-6 years; n=16); group 3 (7-10 years; n=12); group 4 (11-14 years; n=16); and group 5 (15-18 years; n=19). The TM group comprised 44 patients with mild TM (mean age 9.64±4.87 years) and 26 patients with severe TM (mean age 10.23±6.39 years).

### Testicular Volume Measurement

In patients with TM, the median IQR volume of the right and left testis were 1.50 (0.74-4.62) and 1.48 (0.77-4.94) mL, respectively. The median IQR volume of the right and left testis in boys without evidence of TM were 3.04 (0.82-14.76) and 2.01 (0.81-14.55) mL, respectively. The average testicular volume in the TM group was 1.44 (0.70-4.68) mL and 3.09 (0.84-14.65) mL in the non-TM group.

The right, left and average testicular volume increased significantly with increasing age and this was not dependent on the presence of TM (r=0.821, r=0.781, and r=0.827, respectively; $p<0.0001$ for all). The age-specific distribution and comparison of the right, left, and average testicular volume in the TM and non-TM groups are presented in Table 1. In the age-specific comparison, significantly lower testicular volumes were found in boys above 11 years with TM compared to those without TM ($p<0.01$ for all). However, there was no statistically significant association between the overall right, left, and average testicular volume in boys with TM compared to those without TM ($p=0.074$, $p=0.091$, $p=0.096$; respectively). Although it was statistically insignificant, a trend towards lower testicular volume was observed in patients with TM (Figure 2).

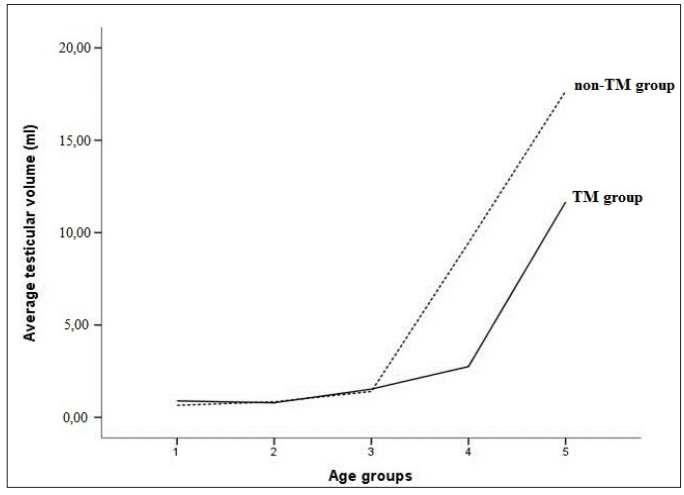


**Figure 2.** Comparison of the testicular volume (mL) between boys with testicular microlithiasis (TM group) and without testicular microlithiasis (non-TM group) among different age groups [Group 1 (0-2 years), Group 2 (3-6 years), Group 3 (7-10 years), Group 4 (11-14 years), Group 5 (15-18 years)]

TM: Testicular microlithiasis

**Table 1. Ultrasound-based testicular volume in the TM and non-TM groups according to age groups**

| Age groups (years) | TM group | | | non-TM group | | | p-value |
|---|---|---|---|---|---|---|---|
| | Right testicular volume (mL) | Left testicular volume (mL) | Average testicular volume (mL) | Right testicular volume (mL) | Left testicular volume (mL) | Average testicular volume (mL) | |
| **0-2** | 0.86 (0.63-1.51) | 0.83 (0.43-1.30) | 0.85 (0.43-1.43) | 0.63 (0.52-0.65) | 0.61 (0.45-0.76) | 0.63 (0.48-0.70) | $p^a$ =0.084<br>$p^b$=0.406<br>p=0.180 |
| **3-6** | 0.65 (0.46-1.04) | 0.80 (0.61-0.99) | 0.70 (0.59-1.14) | 0.62 (0.46-1.02) | 0.82 (0.64-0.95) | 0.73 (0.58-0.98) | $p^a$=0.910<br>$p^b$=0.651<br>p=0.940 |
| **7-10** | 0.87 (0.42-1.56) | 1.26 (0.47-1.80) | 1.06 (0.47-1.66) | 1.08 (0.86-1.23) | 1.13 (0.84-1.40) | 1.04 (0.92-1.27) | $p^a$=0.386<br>$p^b$=0.817<br>p=0.729 |
| **11-14** | 2.22 (1.24-3.83) | 2.26 (1.18-4.86) | 2.26 (1.32-4.38) | 8.99 (6.24-12.02) | 6.69 (4.53-12.07) | 8.19 (5.54-11.60) | $p^a$<0.0001<br>$p^b$=0.002<br>p<0.0001 |
| **15-18** | 12.33 (4.41-18.61) | 12.52 (4.24-18.18) | 12.43 (4.28-17.32) | 18.32 (14.84-19.7) | 17.06 (14.44-20.3) | 16.92 (14.49-20.01) | $p^a$=0.014<br>$p^b$=0.010<br>p=0.014 |
| **0-18** | 1.50 (0.74-4.62) | 1.48 (0.77-4.94) | 1.44 (0.70-4.68) | 3.04 (0.82-14.76) | 2.01 (0.81-14.55) | 3.09 (0.84-14.65) | $p^a$=0.074<br>$p^b$=0.091<br>p=0.096 |

TM: Testicular microlithiasis; Data are expressed as median [interquartile range (IQR) 25-75]; p-values for comparing testicular volume between the TM and non-TM groups; $p^a$-values for comparing the right testicular volume; $p^b$-values for comparing the left testicular volume; p-values for comparing the average testicular volume; p-values were obtained using the Mann-Whitney U test; p-value <0.05 was considered significant

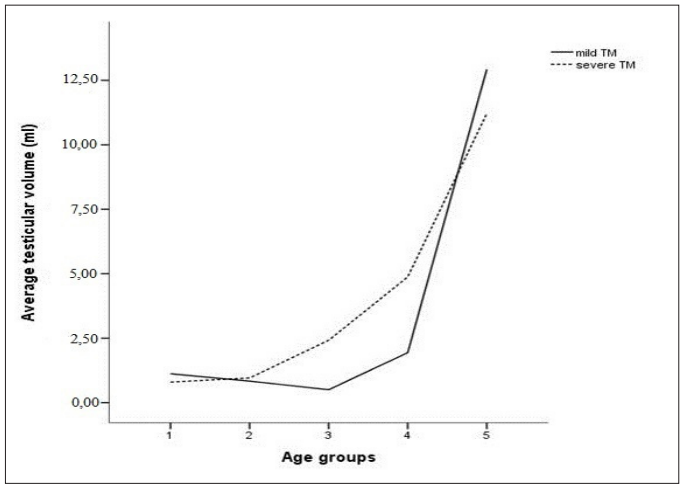


**Figure 3.** Comparison of the average testicular volume (mL) between boys with 'mild' testicular microlithiasis ('mild TM') and 'severe' testicular microlithiasis ('severe TM') among different age groups [Group 1 (0-2 years), Group 2 (3-6 years), Group 3 (7-10 years), Group 4 (11-14 years), Group 5 (15-18 years)]

TM: Testicular microlithiasis

The median values for the overall right, left, and average testicular volume in patients with 'severe TM' (2.90, 3.12, and 3.01 mL; respectively) and 'mild TM' (1.35, 1.33, and 1.35 mL; respectively) were not significantly different (p=0.072, p=0.148, p=0.106; respectively). The age-specific distribution and comparison of the right, left, and average testicular volume in the 'mild TM' and 'severe TM' groups are presented in Table 2. Significantly lower testicular volumes were found in 11-14-y-old boys with 'mild TM' than in those with 'severe TM' (p≤0.003). Although insignificant, the right, left and average testicular volumes were lower in older boys (≥15 years) with 'severe TM' compared to those with 'mild TM' (p=0.652, p=0.334, p=0.485; respectively). The overall right, left, and average testicular volumes in patients with 'severe TM' and non-TM groups were not significantly different (p=0.270, p=0.217, p=0.238; respectively). The distribution of the testicular volume measurements in boys with 'mild TM' and 'severe TM' according to age groups is summarized in Figures 3 and 4.

**Table 2. Ultrasound-based testicular volume in the 'mild TM' and 'severe TM' groups according to age groups**

| Age groups (years) | 'Mild TM' group | | | 'Severe TM' group | | | p-value |
|---|---|---|---|---|---|---|---|
| | Right testicular volume (mL) | Left testicular volume (mL) | Average testicular volume (mL) | Right testicular volume (mL) | Left testicular volume (mL) | Average testicular volume (mL) | |
| **0-2** | 1.07 (0.56-1.55) | 0.87 (0.35-1.36) | 0.96 (0.48-1.44) | 0.86 (0.74-1.25) | 0.83 (0.63-1.11) | 0.85 (0.69-1.18) | $p^a$=0.500<br>$p^b$=0.811<br>p=0.646 |
| **3-6** | 0.55 (0.37-1.00) | 0.79 (0.51-0.87) | 0.63 (0.53-0.83) | 0.99 (0.65-1.07) | 0.87 (0.69-1.46) | 0.94 (0.69-1.23) | $p^a$=0.211<br>$p^b$=0.152<br>p=0.141 |
| **7-10** | 0.73 (0.43-1.43) | 1.16 (0.48-1.75) | 1.04 (0.48-1.52) | 1.50 (0.25-1.68) | 1.55 (0.99-1.72) | 1.53 (0.18-1.65) | $p^a$=0.215<br>$p^b$=0.456<br>p=0.296 |
| **11-14** | 1.76 (0.99-2.58) | 1.83 (1.07-2.60) | 1.70 (1.08-2.32) | 4.70 (3.40-5.84) | 5.26 (3.72-6.33) | 5.00 (3.56-6.06) | $p^a$=0.001<br>$p^b$=0.003<br>p=0.002 |
| **15-18** | 16.30 (6.92-18.16) | 13.88 (8.50-18.92) | 15.28 (7.71-18.41) | 8.84 (3.78-18.84) | 8.90 (3.85-15.60) | 8.87 (3.84-17.46) | $p^a$=0.652<br>$p^b$=0.334<br>p=0.485 |
| **0-18** | 1.35 (0.60-3.58) | 1.33 (0.63-3.55) | 1.35 (0.62-3.60) | 2.90 (0.96-6.08) | 3.12 (0.83-5.86) | 3.01 (0.86-5.85) | $p^a$=0.072<br>$p^b$=0.148<br>p=0.106 |

TM: Testicular microlithiasis; 'mild TM' (5-20 microliths in one transducer field); 'severe TM' (>20 microliths in one transducer field). Data are expressed as median [interquartile range (IQR) 25-75]; P-values for comparing testicular volume between the 'mild TM' and TM and 'severe TM'; $P^a$-values for comparing the right testicular volume; $p^b$-values for comparing the left testicular volume; p-values for comparing the average testicular volume; P-values were obtained using the Mann-Whitney U test; p-value <0.05 was considered significant

