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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.

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

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The 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 the editor.

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

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

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

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

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

Review Articles: Extent investigation writings, including the 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 on the number of references. Related information is available in the following article; Burney RF, Tintinalli JE: How to write a collective review. Ann Emerg Med 1987;16:1402.

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

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

Type of article	Abstract	Word count*	Number of authors	Number of references	Table/ figure
Original Research Articles	250	2000 to 5000 20 double-spaced pages)		40	7
Case report	150	2000 (8 double-spaced pages)	5	15	3
Abstract Reports		3000 (8 double-spaced pages)		15	3
Concepts	150	4000 (16 double-spaced pages)			
Review Articles	250	2000 to 5000 (20 double-spaced pages)	3	50	3
Evidence-based Information		4000 (15 double-spaced pages)			
Letter to the editor	-	1500 (6 double-spaced pages)			-

MANUSCRIPT SUBMISSION

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

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

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Journal:

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

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

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

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

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

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

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seperate file, and not embedded in the text file. Electronic photographs, radiographs, CT scans, and scanned images must have a resolution of at least 300 dpi and 1200x960 pixels. If not obligatory any text typewritten on the figures should be avoided.

Figure legends: Include legends for all figures. Legends should appear on a separate page after the tables, should be brief and specific, and should include magnification and the stain used. Abbreviations and symbols used in the figures must be denoted in the legend.

References

References should be written in compliance with Vancouver style (see.:https://www.ncbi.nlm.nih.gov/books/NBK7256/). Authors are responsible for the accuracy of the references. While writing references, the below-indicated rules should be attentively observed.

References cited in the text

References cited in the text should be numbered in order of their use in the text, and the list of references should be presented accordingly. The number of the reference should be indicated in paranthesis and as a superscript. If more than one reference is used, then a comma (,) should be placed between references.

Sample cited statements in the text:

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).

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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.

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.

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

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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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PEER REVIEW, PUBLICATION ETHICS AND MALPRACTICE STATEMENT

Peer-review

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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Invited Review

Childhood Organ Transplantation

Çocukluk Çağı Organ Transplantasyonları

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ABSTRACT

Organ transplantation has significantly changed the life expectancy of patients with advanced organ failure. The quality of life with transplanted organs and their impact on growth have become more critical for children as they have a much longer life expectancy and will be experiencing growth stages. Solid organ transplantation techniques, which were used only experimentally in animals until the middle of the 20th century, have become a treatment option in the 21st century. Particularly with the discovery of new immunosuppressive drugs in the 1960s, transplantation has gained impetus as a viable therapeutic option. Examination of the biopsies taken from the transplanted organ is an important factor that ensures early recognition of rejection findings and can prolong the life of the organ. In this review, the historical development of transplantation, the mechanisms involved in tissue rejection, rejection evaluation criteria, and the main differences between childhood and adult organ transplantations are briefly reviewed.

Keywords: Solid organ transplantations, transplantation pathology, rejection criteria, childhood, and adulthood transplantations

ÖZ

Organ nakli, ilerlemiş organ yetmezliği olan hastaların yaşam beklentisini önemli ölçüde değiştirmiştir. Nakledilen organlarla sürdürülen yaşamın kalitesi ve nakil sonrası kullanılan ilaçların büyüme üzerine etkisi, çok daha uzun bir yaşam beklenen ve büyüme evresinde olan çocuklar için daha kritik hale gelmiştir. Yirminci yüzyılın ortalarına kadar sadece hayvanlarda deneysel olarak kullanılan solid organ nakli teknikleri, 21. yüzyılda bir tedavi seçeneği haline gelmiştir. Özellikle 1960'lı yıllarda immün sistemi baskılayan yeni ilaçların bulunmasıyla birlikte transplantasyon önemli bir ivme kazanmıştır. Transplante organdan alınan biyopsilerin incelenmesi ret bulgularının erken fark edilmesini sağlayıp, organın ömrünü uzatabilen önemli bir unsurdur. Bu derlemede transplantasyonun tarihsel gelişimi, doku reddinde rol oynayan mekanizmalar, rejeksiyon değerlendirme kriterleri ile çocukluk çağı ve erişkin organ transplantasyonları arasındaki temel farklar kısaca gözden geçirilmiştir.

Anahtar kelimeler: Solid organ transplantasyonları, transplantasyon patolojisi, rejeksiyon kriterleri, çocukluk çağı ve erişkin dönem transplantasyonları

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INTRODUCTION

Although dreams of transplantation go back to the 3rd century AD, this vision only came true at the end of the 20th century[1]. The first successful human organ transplantation was kidney transplantation from a twin performed by Joseph Murray in the year 1954 which allowed the recipient to live for 8 more years[1,2]. With the discovery of immunosuppressive drugs, organ transplantation has gained momentum since the 1960s[2-4]. Pediatric organ transplantation has greatly improved the management and survival of treatment-resistant pathological conditions in children with end-stage organ failure and is now considered the treatment of choice when clinically appropriate. In the pediatric age group, various organs and tissues including the heart, kidney, liver, lung, intestines, pancreas and bone marrow can be transplanted[5-7].

When compared with relevant official records on transplantation, in the USA, which has the largest patient series in solid organ transplantation, approximately 20 thousand renal transplantations, almost all of them from deceased donors, and about 7,000 liver transplantations, of which about 1/4 of them from living donors, were performed in 2017[4].

In childhood, organ transplantation can represent as a challenging and complex treatment option, especially due to the extent of surgical intervention, immune system response, use of immunosuppressive drugs, and the unfavorable effects of all these processes on growth,

skeletal development and quality of life. Advances in immunosuppressive therapies have significantly improved patient care and graft survival rates, but the long-term risks of these therapeutic interventions are not well known due to the lack of randomized clinical trials performed in the pediatric population[5-7].

The aim of this review is to examine the differences between organ transplantations performed in pediatric age and in adults after briefly summarizing the mechanisms that play a role in organ rejection and criteria of rejection.

MOLECULES THAT PLAY A ROLE IN ALLOGRAFT REACTIVITY

1) Major histocompatibility complex (MHC)

The MHC, which plays the most important role in tissue rejection, was identified by Jean Dausset in the 1950s and is termed as human leukocyte antigen (HLA)[1,2]. The MHC system encodes for two major protein groups in humans: Class I (HLA-A; B; C) and Class II (HLA-DP, DQ, DR). MHC class I transmembrane molecules are found in almost all nucleated cells, while MHC class II molecules are mainly found in B lymphocytes, macrophages and dendritic cells. However, some immunomodulatory molecules such as interferon gamma (IFN-γ) can increase MHC class II expression on many cell surfaces, especially endothelium, epithelial cells, and T lymphocytes. Normally, MHC class I molecules present antigenic peptides such as viral antigens and oncogenic products to T lymphocytes. MHC class I molecules and the antigenic peptide complex are recognized by specific cytotoxic CD8 (+) T lymphocytes. T cells are stimulated and these infected cells or those altered by an oncogenic effect are destroyed. On the other hand, MHC class II cells present exogenous antigenic peptides in the extracellular pool to CD4 (+) helper T lymphocytes. However cross presentations are also possible. MHC molecules are highly polymorphic, which provides a high level of immunity against pathogens that constantly mutate[8,9].

2) Minor histocompatibility antigens (MiHAs)

MiHAs are small peptides that coexist with MHC class I or class II molecules on the cell surface. Because of their polymorphic structure and their expression on the hematopoietic cell surface, they are effective in the molecular presentation of self to the immune system. Even differences in a single amino acid can be detected by immunoreactive T cells and can cause graft-versus-host disease (GVHD) in HLA-matched allogeneic stem cell transplantation. While MHC antigens can be detected by both B and T lymphocytes, the response to MiHC antigens appears to be strictly T cell mediated. Although more than one hundred minor tissue compatibility antigens have been identified and sequenced, little data are available regarding the role of MiHA variations in the development of GVHD[10,11].

3) Tissue specific antigen (TSA)

As its name suggests, TSAs are antigens specific to different tissues or organs[1]. Central tolerance to TSAs is under tight thymic control, and autoimmune diseases develop when this control is disrupted. Although their role in transplantation immunobiology is still not fully elucidated, TSAs are known to enhance the host's immune response to the allograft. For example, myosin, which has not normally immunogenic characteristics, becomes immunogenic only when the transplant organ is injured, leading to an organ-specific response[1,2].

4) Donor specific antibody (DSA)

DSAs are recipient antibodies that can bind to MHC class I and II molecules in the transplanted organ, potentially causing graft injury. DSAs are formed by prior exposure to foreign antigens due to various blood product transfusions, previous pregnancies, autoimmune and viral diseases, and are present at the time of transplantation. However, *de novo* DSAs are formed after transplantation in response to genetically disparate HLA molecules of the new donor organ; young age is a risk factor for increased DSA formation. For the early detection of antibody-mediated rejection, regular measurement of DSAs in the blood is necessary. These antibodies often target endothelial cells thereby initiating a reaction with complement in the vascular wall, activating the coagulation cascade and releasing inflammatory mediators. The complement fragment, C4d, is formed during complement activation by the classical pathway. However, unlike other complement fragments, it does not disappear quickly and can be observed in the vessel wall for at least a few days. In most biopsies of transplanted organs or tissues, immunohistochemically detected C4d positivity on capillaries is accepted as additional evidence of an antibody-mediated rejection[1,2,12,13].

TISSUE REJECTION MECHANISMS

The reactions that occur because of different interactions of MHC, MiHA and TSA between recipient and donor are in the form of host-versus-graft (HVG) reactivity or GVHD. In HVG reactivity, the host immune

system recognizes these foreign MHC, MiHA, and TSAs antigens after organ transplantation. If this reaction cannot be prevented, the result is allograft rejection. Organ rejection is generally classified as hyperacute, acute, subclinical, and chronic. Hyperacute rejection develops within minutes or hours and is dependent on the presence of pre-existing antibodies. Acute and subclinical response is usually T cell and/or antibody-mediated rejection and develops within a few days or months. It can be reversible with immunosuppression. Chronic rejection develops months to years after transplantation and is typically unresponsive to treatment. In a sense, chronic rejection is closely related to the functional lifespan of the transplant organ. In the direct pathway, donor MHC molecules are presented to CD4 and CD8 T lymphocytes by donor-antigen presenting cells. The indirect mechanism develops more slowly and is effective in situations that predispose to chronic rejection. The third pathway is the semidirect pathway. In this pathway, recipient dendritic cells present donor MHC molecules to T lymphocytes. NK and NKT cells also play a role in organ rejection[1,14-16].

GVHD is a critical complication that is more common in organ transplantations involving massive hematopoietic cells such as hematological stem cell transplantation or multivisceral organ transplantation. GVHD can also develop acutely or chronically. The target organs of acute GVHD are mainly skin, liver, intestines, lung and lymphoid tissues. Inflammatory cytokines (e.g., tumor necrotizing factor alpha, interleukin 1), microbial lipopolysaccharides and necrotic cells that pass into the circulation from damaged organs are partly responsible for this stimulation. This activation facilitates the recognition of MHC and MiHA molecules by mature donor T cells and NK cells[1,14-16].

GRAFT INJURY MECHANISMS

1) Ischemia and innate immune activation: These complications are more frequently seen in transplantation from cadaveric donors. Increase of MHC and MiHA molecules and many molecular mechanisms such as Toll-like receptors and heat shock proteins play a role[1].

2) Acute rejection: It can develop as antibody - or T cell-mediated rejection.

a) In acute antibody-mediated rejection, anti-HLA antibodies or DSAs may develop in the recipient due to transfusions, pregnancy, or previous transplantation. These antibodies are typically IgM and IgG, with activation of many mechanisms. The presence of C4d and less commonly C3d are indicators of antigen-antibody interaction[1].

b) T cell-mediated rejection is the most common form of acute rejection observed in allografts. It is characterized by the collection of mononuclear cells, mainly lymphocytes and macrophages, in the connective tissue elements such as the interstitial areas. These inflammatory cells, led by T cells, attack mainly vascular structures and epithelial cells. The main targets are tubules in the kidney, bile tubules in the liver, and crypt epithelium in the gastrointestinal tract. Although T lymphocytes play the leading role, NK/NKT cells, monocytes/macrophages, plasmacytes, eosinophils and B lymphocytes also participate in this process. Since one of the important mechanisms working in T cell-mediated rejection involve some enzymes from the perforin/granzyme family used by CD8 (+) T lymphocytes and NK cells, their immunohistochemical detection in tissue or urine supports rejection diagnosis. In some studies, it has been reported that the presence of CXCR3 and CCR5 chemokines were evidence of rejection and their blockage is important in preventing T-cell mediated rejection[1,2,17].

3) Chronic rejection is also an important cause of recipient morbidity and mortality. Although acute rejection rates have been significantly reduced by immunosuppressive treatments developed in the last 20 years, the accumulation of fibroelastic material in various compartments of organs due to obliterative vascular injury, which is a chronic rejection symptom, is not prevented. The typical lesion is progressive arteriopathy involving small and large muscular arterial walls. It differs from atherosclerosis with its diffuse distribution, minimal lipid deposition, and lamination with adventitial scarring with intimal hyperplasia. All immunological processes between donor and recipient, from ischemia-reperfusion injury to acute and subclinical rejection episodes, affect chronic rejection. This process is regeneration and the fibroplasia phase that follows regeneration. Many chemokines aid in fibrosis. Although transforming growth factor-beta (TGF-β) is mainly responsible for this process, angiotensin II and plasminogen activator inhibitor are also involved[1,2].

4) Post-transplant infections are mainly caused by cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus (HHV-6, 7, 8) and hepatitis C virus (HCV)[1,2].

5) Recurrent and/or *de novo* immune disease are most evident in kidneys, and focal segmental

glomerulosclerosis (FSGS); immunoglobulin A (IgA) nephropathy, membranoproliferative glomerulonephritis, membranous nephropathy, fibrillary GN, dense deposit disease, anti-glomerular basement membrane disease, and lupus nephritis are the most common diseases that develop in allografts[1,2].

6) Drug toxicity also causes very different clinical and histopathological changes[1,2].

TRANSPLANTATION PATHOLOGY

Today, there are different grading systems in transplantation pathology for each organ used. Banff classification is the most famous of these grading systems used in different organ transplantation pathologies, mainly in renal transplantations. Banff is a settlement located in the Canadian province of Alberta, and after the transplantation committee held its first meeting in Banff in 1991, Banff grading began to be used as the principal scoring system[18].

1) Evaluation of Kidney Biopsies

The latest Banff 2019 update is used for evaluation of kidney transplant biopsies. Zero-day biopsy sampling is often performed in order to make comparisons in subsequent evaluations. For ideal evaluation, at least 10 glomeruli and 2 arteries should be found in the biopsy material. The changes observed in the allograft are evaluated in 6 categories according to the Banff grading system as follows: normal; antibody-mediated rejection; borderline changes (suspect T-cell-mediated rejection); T-cell-mediated rejection; interstitial fibrosis/tubular atrophy, and other changes. In antibody-mediated rejection (humoral immunity) the capillaries around the kidney tubules are targeted. Glomeruli are also the main targets as they are made up of capillary tufts. Evidence of the presence of circulating alloantibodies is the circumferential deposition of C4d in the glomerular and peritubular capillaries. To be able to say antibody-mediated rejection, all three groups of changes such as the presence of tissue injury findings mainly affecting the capillaries (g, ptc or v>0), C4d positivity showing antibody association with the vascular endothelium, and the presence of DSA developed serologically against HLA or other antigens should be present in combination. Category 3, described as borderline changes in the Banff classification, is suspected acute T cell-mediated rejection. Intimal arteritis should not be found. Because in the presence of intimal arteritis, the diagnosis is cellular rejection. Minimal interstitial infiltration (i0 V i1) is accompanied by tubulitis (t1, t2, t3) or with minimal tubulitis (t1) in the infiltration phase (i2, i3). With T cell-mediated (cellular rejection) rejection, T lymphocytes target the tubules and the endothelium of the arteries. Its histopathological appearance is indistinguishable from tubulointerstitial nephritis[1,2,18-20].

2) Evaluation of Cardiac Biopsy Materials

The most common diseases leading to heart transplantation are nonischemic cardiomyopathy (50%) and ischemic diseases in adults (1/3). In infants, approximately 50% of the cases of congenital heart diseases and 40% of the cases of cardiomyopathy cause organ loss. Cardiomyopathy is the most common cause of organ loss in 60% of older children. In developed centers, 1-, and 5-year survival rates are 81%, and 69%, respectively. Endocardial biopsies are the gold standard for assessing heart transplant complications. With the transjugular approach, the right heart is approached, and sampling is made from the interventricular septal region. Since the lesions are not diffusely distributed in rejection, at least 3-4 samples are taken. Biopsies per protocol are performed at the time of transplantation to exclude myocarditis, ischemic injury, and other causes. Follow-up protocol biopsies are performed once a week in the first month, every 2 weeks in the second month, and every 4 to 8 weeks between 3-12 months. Evaluation is done according to the International Society of Heart and Lung Transplantation-Working Formulation (ISHLT-WF) system. Cardiac transplant rejection types were divided into humoral and cellular rejection categories in 2011 as was done in other organ transplants. This system was last updated in 2013. In order to be able to say that sufficient biopsy material is obtained for classification, at least 3 specimens should be obtained, and >50% of at least one specimen should contain myocardial tissue. Insufficient number of biopsy specimens, or specimens containing only endocardial tissue, thrombus, previous biopsy site scar or adipose tissue prevent proper histopathological evaluation[1-3,21,22].

Bacterial and viral agents are responsible for the majority of infectious complications, followed by fungal, and parasitic agents. *Toxoplasma bradyzoites* can be observed in sarcoplasmic cysts. Viral inclusions are found in capillary endothelial or perivascular cell nuclei rather than muscle cell nuclei. The presence of adipocytes together with cuboidal mesothelial cells in biopsy materials indicates myocardial wall perforation. Fat tissue alone is not diagnostic. Since adipose tissue is a component of the epicardial layer, fat tissue can also be found in the endocardium in obese individuals

and steroid users. However, if biopsy material contains mesothelial cells, then the biopsy material is taken from the epicardium. It is also possible to see dystrophic calcifications that fill the entire muscle cell cytoplasm.