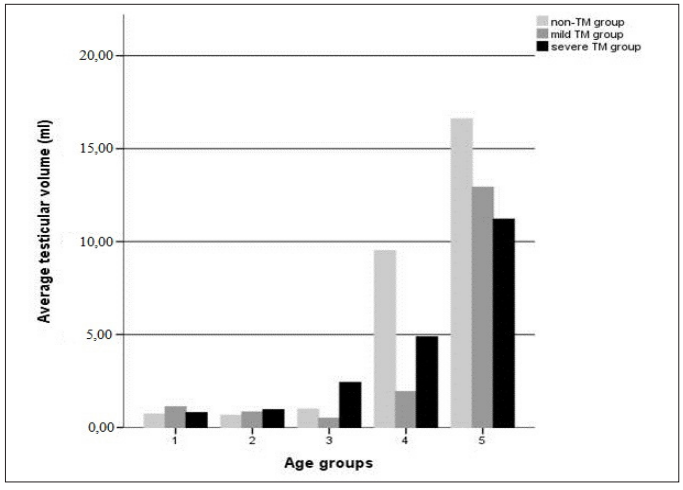


**Figure 4.** Testicular volume (mL) distribution in children without testicular microlithiasis (non-TM group), 'mild TM', and 'severe TM' among different age groups [Group 1 (0-2 years), Group 2 (3-6 years), Group 3 (7-10 years), Group 4 (11-14 years), Group 5 (15-18 years)]

TM: Testicular microlithiasis

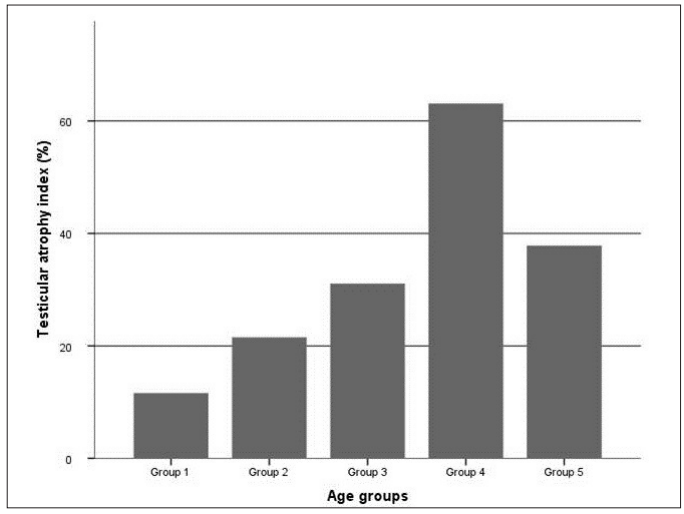


**Figure 5.** The distribution of the mean testicular atrophy index according to age groups [Group 1 (0-2 years), Group 2 (3-6 years), Group 3 (7-10 years), Group 4 (11-14 years), Group 5 (15-18 years)]

The mean TAI was 36% and ranged from 0% to 93%. Twenty-six patients (37%) had a mean TAI of more than 50%. The significantly higher TAI values were found in the older age groups (>11 years) (p=0.037) (Figure 5). TAI was not significantly different between the mild and severe TM subgroups (p=0.747).

## DISCUSSUON

The present study reported no significant association in the overall testicular volume between boys with TM and controls, however, in the age-specific comparisons, significantly lower testicular volumes were found in boys above 11 years with TM compared to those without TM. Moreover, significantly lower testicular volumes were found in 11-14-y-old boys with 'mild TM' than in those with 'severe TM'. The testicular volumes were lower in older boys (≥15 years) with 'severe TM' compared to those with 'mild TM', however, these changes were not significant.

TM is the deposition of microcalcifications in the seminiferous tubules which are visualized as small, non-shadowing, and hyperechogenic foci on testicular ultrasound [1,2]. The first case of TM was reported in a 4-year-old boy by Priebe and Garret in 1970 [17]. The ultrasonographic appearance of TM was first noted by Doherty et al. [18] in 1987. TM is usually bilateral, asymptomatic, and found incidentally on ultrasonographic imaging [1,2]. TM does not seem to be related to testicular malignancy during childhood, however, in the presence of risk factors, an association between TM and testicular malignancy has been confirmed in adults. A potential relationship between testicular atrophy and TM in childhood might increase the risk of testicular malignancy and infertility in adulthood [1,19,20]. Therefore, we aimed to determine the effect of the presence of TM on testicle volume by comparing the testicular volumes between boys with TM and controls.

In our study, the average testicular volumes were 1.44 mL in boys with TM and 3.09 mL in the age-matched subjects without TM. Although there was no statistically significant association between the testicular volume in boys with TM compared with those without TM, there was a tendency for a lower testicular volume in patients with TM. Bayramoglu et al. [13] measured the testicular volume in 23 pediatric patients with bilateral TM (median age 12 years, age range, 5-14 years) using ultrasound and reported no significant difference in the testicular volume between the control and study groups (2.3 mL vs. 3.0 mL, p=0.320, respectively). Cebeci et al. [11] found TM in 9 (36%) patients with Down syndrome and showed that their testicular volumes did not change significantly compared to the control group. Goede et al. [9] investigated the TM in 79 subjects (mean age 8.44 years, age range, 2.0-19.3 years) with Down syndrome and demonstrated smaller testicular volumes in boys with TM than in those without TM.

In the study by Pedersen et al. [4], the testicular volume measurements of 91 patients with TM (median age 48 years, age range, 19-94 years) were compared with those of age-matched control subjects. The testicular volume in patients with TM tended to be lower compared to those without TM, however, this difference did not reach statistical significance (14.3 mL vs. 14.5 mL, p=0.370, respectively) [4]. The median testicular volumes in adult patients with 'limited' TM (<5 microliths) and 'classic' TM (≥5 microliths) were reported as 20.5 mL and 15.5 mL, respectively, by Von Eckardstein et al. [21].

We also found no significant differences in the testicular volume between subjects with 'mild TM' (5-20 microliths) and 'severe TM' (>20 microliths). This result agreed with a previous study by Bayramoglu et al. [13], who described no significant difference in the testicle volume between boys with ≤15 microliths and those with >15 microliths (p=0.210). According to Pedersen et al. [4], severe testicular atrophy (≤8 mL) was more often seen in adult patients with TM compared to controls (p=0.02). In our study, significantly higher TAI values were found in boys above 11 years of age (p=0.037).

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and testicular volume are good indicators of the hormonal and spermatogenic function of the testicles [22-25]. Ruiz-Olvera et al. [22] reported significant differences in the testicular volume between controls and patients with primary or secondary hypogonadism. Several studies suggested that the FSH, LH, and testosterone levels were significantly lower in major thalassemia patients with secondary hypogonadism [23-25]. In the study by Fariborzi et al. [23], 3.2% of 62 beta-thalassemia adult patients had testicular volume under 4 mL. Hypogonadism and TM were seen in 22.6% and 4.8% of the patients, respectively [23]. Ohana Marques Coelho de Carvalho et al. [26] found testicular adrenal rest tumors in six and TM in two out of 12 patients with a history of congenital adrenal hyperplasia by ultrasound, suggesting a possible link between congenital adrenal hyperplasia and TM.

### Study Limitations

Several limitations deserve comments. The present study was a single-institution retrospective study. The exclusion criteria for controls were the same as for the study group, however, some subjects in the control group were admitted to the hospital because of scrotal or groin pain. Further comparison and discussion of our results were limited since most studies in the literature were conducted on adults, subjects with Down syndrome, and only one, so far, was conducted on a small sample of children. Our study was not designed to evaluate the association between the testicular volume and hormonal status or hypogonadism, thus, further studies including more participants are needed to assess this topic. Furthermore, we did not consider the pubertal status of the boys in the analysis which could be addressed in future studies.

## Conclusion

In conclusion, we found no significant association in the testicular volume between boys with TM and age-matched controls, however, a trend towards lower testicular volume was observed in boys with TM. Although insignificant, a lower testicular volume was detected in children above 15 years of age with 'severe TM' compared to those with 'mild TM'. These findings might suggest that clinical follow-up could be considered in patients over 15 years of age with "severe TM >20 microliths".

### Ethics

**Ethics Committee Approval:** This comparative and cross-sectional study conformed to the Declaration of Helsinki and was approved by the University of Health Sciences Turkey, İzmir Dr Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Clinical Research Ethics Committee (approval number: 2021/16-06, date: 21.10.2021).

**Informed Consent:** Since our study had a retrospective design, informed consent was not obtained from the patients.

**Peer-review:** Externally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: E.Ç., B.Ö., Concept: E.Ç., B.Ö., Design: E.Ç., B.Ö., Data Collection and/or Processing: E.Ç., Analysis and/or Interpretation: E.Ç., B.Ö., Literature Search: E.Ç., Writing: E.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.2022.36604
J Dr Behcet Uz Child Hosp 2022;12(2):184-190

Original Article

# Evaluation of Changing Drug Preferences During the COVID-19 Pandemic in a Tertiary Childrens Hospital

## *Bir Üçüncü Basamak Çocuk Hastanesinde, COVID-19 Pandemisi Sırasında Değişen İlaç Tercihlerinin Değerlendirilmesi*

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## ABSTRACT

**Objective:** There is currently no drug that is effective against the coronavirus disease-2019 (COVID-19) and no consensus was present regarding the treatment. In this cross-sectional study, we aimed to evaluate the progress of the treatment process of patients with COVID-19 since the first day of pandemic in our country and the changes in the process.

**Method:** This single-center cross-sectional study was conducted from March 11 through November 30, 2020, in University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, a 400-bed tertiary care hospital in İzmir, Turkey. Treatment options in all hospitalized children with COVID-19 were evaluated.

**Results:** Evaluation of our clinical treatment algorithm from March to December, it was seen that the majority of the patients did not need any specific treatment and recovered only with supportive treatment. Because of the recommendations of the COVID-19 guidelines, no efficacy has been detected during the oseltamivir treatment and there was a significant decrease in use of azithromycin and hydroxychloroquine. Favipiravir is still the first choice of drug for patients with COVID-19.

**Conclusion:** World Health Organization, the Infectious Diseases Society of America, and Surviving Sepsis guidelines indicate that their investigational treatments should only be used in certain clinical trial setting. Supportive care is still the main therapeutic option in COVID-19.

**Keywords:** Antiviral drug, coronavirus disease-2019 (COVID-19), pandemic, favipiravir

## ÖZ

**Amaç:** Günümüzde koronavirüs hastalığı-2019'a (COVİD-19) karşı kanıtlanmış etkili bir ilaç ve de tedavi konusunda fikir birliği yoktur. Bu nedenle, kesitsel çalışmada, ülkemizde pandeminin ilk gününden itibaren COVİD-19 hastalarının tedavi sürecinin ilerleyişini ve süreçteki değişiklikleri değerlendirmeyi amaçladık.

**Yöntem:** Bu tek merkezli kesitsel çalışma, 11 Mart-30 Kasım 2020 tarihleri arasında İzmir, Türkiye'de 400 yataklı üçüncü basamak bir hastane olan Sağlık Bilimleri Üniversitesi Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahi Eğitim ve Araştırma Hastanesi'nde gerçekleştirildi. Hastanede yatan tüm COVİD-19'lu çocuklarda tedavi seçenekleri değerlendirildi.

**Bulgular:** Mart-Aralık ayları arasında klinik tedavi algoritmamız değerlendirildiğinde, hastaların çoğunluğunun herhangi bir spesifik tedaviye ihtiyaç duymadığı ve sadece destek tedavisi ile iyileştiği görüldü. COVİD-19 kılavuzlarının önerileri hızla güncellenmiş ve nihayetinde oseltamivir tedavisinin etkinliği olmadığı saptanmıştır. Bununla birlikte pandemi ilk günlerinden bu yana azitromisin ve hidroksiklorokin kullanımında belirgin azalma olmuştur. Favipiravir ise COVİD-19 hastaları için hala ilk ilaç seçimidir.

**Sonuç:** Kılavuzlarda, tüm tedavi alternatiflerinin yalnızca belirli klinik araştırma ortamlarında kullanılması gerektiğini göstermektedir. Destekleyici bakım, COVİD-19'da hala ana tedavi seçeneğidir.

**Anahtar kelimeler:** Antiviral ilaç, koronavirüs hastalığı-2019 (COVİD-19), pandemi, favipiravir

**Received:** 02.11.2021
**Accepted:** 14.04.2022

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**Cite as:** Cem E, Kıymet E, Böncüoğlu E, Şahinkaya Ş, Yılmaz Çelebi M, Düzgöl M, Akaslan Kara A, Arıkan K, Bayram N, Devrim İ. Evaluation of Changing Drug Preferences During the COVID-19 Pandemic in a Tertiary Childrens Hospital. J Dr Behcet Uz Child Hosp. 2022;12(2):184-190

## INTRODUCTION

Since the beginning of the pandemic, coronavirus disease-2019 (COVID-19) has progressed in very different clinical courses in children. Children were reported to have lower number of symptoms compared to adults and the attributable mortality rates in the children are extremely lower compared to adults [1]. Currently, effective drug against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is not available. Because of the changes in clinical findings over time and the detection of new symptoms, and demonstration of wide variance between countries in terms of treatment guidelines, these guidelines have been updated in time [2]. Lack of evidence concerning both the efficacy and possible harmful effects of these medications, and also the unfavourable risk - to - benefit ratio of supportive care as the primary management strategy used for most pediatric patients especially outside the setting of a clinical trial required urgent action [3]. On May 01, 2020, the Food and Drug Administration (FDA) issued an emergency use authorization for the investigational antiviral drug remdesivir for the treatment of suspected or laboratory-confirmed COVID-19 in adults and children hospitalized with severe disease [4].

There is no consensus on the treatment for COVID-19 in children as well as adults and current management is still controversial. Many studies have reported that children with severe and critical COVID-19 were treated with supportive care alone, whereas numerous ongoing studies for adults are trying to determine whether any pharmacological treatment is available [5,6].

This cross-sectional study aimed to evaluate drug options used for the treatment of pediatric patients with COVID-19 for about a year from the onset of the pandemic, and the variability of this treatment process according to clinical experience.

## MATERIALS and METHODS

This single-center cross-sectional study was conducted from March 11, 2020 through November 30, 2020, in pandemic clinics, which is a tertiary childcare hospital in İzmir, Turkey. All patients diagnosed with COVID-19 under the age of 18 were included in the study. Patients diagnosed with Multisystem Inflammatory Syndrome in Children were excluded from the study.

The patients were diagnosed as COVID-19 based on the presence of clinical characteristics consistent with COVID-19 in children, SARS-CoV-2 polymerase chain reaction (PCR) positivity detected in nasopharyngeal swab samples, and/or the presence of SARS-CoV-2 antibodies as of August 1, 2020 [7].

Data of the patients were collected from medical records, including information on demographic and clinical characteristics (age, gender, symptoms, medical history), underlying diseases or comorbidities (i.e., heart disease, chronic lung disease, developmental delay, hematological disease, epilepsy), the results of chest X-ray and thorax computerized tomography, the indications for the treatment applied, clinical outcomes with the admission date, the time elapsed from the disease onset to the confirmation of the diagnosis and length of the hospital stay.

### Statistical Analysis

Collected data were analyzed with SPSS Software version 20 (IBM Corporation, Armonk, NY, USA). Categorical variables were analyzed using relative frequencies, while continuous variables using median or mean (depending on whether they show normal distribution) values. Categorical variables such as ratio of underlying disease, and ratio of pulmonary involvement were compared using Pearson $\chi^2$ and Fisher's Exact tests. The significance level was taken as $p \leq 0.05$.

Study protocol was approved by the University of Health Sciences Turkey, İzmir Dr Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Clinical Research Ethics Committee (decision no: 474, date: 17.12.20).

## RESULTS

The study enrolled 301 hospitalized children with COVID-19 including 156 (51.8%) male, and 145 (48.2%) female patients with an overall mean age of 98.3±67.6 months (range 1 month to 17 years). Seventy-seven (25.6%) patients had, while majority of the patients (224/301, 74.4%) had not an underlying disease. The most common concomitant chronic medical conditions were neuro-developmental diseases (mental motor retardation, developmental delay, and cerebral palsy) (21/77, 27.2%), followed by asthma (9/77, 11.6%), epilepsy (8/77, 10.3%), and obesity (8/77, 10.3%). The most common underlying diseases were congenital heart diseases (n=7, 9%) and malignancy (n=7, 9%), followed by other rheumatologic diseases, hematologic diseases, and metabolic diseases (Table 1).

Pulmonary involvement was found in 80 (26.5%) of the 301 COVID-19 patients. Eighty-seven (28.9%) patients received any drug for COVID-19 according to

the national guideline, and the remaining patients were followed up without specific treatment. Twenty-four (31.2%) with, and 63 (28.1%) patients without underlying diseases were treated with antiviral agents, with a statistically significant intergroup difference regarding antiviral drug usage (p>0.05). Significantly higher number of (n=44: 53.8%) patients with pulmonary involvement used antiviral drug compared to the patients without (p<0.001).

The drugs administered during the pandemic included hydroxychloroquine (HCQ), oseltamivir, azithromycin, and favipiravir. Because of the varying clinical approaches, at the beginning of the pandemic oseltamivir was used only in three patients. Two of these patients were given oseltamivir in combination with the HCQ and azithromycin. Azithromycin was used in 23 (7.6%) patients. The use of azithromycin was at its peak during April (n=14/37: 37.8%) and May (n=5/33: 15%). Following April, these treatment strategies were discontinued in subsequent months due to changes in knowledge and experiences with COVID-19. Only one patient per month received azithromycin treatment in the months that followed. HCQ treatment was utilized in a total of 68 patients. Its use peaked in May (n=16/68: 23.5%), but gradually decreased in ongoing months and discontinued throughout the pandemic. During this period, use of favipiravir became prominent for patients older than 12 years of age in our clinical practice and two of 24 patients (8.3%) in September and 15 of the 82 patients (18.3%) in November were treated with favipiravir. Monthly distribution of specific antiviral therapies used is shown in Figure 1.

A total of 81 patients received antibiotic therapy for respiratory tract infections, the most commonly used antibiotics were 3rd generation cephalosporin (n=40 :13.2%), amoxicillin - clavulanate (n=32 :10.2%), and ertapenem (n=18 :6%). Considering the distribution of antibiotics by months the distribution rates were the highest in March (n=1/1 :100%) and April (n=18/37 :48.6%). There was also a decrease in the rate of antibiotic use in the following months.

Most of the patients (n=214 :71 %) were followed up without specific treatment for COVID-19. Consequently, the number of patients without any specific medications increased in the following months. While the rates of supplying nonspecific treatment ranged from 60% to 68% from March to August, the rates of patients without specific treatment increased to 81-86% in the final three months of the study (Figure 2).

## DISCUSSION

In this study, our treatment experiences for the hospitalized children with COVID-19 were reviewed. While at the beginning of the COVID-19 pandemic, HCQ was used in 9-16% of the patients, this rate decreased dramatically during the subsequent months. Moreover, after the recommendation of favipiravir use for COVID-19 in September 2020 by the national and international guidelines, it was administered in 8.2% of the patients in November 2020. In addition, the rates of the patients followed up with only supportive treatment increased during the following months of the pandemic as a result of our clinical experiences accumulated during the course of the disease. Treatment approaches did not differ according to the concomitant diseases of the

**Table 1. Clinical and demographic characteristics of 301 patients enrolled to the study**

| Characteristics and underlying medical conditions | Patients, n (%) |
|---|---|
| Age (months), mean | 98.3±67.6 (1 month -17 years) |
| Female, n (%) | 145 (48.2) |
| Comorbities, n=77 | |
| Neurodevelopmental diseases, n (%) | 21 (27.2) |
| Asthma, n (%) | 9 (11.6) |
| Epilepsy, n (%) | 8 (10.3) |
| Obesity, n (%) | 8 (10.3) |
| Congenital heart diseases, n (%) | 7 (9) |
| Malignency, n (%) | 7 (9) |
| Rheumatological diseases, n (%) | 4 (5.1) |
| Miscellaneous*, n (%) | 13 (16.9) |

*Including neutropenia, idiopatic thrombocytopenia, metabolic diseases, Down syndrome, psoriasis, immune deficiency, Becker's muscular dystrophy, type 1 diabetes mellitus

patients, while significantly higher number of patients with pulmonary involvement were treated with antiviral agents (p<0.001).