Quilty effect is a condition that was first described by the surname of the patient and is characterized by the presence of lymphoplasmacytic infiltration in the endocardium. Although cyclosporine has been claimed to have an effect, this issue has not been proven. It is seen in 69% of children and 49% of adults who underwent heart transplantation and is considered an insignificant finding[1,2].

For the diagnosis of antibody-mediated rejection, complement dissociation/degradation products are examined. The presence of C3d and C4d complements is important for the establishment of diagnosis. Serum concentrations of firstly C3, then C4 rise. HLA-DR, fibrin and Igs are unreliable diagnostic parameters. As an important criterion, myocardial capillaries should be stained all around for C3d and C4d. CD68- positive macrophages can be found in the interstitium. However, the presence of CD68 (+) macrophages in myocardial capillaries is an important parameter[1,2]. Cardiac allograft vasculopathy is not specific to the heart and can be seen in all organs. It may be confused with atherosclerosis. It may develop in a few months, or it may take years to develop. It is characterized by intimal proliferation and unlike atherosclerosis, the elastic internal lamina is intact[22].

3) Evaluation of Lung Biopsies

Success rates in lung transplantation have increased within the last 20 years. Bilateral transplantation is performed in most cases. According to the latest data, the average recipient survival time is 5.5 years. For those who survived the first year, survival time extends to 7.7 years, while 5-year survival rate in these cases is 53%. The longest survival time is 7.1 years in cystic fibrosis cases, and the shortest one is 4.3 years in cases with idiopathic pulmonary fibrosis. Today, 3-month, and 1 -year survival rates have increased from 81% to 90%, and from 70% to 81%, respectively[1,2,21].

Three types of early-onset complications may develop after lung transplantation. Primary graft dysfunction develops due to severe ischemia-reperfusion injury. Like acute respiratory distress syndrome, it is the most important cause of post-transplant mortality and morbidity. Survivors often develop bronchiolitis obliterans. Hyperacute rejection occurs when preformed DSA levels are increased, and these antibodies rapidly attack the organ allograft. Antigen-antibody complexes bind to the endothelium, activating complement and causing massive vascular injury in the lung. Since immunological compatibility between donor and recipient is typically not an issue, this is a rare complication. The incidence of airway complications has decreased with the improvement of surgical techniques (7-18%). The rapamycin derivative sirolimus can be a common causative agent. These complications do not develop due to steroids. Necrosis secondary to ischemia and reparative mucosa manifesting as squamous metaplasia are observed[1].

Acute cellular rejection has a frequency of 36-55% in the first year. It is characterized by the presence of lymphocytes around the epithelium and vessels. The detection rate with bronchoscopic biopsy is 80%. For evaluation, at least 5 pieces of lung tissue with alveoli must be examined and at least 100 alveoli must be evaluated. The grading system proposed by The ISHLT-WF is also used for grading lung transplantation biopsies. The existence of acute antibody-mediated rejection for the lung is still controversial. Donor-specific antibodies must also exist. But it is unclear how C4d will be evaluated. Findings are confused with infection, acute cellular rejection, preservation injury and many other conditions[1,21-23].

Chronic lung allograft dysfunction develops in 50% of 5-year transplants. Lymphocytes that cause airway inflammation in cases of acute cellular rejection can lead to mucosal damage and ulceration, along with granulation tissue. This process may occur within a few months following transplantation though it usually develops after 16-20 months. There is patchy involvement. Fibrotic tissue is characterized by type III collagen. Perioperative infections may be caused by actinomyces, staphylococci, and pseudomonas. Most infections that develop in the first month after transplantation are of bacterial origin. Between 1 and 6 months after transplantation, the incidence of bacterial infections decreases, while that of opportunistic infections increases. After 6 months, viral infections may develop and lead to a more severe course. Mycoplasma infections may also occur[1,2,23].

Sarcoidosis is the most common primary disease that relapses. Since cancer can also recur, the presence of carcinoma is a contraindication for transplantation. However, those with 5-year disease-free survival are included in the transplantation list[1,2].

4) Evaluation of Intestinal and Multivisceral Transplantation Biopsies

Transplantation may be performed as isolated intestinal transplantation, or as total/multivisceral (stomach, small and large intestine, liver, pancreas, and spleen) transplantation. Some studies report better results with total transplantation. A very small proportion of intestinal transplantation is performed from a living donor. Potential complications that may develop after intestinal transplantation are acute and chronic rejection, infection, post-transplant lymphoproliferative disorder (PTLD), GVHD, renal dysfunction, bowel perforation, anastomotic leakage, and pancreatitis. Histopathological evaluation should be performed in consideration with the patient's history, clinical findings, and previous biopsy results whenever possible. One or 2 biopsies should be taken from each region and since the lesions are not diffusely distributed, sections obtained from different levels should be examined. GIS transplants should be urgently performed since many posttransplant complications can rapidly lead to allograft dysfunction and failure[1,2].

Preservation damage develops due to ischemia-reperfusion injury. In the early period in a mild lesion, congestion and dehiscence of the surface epithelium are observed in the absence of significant inflammation, edema and swelling of the villi. In its more advanced form, mucosal hemorrhage and deep epithelial necrosis are observed. Findings in the stomach are not well defined[1-3,8].

Clinical and endoscopic correlation is very important for the recognition of acute rejection. Relevant symptoms include increased stool output from the stoma, bloating, and fever. But all these findings are also caused by infections. Morphologically detected rejection without clinical findings is called subclinical rejection[1].

Antibody-mediated rejection: Hyperacute and accelerated acute rejection develop in a few hours or days after transplantation dependent on the presence of high levels of DSAs. Significant bleeding, PMN margination around the vessels, and vascular congestion are seen. Vasculitis can be a very important finding, but it is not usually seen in superficial mucosal biopsies. However, all these listed findings are also observed in ischemia, nonspecific infections and mechanical vascular problems. Therefore, the presence of immunohistochemically detected accumulation of Igs along the interstitium or vessels, and also accumulation of C4d and C3d along small capillaries, and arterioles are important criteria.

Scoring is done as follows[1,2]:

0: Lack of any significant congestion and extravasation.

1: Changes involving 10-40% of the entire tissue obtained.

2: Changes involving 40-70% of the entire tissue obtained.

3: Changes involving more than 70% of the entire tissue obtained.

Acute T cell-mediated (cellular) rejection is the most frequently encountered form of acute rejection in all gastrointestinal and visceral organ transplantations. Lymphocytes play the leading role. Lymphocytes target crypts, glandular structures as well as muscle, endothelial and even nerve cells. Parenchymal metaplasia is observed. CD4- and CD8- positive cells, more often cytotoxic T lymphocytes underlie acute cellular rejection. Apoptosis of crypt epithelial cells is the most common change. CD8 (+) T cells lead to apoptosis via granzyme B/perforin-dependent granular exocytosis pathway FAS/FAS-L dependent cytotoxicity. In animal models, cells other than cytotoxic T lymphocytes have been shown to contribute to apoptosis of crypt epithelial cells. In the grading system, the most important finding is the presence of apoptotic body. The presence of less than 6 apoptotic bodies in 10 crypts is not considered a rejection. In severe acute cellular rejection, extensive morphological distortion, gland destruction, granulation tissue formation with widespread presence of neutrophils and eosinophils, and mucosal peeling with fibrinopurulent exudate are observed. Infections should always be considered in the differential diagnosis of acute cellular rejection. However, as an important corollary concerning differential diagnosis, apoptosis does not increase even in severe infections. The same grading system is also used in the evaluation of rejection in small intestine transplants containing a colonic segment. In gastric transplantation, the grading is slightly different[1-3,8,24,25].

Chronic rejection is also known as chronic allograft enteropathy. In this case, treatment-resistant, progressive protein-losing enteropathy occurs. In endoscopic examination, loss of villi is observed. Pathognomonic findings of chronic rejection are intimal thickening of arteries, medial hypertrophy and adventitial fibrosis. Since mucosal biopsy materials generally do not contain large vessel architecture, mucosal biopsy has a limited diagnostic value. The presence of chronic injury characterized with fibrosis, crypt loss, distortion,

and architectural changes associated with clinical and endoscopic findings may indicate chronic rejection[1-3,24,25].

5) Evaluation of Liver Transplantation Biopsies

While the most common indications for liver transplantation in the USA are HCV infection, alcoholic and non-alcoholic steatohepatitis, chronic HBV infection still predominates in Asia. Ideal donors are deceased persons under 40 years of age, with brain death due to trauma, without cardiovascular, chronic liver disease, and steatosis, but with intact circulation until the time of transplantation. Use of significantly macrosteatotic (>40%) cadaveric organs, those exposed to cold ischemia for more than 12 hours, or organs taken from deceased individuals over 60 years of age are more often contraindicated for transplantation. Living donor transplantations carry a risk of 0.2% mortality and 25% morbidity. Complications following transplantation include vascular complications such as preservation (ischemia/reperfusion) injury, portal hyperperfusion or small allograft syndrome, hepatic artery thrombosis, portal vein thrombosis, hepatic vein and vena cava complications, and biliary tract complications[1-3].

Rejection in the liver is generally considered as acute antibody-mediated rejection, T-cell-mediated rejection, and chronic rejection, as in other solid organ transplantations. The liver is more resistant to antibody-mediated rejection associated with anti-MHC 1 and 2 antibodies compared to the lung and heart. Antibody-mediated rejection usually develops in the first few weeks after transplantation in cases with incomplete ABO compatibility. There is a high titer of DSA in the circulation. Since full ABO compatibility in liver transplantation is only required in the USA, and other countries in the American continent, antibody-mediated rejection is observed more frequently in Asian countries. Hyperacute perfusion can also be seen in the liver which develops following a bleeding episode. In acute rejection, levels of serum bilirubin and parameters of liver function tests rise within a few days or weeks following transplantation. Although it is difficult to distinguish this condition from preservation damage and biliary stricture, increased isoagglutinin levels and C4d staining aid in diagnosis. The O'Leary criteria, updated in 2014, are used for grading acute antibody-mediated rejection. The diagnosis of chronic antibody-mediated rejection is somewhat more uncertain. However, as a rule, signs of acute antibody-mediated rejection should be accompanied by fibrosis, which is a sign of chronic injury[1,2].

Early (<6 months) onset T-cell-mediated rejection affects approximately 30% of cases and develops 5-30 days after transplantation. Risk factors include type of immunosuppressive therapy, being young and healthy (child N, creatinine N), HLA-DR incompatibility, autoimmune hepatitis in the recipient, primary sclerosing cholangitis-like immune deviations, prolonged cold ischemia, elderly donor, and HLA-C genotype. It rarely causes allograft failure or permanent damage and responds well to treatment. Late onset (>1 year) type is usually associated with inadequate immune suppression, DSA development, and can lead to organ failure. It is somewhat similar to chronic hepatitis with a late onset associated with a slightly lower number of blastic lymphocytes, increased interface and lobular activity, milder venous subendothelial but more intense perivenular inflammation. As a rule, in T cell-mediated rejection (especially in the early-onset type), mixed portal inflammation consisting predominantly of activated/blastic lymphocytes, subendothelial inflammation (endothelialitis) in the portal or terminal hepatic venule, and bile duct inflammation-damage must be present. A majority of the lymphocytes are of the CD8 phenotype. The BANFF grading system also indicates the severity of the lesion. According to this system, presence of portal inflammation that does not meet the diagnostic criteria of acute rejection criteria is called indeterminate. In mild cases of acute rejection (grade 1), rejection infiltrate is confined only within some portal spaces. In grade 2, it is present in most portal spaces. In grade 3, the perivenular infiltrate extends beyond the portal area is found. In acute rejection, some centers utilize the rejection activity index. Accordingly, all three findings are scored separately[1-3,26,27].

Signs of chronic rejection include ductopenia, obliterative arteriopathy and perivenular fibrosis. Typical chronic rejection can be defined as early or severe. It causes permanent damage to bile ducts, arteries and veins. Previously, signs of chronic rejection had developed in the first years after transplantation. While the rate of chronic rejection was 15-20% in a 5-year transplant in the 1980s, today this rate has decreased to 3-5%. It is mostly seen in patients who are transplanted without full histocompatibility, HCV (+) patients who are receiving immune activator therapy such as interferon alpha, and those whose immunosuppressive dose is reduced due to lymphoproliferative disease. Risk factors are considered in 2 groups as alloantigen-related and non-alloantigen-related. The most important non-immunological risk factor is the donor age above 40 years[1,2,26-28].

6) Evaluation of Pancreas and Pancreatic Islet Transplantation Biopsies

Pancreas allograft transplants are most commonly performed in type 1 diabetes where hypoglycemia attacks cannot be controlled with ensuing progression of vascular and renal complications. Some pancreas and kidney transplantations are applied synchronously. Only 5-6% of the cases are type 2 diabetes patients. A very small group have a large benign tumor or a dysfunctional organ due to recurrent chronic pancreatitis. Organ or islet autotransplantation should be considered, especially when organ removal is required due to the presence of a benign tumor[1,2].

The first pancreas transplantation was performed in 1966, and the patient lived for a week without the need for insulin, but died in the second week due to pulmonary embolism secondary to pancreatic fistula and pancreatitis-like complications. Many methods have been tried for the drainage of the exocrine pancreas. While only 100 pancreatic transplantations were reported in the world until 1980, this number increased to over 30 thousand in the early 2010s. In the previously tried technique venous drainage was diverted into the iliac vein and exocrine secretion into the native duodenum. Eventually, drainage of exocrine secretion into the bladder was predominantly utilized. Despite side effects of this application, such as hematuria, urinary leakage, recurrent infection, it is useful in monitoring of urine amylase levels. Indeed a decrease of more than 25-50% in post-implant amylase levels is indicative of rejection. Nowadays, enteric drainage is preferred due to its suitability for the physiological condition, and the portal system or iliac vein is used for venous drainage. Elevated creatinine levels in synchronous pancreas and kidney transplants is a finding suggestive of rejection. The increase in amylase-lipase-like exocrine pancreatic enzymes in the blood is an indicator of exocrine acinar cell damage and the levels of these enzymes increase in rejection. However, they are also elevated in inflammatory conditions such as acute pancreatitis. Similarly, hyperglycemia is also seen. Today, less than 10% of successfully transplanted pancreas is lost to acute rejection. However, 5-year organ survival rate is around 40-50%[1,2,29-31].

The diagnostic sensitivity of biopsy in acute rejection is 80%. Due to the non-specific nature of laboratory tests, needle core biopsy is the gold standard diagnostic method for acute rejection. Ultrasonography or computed tomography-guided percutaneous needle biopsy technique was first used in the 1990s, and sufficient biopsy material can be obtained in 85-90% of cases. The rate of serious complications such as bleeding is 2-3% which does not cause organ loss. However, intestinal loop may prevent percutaneous biopsy in patients who are undergoing intestinal drainage. In these cases, laparoscopic biopsy can be performed. In patients undergoing bladder drainage, cystoscopic biopsy can be performed instead of percutaneous core biopsy. The rate of obtaining sufficient tissue specimen with this technique has been reported to be between 57-80%. However, this method is more invasive and expensive. It has been reported that in patients who have recently undergone enteric drainage, pancreatic graft tissue has been found in the proximal jejunum, which is the site of anastomosis, and sufficient material is sampled in 75% of these cases. It is recommended that the biopsy specimen should contain at least 3 pancreatic lobules with accompanying interlobular spaces.

Typically, veins, branches of the pancreatic ducts, and arterial branches are observed in the interlobular space. For best results, at least 2 H&E stained sections from different levels should be examined. All biopsy specimens should be subjected to C4d staining. Humoral rejection should be considered, especially in cases where hyperglycemia develops without any other finding in biopsy or if there is interacinar capillary margination of PMN and other inflammatory cells. Insulin and glucagon staining are also required to demonstrate selective beta cell loss due to recurrence of an autoimmune disease in patients undergoing biopsy for hyperglycemia. Animal experiments have shown that renal and pancreatic transplant rejections coexist in most of the cases (65%). However, their rejection rates can be different. In a large series, the pancreas was singularly rejected in 22% and only the kidney in 13% of the cases. Therefore, it is recommended to perform separate biopsies. Controversial results have been reported regarding the benefit of protocol biopsies and accelerated treatment applied when rejection is detected[1,2,29-31].

Acute allograft rejection mechanisms in pancreas transplantations are not different from those observed in other solid organ transplantations. MHC class I and II molecules are expressed differently in different regions of the pancreas. Normally MHC class II molecules are not involved in this rejection. MHC class I molecules are expressed strongly in the ductal epithelium and weakly in the islets. They are not expressed in normal acinar cells. In acute rejection, acinar cells show overexpression of both MHC class I and II cells. MHC class

II overexpression is also observed in ductal epithelium and endothelium, while MHC class I is only seen in beta cells. The leading cells in cellular rejection are T lymphocytes, monocytes and eosinophils. Cytotoxic T lymphocytes exert their function via perforin, granzyme-like enzymes and FAS ligands. In antibody-mediated rejection, antibodies accumulating on the vessel wall directly stimulate the complement cascade or antibody-dependent cell-mediated toxicity, leading to vascular injury, necrosis, thrombosis, and parenchymal necrosis. Hyperacute rejection, which is a much more severe reaction, also occurs by a humoral mechanism, as described previously. Various rejection patterns develop dependent on different patterns of MHC distribution, and vascularization as well as resistance to ischemia. Animal experiments have shown that the main target of T cell-mediated rejection is the acinar lobules. In chronic rejection, there is fibrosis caused by chronic vascular injury. Islet cells are not directly affected by T cell and antibody-mediated rejection[1,2].

Studies in animal models have shown that acute rejection progresses with inflammatory infiltration in the interstitium, which also involves heterogeneous small vessels and ducts. Acinar inflammation and acinar cell apoptosis may be also observed. More severe forms include intimal arteritis, necrotizing vasculitis, thrombosis with gradual formation of parenchymal necrosis. Although islets of pancreas are not on target, hyperglycemia occurs because islets are affected by extensive parenchymal necrosis. A six-level grading system originally developed by the University of Maryland was used to evaluate pancreatic transplantation pathology. However, this grading system was not successful in the evaluation of the pancreatic transplantation pathology due to the similar histopathological features of grades 2 vs 3 and 4 vs 5. Today, the Banff grading system, which was updated in 2011, is used[1,2,29-31].