Today, there is still no targetted treatment for pediatric COVID-19 cases and experiences are ever changing. The Ministry of Health firstly published the guideline for pediatric patients on March 23, 2020 in Turkey [8] and the treatment algorithm in our clinic was planned according to this guideline. In the first six months of the pandemic, the number of hospitalized-patients was high due to the follow-up of the clinical findings and the uncertainty about the prognosis. Simultaneously, greater number of patients received higher doses of targeted drugs and

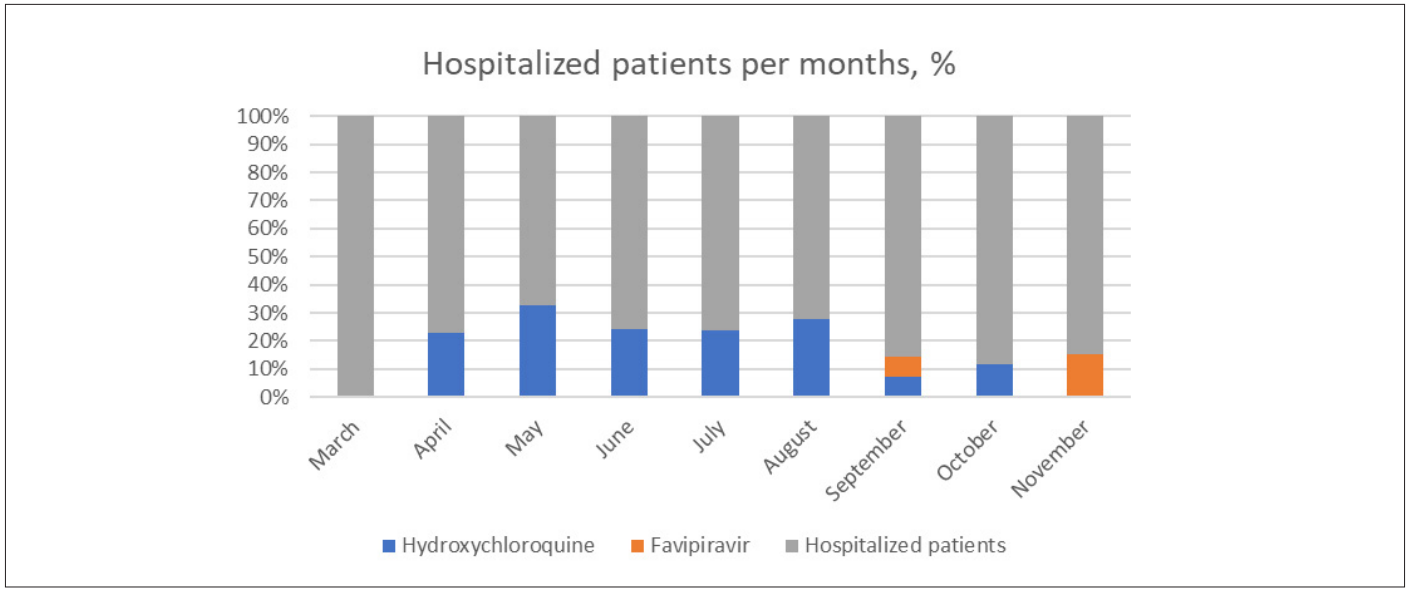


**Figure 1.** Monthly disease - specific therapies

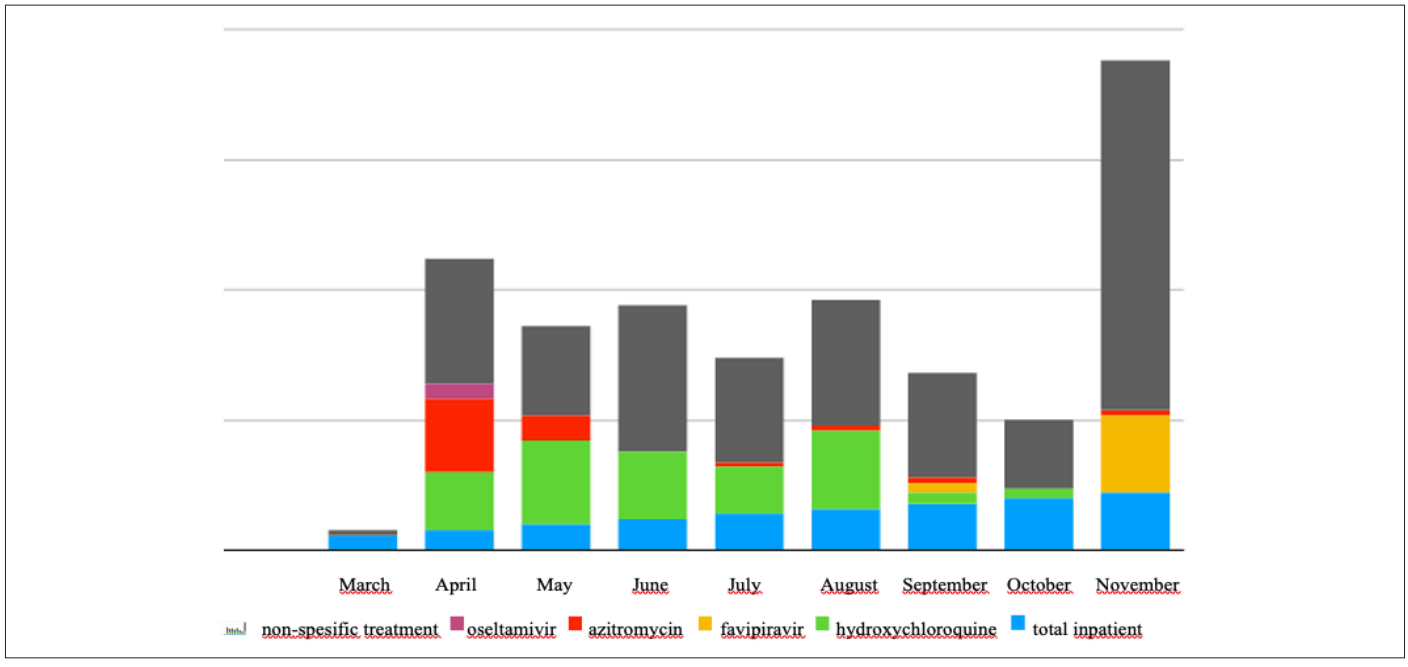


**Figure 2.** Rates of patients without specific treatment

drug combinations in accordance with guidelines. Our treatment approaches have evolved due to the rising number of patients and increased clinical experience. There was a significant decrease in the targeted drug used and the number of in-patients in the last three months according to the recent guidelines.

Treatment process for COVID-19 varies in different countries, and the the World Health Organization (WHO) has published several interim reports based on previous human coronavirus outbreaks [9]. In these temporary WHO guidelines, the COVID-19 treatment recommendation is mostly supportive and treatment decisions should be made on a case-by-case basis, such as starting antimicrobial treatment in selected cases [10].

The clinical manifestation of COVID-19 may be like other upper respiratory tract infections [3]. During the initial phase of pandemic, oseltamivir was administered in compliance with the guidelines, since COVID-19 could not be distinguished from influenza virus infection, increased incidence rates of co-infections with influenza and COVID-19 were reported [11]. The guideline of Turkish Ministry of Health published on April 2, 2020, recommended addition of oseltamivir to the treatment in cases where COVID-19 disease cannot be distinguished from seasonal influenza infection; and the drugs HCQ ± azithromycin, lopinavir/ritonavir were included in the treatment protocol. However, due to global studies, our guideline was updated periodically and lastly published on September 26, 2020 [12]. COVID-19 guideline released by the Ministry of Health for the treatment of pediatric patients was updated as HCQ, favipiravir, lopinavir/ritonavir. Since oseltamivir failed to be effective against COVID-19, it was excluded from the guidelines after the influenza session passed away [13].

In the early stages of the pandemic, HCQ was preferred in patients with widespread lung involvement at our center. Initially, The Infectious Diseases Society of America (IDSA) recommended the usage of chloroquine with or without azithromycin, lopinavir-ritonavir, tocilizumab, and convalescent plasma in the context of clinical studies [14], while advocating against azithromycin treatment with HCQ due to the increased risk of prolongation of the Q-T interval. In the following days, also azithromycin was removed from the current Ministry of Health treatment guidelines, and our practice [12].

While HCQ has antiviral and immunomodulatory efficacy, the benefits of its use for COVID-19 are still controversial in practice [15]. In the study of Gautret et al. [16], the efficacy of co-administration of HCQ and azithromycin was evaluated and it was emphasized that a higher rate of PCR negativity was detected on days 3 to 6 after HCQ treatment. In this study, the usage of azithromycin with HCQ was associated with more viral clearance in a short period of time than HCQ alone [16]. However, an observational study found no evidence of antiviral clearance with HCQ and azithromycin in 11 hospitalized patients and it was stated that QTc prolongation, which is a side effect of both drugs, may be seen at a higher rate [17].

In a randomized, double-blind, phase 2b study, Borba et al. [18] indicated the safety and efficacy of low/high HCQ dosage regimens in 81 patients with severe COVID-19 infection. While all patients received a combination of ceftriaxone and azithromycin, 89.6% of the patients received only oseltamivir. As a result of the study, it was found that mortality and complications as QT prolongation were more common in those receiving high-dose HCQ treatment. The combination of high-dose chloroquine, azithromycin, and oseltamivir might cause an increase in mortality rates [18]. According to the conclusions of these clinical studies and relevant publications, HCQ and azithromycin combination therapy was discontinued after April 2020.

Antiviral agents such as oseltamivir, ribavirin, remdesivir, lopinavir/ritonavir have been used to reduce viral load without any significant treatment benefit [19]. According to the WHO guideline published on December 17, 2020, children were not included in the randomized controlled trials for HCQ, lopinavir/ritonavir, and remdesivir [20]. However, remdesivir has been approved by the FDA for the treatment of suspected or laboratory-confirmed COVID-19 disease in adults and children who have been hospitalized with severe disease [7].

In the open-label comparative controlled study by Cai et al., [21] favipiravir was preferred due its faster viral clearance and improved chest computed tomography changes compared to lopinavir/ritonavir [21]. However, this was not a randomized and double-blinded study as most of the other studies in the literature. The efficacy of favipiravir against COVID-19 has not been proven in the other two randomized clinical trials [22,23] and studies on its efficacy are still ongoing. During the first 6 months of the pandemic, favipiravir, which is not approved for children under 12 years of age, has become a prominent treatment in our clinical practice since September 2020, after it was recommended in the international guidelines. However, WHO, IDSA, and Surviving Sepsis

guidelines generally agree that all of these investigational treatments should only be evaluated on a patient-by-patient basis [24,25].

### Study Limitations

There are several limitations to our study. As a retrospective study, it has limitations when compared to randomized clinical trials, and it only included patients from one single center. Moreover, in the current study, treatment options were not evaluated according to clinical severity of COVID-19, and the concomitant chronic medical conditions of the patients. However, it should be emphasized that the study is one of the rare studies in which the changes in treatment strategies as a result of modifications in recommendations of guidelines during the ongoing pandemic and also the enhanced observations about the recently emerged disease of the current medical center were taken into consideration in pediatric patients with COVID-19.

## CONCLUSION

In conclusion, based on our observations from the onset of the pandemic to December 2020, despite some of the proposed antiviral drugs, the majority of the children with COVID-19 did not get any treatment. Over the course of a year, our therapeutic approaches have changed considerably, but there is still a paradox about the specific treatment due to a lack of evidence and data coming from randomized studies. However, supportive care of pediatric cases is still the main therapeutic option as in the literature.

### Ethics

**Ethics Committee Approval:** Study protocol was approved by the University of Health Sciences Turkey, İzmir Dr Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Clinical Research Ethics Committee (decision no: 474, date: 17.12.20)

**Informed Consent:** Cross-sectional study.

**Peer-review:** Internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: E.C., E.K., E.B., Ş.Ş., M.Y.Ç., M.D., A.A.K., K.A., N.B., İ.D., Concept: E.C., Design: E.C., N.B., İ.D., Data Collection and/or Processing: E.C., E.K., E.B., Ş.Ş., M.Y.Ç., M.D., A.A.K., K.A., Analysis and/or Interpretation: E.C., N.B., İ.D., Literature Search: E.C., E.K., E.B., Ş.Ş., M.Y.Ç., M.D., A.A.K., K.A., Writing: E.C., N.B., İ.D.