FOREMOST DIFFERENCES BETWEEN SOLID ORGAN TRANSPLANTATIONS PERFORMED IN CHILDHOOD AND ADULTHOOD

When the data collected from transplantation centers all over the world are reviewed, an annual increase of approximately 6% is observed in the number of solid organ transplantations from the beginning of the century until 2020. However, solid organ transplantations decreased approximately 17.5% in 2020 due to the impact of the coronavirus disease-2019 pandemic. According to the data of the World Health Organization, more than 150,000 transplants were performed all over the world in 2019 and more than 130,000 in 2020. It is thought that these figures constitute less than 10% of the cases in need of organ transplantation. Mostly kidney (37%) and liver transplants (21%) have been performed. The proportion of pediatric cases receiving solid organ transplants is quite low[32].

The pediatric liver transplant rate has remained stable over the past 5 years. Most liver transplants are from deceased donors, with childhood liver transplant rates reported as 5-6% in North America, 11% in Europe and 17% in Australia. In contrast, 35% of all liver transplants in Japan were performed on children, and almost all were performed from living donors. In a study in which Ege University liver transplant cases from Turkey were presented, the pediatric transplant rate was 18.7%, and it was stated that more than half of the transplants were from living donors, especially after 2000[28,32-34].
Liver transplantation has been very successful in the treatment of children with end-stage liver disease and has provided many years of disease-free survival. Donor shortage, which is the main limitation of transplantation, has been overcome due to innovative surgical techniques such as split-liver or living-donor transplantation. Today, organ transplantation is performed in pediatric cases with almost no waiting list mortality. While formerly the focus of care for children with end-stage liver disease was to perform liver transplantation, today the main aim is to prevent complications related to immunosuppression and to ensure normal growth[35].

In the first years of kidney transplantation, lower graft and patient survival rates were reported in pediatric cases compared to adult cases. In the last 20 years, the success rates have increased considerably, and the 5-year survival rates have been reported as 94% in pediatric renal transplantations from a living donor, and 77-85% in deceased donor transplantation. Many studies have reported that graft survival in pediatric cases is lower than in adults, secondary to poor adherence to drug regimens, side effects of drugs, and a higher rate of recurrent disease. However, it is reported that no difference is observed in terms of patient survival between adult and pediatric kidney transplantations.

Although the clinical process is similar in pediatric and adult patients, the causes leading to end-stage organ failure differ in several aspects, such as the types of complications, optimal donor selection, growth-related problems, associated comorbidities, adherence to drug regimens, and their effects on growth and

development. While the causes of kidney failure in adults are usually diabetic nephropathy, hypertension, autosomal dominant polycystic kidney and chronic glomerulonephritis, the causes of kidney function loss in pediatric patients are mostly FSGS, renal dysplasia and urological disorders due to urinary system anomalies. In addition, recurrent glomerulonephritis in allografts, especially recurrent FSGS is more commonly seen in pediatric patients, and it is an important complication that determines the long-term outcome of the transplant[5,33].

Cardiovascular complications are among the most important complications following pediatric kidney transplantation, and cardiovascular mortality in children is 100 times higher than age-adjusted healthy pediatric population. Cardiovascular disease accounts for 11% of the causes of death after kidney transplantation in pediatric patients. Various metabolic conditions that develop during dialysis, such as obesity, hyperglycemia, hypercholesterolemia, and hypertension, tend to persist after transplantation. In addition, donor-recipient size mismatch is an important factor that increases the pathological cardiac burden in pediatric lung transplantation. Pediatric donors are few among the donor population and pediatric donors are not always suitable as pediatric recipients due to the technical difficulties of anastomosis with small vessels and the risk of thrombosis at the anastomosis site. Therefore, in pediatric patients the kidney is usually obtained from adult donors and donor-recipient size mismatch is a common challenge, especially in infants and young children. Donor-recipient size mismatch usually results in graft hypoperfusion and delayed graft function[5].

Heart transplantation is a valid treatment for end-stage heart disease in both adults and children. Survival after heart transplantation from birth to the age of 18 is excellent, and this rate is reported to be over 65% for all age groups[6]. Although survival rates in pediatric cases are comparable to those of adults, there are important differences regarding indications, assessment, surgical technique, and post-transplant management. Indications for transplantation in pediatric patients include metabolic and genetic diseases leading to cardiomyopathy and congenital heart disease. Since mitochondrial and metabolic diseases are among the etiological factors, during the evaluation process, metabolic examinations should also be done. In the presence of phenotypic features of genetic anomalies or family history, genetic studies should be performed. Most importantly, if children referred for transplantation due to congenital heart disease had previously undergone multiple palliative surgical interventions, then the success of pediatric heart transplantations is reduced. The main problems to be experienced in pediatric cases after heart transplantation are the inadequacy of education and social support, the disruption of growth and development, and the need for psychosocial assistance of the patient and family in relation to their future expectations[6].

Wever-Pinzon et al.[22] investigated 52,995 heart transplantations and reported that the causes of death differed significantly with the age of the recipient at the time of transplantation. The lowest ten-year survival rates were found in patients aged 60 to 69 years (49%), and ≥70 years (36%). Whereas, acute rejection, cardiac allograft vasculopathy and graft failure were observed at high rates in the youngest patient group. While the risk of death due to infection and malignancy was high in elderly recipients, the risk of death from renal failure was low in young recipients. Cause-specific death profiles in this study suggested the possible impact of inadequate immunosuppression in younger and excessive immunosuppression in older recipients. Since there was no pediatric case in the study, no comment was made on cause-specific mortality rates[22].

Lung transplantation in children has been performed since the 1980s. Currently, pediatric lung transplantation has provided a clear survival advantage and improved quality of life in well-selected children with end-stage lung disease[1-3]. It has been reported that over 100 pediatric lung transplants are performed worldwide each year, and over 2400 procedures have been performed in children to date. However, the number of centers performing pediatric lung transplantations have remained almost unchanged in recent years. Conventionally, centers performing pediatric lung transplants are mostly located in North America, Europe, and Australia, although successful cases of pediatric lung transplants have also been reported in Asia and South America. Cystic fibrosis remains the most common primary indication for pediatric lung transplantation, although indications vary considerably with patient age. Pulmonary hypertension and surfactant disorders in infants are the main indications. Cystic fibrosis and idiopathic pulmonary hypertension are the most common indications for lung transplantation in children aged one to 10 years. In adolescents (11-17 years of age), cystic fibrosis is by far the most common disease leading to lung transplantation, especially in centers outside of North America. However, just like other

solid organ transplantations, the surgical approach in lung transplantation is more challenging in children. In addition, the effects of immunosuppression on the developing immune system in these patients and its psychosocial effects, especially in adolescents, should be considered[1-3,36].

Growth retardation is a common problem in pediatric patients after solid organ transplantation. It is known that the growth and development of living related donor kidney recipients is better than that of cadaveric donor graft recipients. In pediatric renal transplantation, transplantation before 6 years of age, refraining from steroid treatment and use of recombinant human growth hormone (rhGH) have positive effects on growth of these children. However, the use of rhGH was found to be associated with an increased incidence of renal cell carcinoma and acute rejection in patients with a history of acute rejection[5].

Almost 75% of adolescents tend not to comply with treatment regimens, and this is an important factor that can lead to organ loss in pediatric transplants. In general, a child's transition to adulthood is a very labile period, and while the rate of one-year survival after transplants made during this period is very good, long-term transplant results have been disappointing. Non-compliance with the use of immunosuppressive drugs is one of the most important factors contributing to graft rejection and loss in adolescents. Therefore, adherence to treatment should be monitored with objective methods such as close monitoring of blood levels of drugs and extensive use of electronic devices[5].

PTLD is an abnormal lymphocyte proliferation seen in immunocompromised patients receiving transplantation. Histopathological findings range from infectious mononucleosis-like disease to the development of non-Hodgkin lymphoma. Since a longer posttransplant survival is expected in pediatric cases, PTLD poses a crucial challenge. Risk factors for PTLD include EBV seronegative status of recipients, use of calcineurin inhibitors and antilymphocyte antibodies, number of methylprednisolone pulses administered, presence of CMV infection, young age, and acute graft rejection events. While the incidence of PTLD in adults is 1%, it has been reported up to 49% in EBV-seronegative pediatric patients. The risk of non-PTLD malignancy in kidney transplanted children was also found to be 6.7 times higher than in a healthy pediatric population. Renal cell carcinoma is the most common type of non-PTLD malignancy observed[5,32-34].

CONCLUSION

In summary, solid organ transplantation performed in children differs from adults in several aspects including clinical features, causes of organ loss, types of complications, selection of optimal donors, growth problems, drug incompatibility, transition to adulthood, and effects on the child's development. Therefore, for the success of pediatric organ transplantation, a multidisciplinary approach with effective intra-and inter-institutional coordination between pediatricians and pediatric subspecialists, gastroenterologists, cardiologists, pulmonologists, urologists, transplantation surgeons, immunologists, pathologists, social workers, pharmacists, and clinical coordinators conveys critical importance.

Ethics

Peer-review: Internally peer reviewed.

Author Contributions

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

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Original Article

Does Endotracheal Suctioning Affect Bispectral Index and Ramsay Sedation Scores in Pediatric Intensive Care Patients?

Çocuk Yoğun Bakım Hastalarında Endotrakeal Aspirasyon Bispektral İndeks ve Ramsey Sedasyon Skorlarını Etkiler mi?

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ABSTRACT

Objective: A particular electroencephalography parameter known as the bispectral index (BIS) is one of the objective methods used to assess sedative and hypnotic effects. The aim of this study is to monitor the level of consciousness of patients who underwent sedation-analgesia in pediatric intensive care unit (PICU) and to evaluate how their sedation levels were affected by painful procedures by examining BIS and Ramsey Sedation Scale (RSS).

Method: This prospective observational study was held 43 pediatric patients who were hospitalized in the 24-bed university-affiliated tertiary PICU. BIS, RSS and vital signs were recorded both before and after the endotracheal suctioning procedure. Patients were divided into two groups according to the chosen analgesic which was fentanyl or morphine. Percentage change (BIS, electromyography activity, signal quality index, RSS score, heart rate, oxygen saturation) of before/after endotracheal suctioning was calculated.

Results: The increase in BIS value, increase heart rates and decrease RSS of patients with endotracheal suctioning were found to be significant ($p<0.01$, $p=0.01$, $p<0.001$ respectively). Percentage changes were compared between two groups and there was no significant difference between morphine and fentanyl group.

Conclusion: Even in patients receiving strong analgesic agents like opioids, any painful procedures such as endotracheal suctioning increase the BIS values of the patients and disrupt their comfort. Continuous BIS value tracing may be more beneficial than clinical scoring systems on sedation monitoring and patient comfort. We also suggest further studies with different groups of analgesic agents should be conducted.

Keywords: Analgesia, pain, pediatrics, critical care, conscious sedation, bispectral index

ÖZ

Amaç: Bispektral indeks (BİS) olarak bilinen belirli bir elektroensefalografi parametresi, sedatif ve hipnotik etkileri değerlendirmek için kullanılan objektif yöntemlerden biridir. Bu çalışmanın amacı, çocuk yoğun bakım ünitesinde sedasyon-analjezi uygulanan hastaların bilinç düzeylerinin izlenmesi ve ağrılı işlemlerden sedasyon düzeylerinin nasıl etkilendiğinin, BİS ve Ramsey Sedasyon Skalası (RSS) ile değerlendirilmesidir.

Yöntem: Bu prospektif gözlemsel çalışma, 24 yataklı üniversiteye bağlı üçüncü basamak pediatrik yoğun bakım ünitesinde yatan 43 çocuk hasta üzerinde yapıldı. Endotrakeal aspirasyon prosedüründen önce ve sonra BİS, RSS ve vital bulgular kaydedildi. Hastalar seçilen analjeziklere göre fentanil veya morfin olmak üzere iki gruba ayrıldı. Endotrakeal aspirasyon öncesi/sonrası yüzde değişimi (BİS, elektromiyografi aktivitesi, sinyal kalite indeksi, RSS skoru, kalp hızı, oksijen satürasyonu) hesaplandı.

Bulgular: Endotrakeal aspirasyon yapılan hastaların BİS değerindeki artış, kalp atım hızındaki artış ve RSS'deki düşüş anlamlı bulundu (sırasıyla $p<0,01$, $p=0,01$, $p<0,001$). BİS ve RSS'deki yüzde değişimler ise iki grup (morfin ve fentanil grupları) arasında karşılaştırıldığında anlamlı fark bulunmadı.

Sonuç: Opioidler gibi güçlü analjezik ajanlar alan hastalarda bile endotrakeal aspirasyon gibi ağrılı işlemler hastaların BİS değerlerini yükseltmekte ve konforlarını bozmaktadır. Sürekli BİS değer takibi, sedasyon izleme ve hasta konforu konusunda klinik puanlama sistemlerinden daha faydalı olabilir. Ayrıca farklı analjezik ajan gruplarıyla daha ileri çalışmaların yapılmasını öneriyoruz.

Anahtar kelimeler: Analjezi, ağrı, pediatri, yoğun bakım, bilinçli sedasyon, bispektral indeks

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INTRODUCTION

In intensive care, sedation and analgesia are very important for the effectiveness and success of treatment. Even just being in the intensive care unit (ICU) can cause anxiety and stress in patients. In patients receiving invasive and non-invasive respiratory support, this anxiety and stress lead to an increase in the oxygen consumption of the myocardium as well as patient-ventilator asynchrony, and the barotrauma in the lungs. Effective sedation and analgesia may shorten and facilitate the treatment of critically ill patients. A comfortable ICU stay with easy awakening should be defined as a goal in intensive care patients[1].

Since subjective scoring systems used to monitor patients' sedation levels may vary depending on the individual, objective evaluations provide us with a more realistic level of sedation. One of the objective methods, bispectral index (BIS), is a special electroencephalography (EEG) parameter used to quantify sedative and hypnotic effects. The BIS is an analysis method that examines the correlations between sinus wave components and it specifically shows the quantitative level of synchronization in the bispectral EEG[1]. BIS monitor detects EEG signals with the electrodes applied to the forehead and temporal region and it provides information on the signal quality index (SQI), suppression ratio, electromyography activity (EMG) and the raw EEG waveform. BIS values are updated to reflect the correct value from 0 (deep sedation) to 100 (awake)[2]. The SQI gives information about the adequacy of the EEG signal. A higher value of SQI indicates better signal. When the SQI is above 50%, this indicates sufficient EEG transmission, however, in most studies, it is aimed to have an SQI which is over 80%. Electromyographic power shows the EMG effect on BIS elevations. For example, being above 50 decibels may cause serious interactions with BIS[3]. Ramsay sedation scale is the most widely used sedation scale in critically ill children. It allows us to visually assess the state of consciousness of patients in 6 categories, from full sleep to agitated awake (Table 1)[4]. The aim of this study is to demonstrate how painful procedures altered the BIS values and Ramsey Sedation Scale (RSS) scores of pediatric ICU patients.

Table 1. Ramsay sedation scale

Clinical score	Level of activity
1	Awake; agitated or restless, or both
2	Awake; cooperative, oriented and tranquil
3	Awake; only responds to verbal commands
4	Asleep; brisk response to a light glabellar tap or loud auditory stimulus
5	Asleep; sluggish response to a light glabellar tap or loud auditory stimulus
6	Asleep; no response to a light glabellar tap or loud auditory stimulus

MATERIALS and METHODS

This was a prospective observational study conducted in the pediatric patient population, ages 1 month to 18 years, who were hospitalized in the pediatric ICU of University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital from August 2017 to August 2018. The study was approved by the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Ethics Committee (protocol no: 2017/157, date: 06.07.2017) and written informed consent was obtained from the parents of all participating subjects. The sample size was calculated to be 43 patients with a 0.76 effect size, 95% power, and 5% type-1 error using the G*Power program (version 3.0). The study included 43 patients who required mechanical ventilation and were under sedation-analgesia. Patients with epilepsy, neurodegenerative diseases with acute or chronic seizure activity, muscular diseases, postoperative patients, patients who received neuromuscular blockage, patients who required more than two additional sedative bolus doses after suctioning during a 6-hour period, and patients who had more than one suctioning per hour due to excessive secretions were excluded from the study.

Sedation level was evaluated by both RSS score, and BIS monitoring. Pediatric 4-sensor probe (Covidien IIC, Mansfield, USA) compatible monitor (Philips Medizin Systeme, Boeblingen, Germany) was used. The probes were placed one by one after the patient's skin was cleaned with 70% alcohol. The

first probe was placed 1 cm above the nasal root, the second probe right next to the first probe, the fourth probe was placed parallel to the eyebrow and the third probe was placed between the lateral part of the eye and the hairline.

Either midazolam-fentanyl or midazolam-morphine standardized infusions were given to 43 mechanically ventilated patients who needed sedation. Initially, both groups of patients received an intravenous infusion of midazolam at a dose of 0.1 mg/kg/hour after an intravenous bolus of 0.1 mg/kg midazolam. In some of the patients, IV Fentanyl infusion (1 mcg/kg/hour) was added to continuous IV midazolam infusion whereas in some of the patient's IV Morphine infusion (0.025 mg/kg/hour) was added. Preference for fentanyl or morphine was based on the clinical decision of the physician. Ramsay score, and infusion rates were adjusted to obtain a Ramsay score above 4 and a BIS level at 70 before the beginning of the protocol. We chose this value because BIS values of 40-70 have been suggested for adequate sedation[5].

Pediatric BIS probes were attached to the patients according to the previously described technique. One hour after the onset of infusion; EMG, BIS, SQI values, RSS score and vital measurements of the patient were recorded as the baseline value. The same values were recorded during the follow-up of the patients, immediately before and after the suctioning. Endotracheal suctioning was performed by the same physician after 30 seconds of preoxygenation with a suction catheter of appropriate caliber in the accompaniment of another healthcare provider. While the BIS values were noted by the physician, the RSS was noted for all patients by the other physician who was censored for the BIS values in order to avoid bias. In addition, the nurse who applied the scale together with the service senior nurse was also present for each patient as an observer.

Patients were divided into two groups according to the chosen analgesic which was fentanyl and morphine. Percentage change of BIS, EMG, SQI, RSSs, HR, oxygen saturation (SpO_2) values between before and after endotracheal suctioning was calculated [(after suctioning-before suctioning/before suctioning)*100].

The Statistical Package for Social Sciences (SPSS) software for Windows 21.0 was used to analyze the data. The results are presented as either the mean and standard deviation or median and interquartile range, depending on the distribution of the data. Paired samples t-test was used for normally distributed data, and the Wilcoxon signed-rank test was used for data that was not normally distributed. Descriptive analytics were also performed using the median and quartile intervals for non-normally distributed variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic data and baseline measurements after sedoanalgesia at the first hour of BIS, SQI, EMG, pulse, SpO_2, body temperature and RSS scores are given in Table 2. Simultaneous EMG, BIS values of patients with SQI values of 85 and above were recorded and included in the study.

There was a moderately significant negative correlation between the BIS value measured at the first hour and the RSS evaluated simultaneously ($r=-0.782$, $p<0.001$). Patients with high BIS scores had lower RSS scores, vice versa the patients with lower BIS scores had higher RSS scores. When compared, there was a significant increase in the BIS data obtained before

Table 2. Demographic characteristics and baseline measurements of patients

Demographics	**n=43**
Gender	**n (%)**
Male/female	19 (44.2)/24 (55.8)
Age	**Median; (IQR)**
Months	12; (28)
PICU admission diagnoses	**n (%)**
Respiratory failure	18 (41.9)
Cardiac failure	8 (18.6)
Septic shock	11 (25.6)
Metabolic crisis	4 (9.3)
Hematological disease	2 (4.7)
Analgesia	**n**
Fentanyl/morphine	25/18
Baseline measurements	**Median; (IQR)**
BIS	59 (13)
EMG	32 (8)
SQI	95 (6)
RSSs	5 (1)
HR	108 (35)
SpO_2	95 (2)

HR: Heart rate (per minute), SpO_2: Oxygen saturation (%), BIS: Bispectral index, EMG: Electromyographic activity, SQI: Signal quality index, RSSs: Ramsey Sedation Scale score, IQR: Interquartile range

and after the endotracheal suctioning procedure (p<0.001). There was no significant difference between EMG and SQI values before and after suctioning (p=0.206, p=0.214). There was a significant decrease in RSS scores (p=0.001). No significant difference was found in the SpO_2 values of the patients related to suctioning (p=0.75). Heart rate values showed an increase with endotracheal suctioning. This increase was statistically significant (p<0.001) (Table 3).

Changes of BIS and RSS values were compared between fentanyl and morphine groups, too. There was no significant difference between morphine and fentanyl group (Table 4).

Table 3. Comparison of measurements before/after endotracheal aspiration

Measures/ scores	Before suctioning Median (IQR)	After suctioning Median (IQR)	p-value
BIS*	56 (16)	66 (15)	<0.001
EMG*	35 (9)	34 (10)	0.206
SQI*	95 (6)	93 (6)	0.214
RSSs*	5 (1)	5 (2)	0.001
HR*	110 (42)	130 (40)	<0.001
SpO_2*	96 (2)	95 (4)	0.75

*Wilcoxon t-test, HR: Heart rate (per minute), SpO_2: Oxygen Saturation (%), BIS: Bispectral index, EMG: Electromyographic activity, SQI: Signal quality index, RSSs: Ramsey Sedation Scale score, IQR: Interquartile range

Table 4. Comparison of changes in BIS, EMG, SQI, RSS, HR, BIS percentages before and after endotracheal aspiration between Fentanyl and Morphine groups

Measures/ scores PC**	Fentanyl group Median (IQR)	Morphine group Median (IQR)	p-value*
BIS	10.52 (16.11)	10.52 (11.85)	0.721
EMG	0 (15.71)	-2 (21.92)	0.117
SQI	0 (7.7)	0 (4.75)	0.489
RSSs	0 (22.50)	0 (5)	0.341
HR	11.45 (8.44)	9.57 (8.51)	0.571
SpO_2	0 (1.58)	-0.53 (3.16)	0.084

*Mann Whitney-U Test, **PC: Percentage change before/after endotracheal suctioning, HR: Heart rate (per minute), SpO_2: Oxygen saturation (%), BIS: Bispectral index, EMG: Electromyographic activity, SQI: Signal quality index, RSSs: Ramsey Sedation Scale score, IQR: Interquartile range

DISCUSSION

The aim of sedation in critically ill patients is to ensure that the patient remains calm, does not feel pain, and continues to breathe spontaneously; thus helps to reduce the length of stay in ICU and duration of mechanical ventilation. However, the clinician should avoid the undesired side effects of excessive sedation. Amnesia is probably another useful goal of sedation therapy so that the patient has no recall of unpleasant events or surroundings[6-9]. Prospective studies reported that BIS index values between 40-60 indicate sufficient hypnotic effect during general anesthesia[10].

In the follow-up of sedation levels of critically ill patients in ICU; different objective and subjective measurements such as clinical scales, hemodynamic changes, BIS, EEG and auditory evoked potentials are used[11,12].

In our study, we observed that BIS, HR and RSSs were changed by endotracheal suctioning. We found that sedation levels decreased. In a study, Brocas et al.[13] reported additional dose of analgesia was required during endotracheal suctioning. We suppose that giving an additional dose of analgesic before painful procedures will prevent deterioration of sedation level and patient comfort. Benzodiazepines and opioids are the most commonly used agent for sedation and analgesia in pediatric ICU (PICU). It is known that the BIS is not correlated with the level of analgesia but with the level of sedation[14]. In ICU patients, there is an increase in BIS index in nociceptive stimuli such as tracheal suctioning. This increase is related to a central noradrenergic stimulation causing cortical alertness. The effect of different sedative drugs (benzodiazepine or opioid) on BIS may be different. The addition of opioids to sedation in the presence of painful stimuli may suppress the increase in the BIS index[15].

In our study, we examined all patients who received fentanyl or morphine for analgesia in addition to midazolam which is the first line sedative agent that routinely used in our PICU. Moreover, we assessed the subgroups according to the analgesic agent (morphine group/fentanyl group). We found that the percentage changes before and after suctioning were not different between the groups. The midazolam infusion doses we started for sedation were different between patients, but opioid doses were started as standard, so we compared percentage changes. Considering that opioid-derived analgesics have similar effects, differences may occur between analgesics of different effects. Chun et al.[16]

evaluated the effects of dexmedetomidine-ketamine (DK) against dexmedetomidine-midazolam-fentanyl (DMF) combinations on sedation/analgesia quality and recovery profiles for monitored anesthesia. Patients during a port catheter insertion were given DK in one group and DMF in the other. BIS was used to monitor anesthesia activity, as well as extra sedation demands, waking time, and cardiorespiratory variables. According to the authors, superior data were obtained in the second group[16].

BIS monitoring is being used in the operating rooms and adult ICUs in order to monitor the level of anesthesia applied. In a study conducted in the PICU, it was found that there was a significant correlation between sedation scoring and BIS in order to determine the sedation levels of the patients in the PICU so that authors concluded that BIS monitoring could be useful in PICUs[17]. A survey conducted in England revealed that only 2% of ICUs were using BIS while 88% of them used sedation scales. Regarding the scales, 66% of them were RSS and 5% of them were Richmond agitation sedation scale[18]. We follow the sedation levels of patients with RSS in our PICU. In addition, we use BIS as an objective measurement method in some critical patients who receive neuromuscular blocking agents and whom RSS is inapplicable.

One of the major challenges in BIS follow-up is keeping the probes stable on the patient's skin. The signal quality deteriorates during sweating and routine interventions. In our study, SQI values were monitored carefully in order to obtain accurate results during BIS follow-up. The BIS values were obtained when SQI levels are higher than 85[19]. Moreover, in our study population, we did not have any RSS scores below 4 which is an unavoidable reason for adequate sedation in mechanically ventilated pediatric patients. It may be more appropriate to include mildly sedated patients in future studies to determine the correlation of lower scores with BIS values. Furthermore, we expect that monitoring a continuously updated value on the monitor will allow clinicians and health personnel to intervene at the appropriate moment. Both high dose and inadequate sedation will adversely affect our treatment outcomes. During our study, the nurses who took care of the patients reported that sedation monitoring with a numerical scale is more comfortable than RSS. The use of BIS may facilitate nurse-led sedation in PICU.

Study Limitations

One limitation of our study is its single centered design. Another limitation is that only RSS is used for clinical sedation scoring. In terms of patient comfort, the effects of endotracheal suctioning can be evaluated by using comfort scale. As far as we can determine our study was the first study to evaluate the effect of sedation with pain stimulus and BIS monitoring and clinical scores in the PICU.

CONCLUSION

Our recent study investigated the effects of painful procedures, such as endotracheal suctioning, on patients receiving sedation and strong pain medications like opioids in an intensive care setting. To our surprise, we found that these procedures significantly increased the BIS values of the patients and disrupted their comfort, despite the use of sedation and strong pain medications. While our study focused solely on the impact of endotracheal suctioning, it is important to consider that similar results may not be seen with other painful interventions.

The use of BIS monitoring in intensive care patients is a topic of ongoing debate, with some arguing that it can effectively reduce the need for sedative medications, prevent self-extubations, and ultimately decrease the cost and length of stay in intensive care. However, there is a lack of data specifically on the use of BIS monitoring in pediatrics, and there are still several questions that need to be addressed regarding its impact on patient comfort, long-term neurophysiological function, and overall patient outcomes. Further research is necessary to fully understand the benefits and limitations of using BIS monitoring in this patient population.

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Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Ethics Committee (protocol no: 2017/157, date: 06.07.2017).

Informed Consent: Written informed consent was obtained from the parents of all participating subjects.

Peer-review: Externally peer reviewed.

Author Contributions

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

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Original Article

Pseudo-Bartter Syndrome in Patients with Cystic Fibrosis and Clinical Features

Kistik Fibrozis Hastalarında Psödo-Bartter Sendromu ve Klinik Özellikleri

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ABSTRACT

Objective: Pseudo-Bartter syndrome (PBS) is a complication of cystic fibrosis (CF) accompanied by electrolyte disorders. We aimed to compare the clinical features of patients diagnosed with CF with or without PBS in our clinic.

Method: One hundred twenty-eight patients with the diagnosis CF data was recorded. Clinical features, diagnostic test results, colonization status, complications and genetic test results were compared in patients with and without PBS.

Results: Totally 128 patients who were regularly followed diagnosis CF January 2017 and May 2022 and 18 of them (14%) developed PBS. Median age of CF diagnosis was significantly lower in patients with PBS (p<0.003). There was a significant difference between the two groups in terms of colonization. In the group with PBS, the chronic respiratory tract colonization was detected more. There were no significant differences for age, gender, weight, height, sweat test. The most common genetic mutation was c1521_1523delCTT (p. F508Del).

Conclusion: PBS was the most common finding in our patients with CF. It may be exacerbated by the warm weather conditions in our country. It may be a clue for early diagnosis of CF.

Keywords: Cystic fibrosis, Pseudo-Bartter syndrome, complications

ÖZ

Amaç: Psödo-Bartter sendromu (PBS) kistik fibrozis (KF) hastalığının elektrolit bozukluğu ile seyreden bir komplikasyonudur. Kliniğimizde KF tanısı olan, PBS gelişen ve gelişmeyen hastaların klinik özelliklerini karşılaştırmayı hedefledik.

Yöntem: Kistik fibrosis tanısı olan 128 hastanın verileri kayıt edildi. PBS gelişen ve gelişmeyen hastaların klinik özellikleri, tanısal test sonuçları, kolonizasyon durumları, komplikasyonları ve genetik sonuçları karşılaştırıldı.

Bulgular: Kistik fibrozis tanısıyla Ocak 2017-Mayıs 2022 tarihleri arasında kliniğimizde düzenli takip edilen 128 hastamız olup bunların 18'inde (%14) PBS gelişti. Hastaların ortalama tanı yaşı PBS olanlarda anlamlı olarak daha düşüktü (p<0,003). Yaş, cinsiyet, ağırlık, boy, ter testi, kronik solunum yolları bakteriyel kolonizasyonları ve KF komplikasyonlar arasında anlamlı farklılık yoktu. En sık görülen genetik mutasyon deltaF508 idi.

Sonuç: PBS kistik fibrozis hastalarımızda en sık görülen bulguydu. Ülkemizde sıcak hava koşulları buna neden olabilir. Kistik fibrozis hastalığının erken tanısı için ip ucu olabilir.

Anahtar kelimeler: Kistik fibrozis, Psödo-Bartter sendromu, komplikasyon

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INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessively inherited disease with an incidence of on in every 2,000-3,000 births[1]. In the United States, the mean life expectancy for the patients born with CF in 2018 was 47.4 years. According to 2017 data from the Turkish National Cystic Fibrosis Data Registry System, in 1,170 of patients, 23% of diagnoses were made by the neonatal screening program[2]. Mutation of the CF transmembrane conductance regulator (CFTR) protein, which is a complex chloride channel regulator protein that exists in all exocrine tissues, causes CF. Irregular transportation of ions like sodium, chloride and bicarbonate (HCO_3) results in thick viscous secretions in lungs, pancreas, liver, intestines and genital system and increases the amount of salt in sweat glands[3,4]. Chronic cough, phlegm and

wheezing are among respiratory tract findings. As the disease progress, it causes damage to the walls of the bronchus and bronchiectasis develops[5].

Respiratory tract infections due to pathogenic bacteria occur at early ages. *Staphylococcus aureus, Hemophilus influenza* and *Pseudomonas aeruginosa* are type of microorganisms that frequently colonize the respiratory tract[6]. Pseudo-Bartter syndrome (PBS) is a known complication of cyctic fibrosis the clinical presentation of hyponatremia, hypokalemia, hypochloremia and metabolic alkalosis[6]. Unlike Bartters syndrome, chloride excretion in urine is low. PBS is generally seen in infancy and in regions with warm climate[7]. Risk factors for developing PBS include warmer climate conditions, vomiting, diarrhea and respiratory tract infections[7]. Individuals with CF lose the increased amount of sodium and chloride, they cannot compensate for this loss due to reasons like malnutrition and this causes development of PBS. The purpose of this study was to compare the clinical features of patients with and without PBS to determine the risk factors for PBS development[8].

MATERIALS and METHODS

All data were recorded for 128 patients who were diagnosed with CF and regularly followed between January 2017 and May 2022. The diagnosis of CF was made with two criteria: (a) chloride concentration higher than 60 mmol/Lin two sweat-tests, and (b) one sweat-test higher than 60 mmol/L and two mutations related to the disease in DNA sequence analysis. If the sweat-test was lower or equal to 60 mmol/L, two different disease-related mutations with typical clinical features of CF were considered CF. If patients had hyponatremia (<134 meq/L) (severe <125, light-medium >125), hypochloremia (<100 meq/L), hypopotassemia (<3.4 meq/L) and metabolic alkalosis (HCO_3 >27) with dehydration but without renal tubulopathy, they were diagnosed with PBS.

Sex, present age, age at the time of diagnosis, height, weight and body mass index (BMI) z-scores were recorded. Accompanying complications (bronchiectasis, allergic broncho-pulmonary aspergillosis, diabetes mellitus, chronic liver disease), culture from sputum and existence of colonization were studied. Genetic results were scanned taking Clinical and Functional Translation of CFTR (CFTR2) and CFTR France database as references. Arterial blood gas and biochemical parameters (sodium, potassium, chlorid). Informed consent was obtained from all the patients and/or their parents and ethics committee approval was provided by Ege University Ethical Committee for Medical Research for the study (decision no: 22-6.1T/49, date: 23.06.2022) were recorded for patients who developed PBS.

Statistical Analysis

The IBM SPSS statistics 20.0 for Windows (Chicago, IL) was used for the statistical measurements. The χ^2 test was used for nominal variables. The data was expressed as mean ± standard deviation. Student t-test was used if parametric conditions were obtained if not, the Mann-Whitney U test was used. The Kolmogorov-Smirnov/ Shapiro-Wilk test was applied to test the normal distribution of the numerical variables. P-value of less than 0.05 was considered significant.

RESULTS

In our clinic, 18 (14%) of 128 CF patients who were followed developed PBS. Of all patients, 70 (55%) were male and 58 (45%) were female. Of the 18 patients with PBS 11 (61%) were male. The median age of diagnosis for CF was median 24 months [minimum (min): 1; maximum (max): 86]. The age of patients median PBS was 18 months. The median age of patients without PBS was median 49 months (min 1; max 168). Between the two groups, the mean age of CF diagnosis was significantly lower for the group with PBS (p=0.003). There was no statistical difference between the current age of patients (p=0.060). The age of patients median PBS was 18 months.

Mean Z-score for weight was -1.25±2.50 in the group with PBS and -1.08±1.63 in the group without PBS (p=0.782). Mean Z-score for BMI was-0.93±2.5 in the group with PBS and -0.81±1.71 in the group without PBS (p=0.719). There were no differences between weight and BMI of the groups. Sweat-test results were lower in the group with PBS, but there was no significant difference. When the immunoreactive trypsinogen screening test was compared between the two groups, it was positive in 4 patients (22.2%) in the PBS group, while it was positive in 27 patients (24.5%) in the non-PBS group (p=0.072).

There was a significant difference between the two groups in terms of bacterial colonization of chronic respiratory tract. While the colonization rate was 50.0% in the PBS group, it was 21.8% in the non-PBS group (p=0.021). Colonization distribution was *P. aeruginosa* for 22.2% of patients with PBS while 10.0% non-PBS patients (p=0.041), colonization was *S. aureus* for 16.7% of patients with PBS while 9.1% non-PBS group (p=0.061), *S. aureus* and *P. aeruginosa* colonization together rate was 11.1% PBS patients while 2.7% non-PBS patients

(p=0.032), there was a significant difference between the two groups.

Although not statistically meaningful, male patient rates were higher, weight, height, BMI Z-scores were lower and development of ABPA and diabetes were more frequent in patients with PBS. When the drugs that both groups use regularly are examined, they included dornase alpha, multi-vitamin, pancreatic enzymes, B2 agonist, inhaler steroids and inhaler antibiotics. There were no significant differences detected. Table 1 presents the comparison of clinical and demographic features of PBS and non-PBS patients.