**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.2022.83584
J Dr Behcet Uz Child Hosp 2022;12(2):191-196

Original Article

# Hematuria in Patients with Congenital Coagulation Factor Deficiencies

## *Konjenital Kanama Bozukluklarında Hematüri*

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## ABSTRACT

**Objective:** Inherited bleeding disorders are a group of congenital coagulopathies, arising due to protein deficiencies, which affect clotting, platelet function or fibrinolysis. Spontaneous gross hematuria or subclinical microscopic hematuria, which are often detected by chance, are relatively common in patients with coagulopathies. This study aims to evaluate the incidence, the causes and treatment modalities of hematuria in congenital factor deficiencies.

**Method:** Data concerning the type of coagulation defect, the level of deficient factor, age of the patients with hematuria episodes, presence of inhibitor, ongoing treatment modality, the etiology of hematuria, the treatment approach to hematuria, the number of hematuria episodes has been collected from medical records and hemophilia dairies of patients between 1985 and 2015 and confirmed by phone calls. Six hundred twenty nine patients with congenital factor deficiencies followed were evaluated retrospectively.

**Results:** Hematuria was seen in 10.1% of hemophilia A, 15.5% of hemophilia B, 3.8% of von Willebrand's disease patients and 7% of patients with other factor deficiencies. Hematuria was seen in 2 mild, 20 moderate and 29 severe factor VIII and IX deficiencies. In 7 of these patients inhibitor was positive. While no etiological reason for hematuria could be identified in 78% of these patients, 15.3% had nephrolithiasis, 1.7% had post streptococcal acute glomerulonephritis, 3.4% had urinary tract infection and 1.7% had a renal cyst.

**Conclusion:** The study demonstrates that hematuria is relatively common in factor deficiencies, whereas further studies are needed to elucidate the causes and effects on renal function in children with coagulation deficiencies.

**Keywords:** Macroscopic hematuria, congenital coagulation deficiencies, hemophilia

## ÖZ

**Amaç:** Kalıtsal kanama bozuklukları, pıhtılaşmayı, trombosit fonksiyonunu veya fibrinolizi etkileyen protein eksiklikleri nedeniyle ortaya çıkan bir grup hastalıktır. Genellikle tesadüfen saptanan spontan gros hematüri veya subklinik mikroskobik hematüri nispeten yaygındır. Bu çalışmada kanama bozukluklarında hematürinin görülme sıklığı, nedenleri ve tedavi yöntemlerinin değerlendirilmesi amaçlanmıştır.

**Yöntem:** Kalıtsal kanama bozukluğu tanısı ile takip edilen 629 hastanın retrospektif olarak değerlendirildi. 1985 ve 2015 yılları arasındaki hastaların tıbbi kayıtları ve hemofili günlükleri ve telefon görüşmeleri ile teyit edildi. Pıhtılaşma bozukluğunun tipi, eksik faktör düzeyi, hematüri atakları olan hastaların yaşı, inhibitör varlığı, devam eden tedavi şekli, hematürinin etiyolojisi, hematüriye tedavi yaklaşımı, hematüri atak sayısı incelendi.

**Bulgular:** Hematüri, hemofili A'nın %10,2'sinde, hemofili B'nin %15.6'sında, von Willebrand hastalığı olan hastaların %3,9'unda ve diğer faktör eksikliği olan hastaların %7,5'inde görüldü. Yirmi dokuz şiddetli, 20 orta ve 2 hafif faktör VIII ve IX eksikliğinde hematüri görüldü. Bu hastaların 7'sinde inhibitör pozitifti. Bu hastaların %78'inde hematüri için herhangi bir etiyolojik neden saptanamazken, %15,3'ünde nefrolitiazis, %1,7'sinde streptokok sonrası akut glomerülonefrit, %3,4'ünde idrar yolu enfeksiyonu ve %1,7'sinde böbrek kisti vardı.

**Sonuç:** Çalışma, faktör eksikliklerinde hematürinin nispeten yaygın olduğunu gösterirken, pıhtılaşma eksikliği olan çocuklarda böbrek fonksiyonu üzerindeki nedenleri ve etkileri aydınlatmak için daha fazla çalışmaya ihtiyaç vardır.

**Anahtar kelimeler:** Makroskobik hematüri, konjenital koagülasyon defekti, hemofili

**Received:** 19.10.2021
**Accepted:** 28.04.2022

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**Cite as:** Karadaş N, Balkan C, Yılmaz Karapınar D, Aydınok Y, Kavaklı K. Hematuria in Patients with Congenital Coagulation Factor Deficiencies. J Dr Behcet Uz Child Hosp. 2022;12(2):191-196

## INTRODUCTION

Inherited bleeding disorders are a group of congenital coagulopathies which occur as a result of protein deficiencies, which affect clotting, platelet function or fibrinolysis.

Hemophilia A (HA), hemophilia B (HB), von Willebrand's disease (VWD) and other rare congenital coagulation deficiencies (factor V, VII, V+VIII, X, XI deficiencies, etc.) are the most frequently seen inherited bleeding disorders in the general population, varying between 1 in 10.000 to 1 in 2 million [1-5].

Spontaneous gross hematuria or subclinical microscopic hematuria, which is often detected incidentally, is relatively common in patients with hemophilia. In two studies of hemophiliacs, 66% of the patients had a history of hematuria [6,7]. Hematuria is usually considered to be in benign nature, unless accompanied by ureteral clots. Hematuria can have several causes including history of analgesics (anti-inflammatory drugs), calcium and vitamin D intake, stress, trauma, and exercise [8,9]. The etiology of spontaneous hematuria is not known.

This study retrospectively evaluates the incidence and causes of macroscopic hematuria in patients with hemophilia and the other bleeding disorders and the general approach to hematuria in our clinic.

## MATERIALS and METHODS

Six hundred twenty nine patients with congenital factor deficiencies, who were being followed up in a tertiary university hospital, were retrospectively searched for reported macroscopic hematuria episodes. Data from the period 1985 to 2020 was collected from medical records and hemophilia dairies of patients and confirmed by phone calls. The name of the coagulation deficiency, the deficient factor level, presence of inhibitor, gender, age, the history of bleedings, the examinations to rule out the etiologies of hematuria, the reasons of bleedings, the ongoing treatment and the medical approach to bleedings were noted from the medical records. Although urine analysis had been performed to all patients with hematuria, urine culture had been performed only for ten patients. Abdominal ultrasonography was performed on 52 patients and further investigations were done according to the history and physical examination in few patients. Within the scope of this study, the age of the patients with hematuria, the etiology of hematuria, the treatment approach to hematuria, the numbers of hematuria episodes were collected.

The study was approved by Ege University Faculty of Medicine Medical Research Ethics Committee (decision number: 21-5T/118, date: 20.05.2021).

The severity of hemophilia was categorized as severe if the baseline clotting FVIII or FIX activity was ≤1%, moderate if >1-5% and mild if >5-30% [9]. The type of VWD was categorized according to International Society of Thrombosis and Hemostasis-Scientific and Standardization Committee [10]. The inhibitor titer was measured at least once-a-year and viral serology was tested routinely once a year.

### Statistical Analysis

The data analyzed using SPSS (Statistical Package for Social Sciences) statistical program (version 17.0). Mean values and standard deviation were calculated for continuous variables. Categorical variables were compared by using chi-square test. Since the groups were not normally distributed, Mann-Whitney U test was applied to make a comparison in terms of hematuria frequency and incidence between severe, moderate and mild deficiencies and between inhibitory positive and negative hemophilia. The differences between HA, HB and VWD were analyzed using Kruskal-Wallis variance analysis and the findings were considered as significant when the case was $p<0.05$.

## RESULTS

A total of 629 [mean age: 17.1±14.7 (1-45) years, 549 (87%) of them males, mean follow-up time: 16.1±11.7 years] medical records, consisting of 383 (60.9 %) HA [185 (48.3%) severe; 56 (14.7%) moderate; 142 (37%) mild HA], 77 (12.2%) HB [52 (67.5%) severe; 15 (19.5%) moderate; 10 (13%) mild HB], 99 (15.7%) type-1, 16 (2.5%) type-2 and 14 (2.2%) type-3 VWD, 16 (2.5%) FVII deficiency, 8 (1.3%) FV deficiency, 5 (0.8%) FX deficiency and 11 (1.7%) other rare factor deficiencies were examined. Thirty four of the patients with hemophilia (31 HA and 3 HB) had inhibitory.

Macroscopic hematuria was seen in 59 (9.4%) of 629 patients with coagulation deficiencies. Hematuria was seen in 39 (10.2%) of 383 HA patients, 12 (15.6%) of 77 HB patients, 5 (3.9%) of 129 VWD patients, 3 (7.5%) of 40 patients with other factor deficiencies (Table 1). There was no significant difference in terms of hematuria incidence between HA and HB ($p=0.207$). The VWD patients had a significantly lower hematuria incidence compared to

HA and HB patients (p=0.002). The mean age of the first hematuria attack was 15.5±6.9 (3-34) years.

While 34 patients had only one hematuria episode, 8 patients had two episodes, 4 patients had 3 episodes, 3 patients had four episodes and 10 patients had five and more episodes. None of the patients had history of trauma. Hematuria incidence was higher in patients with moderate and severe (15.9%, 49/308), compared to mild (1.9%, 3/155) factor VIII and IX deficiencies (p=0.012). Inhibitory was positive in 7 of these patients (6 HA and 1 HB). The number of bleeding episodes was not significantly higher in patients with inhibitor compared to patients without inhibitor (p>0.782).

Although there was no significant correlation between hematuria frequency and the factor levels (r=0.27, p>0.645), the hematuria incidence was significantly higher in severe and moderate hemophilia patients compared to mild hemophilia patients (p=0.01).

The ages of the patients with hematuria were between 3 and 34 years, with a mean age of 15 years. All of the patients with hematuria were above five years old except a three-year old female, who had hematuria because of a urinary tract infection, and a 4-year old male, who had hematuria because of nephrolithiasis.

In most of the bleeding episodes, patients didn't report pain, except nine patients with nephrolithiasis. Ultrasonography was performed on 52 of 59 patents with hematuria. While nephrolithiasis was seen in 9 patients (15.3%), pyelocaliectasis was seen in 1 patient (1.7%) and 1x1cm sized renal cyst in the upper renal parenchyma was seen in 1 patient (1.7%), all with hematuria, and no significant findings were observed in ultrasonography in 41 of 52 patients (78.8%). The stone was located either in renal pelvis or in ureter and none of them exceeded 1 cm diameter and no obstruction was seen in any of the patients with nephrolithiasis.

No reason could be identified in 46 (78%) of these patients.