When the complaints related to PBS are investigated, they included nausea-vomiting in 14 (48.2%), fever in 9 (31.0%), diarrhea in 6 (20.7%), stomachache in 4 (13.8%) and cough in 2 cases (6.9%). Hospitalization duration for the PBS group was 5.2±2.1 days and acute pulmonary exacerbation accompanied the clinical representation of PBS in three patients (16.6%). Culture from sputum of three patients (16.6%) had breeding and there was also acute pulmonary exacerbation accompanying PBS. All of the patients administered had parenteral fluid therapy. The patients with breeding in sputum culture were administered 14 days of antibiotic treatment along side fluid therapy. Sputum culture of two patients had *P. aeruginosa* growth; therefore, they were treated with meropenem and amikacin. One patient had *S. aureus* growth and was treated with ceftazidime and amikacin. Acute pulmonary exacerbation diagnosis was made with respiratory distress, increased cough and sputum. Four patients (3.12%) attended with PBS clinical findings and

Table 1. Clinical and demographic comparison of Pseudo-Bartter syndrome and non- Pseudo-Bartter syndrome patients

	Patients with PBS, (n=18)	**Patients without PBS, (n=110)**	**p-value**
Age of CF diagnosis (months)[a]	24 (1-86)	49 (1-168)	0.003
Current age (months)[a]	84 (4-144)	96 (5-189)	0.060
Sex (M/F)	11/7	59/51	0.061
z-score for weight[b]	-1.25±2.50	-1.08±1.63	0.782
z-score for height[b]	-1.28±1.75	-1.24±1.60	0.921
z-score for BMI[b]	-0.93±2.5	-0.81±1.71	0.719
1st sweat chloride test mmol/L[b]	75.22±24.96	86.50±32.20	0.126
2nd sweat chloride test mmol/L[b]	69.66±28.44	82.30 ±29.11	0.110
Neonatal screening positivity, n (%)	4 (22.2)	27 (24.5)	0.072
Colonization, n (%)	9 (50.0)	24 (21.8)	0.021
Pseudomonas aeruginosa, n (%)	4 (22.2)	11 (10.0)	0.041
Staphylococcus aureus, n (%)	3 (16.7)	10 (9.1)	0.061
P. aeruginosa and *S. aureus*, n (%)	2 (11.1)	3 (2.7)	0.032
Complication			
Bronchiectasis, n (%)	2 (11.1)	14 (12.7)	0.651 0.464
Chronic liver disease, n (%)	1 (5.5)	7 (6.3)	
Diabetes mellitus, n (%)	1 (5.5)	2 (1.8)	
ABPA, n (%)	1 (5.5)	2 (1.8)	
Drugs used			
Dornase alpha, n (%)	15 (83.3)	77 (89.5)	0.544
Multivitamin, n (%)	15 (83.3)	75 (87.2)	0.496
Pancreatic enzyme, n (%)	14 (77.7)	74 (86.0)	0.532
B2 agonist, n (%)	9 (50.0)	34 (39.5)	0.461
Inhaled steroid, n (%)	3 (16.6)	12 (13.9)	0.472
Inhaled antibiotic, n (%)	3 (16.6)	21 (13.9)	0.634

[a]median, interquartile range, [b]mean ± standard deviation, CF: Cystic fibrosis, PBS: Pseudo-Bartter syndrome, BMI: Body mass index, ABPA: Allergic bronchopulmonary aspergillosis, M/F: Male/female

were diagnosed with CF. The majority of attendances due to PBS occurred during the summer season (72%).

During admission, mean serum levels were sodium 125.5 meq/L (min: 112.4; max: 132.3), potassium 2.4 meq/L (min: 1.9; max: 3.2) and chloride 71.4 (min: 56.2; max: 86.3). Mean pH of arterial blood gas was 7.50 (min: 7.46; max: 7.65) and mean HCO_3 levels were 35.9 (min: 28.1; max: 48.6). Of patients, 72.2% (n=13) had their PBS exacerbations in the summer.

Severe hyponatremia (Na <125) was detected in 10 (55.6%) of the patients with PBS at the time of admission. Two patients with serum sodium levels of 120 meq/L and 115 meq/L were admitted to hospital with hyponatremic convulsions. When the demographic and clinical characteristics of patients with and without severe hyponatremia were compared, the number of hospitalization days and chronic bacterial colonization were found to be higher in patients with severe hyponatremia, but there was no statistically significant difference. Clinical features patients according to their sodium levels shown in Table 2.

With the PBS table, 18 patients attended 29 times. Of patients, 72.2% (n=13) had PBS attacks in the summer months. For the 29 PBS attendances in the table, information about salt use was accessed during attendance for 20 cases and 60% of patients did not use salt. One patient had PBS recur four times, one had PBS recur three times and 6 patients had PBS recur twice. When patients with and without recurrent PBS are compared, the chronic bacterial colonization rates were significantly high for patients with recurrent PBS (p=0.031). The annual mean pulmonary exacerbation number for patients with recurrent PBS attacks was four, while it was two for patients with a single attack.

There were 17 different mutations in 34 different alleles in patients with PBS. The most common mutation was homozygote c.1521_1523delCTT (F508del) with 4 (11.76%) alleles. The other common mutations were c.2052delA (2184delA), and c.3131A>G (E1044G). Genetic mutations of the patients with PBS are shown in Table 3.

DISCUSSION

PBS can be the first form of hospital admission for CF. PBS frequency was 14% in our study. This rate was 10% in a study in our country using Cystic Fibrosis Data Registry System data, while Dahabreh et al.[9] found 9% and 16.8% in another study in Spain.

Age of CF diagnosis and current age of patients with PBS were significantly younger than non-PBS cases. The study found mean age of diagnosis of the patients with PBS was 0.77±1.70 years using the Cystic Fibrosis Data Registry System in our country, which was similar to our study. In this age group, feeding with low-sodium breast milk may be associated with increased tendency toward dehydration[9]. In countries without a newborn screening program, PBS may be an early symptom that indicates CF, before patients develop permanent pulmonary defect[10,11]. Educating physicians about differential diagnosis for patients admitted with hypokalemic metabolic alkalosis is a necessity[12].

Z-score weight, height and for BMI was higher in the non-PBS group. Patients with PBS have more diarrhea and vomiting, which causes feeding disorder and their increased metabolic requirements which affects growth and development. Additionally, as *P. aeruginosa* and *S. aureus* colonization was more frequent in the PBS group, frequent pulmonary infections may be associated with disrupted nutrition.

Beginning in 2015, heel lance blood samples began to be scanned for immune reactive trypsinogen because CF causes serious pulmonary and metabolic problem our country. The first and second sweat-test results of

Table 2. Clinical features of Pseudo-Bartter syndrome patients according to their sodium levels

	Sodium <125 (n=10)	Sodium >125 (n=8)	p-value
Age of CF diagnosis (months)[b]	6.4±1.2	12.2±2.2	0.302
Sex (M/F)	6/4	5/3	0.522
Colonization (n, %)	5 (50)	4 (50)	0.264
z-score for height[b]	-1.79±2.11	-1.52±1.72	0.567
z-score for weight[b]	-1.22±1.90	-1.12± 1.63	0.361
1st sweat test mmol/L[b]	72.21±24.61	68.12±22.54	0.384
2nd sweat test mmol/L[b]	65.45±19.11	66.33±21.36	0.435
Hospital stay/day[a]	11 (4-18)	6 (3-8)	0.061

[a]median, interquartile range, [b]mean ± standard deviation, CF: Cystic fibrosis, M/F: Male/female

patients with PBS were low. In a multi-center study in our country, chloride levels were low on the 1st and 2nd sweat tests, similar to our study. The authors speculated that the reason could be chronic chloride loss from sweating and this may result in low serum chloride concentration. We think that chronic chloride loss from sweat is higher in patients with PBS.

Opportunistic pathogens like *S. aureus* and *P. aeruginosa* colonize the respiratory tracts of CF patients[13,14]. In all of the CF patients, the *P. aeruginosa* colonization rate was 11.8%, *S. aureus* colonization rate was 10.2% and the *P. aeruginosa* and *S. aureus* colonization rate was 4%. These rates are similar to another study in our country. In Europe, the chronic *P. aeruginosa* infection rate was between 14.29% and 62.16%. The group with PBS syndrome had higher chronic bacterial colonization in the lungs. For this, increased mucous density and viscosity with electrolyte changes and dehydration and the higher accompanying complications in the PBS group may be associated with the higher regression in growth development. According to 2019 European data, the chronic *Pseudomonas* infection rate changes between 15.38% and 67.5%. *P. aeruginosa* and *S. aureus* colonization was more frequent in the group with PBS compared to non-PBS cases. This may be related to patients diagnosed at early ages and being younger. Bronchiectasis and chronic liver disease were more frequent among the non-PBS group. There were no significant differences for complications between the two groups.

The majority of attendances due to PBS occurred during the summer season. This may be associated with our country being located in a hot climate belt and greater tendency for dehydration due to patients not increasing fluid and sodium intake during the summer months.

Eight patients had repeated PBS attacks and bacterial colonization rates were higher in these patients. Also annual numbers of exacerbations and hospitalization of these patients were higher. They were admitted to hospital with severe hyponatremia. In these patients, often dehydration attacks may increase the viscose secretions in the respiratory tract and may cause more bacterial colonization. As colonization increases, nutrition of the patients may worsen, which can disrupt the salt balance.

PBS is a fatal complication of CF that may cause electrolyte abnormalities, seizures, hypoventilation and arrhythmia[15-17]. It is difficult to provide strict rules about electrolyte supplements needed and the dose and duration of therapy[18-20]. The purpose of the therapy is to ensure normal electrolyte balance. When pulmonary exacerbation, gastroenteritis or over activity is seen, salt

Table 3. Genetic results of patients with Pseudo-Bartter syndrome

C DNA name	Protein name	Legacy name	Phenotype	Genotype information	n	%
c.1521_1523delCTT	Phe508del	deltaf508	disease-causing	homozygote	2	11.76%
				heterozygote	3	8.82%
c.2052delA	Lys684Asnfs*38	2184delA	disease-causing	homozygote	1	5.88%
				heterozygote	2	5.88%
c.3131A>G	p.Glu1044Gly	E1044G	unknown	homozygote	2	11.76%
c.328G>C	p.Asp110His	D110H	disease-causing	heterozygote	2	5.88%
c.3909C>G	p.(Asn1303Lys)	N1303K	disease-causing	heterozygote	2	5.88%
c.1624G>T	p.(Gly542*)	G542X	disease-causing	heterozygote	2	5.88%
c.274G>A	p.(Glu92Lys)	E92K	disease-causing	heterozygote	1	2.94%
c.2988+1G>A	No protein name	3120+1G>A	disease-causing	homozygote	1	5.88%
c.2834C>T	p.(Ser945Leu)	S945L	disease-causing	heterozygote	1	2.94%
c.1399C>T	p.(Leu467Phe)	1531C/T (L467F)	unknown	heterozygote	1	2.94%
c.2195T>G	p.(Leu732*)	L732X	disease-causing	homozygote	1	5.88%
c.254G>A	p.(Gly85Glu)	G85E	disease-causing	heterozygote	1	2.94%
c.1186A>T	p.Asn396Tyr	N369Y	unknown	heterozygote	1	2.94%
c.2339delG	p.Gly780	ValfsX23	unknown	heterozygote	1	2.94%
c.1202G>A	p.(Trp401*)	W401X (TAG)	disease-causing	homozygote	1	5.88%
c.1040G>C	p.Arg347Pro	R347P	disease-causing	heterozygote	1	2.94%

intake should increase. PBS patients were classified as severe (<125) and mild-moderate (>125) according to their sodium levels at admission and the two groups were compared. Mean duration of hospitalization and pulmonary colonization rate of the severe hyponatremia group were insignificantly higher. In the same group, acute bacterial exacerbation was present in patients and it was thought that this situation also lowered the sodium levels.

It is known that recombinant human DNAase (rhDNase) and inhaler hypertonic saline are used to increase mucociliary clearance, improve pulmonary function and decrease pulmonary exacerbations regardless of the severity[21-23]. Studies showed that these are related to decreased pulmonary function. The rates of use for inhaler antibiotics, steroids and B2 agonists were higher in the PBS group. This may be related to the higher chronic bacterial colonization rates and the more frequent pulmonary flare-ups. There were no significant differences between the two groups regarding the drugs they used.

The most common mutation in patients with PBS was F508. In a study using the National Cystic Fibrosis Data Registry system, the deltaF508 rate in our country was 28.4%. Other common mutations were c.2052delA and c.3131A>G. Three (10.8%) patients with PBS but also with normal CFTR genetic scanning were diagnosed with CF by using multiplex ligation-dependent probe amplification. The deltaF508 frequency in non-PBS group was 21.4%. Research in China observed the most frequent mutation in CF patients with PBS was c290G>A, while this mutation was not observed in our PBS patients. Mutation rates between the two groups were similar. This suggests that PBS is independent of mutations[24-26].

Study Limitations

Although all of the follow-up patients in our clinic were included the study, there are some limitations. Since our study is one centered, we have few patients. However as the patient count increases we will continue our work. Due to the data being analyzed by scanning files retrospectively, only limited information was reached.

CONCLUSION

In the conclusion PBS is a common finding of CF. Since CF is included in the newborn screening program, it should be kept in mind that asymptomatic patients are also followed in our country and the first clinical finding in these patients may be PBS. It is seen often in warm climate countries. CF should be considered for patients that attend with clinical manifestations of PBS and further examinations should be performed if suspected. Patients with CF diagnosis should be informed about PBS and warned about regular salt intake.

Ethics

Ethics Committee Approval: Ethics committee approval was provided by Ege University Ethical Committee for Medical Research for the study (decision no: 22-6.1T/49, date: 23.06.2022).

Informed Consent: Informed consent was obtained from all the patients and/or their parents.

Peer-review: Externally peer reviewed.

Author Contributions

Surgical and Medical Practices: M.M.Ö., H.D.Ş., M.B., F.Ç., B.D., E.D., F.G., Concept: M.M.Ö., H.D.Ş., M.B., F.Ç., B.D., E.D., F.G., Design: M.M.Ö., H.D.Ş., M.B., F.Ç., B.D., E.D., F.G., Data Collection or Processing: M.M.Ö., M.B., F.Ç., B.D., E.D., Analysis or Interpretation: M.M.Ö., H.D.Ş., F.Ç., B.D., E.D., F.G., Literature Search: M.M.Ö., H.D.Ş., M.B., F.Ç., E.D., Writing: M.M.Ö., H.D.Ş., M.B., E.D., F.G.

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Original Article

Complaints, Endoscopic and Histopathological Findings in Children with *Helicobacter pylori* Infection: Are There Any Correlations with Each Other?

Helicobacter pylori Enfeksiyonu Olan Çocuklarda Şikayetler, Endoskopik ve Histopatolojik Bulgular: Birbirleriyle Korelasyon Var Mı?

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ABSTRACT

Objective: There is no significant clinical manifestations indicating *Helicobacter pylori* (*H. pylori*) infection in pediatric population. In this study, the most common complaints, endoscopic and histopathological findings in children with *H. pylori* infection were evaluated and their correlation with each other was explicated.

Method: Patients between 3-18 years, who had documented *H. pylori* infection, were enrolled in this study. Gastric biopsies were taken in all patients for rapid urease test (RUT) and histopathological examination, activity of gastritis, chronic inflammation, and Sydney classification was used to evaluate intensity of *H. pylori*, grade of atrophy, and intestinal metaplasia. Demographic characteristics, complaints, endoscopic findings and Sydney scores of the patients were recorded.

Results: A total of 339 patients (183 females) were enrolled in the study. The most common complaints were dyspepsia and epigastric pain. Ninety percent of the patients had antral pathology, including antral nodularity in 78% of the cases. Relation between dyspepsia, epigastric pain, and antral nodularity was found to be statistically significant. In histopathological examination, intensity of *H. pylori* is found to be increasing with age. RUT was positive in 89.4% of the patients and relation between RUT results and the intensity of *H. pylori* was statistically significant. Highly significant correlations were detected between macroscopic changes in the antrum, the intensity of *H. pylori* and gastritis activity ($p \leq 0.0001$, $p=0.01$, respectively).

Conclusion: The most common complaints of children with *H. pylori* infection were epigastric pain and dyspepsia. There were significant relations with these complaints, antral macroscopic changes and intensity of *H. pylori*, which increases with age. As a result, dyspepsia and epigastric pain are related with antral changes. Considering that antral changes are also associated with *H. pylori* intensity, gastritis activity and chronic inflammation, early eradication of the *H. pylori* infection can be recommended to prevent long-term complications in children with *H. pylori* infection.

Keywords: Children, endoscopic findings, *Helicobacter pylori*, histopathological findings

ÖZ

Amaç: Pediatrik popülasyonda *Helicobacter pylori* (*H. pylori*) enfeksiyonunu gösteren önemli bir klinik durum yoktur. Bu çalışmada, *H. pylori* enfeksiyonu olan çocuklarda en sık görülen şikayetler, endoskopik ve histopatolojik bulgular değerlendirilmiş ve birbirleri ile korelasyonu ortaya konmuştur.

Yöntem: Bu çalışmaya 3-18 yaş arası, belgelenmiş *H. pylori* enfeksiyonu olan hastalar alındı. Tüm hastalardan hızlı üreaz testi (RUT) ve histopatolojik inceleme için mide biyopsisi alındı. *H. pylori* yoğunluğunu, gastrit aktivitesini, kronik enflamasyonu, atrofiyi ve intestinal metaplaziyi değerlendirmek için Sydney sınıflandırması kullanıldı. Hastaların demografik özellikleri, şikayetleri, endoskopik bulguları ve Sydney skorları kaydedildi.

Bulgular: Çalışmaya toplam 339 hasta (183 kadın) alındı. En sık şikayetler dispepsi ve epigastrik ağrı idi. Hastaların %90'ında antral patoloji mevcuttu ve bunların %78'i nodülarite idi. Dispepsi, epigastrik ağrı ve antral nodülarite arasındaki ilişki istatistiksel olarak anlamlı bulunmuştur. Histopatolojik incelemede yaşla birlikte *H. pylori* yoğunluğunun arttığı tespit edildi. Hastaların %89,4'ünde RUT pozitifti ve RUT ile *H. pylori* şiddeti arasındaki ilişki

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istatistiksel olarak anlamlıydı. Antrumdaki makroskopik değişiklikler ile *H. pylori* yoğunluğu ve gastrit aktivitesi arasındaki ilişkiler oldukça anlamlıydı (sırasıyla p≤0,0001, p=0,01).

Sonuç: *H. pylori* enfeksiyonu olan çocukların en sık şikayetleri epigastrik ağrı ve dispepsi idi. Bu şikayetler, antral makroskopik değişiklikler ve *H. pylori*'nin şiddeti ile anlamlı ilişkiler vardı ve yaşla birlikte *H. pylori*'nin şiddeti artmaktaydı. Bu şikayetler antral değişikliklerle ilişkili olduğundan ve bu değişiklikler gastrit aktivitesi, kronik enflamasyon ve artan *H. pylori* yoğunluğu ile ilişkili olduğundan, bu çocuklarda uzun dönemli komplikasyonlardan korunmak için erken eradikasyon önerilebilir.

Anahtar kelimeler: Çocuklar, endoskopik bulgular, *Helicobacter pylori*, histopatolojik bulgular

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a microorganism associated with serious gastric diseases such as chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, adenocarcinoma of the stomach and considered as a great health problem especially in developing countries due to its high prevalence rates[1,2]. Bacteria are commonly acquired in the first years of life by inter-family transmittance and cause a lifelong chronic infection[3,4]. The prevalence of *H. pylori* was 20% to 79% in children in cohort studies using non-invasive direct detection methods and prevalence estimates appear to increase with age and in developing countries[5]. *H. pylori* infection may be asymptomatic in most of the patients in pediatric population[6]. Recent studies have shown that children infected with *H. pylori* have gastrointestinal complaints such as, chronic or recurrent abdominal pain, epigastric pain, anorexia, weight loss, dyspepsia, vomiting, gastrointestinal bleeding demonstrated as hematemesis or melena[5,7]. Although recurrent abdominal pain is a common symptom among children at school age, it cannot definitely be associated with *H. pylori* infection[6].

Although *H. pylori* infection commonly causes diffuse antral gastritis and pangastritis in pediatric age group, endoscopic findings may be normal in 50% of the patients, Nonetheless, antral nodularity, believed to appear due to lymphoid hyperplasia caused by *H. pylori* infection, is more commonly seen as a specific endoscopic finding[8]. Guarner et al.[9] reported in their 10 years long compilation of diagnostic methods, that endoscopy with histopathological assessment is the only and the most effective diagnostic method for *H. pylori* infection and its associated lesions. Although some studies have shown that *H. pylori* infection may not alter the normal histopathology in children, children colonized with *H. pylori* most commonly develop chronic gastritis[8,10]. Histological findings include infiltration of gastric mucosa with domination of plasma cells and lymphocyte. The organism can be identified with Giemsa, Diff-Quick, periodic acid Schiff-Alcian blue or hematoxylin and eosin dyes[11,12]. In Sydney classification *H. pylori* gastritis is defined, and the intensity of *H. pylori,* gastric activity, and severity of inflammation, antral atrophy and intestinal metaplasia are graded[12]. In this study, the most common complaints, endoscopic and histopathological findings in children with *H. pylori* infection were evaluated and their correlation with each other was explicated.

MATERIALS and METHODS

The study was conducted according to the principles of World Medical Association Declaration of Helsinki (ethical principles for medical research involving human subjects) and approved by the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospitals Clinical Research Ethics Committee (decision no: 4, date: 22.08.2014). Children between 3-18 years of age, applied to pediatric gastroenterology outpatient clinic within the last two years who had not used any antibiotics, non-steroid anti-inflammatory drugs or gastric acid inhibitors within the last three months and underwent upper gastrointestinal tract endoscopy due to different complaints including dyspepsia, epigastric pain, reflux symptoms, growth retardation, malabsorption findings, resistant iron and/or vitamin B12 deficiency and suspicion of gastrointestinal bleeding were enrolled in the study. Patients' informed consent was obtained from their parents and under the deep sedation applied by anesthesiologist, esophagogastroduodenoscopy was performed with age- appropriate size Fujinon video endoscope (Alternup Medical, France). Macroscopic findings seen at esophagus, stomach or duodenum were noted by performing endoscopist.

During the endoscopy procedure, four tissue biopsy specimens were obtained both from gastric corpus and antrum. One biopsy specimen from both sites were used for rapid urease test (RUT), other three biopsy specimens from each region were sent to pathology laboratory in 10% neutral buffered formalin solution. Tissue samples were prepared in the laboratory and stained with hematoxylin-eosin and May Guenwald-Giemsa staining solutions and evaluated by an experienced specialist. Findings of *H. pylori* gastritis revealed in histopathologic evaluation were scored by Sydney classification[12]. Intensity of *H. pylori,* gastric activity, severity of inflammation,

antral atrophy, and intestinal metaplasia were graded based on Sydney classification system. Accordingly, 1) intensity of *H. pylori* defined by the percentage of bacteria on mucosa, 2) gastric activity defined by the quantity of polymorphonuclear leukocytes, 3) severity of inflammation indicated by the increase in the number of mononuclear cells (lymphocytes, plasma cells, monocytes, mast cells, and eosinophils) on the mucosa were graded as absent, mild (grade 1), moderate (grade 2), and severe (grade 3). Intestinal metaplasia was graded as absent, complete (type 1; 1+), incomplete (type 2 and type 3; 2+) and all findings were recorded[13].

Statistical Analysis

Statistical analyses of the study data were performed by using SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) for Windows v.16.0 program. Besides supplemental statistical methods as frequency, percentage, mean and standard deviation, Student's t-test was used for group median comparison of quantitative data and chi-square test and Fisher's Exact test were used for comparison of qualitative data. Results were evaluated within 95% confidence interval and at a statistical significance level of $p<0.05$.

RESULTS

A total of 339 patients, 183 girls (54%) and 156 boys (46%), who underwent endoscopy for any gastrointestinal complaint and were diagnosed with *H. pylori* infection, were included in the study. Mean age of cases was 12.18±3.51 years (range: 3-17.7 years).

Epigastric pain was the most common complaint in 61.9% of the cases. Dyspeptic symptoms like bloating, early satiety, abdominal distension, and belching were seen in 45.1% of the patients. Both of these complaints were present in 42% of the patients. The other commonly seen symptoms were failure to thrive in 34.5%, reflux symptoms as nausea, vomiting, regurgitation, retrosternal pain, and swallowing difficulty in 21.2%, anemia in 20.4% of the patients. Also 14% of the patients had other complaints such as loss of appetite and diarrhea (Figure 1).

In endoscopic evaluation, esophageal findings were in normal physiological ranges in 182 (53.6%) out of 339 patients. Hyperemia was present in 152 (44.8%) and erosion in 53 (15.6%) patients. Nodularity and ulcer were other esophageal findings. Gastric corpus was normal in appearance in 198 patients (58.4%). Hyperemia was present in 143 (42.2%) and antral nodularity in 36 (10.6%) patients. All of the patients with antral nodularity also had hyperemia.

Antral pathology was present in 308 (90.8%) of 339, hyperemia in 284 (83.7%), gastric erosion in 16 (4.7%), and gastric ulcer in 4 (1.2%) patients. Antral nodularity, mostly accepted as a finding of *H. pylori* infection, was present in 268 (79%) patients. Hyperemia and antral nodularity were seen together in 246 (72.5%) patients. Duodenum was found to be normal in 76% (258) of the patients. Nodularity was present in 10.3% (35) and erosion in 9% (30) patients. Ulcer was found in 24 (7.1%) patients and 4 of these patients had both erosion and ulcer.

Biopsies taken from gastric corpus and antral region have been evaluated histopathologically and *H. pylori* gastritis was scored by Sydney classification. In 74% (251) of the patients lymphoid aggregates or follicles were detected. Lymphoid aggregates were found in 134 (53.3%) and lymphoid follicles in 117 (46.6%) patients. In 51 of these patients both lymphoid aggregates and lymphoid follicles were present. Histopathological findings, especially intensity of *H. pylori* was found to increase with age. When patients were divided into three groups according to age, intensity of *H. pylori* was mild in 83% of 0-6 years group, decreased to 28.2% in 6-12 years group and it was 20.6% in patients aged 12 years and over. Severe *H. pylori* intensity was found to be 17% in 0-6 years group, 30.8% in 6-12 years group and 33.8% in patients aged 12 years and over. Intensity of *H. pylori* differed statistically significantly between age groups ($p=0.021$).

RUT was positive in 303 (89.4%) and negative in 36 (10.6%) patients. A strong statistically significant relationship existed between positive RUT and intensity of *H. pylori* ($p<0.001$).

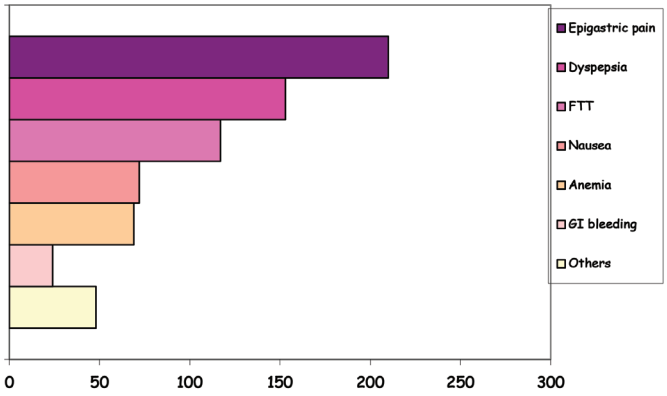

Figure 1. Complaints of patients

GI: Gastrointestinal, FTT: Failure to thrive

Intestinal metaplasia was seen in 8.2% and gastric atrophy in 6.8% of the patients in the study group, without any statistically significant correlation between gastric atrophy or intestinal metaplasia and *H. pylori* intensity, endoscopic findings and complaints.

Complaints and their relations with endoscopic findings were assessed, and extremely, and statistically significant correlations were detected between epigastric pain and antral pathologies (hyperemia, nodularity, erosion and ulcer) (p≤0.0001) (Table 1). There was no significant relationship between epigastric pain and other endoscopic findings. There was also extremely significant relationship between dyspeptic complaints and antral nodularity (p≤0.0001) (Table 2). We did not find any relation between other complaints and endoscopic findings.

When the pathologic or normal appearance of the antrum and histopathological findings were compared; there was statistically significant difference between the group of patients with normal antrum and antral pathologies (hyperemia and/or nodularity, erosion/ulcer); and *H. pylori* intensity and activity (p≤0.0001 and p=0.01, respectively) (Table 3). The relationship between antral nodularity observed in endoscopy and *H. pylori* intensity and chronic inflammation scores was statistically significant (p=0.001 and p=0.001, respectively) (Table 3). The relationship between lymphoid aggregates or lymphoid follicles and antral nodularity was not statistically significant.

Table 1. Association between epigastric pain and antral pathologies

Epigastric pain	Antral pathologies (hyperemia and/or nodularity)		F x^2
	+	-	
+	201 (59.3%)	9 (2.7%)	p≤0.0001
-	104 (30.7%)	25 (7.3%)	
Total	305 (90%)	34 (10%)	

F x^2: Fisher's Exact test

Table 2. Association between dyspepsia and antral nodularity

Dyspepsia	Antral nodularity		F x^2
	+	-	
+	135 (39.8%)	18 (5.3%)	p≤0.0001
-	129 (38.1%)	57 (16.8%)	
Total	264 (77.9%)	75 (22.1%)	

F x^2: Fisher's Exact test

Table 3. Association between antral endoscopic and histopathological findings

Histo-pathological findings	Antral endoscopic findings (hyperemia and/or nodularity)	Sydney scores				
		0	1	2	3	X^2
***H. pylori* intensity**	+	0 (0%)	72 (21.2%)	140 (41.3%)	93 (27.4%)	p≤0.0001
	-	0 (0%)	18 (5.3%)	1 (0.3%)	15 (4.4%)	
Activity	+	1 (0.3%)	159 (46.9%)	126 (37.2%)	18 (5.3%)	p=0.01
	-	2 (0.6%)	15 (4.4%)	15 (4.4%)	3 (0.9%)	
	Antral nodularity	**Sydney scores**				
		0	**1**	**2**	**3**	**X^2**
***H. pylori* intensity**	+	0 (0%)	54 (15.9%)	120 (35.4%)	90 (26.5%)	p=0.0001
	_	0 (0%)	36 (10.6%)	21 (6.2%)	18 (5.3%)	
Inflammation	+	2 (0.6%)	64 (19.5%)	150 (44.2%)	46 (13.5%)	p=0.001
	_	1 (0.3%)	32 (9.7%)	42 (12.4%)	2 (0.5%)	

0= Negative, 1= Mild, 2= Moderate, 3= Severe, X^2: Chi-square test

DISCUSSION

H. pylori infection is one of the most commonly seen infections in the world, thus it is a great public health problem for developing countries. It has been accepted that nearly half of the world's population will be infected with *H. pylori* sometime during their life[5,14] *H. pylori* infection is seen more commonly in some age and ethnic groups but without any gender predominance[15]. In our study, 54% of female and 46% of male patients were infected with *H. pylori* without any significant gender difference.

Studies have shown that the children acquired the bacteria mostly by inter-familial transmission during early childhood, prevalence of the disease increased with age and it may become a lifelong infection if *H. pylori* infection is not eradicated[2,3,15]. Ertem et al.[15] reported that the rate of *H. pylori* infection is 18.2% in children under 4 years of age, and its prevalence increases with age even up to 65% in adolescence group in our country. Similar to these findings, in our study on pediatric patients infected with *H. pylori* between the years of 3 and 18, only 5.5% of the patients were under the age of 6 and 60% were above the age of 12.

H. pylori infection in pediatric population has no characteristic clinical manifestations, and the infection is mostly asymptomatic. On the other hand, gastrointestinal symptoms such as epigastric pain, dyspeptic symptoms (early satiety, bloating, abdominal distension and belching), nausea, lack of appetite, weight loss, treatment-resistance iron deficiency anemia, upper gastrointestinal bleedings described as hematemesis or melena may be manifestations of *H. pylori* infection and may be also associated with organic diseases such as peptic ulcer. It is recommended to perform esophagogastroduodenoscopy to those patients[2,7,16]. In our study, the most common complaint, in correlation with literature findings, was epigastric pain (61.9%), and dyspepsia (45.1%) at indicated rates. Failure to thrive, reflux symptoms like nausea, vomiting, regurgitation, retrosternal pain, dysphagia and anemia were the other common complaints.

In a pediatric study reviewing topographic settlement of *H. pylori* in stomach, bacteria colonization and gastritis findings were specifically observed in antral region[4,17]. In our study, antral pathology was detected in 90% of the patients, where *H. pylori* is localized mostly. Especially in patients with epigastric pain, antral pathology was observed at higher rates during endoscopic examination. Antral nodularity have been reported at varying rates in the study of Koh et al.[18] (50.6%; total n=328) and, Tomić et al.[19] (67.5%). In our study antral nodularity was found in 77.9% of the patients which was at a higher in patients undergoing endoscopy with dyspeptic complaints.

Gastric ulcer in children usually occurs due to etiologic factors unrelated to *H. pylori* and *H. pylori* associated ulcers are seen very rarely[10]. In our patient group, gastric erosion was present in 16 (4.7%) patients and only four patients (1.2%) had gastric ulcer. On the other hand, *H. pylori* infection is the primary etiologic factor in bulbar and duodenal ulcers in pediatric age group. In the study of Rick et al.[20] duodenal ulcer was present in 11 patients out of 51 and all of these patients had *H. pylori* infection. In our study population of 339 patients, 30 (9%) had gastric erosion and 24 (7.1%) had bulbar or duodenal ulcer.

Sensitivity and specificity of RUT in the detection of *H. pylori* in gastric mucosa were found to be 83.4% and 99%, respectively[21]. Madani et al.[22] found that positive RUT correlated with *H. pylori* intensity in gastric mucosa and activity of gastritis. In our study, RUT was positive in 89.4% and negative in 10.6% of the patients and the relation between the positivity of the test and intensity of *H. pylori* was statistically significant.

Histopathologic features of *H. pylori* gastritis were scored by the Sydney classification[12] based on intensity of bacteria, neutrophil activity showing active gastritis, chronic mononuclear inflammation, glandular atrophy and intestinal metaplasia. Tutar et al.[8] showed that *H. pylori* infection may also appear with normal gastric histopathology, but in our study almost all of the patients had *H. pylori*-related chronic active gastritis except three patients had not active gastritis; and about half of our patients had moderate gastritis and chronic inflammation. In the study of Kamada et al.[23] with young adult patients, mostly moderate activity of gastritis and chronic inflammation were detected as in our study (67.9%, and 46.4%, respectively).

In our study group, when all patients were evaluated independent of age, *H. pylori* intensity was mild in 26.5%, moderate in 41.6%, and severe in 31.9% of the patients. When patients were divided into three age groups and evaluated, 17% of 6 years and below group and 33.8% of 12 and above group had severe (3+) *H. pylori* intensity. This increase with age was found to be significant. These findings also support the study of Domşa et al.[24] which stated that the prevalence of *H. pylori* increased gradually with age.

Atrophic gastritis and intestinal metaplasia are primary histopathologic changes which may lead to gastric cancer in years to come[25]. The relationship

between *H. pylori* infection acquired in childhood and development of gastric cancer in adulthood is still unclear. However, the study by Yörgüç et al.[26] on tissue immune markers have shown that the pathogenicity of *H. pylori* in children is higher than in adults. Although the prevalence of intestinal metaplasia is reportedly very low in pediatric studies[8,18]; according to the findings of Kato et al.[27] atrophic gastritis, a precursor of gastric cancer, is significantly more common in children infected with *H. pylori* compared to the uninfected group. Ethnicity-associated genetic factors, environmental factors like nutritional habits or virulence of *H. pylori* seen in some geographic regions may be the reason of high rates of atrophic gastritis especially reported in Far Eastern studies[18,23,27]. In our study group, intestinal metaplasia and gastric atrophy rates were 8.2% and 6.8%, respectively and we could not find any correlation among *H. pylori* intensity, endoscopic findings and complaints.