While nine (15.3%) patients had nephrolithiasis (8 patients with HA/HB, 1 patient with VWD), 1 (1.7%) patient had pyelocaliectasis, 1 (1.7%) patient had post-streptococcal acute glomerulonephritis (PSAGN), 1 (3.4%) patient had a upper urinary tract infection, 1 (1.7%) patient had a renal cyst. Only 1 patient with hematuria had chronic hepatitis C (HCV) infection, and no other viral infection such as hepatitis B or human immunodeficiency virus (HIV) was found.

Regular screening tests such as urine analysis and ultrasonography were not applied in the follow ups.

As HA and HB are X-linked disorders of the coagulation system and the other coagulopathies incidences were lower, hematuria was seen in only 4 girls. Among these 4 female patients; one had factor V deficiency, one had factor VII deficiency and 2 had VWD. While one VWD patient with PSAGN and the other VWD patient with upper urinary tract infection were treated according to the treatment protocols of the diseases, the other two female patients (1 FVII deficiency with renal cyst and FV deficiency), who had severe factor deficiencies, were given hydration and factor replacement therapies.

Four of our patients were diagnosed with congenital coagulation deficiency when the cause of hematuria was being investigated (1 VWD and 3 hemophilia patients).

While eight (13.6%) patients were given only hydration therapy; forty three (72.9%) patients were given both hydration and factor replacement therapy at home (1-2 times factor replacement therapy). Five (8.5%) patients were hospitalized and received hydration and factor replacement therapy not more than three times. Since obtaining the factor was difficult because of the insurance policy of the country on supply of factor derivatives before 1999, two (3.4%) patients had

**Table 1. Number of patients with macroscopic hematuria according to disease group**

| Diagnosis | Number of patients | Percentage | Age | Gender | Number of hematuria | Hematuria percentage |
|---|---|---|---|---|---|---|
| Hemophilia A | 383 | 60.9 | 1-42 y | 383 boys | 39 | 10.2 |
| Hemophilia B | 77 | 12.2 | 1-45 y | 77 boys | 12 | 15.6 |
| VWD* | 129 | 20.5 | 4-38 y | 68 boys | 5 | 3.9 |
| Other rare factor D** | 40 | 6.35 | 3-17 y | 21 boys | 3 | 7.5 |
| Total | 629 | 100 | 1-45 y | 549 boys | 59 | 9.4 |

*VWD: von Willebrand's disease type 1, 2 and 3, **Factor V, FVII, FV+VIII, FX, FXI, FXIII deficiencies

been given fresh frozen plasma and one (1.7%) patient had been given fresh frozen plasma and prednisolone therapy in early years.

## DISCUSSION

Hematuria which is commonly divided into two groups, as macroscopic and microscopic hematuria, is defined as the abnormal presence of red blood cells in the urine. Macroscopic hematuria can be observed from as little as 1 mL of blood in 1 L of urine, therefore the color does not reflect the degree of blood loss [11,12].

Both macroscopic and microscopic types hematuria are common among patients with hemophilia. Incidence of hematuria in patients with hemophilia varies between 9.3% to 66% in the literature [6-8,13]. However its long-term effects on the kidney and renal functions are not well defined. Hematuria is often detected by chance in hemophilia patients. The history of analgesics (anti-inflammatory drugs), calcium and vitamin D intake, stress, trauma, exercise can cause hematuria [8,9]. The etiology of spontaneous hematuria is not known. It is thought to be a reflection of tubular damage caused by circulating immune complexes [11]. In addition, contrary to the general belief that renal disease is a rare complication of hemophilia, Kulkarni et al. [8] has found acute or chronic renal disease in 2.9% of hospitalized hemophilia patients. Kulkarni et al., [8] have investigated data collected from the medical records of 3422 males with hemophilia living in six the United States states between 1993 and 1998. They have examined associations of renal disease with demographic and clinical factors including age, race, hemophilia type and severity, hypertension, diabetes, history of recent renal bleeds, presence of an inhibitor, and infection with HCV or HIV. The study has revealed that HIV infection and hemophilia-related factors including inhibitors and kidney bleeds associated with renal disease in a group of males with hemophilia [8].

The development of asymptomatic gross or microscopic hematuria is relatively common in children. Although the incidence of asymptomatic gross hematuria is unknown, the prevalence of asymptomatic microscopic hematuria in school-age children has been estimated as 0.5% to 2.0% [12,14]. As hemophilia patients have a tendency to bleed, reported macroscopic and microscopic hematuria incidence is between 9% and 66% [6,9,13]. In our study, the hemophilic patients' macroscopic hematuria incidence was found to be 9.4% which is similar to a study which had been performed by Schlussel [15].

The most prominent symptom in VWD is mucosal bleeding (eg, epistaxis, gastrointestinal bleeding and menorrhagia) [16]. Incidence of hematuria has not been reported in the literature. In our study five (3.9%) of 129 VWD patients had a macroscopic hematuria episode. In VWD patients, the etiologies of the hematuria consist of urinary tract infection in one patient, nephrolithiasis in one patient and PSAGN in one patient. One of these patients received the diagnosis of VWD while he was being examined for hematuria.

Macroscopic hematuria has an estimated incidence of 1.3 per 1000 in the pediatric population [17]. Although hematuria origins from the lower urinary tract in most patients, in less than 10% of the cases, hematuria is caused by glomerular bleeding [18]. A clinician should ensure that while avoiding the unnecessary and expensive laboratory tests, serious conditions do not remain unnoticed and provide necessary advice for further evaluation wherever needed. Obtaining the true history of the condition and physical examination are the most important steps in the treatment of hematuria.

In our cohort, the hematuria episodes were seen at between 3 and 34 years of age, with a mean age of 15 years. All of the patients who had hematuria were above 5 years of age except a 3-year old female, who had hematuria because of urinary tract infection and a 4-year old male, who had hematuria because of nephrolithiasis. Hematuria episodes in hemophilia patients, according to the literature, developed after the age of 5 [19].

Among all hemophiliac patients whose medical records were examined, nephrolithiasis was seen in 1.4% of the patients. In general population under the age of 40, this ratio is 4.5/10.000. Nephrolithiasis frequency is much higher in hemophilic patients [20,21]. Ghosh et al., [20] have performed a retrospective study on 474 hemophiliacs aged between 1-64 years (only six of them was over forty years) with two or more episodes of hematuria. Nephrolithiasis had been determined in 1.3 % of the patients with moderate and severe hemophilia. In all these patients, the stone was either in renal pelvis or in the ureter. Also several genes linked to X chromosomes [21] may be responsible for abnormal vitamin D metabolism, and renal tubular acidosis may cause stone formation. One of the most important features in hemophiliacs in developing countries, is repeated joint bleeds that causes osteoporosis and increases calcium resorption of the bone and consequently, elevated calcium in the urine [20]. In line with the existing literature, no significant correlation between deficient factor levels

and hematuria frequency was found in our study [22,23]. But hematuria incidence was higher in patients with moderate and severe hemophilia compared to patients with mild hemophilia (p=0.01). However, the etiology of hematuria in hemophilia is often unclear, and it may be attributed to the underlying coagulation deficiency.

No other infectious agents were found in patients with hematuria. Viral infections may induce hematuria and renal disease in hemophilia patients [8], but the number of infected patients was relatively low in our patient group and incidence of hematuria among them was not significantly higher. Only one (1.7%) patient with hemophilia had chronic HCV infection.

Since the treatment options of hematuria patients with coagulation deficiencies couldn't be determined after such a long period of time we had excluded the treatment modalities.

### Study Limitations

The limitations of our study are; first of all, it is a retrospective study, which was conducted by using the data collected from patient reports. Moreover, the effects of hematuria on kidney function were not assessed, regular screening tests such as urine analysis and ultrasonography were not applied in the follow ups and metabolic evaluation for nephrolithiasis is lacking.

## CONCLUSION

The increased prevalence of macroscopic hematuria among children with congenital coagulation factor deficiencies is shown. Further studies will help to elucidate the causes and effects on renal function.

### Ethics

**Ethics Committee Approval:** The study was approved by Ege University Faculty of Medicine Medical Research Ethics Committee (decision number: 21-5T/118, date: 20.05.2021).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally and internally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: N.K., C.B., D.Y.K., Y.A., K.K., Concept: N.K., C.B., D.Y.K., Y.A., K.K., Design: N.K., C.B., D.Y.K., K.K., Data Collection and/or Processing: N.K., Analysis and/or Interpretation: N.K., C.B., D.Y.K., Y.A., K.K., Literature Search: N.K., C.B., D.Y.K., K.K., Writing: N.K., C.B., K.K.

**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.2022.31549
J Dr Behcet Uz Child Hosp 2022;12(2):197-202

Experimental Work

# L-glutamine Supplemented Nutrition Alleviates Damage Caused by Corrosİve Esophagitis in Rats

## *L-glutamin Destekli Beslenme Ratlarda Koroziv Özefajit Hasarını Azaltmaktadır*

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## ABSTRACT

**Objective:** The primary goal in the treatment of corrosive esophagitis (CE) is to control inflammation and scar reactions. L-glutamine (Gln) is beneficial for the integrity of the intestinal mucosal epithelium and is an amino acid that promotes mature collagen growth. This study was designed to demonstrate the positive results of Gln on injury in corrosive esophagitis.

**Method:** Thirty Sprague-Dawley rats were used in the study. They were divided into 3 groups. CE was formed by dripping 20% sodium hydroxide into the distal esophagus in both groups except the control group (n=10). First group (n=9) was left untreated, while the other group (n=9) was fed orally with the addition of 1 g/kg Gln once a day for 21 days. All rats were sacrificed after 3 weeks. Esophagus of treated and other group rats were examined under light microscope to evaluate collagen deposition, histological damage score and stenosis index.

**Results:** Excess submucosal collagen, muscularis mucosal damage, inflammation and ulceration, which are among the histological damage score parameters, were significantly higher in the untreated group than in the Gln group (p=0.005, p=0.015, p=0.001, respectively). The stenosis index was significantly different (p=0.013). The group treated with Gln had inflammation but no ulceration and necrosis.

**Conclusion:** Our experimental animal study suggests that Gln in nutrition reduces damage in the esophageal mucosa, slows down or partially stops the cellular destruction process that causes stenosis,

**Keywords:** Caustics, corrosives, esophagitis, L-glutamine, esophagial stricture

## ÖZ

**Amaç:** Koroziv özofajit tedavisinde birincil amaç enflamasyon ve skar reaksiyonlarını baskılayarak kontrol etmektir. L-glutamin, bağırsak mukozal epitelinin bütünlüğünü ve matür kollajen büyümesini destekleyen bir amino asittir. Bu çalışma, L-glutamin'in korozif özofajitte oluşan özofagial hasar üzerindeki olumlu sonuçlarını değerlendirmek için tasarlanmıştır.