There are many studies analyzing the coexistence and relation between antral nodular gastritis and *H. pylori* infection in pediatric population[18,27-29]. These studies support the association of antral nodular gastritis with *H. pylori* intensity, increased activity of gastritis and chronic inflammation. Correspondingly, our study has shown that antral nodularity is especially associated with *H. pylori* intensity and chronic inflammation. In addition to these findings, Yang's[30] recent study has also revealed that nodular gastritis may indicate gastric MALT in children with *H. pylori* infection and that the degree of antral nodularity is also correlated with severity of MALT.

In their study, Kato et al.[27] determined that the intensity of *H. pylori* was significantly higher in patients with duodenal ulcer relative to the patients with gastric ulcer. In our study group, only four patients had gastric ulcer and severe (grade 3) *H. pylori* intensity. Besides, gastric activity and inflammation according to Sydney classification system of gastritis 7.1% of the patients had duodenal ulcer and no significant correlation was found between duodenal ulcer and *H. pylori* intensity in this group.

Study Limitations

Based on literature findings, the relation between complaints and histopathologic findings in pediatric or adult age group with *H. pylori* gastritis has not been investigated so far. Although retrospective design and limited number of patients were the limitations of our study; we found some significant correlations between the endoscopic and histopathological findings and the complaints of the patients. According to the findings of our study; epigastric pain was significantly related with antral pathologies such as hyperemia, nodularity, erosion and ulcer. There was also statistically significant relationship between dyspeptic complaints and antral nodularity. Other complaints such as heartburn, failure to thrive, gastrointestinal bleeding or anemia were not correlated with endoscopic findings.

CONCLUSION

Especially in regions with relatively higher *H. pylori* prevalence, children may acquire *H. pylori* infection at an early age and most of them develop gastritis. The most common complaints of these children with *H. pylori* infection are epigastric pain and dyspepsia. According to our findings there is a significant relation with these complaints and morphological changes in antrum such as hyperemia, nodularity, erosion or ulcer. These antral pathologies are also significantly related to *H. pylori* intensity, activity of gastritis and chronic inflammation scores determined at histopathological evaluation.

As a result; since these complaints are related with antral morphological changes and these changes are related with intensity of *H. pylori,* which is increasing with age, early eradication of *H. pylori* infection can be recommended in these children in order to prevent from its long- term complications, such as peptic ulcer, gastric atrophy and even possibly gastric cancer, and MALT lymphoma later in life.

Ethics

Ethics Committee Approval: The study approved by the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospitals Clinical Research Ethics Committee (decision no: 4, date: 22.08.2014).

Informed Consent: Patients' informed consent was obtained.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: G.K., E.P., M.Ç., T.K.O., Ş.M.K., N.G., Concept: G.K., E.P., M.Ç., T.K.O., Ş.M.K., N.G., Design: G.K., E.P., M.Ç., T.K.O., Ş.M.K., N.G., Data Collection and/or Processing: G.K., E.P., T.K.O., Ş.M.K., N.G., Analysis or Interpretation: G.K., E.P., T.K.O., Ş.M.K., N.G., Literature Search: G.K., E.P., M.Ç., T.K.O., Ş.M.K., N.G., Writing: G.K., E.P., M.Ç., T.K.O., Ş.M.K., N.G.

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Original Article

The Effectiveness of Rectal Suction Biopsy in the Diagnosis of Hirschsprung's Disease

Hirschsprung Hastalığı Tanısında Rektal Aspirasyon Biyopsisinin Etkinliği

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ABSTRACT

Objective: In order to analyse the adequacy, sensitivity, and specificity of samples obtained with rectal suction biopsy (RSB) as the standard histopathological diagnostic method in patients with suspected Hirschsprung's disease (HD).

Method: This study was carried out between November 2016 and March 2018 with 24 suspected HD patients aged 0-3 years. After calculating rectosigmoid indexes (RSIs) according to barium enema images obtained in contrast-enhanced colon graphies, patients with RSI <1 underwent RSB. Clinical features of patients, treatment options, complications, laboratory test results and radiological findings were recorded for further analyses. An expert pathologist evaluated biopsy specimens, and observations were recorded.

Results: There were no ganglion cells in RSB specimens in 10 (41.6%) patients who received the diagnosis of HD later on. Ganglion cells were detected in specimens of 5 (20.8%) patients; thus, the diagnosis of HD was excluded. Inadequate or suspicious biopsy specimens for histopathological evaluation were observed in 9 (37.5%) patients. If the biopsy specimen volume was more voluminous than 4 mm^3, its diagnostic sensitivity and specificity for HD were 80% and 66.67%, respectively (area under the curve=0.789); If the submucosa/mucosa ratio was greater than 0.75 or the submucosa/total specimen ratio was greater than 0.42, then the diagnostic sensitivity and the specificity of the specimen for HD were 86.67% and 66.67%, respectively.

Conclusion: In this prospective cross-sectional study, we demonstrated that RSB in diagnosing HD is a feasible, safe method with high sensitivity and specificity and low complication rates.

Keywords: Hirschsprung's disease, ganglion, rectal aspiration biopsy, rectal biopsy

ÖZ

Amaç: Hirschpsung hastalığı (HH) şüphesi olan hastalarda histopatolojik tanı için standart tanı olarak rektal aspirasyon biyopsi (RAB) örneklerinin yeterliliğini, duyarlılığını ve özgüllüğünü araştırmak.

Yöntem: Çalışma Kasım 2016-Mart 2018 tarihleri arasında prospektif olarak gerçekleştirildi. Çalışmaya yaşları 0-3 arasında değişen HH şüphesi olan 24 hasta dahil edildi. Kontrastlı kolon grafisine göre rektosigmoid indeks (RSI) hesaplandıktan sonra RSI <1 olan hastalara RAB yapıldı. Hastaların klinik özellikleri, tedavi seçenekleri, komplikasyonlar, laboratuvar sonuçları ve radyolojik bulguları ileri analizler için kaydedildi. Biyopsi örnekleri uzman patolog tarafından değerlendirildi ve bulgular kaydedildi.

Bulgular: HH tanısı alan 10 (%41,6) hastanın RAB örneklerinde ganglion yoktu. Beş (%20,8) hastada ganglion hücresi saptandı ve HH tanısı dışlandı. Dokuz (%37,5) hastada histopatolojik değerlendirme için yetersiz veya şüpheli biyopsi örnekleri gözlendi. Biyopsi hacmi 4 mm^3'ten büyükse HH tanısında duyarlılık %80, özgüllük %66,67 (eğrinin altındaki alan=0,789); submukoza/mukoza oranı 0,75'ten büyük veya spesmendeki submukoza oranı 0,42'den büyük ise duyarlılık ve özgüllük oranları sırasıyla %86,67 ve %66,67 idi.

Sonuç: Bu prospektif kesitsel çalışmada, HH tanısında RABnin kolay uygulanabilir, güvenli, duyarlılığı ve özgüllüğü daha yüksek ve komplikasyon oranlarının düşük olduğunu gösterdik.

Anahtar kelimeler: Hirschsprung hastalığı, gangliyon, rektal aspirasyon biyopsisi, rektal biyopsi

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INTRODUCTION

Hirschsprung's disease (HD) occurs in the newborn and childhood period and manifests with signs of intestinal obstruction and constipation[1]. The primary underlying pathology of the disease is the migration deficiency of neural crest cells during the development of the bowels in the fetal period. Functional colonic obstruction in the HD occurs due to a lack of relaxation of an aganglionic colon segment[2-5].

HD occurs in approximately 1 in 5,000 live births, and male to female ratio ranges from 3: 1 to 4: 1[6-8]. Most patients are diagnosed during the newborn period[9]. Sometimes, in infants with short-segment HD, the disease may progress with mild symptoms so that diagnosis may be delayed until childhood. Only up to 10% of patients are diagnosed after age three[10,11].

The diagnosis is made by demonstrating the complete absence of enteric ganglion cells in the submucosal (SM) and myenteric plexuses of the distal colon in pathology preparations. Rectal biopsy could be performed by two methods: full-thickness rectal biopsy (FTRB) and rectal suction biopsy (RSB)[12]. Biopsies must be taken 2 cm above the dentate line to prevent misdiagnosis because there is a physiological aganglionic region below of dentate line[13].

Previous studies reported that anal dilatation, rectal irrigation and barium enema used in diagnosing and treating HD might also cause inflammation and edema in the anorectal mucosa, which may prevent retrieval of sufficient biopsy material required for an accurate diagnosis. However, these confounding factors could not be eliminated due to the retrospective design of these studies[14].

Our study aims to determine the diagnostic accuracy of RSB, the sufficient amount of RSB samples required for histopathological diagnosis, and to estimate the sensitivity and specificity of RSB.

MATERIALS and METHODS

The main findings of this study were obtained between November 15, 2016 and March 15, 2018. After the İzmir Katip Çelebi University Clinical Research Ethics Committee (decision number: 16, approval date: 02.09.2017) was obtained, the data of 24 patients aged 0-3 years with an initial diagnosis of HD who were hospitalized in our clinic for advanced examination and treatment were analyzed prospectively.

Laboratory test results and plain abdominal radiograms of the patients were evaluated. Then contrast-enhanced colon graphies and 24th hour control X-ray graphies (retention graphy) were examined and recorded (Figure 1). Rectosigmoid index (RSI) was calculated for all patients. In the RSI evaluation, the presence of the transition zone and the barium in the intestines on the radiograms obtained after 24 hours were evaluated and recorded. The RSI was calculated by proportioning the diameter of the widest part of the rectum to the diameter of the sigmoid colon on the barium enema graphy. RSI <1 was considered in favour of HD (Figure 2)[15].

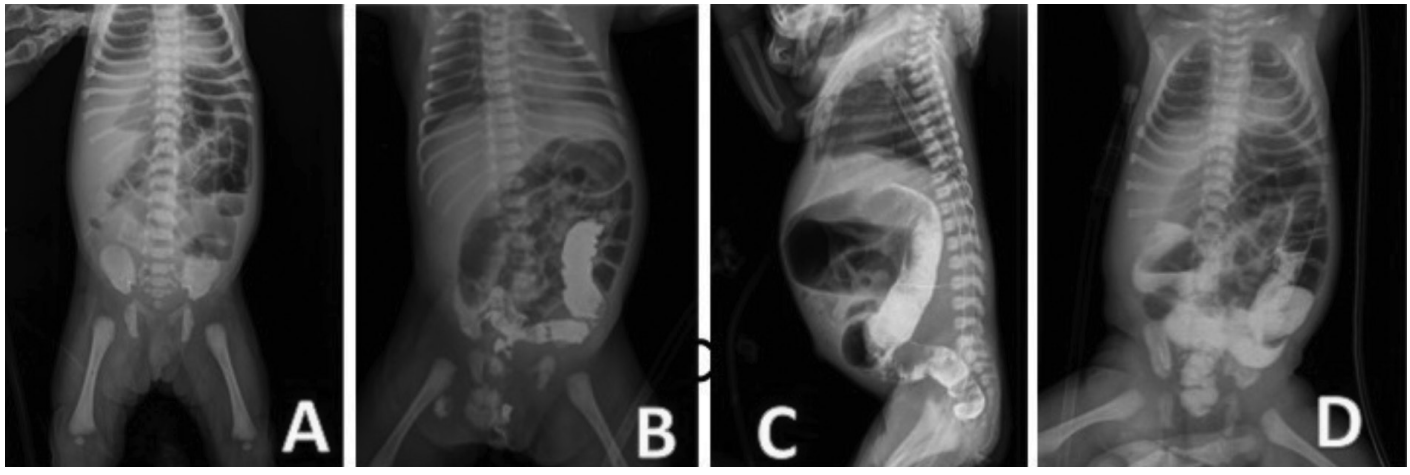

Figure 1. X-ray imaging of the patient with suspected Hirschsprung disease (**A:** Broad-based air-fluid levels in the abdominal radiography of a patient with abdominal distension, **B:** Anterior/posterior barium enema graphy, **C:** Lateral barium enema graphy, **D:** Control X-ray radiography 24 hours after application of barium enema)

After the complaints of these patients, such as abdominal distention and vomiting, were relieved by nasogastric decompression; RSB was performed at least 48 hours after anal dilatation or rectal irrigation procedures. The biopsy was performed at the bedside for the patients younger than 1-year-old without anaesthesia and in the operating room with sedation (0.01 mg/kg IV midazolam) for patients older than 1-year-old.

Rectal suction biopsies were performed with Rbi2 biopsy kit (Aus systems Pty Ltd, Allenby Gardens, Australia). For histopathological diagnosis, two samples were obtained, one from the posterior wall and one from the lateral wall of the affected colonic segment at a level of 2 cm proximal of the anocutaneous line. Negative pressure during the procedure was adjusted to 150 mm H_2O by creating a vacuum of 5 cc in a 10 cc injector[14]. The fresh samples were taken to the pathology laboratory and fixed in 10% formaldehyde solution, and then their paraffin blocks were prepared. Very thin (4-5 µ) sections of paraffin blocks were stained with hematoxylin-eosin and examined under a light microscope at a magnification of 100 - 400 x (Figure 3). Ret-oncoprotein (Figure 3) and neuron-specific enolase staining was performed by immune-histochemical method on samples whose ganglion cells and nerve plexuses could not be discerned during a routine examination. In addition, all sections were evaluated by measuring the mucosal (M) and SM areas in NIS-Elements ver 4.30 digital imaging program of Nikon Eclipse Ni microscope (Figure 3).

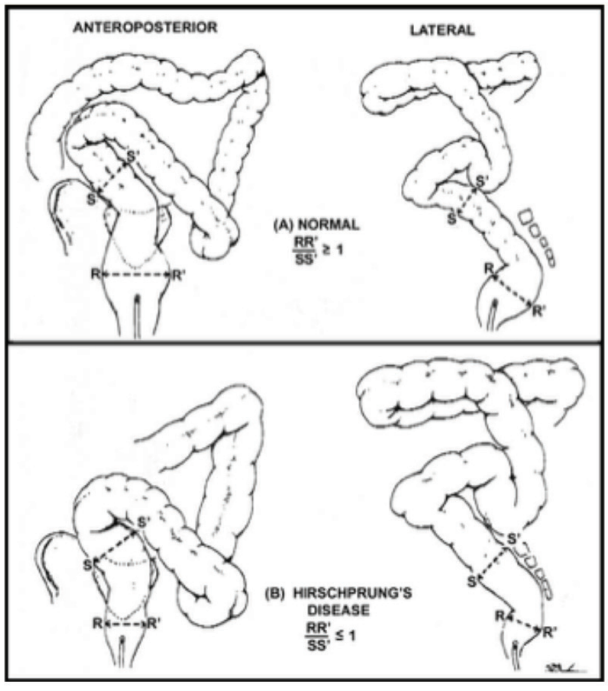

Figure 2. A: Normal RSI measurement. The ratio of the widest diameter (RR') of the rectum to the widest diameter (SS') of the sigmoid colon is greater than or equal to 1. **B:** Abnormal RSI: Hirschsprung's disease: RSI <1

RSI: Rectosigmoid index

Statistical Analysis

The data were evaluated in the IBM SPSS Statistics 25.0 statistical package program. We used numerical values, percentages (%), and mean ± standard deviation for the descriptive statistics. The distribution of numerical variables was evaluated with the Shapiro-Wilk test of normality and Q-Q graphs. Comparisons of the two groups for the normally distributed variables were made with an independent two-sample t-test. The relationship between categorical variables was examined with the exact method of the Pearson chi-square test. The volume of the biopsy specimen resected the SM/mucosa (M) ratio and the percentage of SM (SM%) tissue in the postoperatively resected intestinal segment were determined by receiver operating characteristic (ROC) curve analysis. A p-value of <0.05 was considered statistically significant.

Results

The study population (n=24) consisted of 14 (58%) female, and 10 (48%) male patients. Fifteen (62.5%)

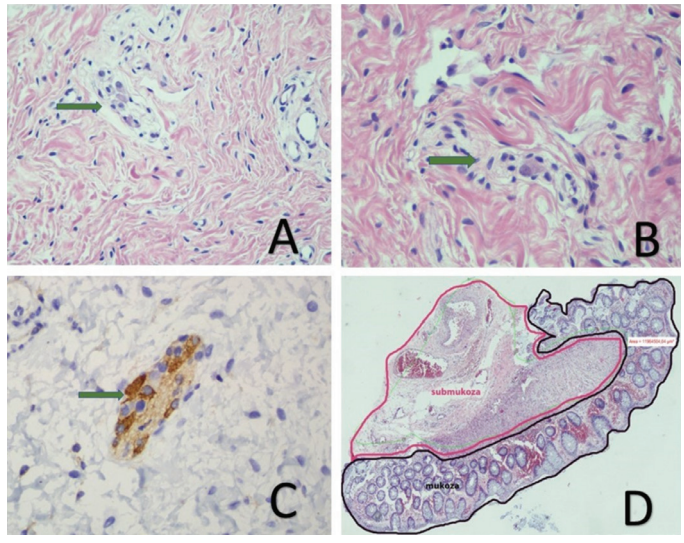

Figure 3. A: Ganglion cells in nerve plexuses (HE × 100), **B:** Ganglion cell (HE × 400), **C:** Ganglion cell stained with ret-oncoprotein (DAB × 400), **D:** Measurement of mucosal/submucosal areas

patients were newborns (0-28 days), and 9 (37.5%) patients were children aged between 1 and 36 months. Meconium output was not observed in 16 (66.7%) patients within the first 24 hours after birth, and 10 (62.5%) of these 16 patients were diagnosed with HD after further examinations and evaluations. RSI was calculated as <1 in 7 (29.1%) and ≥1 in 9 (37.5%) patients. Adequate radiography could not be obtained or evaluated due to previous ileostomy or colostomy in 8 (33.3%) patients. Seven (29.1%) patients with RSI less than one were diagnosed with HD after subsequent examinations.

During the study, none of the patients developed rectal bleeding, intestinal perforation, sepsis or similar complications after RSB at the time of diagnosis.

No ganglion cells were found in the samples of 10 (41.6%) patients in the histopathological evaluation and diagnosed as HD. Ganglion cells were observed in the histopathological examination of the samples in 5 patients (20.8%). Biopsy samples of 9 (37.5%) patients were evaluated and reported as insufficient or suspicious samples for the pathological examination.