**Yöntem:** Çalışmada 30 adet Sprague-Dawley cinsi sıçan kullanıldı. Üç gruba ayrıldılar. Kontrol grubu (n=10) hariç her iki grupta da distal özofagusa %20 NaOH (sodyum hidroksit) damlatılarak korozif özofajit oluşturuldu. Birinci grup (n=9) tedavi edilmeden bırakıldı, diğer grup (n=9) 21 gün boyunca günde bir kez 1 g/kg L-glutamin ilave edilerek ağızdan beslendi. Tüm sıçanlar 3 hafta sonra sakrifiye edildi. Kollajen birikimi, histolojik hasar skoru ve stenoz indeksini değerlendirmek için tedavi edilen ve diğer grup sıçanların yemek borusu ışık mikroskobu altında incelendi.

**Bulgular:** Histolojik hasar skoru parametrelerinden olan aşırı submukozal kollajen, muskularis mukozal hasar, enflamasyon ve ülserasyon tedavi edilmeyen grupta L-glutamin grubuna göre anlamlı derecede yüksekti (p=0.005, p=0.015, p=0.001, sırasıyla). Stenoz indeksi önemli ölçüde farklıydı. (p=0.013). L-glutamin ile tedavi edilen grupta enflamasyon saptandı ancak ülserasyon ve nekroz görülmedi.

**Sonuç:** Çalışmamız, L-Glutamin'in deneysel oluşturulmuş koroziv özefajit de ortaya çıkan mukozal hasarı azalttığını, stenoza neden olan hücresel yıkım sürecini yavaşlattığını veya kısmen durdurduğunu histopatolojik bulgularla göstermiştir.

**Anahtar kelimeler:** Kostik, koroziv, özefajit, L-glutamin, özefagus darlığı

**Received:** 05.01.2022
**Accepted:** 10.03.2022

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**Cite as:** Okur Ö, Diniz G, Arslan OA, Can M, Evciler H, Oral A, Hoşgör M. L-glutamine Supplemented Nutrition Alleviates Damage Caused by Corrosive Esophagitis in Rats. J Dr Behcet Uz Child Hosp. 2022;12(2):197-202

## INTRODUCTION

Corrosive esophagitis (CE) is a devastating health problem in children. Such injuries result in narrowing and even perforation of the digestive tract and more particularly of the esophagus [1]. Mucosal burns are more common in the acute phase and death may occur rarely after perforation. In the long-term, it causes stenosis at the injury site as a late complication [2,3]. The process of corrosive esophageal stricture occurs with reactive inflammation and edema, followed by granulation [4]. There is no consensus on the use of antibiotics and steroids for treatment, and some studies have shown no benefit from their use [5,6].

The primary objective of the medical treatment is to reduce the development of inflammation, fibroplasia, and scar reactions to prevent stricture. For an agent compatible with these basic therapeutic principles, our attention was drawn to the nutritional L-glutamine, which has been shown to contribute favourably to the integrity of the mucosal epithelium of the digestive tract, promote mature collagen growth, and accelerate healing [7,8]. The study was designed to investigate the curative effect of L-glutamine on corrosive burns of the esophagus and to demonstrate that L-glutamine is beneficial in preventing the formation of corrosive esophageal strictures, reducing scar formation and deposition of immature collagen.

## MATERIALS and METHODS

### Ethics Committee Approval

Ethics committee approval was obtained from the Ege University Animal Experiments Local Ethics Committee (decision number: 2010-157, date: 24.12.2010).

### Subjects

Rats were obtained from Ege University Animal Experiments Laboratory. Thirty Spraque-Dawley rats weighing 150 to 250 g were used. The rats were divided into three groups: the unburned esophagus group (control group, n=10), the burned esophagus group (EB, n=10) and the burned esophagus + L-glutamine supplemented group (EB GLN, n=10).

### Animal Model of Corrosive Esophagitis

In this study, Gehanno and Guedon [9] esophageal burn model was used. The feeding of all rats was stopped 12 hours before the experiment. Afterward, anesthesia was administered by intraperitoneal injection of 0.75 mg/100 g ketamine and 0.15 mg/100 g xylazine hydrochloride. The stomach was accessed through laparotomy incision under sterile operating conditions and taken out through the incision. The distal part of the esophagus was suspended together with a 1.5-2 cm segment of the abdominal esophagus using 3/0 Vicryl sutures, and a bulldog clamp was used for the esophagogastric junction. An 8 Fr orogastric feeding tube was inserted from the pharynx into the distal lumen of the esophagus 0.5 cm away from the cardia. A 3/0 Vicryl thread was tied around this feeding tube to prevent proximal reflux.

The distal and proximal ends of the esophagus were clamped, and 1 mL of 20% sodyum hidroksit solution was instilled in the EB and EB-GLN groups. In the other group, 1 mL of 0.9% NaCl solution was given. After 1 minute, the solutions were aspirated and the clamps were released. Subsequently, the abdominal incision was closed full-thickness using a 3-0 silk suture. Afterward, the rats were fed with tap water and an aqueous solution of a standard rat chow delivered without irritating the esophagus. All animals were kept in the same areas throughout the study and were fed with rat chow and water ad libitum.

### Application of L-glutamine and Termination of the Experiment

The dose of L-glutamine for rats was determined as 1 g/kg/day. L-glutamine was given to the EB-GLN group by gavage for 21 days as a single dose. The rats in the control and EB groups also received 0.9% NaCl solution using the same protocol. The experiment was finished after 22 days. The rats were sacrificed by cervical dislocation. The esophagus of the subjects was removed and placed in 10% formaldehyde.

### Histopathological Examination

The samples were examined in paraffin blocks. Sections from paraffin (4-5 μm thick) were stained with hematoxylin and eosin and Gömöri trichrome and examined under a Nikon Optiphot-2 light microscope. Tissue damage was assessed using collagen deposition, ulceration and inflammation scoring, and the stenosis index (SI) [10]. Histopathological evaluation criteria are shown in Table 1. The SI was calculated using the formula SI = wall thickness/lumen diameter. The esophageal lumen was measured using an x4 ocular micrometer lens. Results of esophageal wall thickness and lumen diameters measured from 4 different sites were recorded.

### Statistical Analysis

For the analysis of variance between groups, the Kruskal-Wallis test and Mann-Whitney U test with

Bonferonni correction were used. Histopathological scores were analyzed using $\chi^2$ and Fisher's Exact tests. P<0.05 was considered statistically significant. Statistical calculations were performed using SPSS statistical software version 19.

## RESULTS

### Mortality and Body Weight

Two rats exited on day 6 (EB group) and day 13 (EB-GLN group) were not included in the study. The body weights of all rats were measured at the beginning and end of 3 weeks (Table 2). The control group of unburned rats gained weight. Weight loss in the group with burns that received L-glutamine was lesser compared to those without.

### Results of Histopathological Evaluation

In the histopathological examination, there was little inflammation in 1 out of 10 rats in the control group without any collagen deposition, ulceration, or damage to the muscular mucosa in the tunica muscularis (Figure 1). In the EB group, mild inflammation was detected in six, and significant inflammation, and muscular mucosal damage in three rats. Six rats had ulcerations. Damage or collagen deposition in the tunica muscularis was grade 1 in one rat and grade 2 in the other eight rats (Figure 2).

Two rats in the EB-GLN group had inflammation and damaged muscularis mucosa. No ulceration was observed in the EB-GLN group (Figure 3). Collagen depositions in the tunica muscularis were grade 1 in one rat and grade 2 in two rats in the EB-GLN group where all injuries caused by induced CE were statistically significantly alleviated (p<0.05) compared with the EB group (p<0.05).

Statistically significant differences were observed between all groups regarding the SI (p<0.05). The stenosis indices in the control group and EB-GLN group were significantly lower compared to the EB group (p<0.05). In addition, the SI in the control group did not differ significantly compared to the EB-GLN group (p=-0.624). Comparative results of histopathological examinations and SI are shown in Table 3.

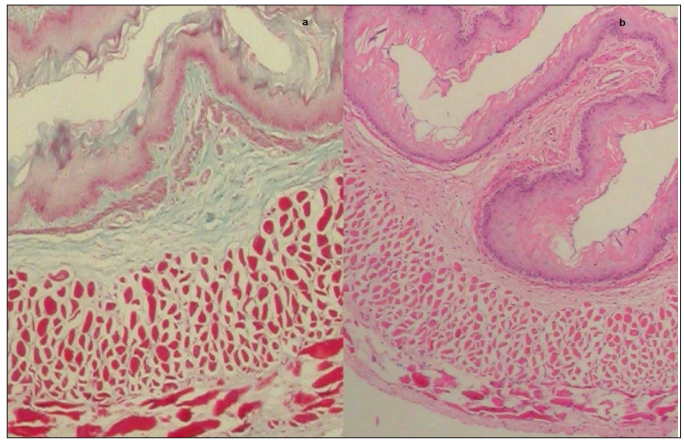


**Figure 1.** The sequence of the oesophagus of the control group. [minimal inflammation in one magnification, x100; staining, (a) Gomori trichrome and (b) hematoxylin-eosin]

**Table 1. Histopathological evaluation criteria**

| | | | |
|---|---|---|---|
| Inflammation | | None | 0 |
| Little | | 1 | |
| Mild | | 2 | |
| Marked | | 3 | |
| Ulceration | | None | 0 |
| Present | | 1 | |
| Increase in collagen deposition | Submucosa | None | 0 |
| | | Mild (submucosal collagen at least twice the thickness of muscularis mucosa) | 1 |
| | | Marked (submucosal collagen more than twice the thickness of muscularis mucosa | 2 |
| | Muscularis mucosa | No damage | 0 |
| | | Damage present | 1 |
| | Tunica muscularis | No damage or collagen deposition | 0 |
| | | Damage present | 1 |

Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model.[10]

## DISCUSSION

Our experimental study aimed to show whether oral L-glutamine supplementation prevents fibrosis and stricture formation after corrosive esophagitis. The hypothesis of the study is based on the anti-inflammatory and protective effects of L-glutamine on intestinal, myocardial and liver tissues demonstrated in previous studies [7,11]. As a potent agent to help heal tissue damage, several meta-analyses have evaluated the therapeutic efficacy of glutamine in other inflammatory

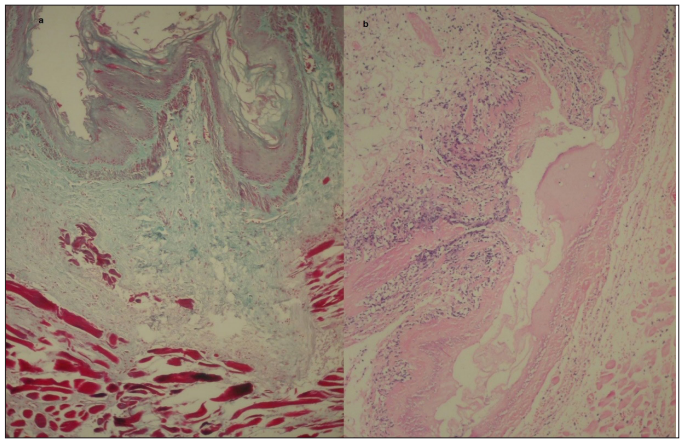

**Figure 2.** Widespread collagen accumulation was observed in the submucosal and muscular layers and inflammation and ulceration in EB group [magnification, x100; staining, (a) Gomori trichrome and (b) hematoxylin-eosin]

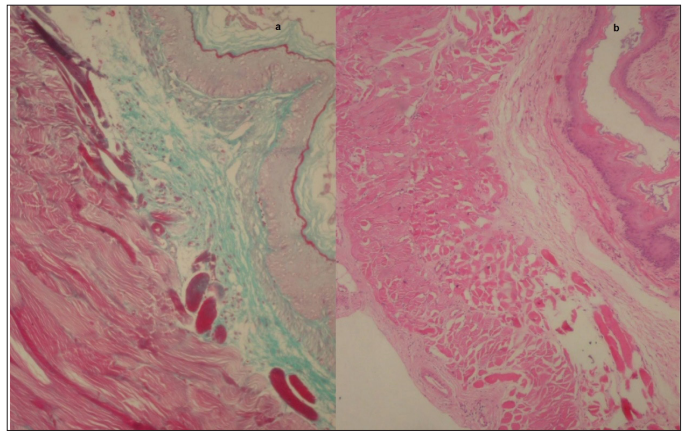

**Figure 3.** Reepithelization, minimal inflammation and regular mucosal, submucosal and muscular structures in EB-GLN Group. A slight increase in collagen accumulation was observed in the muscularis mucosa [magnification, x100; staining, (a) Gomori trichrome and (b) hematoxylin-eosin]

**Table 2. Body weights of the animals in each group**

| Group | Mean weight at day 1 | Mean weight at day 21 | Change (%) |
|---|---|---|---|
| Control | 172.5 g | 173.5 g | 0.59% (+) |
| EB | 164.09 g | 153.5 g | 6.45% (-) |
| EB-GLN | 166.18 g | 164.5 g | 1.01% (-) |

EB: Esophagus burn, EB-GLN: Esophagus burn glutamin administered

**Table 3. Comparison of stenosis index and histopathological evaluations**

| Histopathological findings | Control | EB group | EB-GLN group | P value (EB versus EB-GLN) |
|---|---|---|---|---|
| Inflammation | 1/+<br>9/- | 9/+<br>0/- | 2/+<br>7/- | 0.001 |
| Ulceration | 0/+<br>10/- | 6/+<br>3/- | 0/+<br>9/- | <0.001 |
| Injury of muscularis mucosa | 0/+<br>10/- | 9/+<br>0/- | 2/+<br>7/- | 0.015 |
| Collagen deposition in tunica muscularis | | | | |
| Grade 0 | 10/+ | 0/+ | 6/+ | 0.005 |
| Grade 1 | 0/+ | 1/+ | 1/+ | |
| Grade 2 | 0/+ | 8/+ | 2/+ | |
| Stenosis index | 0.25 (+\-0.05) | 0.72 (+\-0.07) | 0.36 (+\-0.06) | 0.013 |

EB: Esophagus burn, EB-GLN: Esophagus burn glutamin administered

diseases such as Crohn's disease, oral mucositis, and respiratory disease [12,13].

Studies have shown that under stress, serum glutamine level generally decreases and the integrity of the intestinal tract epithelium is impaired [14]. In rats with intestinal ischemia, treatment with glutamine has been shown to suppress lactate dehydrogenase levels and reduce the incidence of bacterial translocation [15]. In rats exposed to hypobaric hypoxia, glutamine treatment has become effective in alleviating intestinal damage by reducing inflammatory cytokines (TNF-$\alpha$ and IL-6) [16]. Accordingly, the antioxidant and anti-inflammatory effect of L-glutamine protects cells against lipid peroxidation, which is the beginning of many pathological stages [17].

Corrosive strictures with alkaline agents occur in three phases. In the first phase - the acute necrotic phase which onsets 1-4 days after an injury - an intense inflammatory reaction in the local tissue and coagulation of intracellular proteins cause cell necrosis. In the second phase (3-12 days following injury); ulceration and granulation tissue fill the defective area. In the final phase of the wound healing process (3rd week of injury); the connective tissue contracts and narrows the esophagus [18]. Fluid therapy, antireflux therapy, antibiotics, and steroid therapy are being currently used to reduce chemical trauma and bacterial infection in the acute phase, prevent fibroblastic activity, and collagen deposition in the long term [5,19].

In our study, we observed that dietary L-glutamine supplementation in the caustic burn group was able to prevent chemical damage by decreasing the deep penetration of chemical damage thro h increasing epithelialization in the esophageal lumen in the acute phase of corrosive damage. Our data have shown that, compared to the EB group, the EB-GLN group had a significantly lower SI, lesser inflammation, and collagen deposition in the submucosa. Histopathological findings correlate with the results. Lesser weight loss was detected in the group that received nutritional support with glutamine compared to the other groups. It has been shown that oral glutamine use may have an important role in reducing weight loss and the need for analgesics in aerodigestive system malignancies with acute radiation toxicities [20].

### Study Limitations

One of the limitations of our study is that although the burn model was created by taking a previously defined model as an example, esophageal stricture formation may not always be observed after this injury. The limited number of subjects used also reduces the probability of esophageal stricture occurring. If contrast imaging could be done, it might create data to support the predictive value of the SI.

## CONCLUSION

In conclusion, our results have shown the efficacy of L-glutamine therapy as an adjunct to conventional therapy in the model of corrosive esophageal burn. L-glutamine has been shown to significantly reduce the degree of fibrosis and improve histopathological damage. Our study's the first experimental research that used L-glutamine in the treatment of corrosive esophageal burns. More detailed animal and/or clinical studies are needed to support these results.

### Ethics

**Ethics Committee Approval:** Ethics committee approval was obtained from the Ege University Animal Experiments Local Ethics Committee (decision number: 2010-157, date: 24.12.2010).

**Informed Consent:** Informed consent is not required.

**Peer-review:** Externally peer-reviewed.

### Author Contributions

Surgical and Medical Practices: Ö.O., G.D., O.A.A., Concept: Ö.O., O.A.A., A.O., Design: Ö.O., O.A.A., A.O., M.H., Data Collection and/or Processing: Ö.O., H.E., M.H., Analysis and/or Interpretation: Ö.O., G.D., Literature Search: Ö.O., O.A.A., M.C., H.E., M.H., Writing: Ö.O., O.A.A., M.C., H.E., A.O.

**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.2022.54926
J Dr Behcet Uz Child Hosp 2022;12(2):203-204

Letter to the Editor

# Immune Thrombocytopenia in Childhood: Before and During the COVID-19 Pandemic

## *Çocukluk Çağında İmmün Trombositopeni: COVID-19 Pandemisi Öncesi ve Sırasında*

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**Keywords:** Immune thrombocytopenia, the COVID-19 pandemic, pathogenesis of immune thrombocytopenia, childhood

**Anahtar kelimeler:** İmmün trombositopeni, COVID-19 pandemisi, immün trombositopeni patogenezi, çocukluk çağı

**Received:** 08.04.2022
**Accepted:** 25.04.2022

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**Cite as:** Akyol Ş, Tüfekçi Ö, Yılmaz Ş, Ören H. Immune Thrombocytopenia in Childhood: Before and During the COVID-19 Pandemic. J Dr Behcet Uz Child Hosp. 2022;12(2):203-204

**To the Editor,**

The coronavirus disease-2019 (COVID-19) pandemic has affected many different disease groups and patients in different ways, as is the case in pediatric hematology [1-3]. In our clinic, we retrospectively analyzed the data of children who were admitted to our clinic between March 2019 and March 2021 with the diagnosis of immune thrombocytopenia (ITP). Outbreak of COVID-19 pandemic in Turkey was legally accepted in March 2020. We aimed to evaluate the possible changes that can be seen in the laboratory findings and disease course of ITP patients during the period of the pandemic.

We divided our patients into two subgroups as those admitted to our clinic before and during the first year of the pandemic. The COVID-19 polymerase chain reaction (PCR) tests were performed in all patients, and those with negative PCR test results were admitted to our clinic during the pandemic. There were 25 ITP patients in the year before the pandemic and 14 during the pandemic period. The number of cases had decreased by almost 45 percent. When we compared the mean and median platelet counts of the pre-and post-pandemic period; it was observed that mean and median platelet counts were lower during the pandemic period without any statistically significant difference [mean: 40.880±38.020/mm$^3$ and 20.500±27.556/mm$^3$, median: 20.000 (3.000-98.000)/mm$^3$ and 10.500 (5.000-39.000)/mm$^3$, respectively, p=0.063]. Remarkably, 40% (n=10) of 25 cases with acute ITP admitted in the pre-pandemic period developed persistent ITP. However, development of persistent ITP during the pandemic period was significantly less frequently seen ie. only 8% (n=1) of 14 acute ITP admissions (p=0.024).

In the etiopathogenesis of childhood ITP, autoimmunity induced with viral infections is a very well-known entity [4]. We may assume that the incidence of various viral infections decreased due to the use of masks, implementation of social distancing, and better hygiene conditions during the pandemic, and therefore lesser number of ITP cases were admitted to the clinics. On the other hand, there were ITP patients diagnosed incidentally before the pandemic period, but during the pandemic period, a significant decrease was observed in the rates of admission to the outpatient clinics and pediatric emergency services of children with various complaints, causing a decline in incidentally diagnosed ITP patients. During the pandemic, patients with ITP had lower platelet counts at diagnosis, which may be due to underlying weakened immune mechanisms or resistance to admission to a hospital due to fear of contracting COVID-19.

In the pandemic period, 92% of patients were diagnosed with acute ITP, and only one of them had persistent ITP. Although our patients had negative COVID-19 PCR test results, since COVID-19 disease can be asymptomatic in children, we don't know whether they have recovered from the disease recently or not [5]. Newly diagnosed ITPs due to COVID-19 disease and vaccination have been reported in the literature [6,7].

In conclusion; in the first period of the pandemic, a decrease was observed in the number of the newly diagnosed ITPs and most cases experienced acute course of the disease. However, we have to admit that one cannot draw definite conclusions based on the limited number of cases in our study. Large-scale studies are needed to evaluate the changes in laboratory findings and the course of ITP developed due to COVID-19 disease and during the pandemic.

### Ethics

**Informed Consent:** This article does not contain any studies with human participants or animals performed by any of the authors. The informed consent was not obtained.

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

Concept: Ş.A., Ö.T., Ş.Y., H.Ö., Design: Ş.A., Ö.T., Ş.Y., H.Ö., Data Collection or Processing: Ş.A., Ö.T., Ş.Y., H.Ö., Analysis or Interpretation: Ş.A., Ö.T., Ş.Y., H.Ö., Literature Search: Ş.A., Ö.T., Ş.Y., H.Ö., Writing: Ş.A., Ö.T., Ş.Y., H.Ö.

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

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

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