FTRB specimens obtained from 4 (16.6%) patients who were evaluated as inadequate and suspicious RSB, and HD diagnosis was excluded in these patients due to the presence of ganglion cells detected during the histopathological examination of the samples. The other 5 (20.8%) patients with insufficient results were discharged after their complaints regressed; thus, the diagnosis of HD was excluded. Afterwards, patients were monitored for six months during periodic follow-up controls in outpatient clinics. During the follow-up period, no findings suggestive of HD were detected, so their families were informed, and these patients were excluded from routine follow-up controls. In addition, all patients were followed up with outpatient clinic controls after discharge for at least six months. Based on the RSB results obtained from 24 patients, the volume of the biopsy specimens, the SM/mucosa (M) ratio and the percentage of SM tissue in the preparation (SM%) were calculated. The results are summarized in Table 1.

When the preoperative RSB and postoperative resection materials were compared, 10 (41.6%) patients with preoperative ganglion-negative RSB results also had aganglionosis in postoperative resection materials. In the histopathological evaluation of samples in 5 (20.8%) patients, ganglion cells in BRS materials were reported. According to these results of 5 patients; due to the disappearance of symptoms in favour of HD during clinical observation, their families were informed after 6 months of outpatient clinic follow-up, and the patients were excluded from the follow-up.

When ROC analysis was performed to compare RSB samples in terms of histopathology and clinical examination results, it was calculated that the volume of the biopsy material greater than 4 mm^3 had 80% sensitivity and 66.67% specificity in the diagnosis of HD [area under the curve (AUC)=0.789]. While the SM/M ratio greater than 0.75 or the SM greater than 0.42 had diagnostic sensitivity, and specificity of 86.67% and 66.67%, respectively (AUC=0.748, and 0.752, respectively) (Table 2).

When the results of RSB were evaluated, samples of 15 (62.5%) patients were sufficient, and 9 (37.5%) patients were insufficient or suspicious. The mean volume of adequate biopsies (8.4 mm^3), SM/M ratio (1.22), and percentage of SM (0.52%) were estimated as indicated.

Table 1. Histopathological evaluation results of biopsy specimens

	Volume (mm^3)	SM/M	SM%
Number of specimens (mean ± SD)	24 (7.04±4.63)	24 (1.05±0.55)	24 (0.47±0.12)
Minimum	3.00	0.36	0.26
Maximum	24.00	2.41	0.70

SD: Standard deviation, SM/M: Submucosa/mucosa

Table 2. ROC analysis results for histopathological evaluations

	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	+PV	-PV	AUC	p-value
SM%	>0.42	86.67 (59.5-98.0)	66.67 (30.1-92.1)	81.3	75.0	0.752	0.011
SM/M	>0.75	86.67 (59.5-98.0)	66.67 (30.1-92.1)	81.3	75.0	0.748	0.013
Volume (cc)	>4	80.00 (51.9-95.4)	66.67 (30.1-92.1)	80.0	66.7	0.789	0.001

+PV: Positive predictive value, -PV: Negative predictive value, AUC: Area under the curve, SM/M: Submucosa/mucosa, CI: Confidence interval

The mean volume of inadequate biopsy specimens (4.7 mm^3), SM/M ratio (0.77), and percentage of SM (0.40) were also determined. In terms of SM% of patients, the difference between groups was statistically significant (p=0.033) (Table 3).

We estimated the diagnostic adequacy rate of RSB as 73.3% in newborns and 44.4% in children aged between one month and three years (Table 4).

DISCUSSION

In this study, we aimed to measure the diagnostic adequacy, sensitivity and specificity of the RSB method applied to obtain rectal biopsy samples, the gold standard diagnostic method for HD. The RSB results obtained for HD were found to be sufficient for diagnosis. Most (62.5%) of the samples obtained using the RSB method had sufficient, and 37.5% had insufficient or suspicious diagnostic accuracy. Different studies have presented variable and insufficient sampling rates when the literature is reviewed. Apart from the discussions on this subject, insufficient sampling rates between 11-50%[16-22] were stated when the studies were considered. What makes these studies different from each other is that the biopsy sampling process was carried out using variable methods. In the literature, in most of the studies with low insufficient diagnostic material rates, 3 or 4 samples were taken in each biopsy procedure, and the biopsy was repeated if the SM tissue was deficient. When the studies were examined in general, it was stated that 1-4 biopsy samples should be taken during biopsy performing process. In many studies, at least three samples were taken[17-22]. Based on our study, two samples were taken from each patient, so our insufficient sampling rate is at an acceptable level. However, this rate was higher compared to other studies in the literature because only two samples in each procedure were obtained, and the patients did not undergo RSB for the second time according to the recently applied protocol in our department. In addition, the biopsy material of our two patients that were reported as suspicious but considered insufficient may have increased this rate.

In our study, when samples containing sufficient and insufficient tissue samples were examined to confirm or exclude the disease in the histopathological examinations of biopsy materials, the average volumes of sufficient and insufficient samples obtained to establish diagnosis were 8.4 mm^3 and 4.7 mm^3, respectively. Although these values were not statistically significant, it is noteworthy that sufficiently higher volumes of samples are required to make an accurate diagnosis. There are limited studies in the literature regarding the volume of samples that should be retrieved for diagnostic purposes. In a different study, the average volume of RSB samples obtained was reported as 14.8±7.8 mm^3[14]. Several studies have reported that an adequate biopsy sample should be at least 3.5 mm in diameter[17,23,24]. The increase in the volume of the samples contributes to the decision-making process, but the depth of the biopsy is also another important diagnostic criterion. In the histopathological examinations of the samples in our study, the average percentages of sufficient and insufficient SM tissue specimens were found to be 52%,

Table 3. Assessment of histopathological adequacy of rectal suction biopsy

	Histopathological adequacy of biopsy	Mean	Standard deviation	p-value
Volume (mm^3)	Insufficient (n=9)	4.77	1.56	0.62
	Sufficient (n=15)	8.40	5.35	
SM/M	Insufficient (n=9)	0.77	0.46	0.55
	Sufficient (n=15)	1.22	0.54	
SM%	Insufficient (n=9)	0.40	0.11	0.33
	Sufficient (n=15)	0.52	0.11	

SM/M: Submucosa/mucosa; SM%: Percentage of submucosal tissue

Table 4. Assessment of histopathological adequacy of rectal suction biopsy according to age groups

			Age groups			
			Newborn	1 month-3 years	Total	p-value
Biopsy adequacy	Insufficient	Number (%)	4 (26.7)	5 (55.6)	9 (37.5)	0.212
	Sufficient	Number (%)	11 (73.3)	4 (44.4)	15 (62.5)	
Total		Number (%)	15 (100)	9 (100)	24 (100)	

and 40%, respectively, with a statistically significant difference between them (p=0.033). Although there are few prospective studies on this issue in the literature, remarkably retrospective studies reported that the rate of SM in the biopsy tissue material should not be less than 35-50% as the inclusion criteria for the samples[14,16,17]. In our study, similar rates were reported with these studies in the literature in terms of both the volume of RSB samples and the relative percentage of SM tissue.

In this study, we found that 73.3% of the biopsy materials obtained from the newborn age group were sufficient, while biopsy specimens retrieved from 44.4% of the patients aged between one month and three years were considered sufficient for histopathological examination. Although this difference is not statistically significant, tissue adequacy rates in RSB were higher in newborns than in older children in our study. When the studies in the literature are examined, different results draw attention when the diagnostic features of RSB according to age groups are evaluated. While some studies reported no difference in the diagnostic yield of RSB between age groups, several studies reported that the insufficiency rate was higher in infants younger than 1.5 months than in infants older than 1.5 months[5,22]. In another study, higher tissue adequacy rates were reported in babies older than a year[25]. According to some studies, it has been stated that the tissue adequacy ratio of RSB decreases after 3 years of age[20,26,27]. When it was desired to evaluate the literature, it was seen that different results prevented the formation of a consensus on this issue. Hypertrophic nerve fibres in the pathological evaluation of the tissue obtained is a positive finding favouring the diagnosis of HD. Previous attacks of enterocolitis and thicker intestinal mucosa could be considered a negative factor and may play a role in obtaining different results in each study. Although the immaturity of the ganglion cells in the neonatal period and the lack of hypertrophic nerve fibres are factors that challenge the pathologist, an experienced pathologist can make a definitive diagnosis with a sample that contains a sufficient amount of SM tissue. It is well-known that the FTRB procedure is more complex, especially in the newborn period, compared to older ages. In our study, a higher material adequacy rate in the newborn period could be achieved with the RSB method, and low complication rates were observed in patients of all ages. These results have shown that the BRS technique is an easily applicable and safe method in the neonatal period.

In patients with an initial diagnosis of HD, methods of anal dilatation and rectal irrigation are often recommended in terms of diagnosis and treatment during the decision-making process for biopsy[4]. We planned a prospective study by eliminating these pre-biopsy procedures for the first time in the literature, with the suspicion that both procedures may have an oedema-forming effect on the rectum wall and may prevent retrieval of sufficient SM tissue during the subsequent RSB. We concluded that our insufficient material rates were parallel to similarly designed previous studies in the literature. As a result, we have revealed that a 48-hour period without anal intervention before RSB does not provide any benefit, and it is possible to perform a biopsy whenever desired. When nine biopsy materials obtained from 24 patients considered insufficient for accurate diagnosis were not included in the evaluation, the diagnostic specificity and sensitivity of RSB performed in 15 patients were found to be 100%. Diagnostic sensitivity and specificity of RSB in HD have been reported as 88-93% and 95-99%, respectively[22,23,28-30]. Similar to our study, Sharp et al.[28] did not report any false negative or positive RSB results.

Compared to previous studies, our study yielded 100% diagnostic accuracy without any margin of error caused by the low number of cases. We consider that excluding suspicious RSB results from the evaluation and working with an experienced pathologist to establish the diagnosis of HD increased the accuracy of the study results. In further prospective randomized, double-blind studies including large-patient populations, it may be expected that our study's sensitivity and specificity rate, which was 100%, will decrease slightly. Therefore, this high rate supports the opinion that the RSB technique should be preferred over FTRB.

CONCLUSION

This study showed that diagnosing HD using the RSB provides a significant advantage over FTRB. It could be performed safely and without anaesthesia due to the very low rate of serious complications in newborn and older children. In addition, if the problem of insufficient amount of biopsy material required for accurate diagnosis is left aside, RSBs sensitivity and specificity rates were very high, leading to the prompt diagnosis of HD. We suggest RSB is the gold standard for diagnosing HD accurately.

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Ethics

Ethics Committee Approval: Approval was obtained from İzmir Katip Çelebi University Clinical Research Ethics Committee (decision number: 16, approval date: 02.09.2017).

Informed Consent: Prospective study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: C.B., M.O.Ö., T.Ö., G.K., Concept: C.B., M.O.Ö., G.K., Design: C.B., M.O.Ö., G.D., T.Ö., A.S., G.K., Data Collection and Processing: C.B., G.D., Analysis and Interpretation: M.O.Ö., Literature Search: C.B., M.O.Ö., G.K., Writing: C.B., M.O.Ö., G.D., A.S., G.K.

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Original Article

Arterial Stiffness and Subclinical Myocardial Dysfunction in Pediatric Asthma: A Novel Approach Using Aortic Propagation Velocity

Çocukluk Çağı Astımında Arteriyel Sertlik ve Subklinik Miyokardiyal Disfonksiyon: Yeni Bir Yaklaşım Olarak Aortik Yayılım Hızının Kullanımı

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ABSTRACT

Objective: Childhood asthma is associated with systemic inflammation, airway inflammation, and cardiac adverse effects such as right ventricular (RV) failure and pulmonary hypertension. Arterial stiffness, an early marker of atherosclerosis, can be assessed using the color M-mode-derived aortic propagation velocity (APV) method. This study aims both to evaluate APV as a measure of arterial stiffness and also subclinical myocardial dysfunction using Doppler echocardiography in children with asthma.

Method: This prospective study evaluated early markers of arterial stiffness and subclinical myocardial dysfunction in children with asthma compared to a control group. The study included 44 children with asthma and 40 healthy controls. Echocardiographic measurements, including tissue Doppler imaging and color M-mode-derived APV were performed to assess ventricular function and arterial stiffness. Pulmonary function tests were also conducted for asthmatic patients.

Results: Our study did not reveal any significant differences in APV, left ventricular function, mitral valve Em/Am ratios, and left heart myocardial performance indices between the asthma group and the control group. However, we observed a significant difference in the peak systolic velocity at the anterior leaflet of the tricuspid valve (tricuspid valve Em velocity), which suggests that diastolic function of the RV performance is impaired in children with asthma.

Conclusion: This study is the first to evaluate APV in young children with asthma and has found no significant correlation between asthma and arterial stiffness or subclinical atherosclerosis. However, it has revealed that children with asthma are more likely to have RV diastolic dysfunction. Further studies are needed to investigate the potential link between childhood asthma and subclinical atherosclerosis.

Keywords: Childhood asthma, arterial stiffness, aortic propagation velocity, diastolic dysfunction, tissue Doppler imaging

ÖZ

Amaç: Çocukluk çağı astımı sistemik enflamasyon ve hava yolu enflamasyonunun yanında sağ ventrikül (SV) yetersizliği ve pulmoner hipertansiyon gibi kardiyak etkilerle de ilişkilidir. Aterosklerozun erken bir belirteci olan arteryel sertlik, renkli M-mod Doppler'den türetilmiş aortik yayılım hızı (APV) yöntemiyle değerlendirilebilir. Bu çalışmada astımlı çocuklarda arteriyel sertliğin bir ölçütü olarak APV'nin ve doku Doppler ekokardiyografi ile subklinik miyokardiyal disfonksiyonun değerlendirilmesi amaçlandı.

Yöntem: Bu prospektif çalışmada astımlı çocuklar kontrol grubuyla karşılaştırılarak arteryel sertliği gösteren erken belirteçler ve subklinik miyokardiyal disfonksiyon değerlendirildi. Çalışmaya 44 astımlı ve 40 sağlıklı kontrol olmak üzere 84 çocuk dahil edildi. Doku Doppler görüntüleme ve renkli M-mod'dan türetilen APV ile ventrikül fonksiyonları ve arteriyel sertlik değerlendirildi. Astımlılara ayrıca solunum fonksiyon testleri de yapıldı.

Bulgular: Astımlı çocuklar ve kontrol grubu arasında APV, sol kalp fonksiyonu, mitral kapak Em/Am oranı ve sol kalp miyokardiyal performans indeksi açısından anlamlı bir fark yoktu. Bununla birlikte, astımlı çocuklarda SV diyastolik disfonksiyonuna işaret eden triküspit kapak Em hızında anlamlı fark izlendi.

Sonuç: Bu çalışma astımlı çocuklarda APV'yi değerlendiren ilk çalışmadır. Küçük çocuklarda astım ile arteriyel sertlik yani subklinik ateroskleroz arasında anlamlı bir ilişki bulunamadı. Bununla birlikte, astımı olan çocukların SV diyastolik disfonksiyonuna sahip olma ihtimalinin daha yüksek olduğu ortaya kondu. Çocukluk çağı astımı ile subklinik ateroskleroz arasındaki potansiyel ilişkiyi araştırmak için daha fazla kontrollü araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Çocukluk çağı astımı, arteriyel sertlik, aortik yayılım hızı, diyastolik disfonksiyon, doku Doppler görüntüleme

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INTRODUCTION

Childhood asthma is a prevailing chronic condition, and recent studies have indicated a rise in systemic inflammation and airway inflammation among individuals with asthma[1,2]. In children with asthma, the ventricular contractility is also influenced by the chronic inflammatory response[3]. In asthmatic patients, right ventricular (RV) failure, pulmonary hypertension, and atrial dilation are among the more frequently observed cardiac effects[4]. Atherosclerosis and asthma are both chronic inflammatory diseases. Inflammation leads to impaired endothelial cell function and chronic inflammation accelerates the development of atherosclerosis[5].

Aortic propagation velocity (APV) measurement using a color M-mode aortic practical way to evaluate the arterial stiffness in the thoracic aorta and is found to be inversely related to the extent of coronary artery disorder[6,7]. APV is more convenient for clinical use than other techniques, including pulse wave velocity, aortic distensibility, and aortic strain. Recent research has indicated that APV is similarly effective as pulse wave velocity and aortic distensibility for the evaluation of arterial stiffness, and it is also simpler to apply and replicate[8].

Asthma is characterized by chronic inflammation, which causes pulmonary vasoconstriction through the release of mediators and cytokines. Pulmonary vasoconstriction is further exacerbated by structural changes in pulmonary vessels due to parenchymal deterioriation, increased cardiac output, and blood viscosity due to hypoxia-induced polycythemia[9]. Asthmatic patients have increased intrathoracic pressure due to excessive breathing efforts, leading to RV afterload, and ultimately, to RV hypertrophy and diastolic dysfunction. RV diastolic dysfunction is a significant prognostic factor[10]. Left ventricular (LV) diastolic dysfunction, on the other hand, is related to the interventricular interaction, increased LV afterload, and decreased LV preload[10].

The use of color M-mode derived APV has not been investigated in children with asthma. Therefore, our aim was to evaluate the early signs of arterial stiffness in asthmatic children by using APV and also determine possible subclinical myocardial dysfunction using Doppler echocardiography.

MATERIALS and METHODS

Between December 2018 and July 2019, we conducted a prospective study at Kütayha Evliya Çelebi Training and Research Hospital. The study involved children who had been diagnosed with asthma at least 6 months before and were followed up at the pediatric allergy outpatient clinic. We also recruited healthy children who were matched by age and sex and visited the pediatric cardiology outpatient clinic as the control group. There were 84 children in the study, 44 in the asthma group and 40 in the control group. The parents provided the informed consent and the Kütahya Health Sciences University Ethics Committee approved the study (decision no: 2018/14-6, date: 14.11.2018). We followed the GINA Strategy 2018 guidelines to evaluate the diagnosis, treatment, and control of asthma symptoms[11].

The study excluded patients who had a respiratory tract infection or asthma attack in the previous month. Children aged 5 to 15 years who attended the pediatric cardiology outpatient clinic with heart murmurs or chest pain made up the control group. All patients underwent routine examinations. We also excluded participants who had a chronic lung disease, an atopic, rheumatologic, or autoimmune disease, were exposed to secondhand smoke, or had an asthma attack in the previous month. We carried out echocardiographic measurements for eligible participants in both groups, and pulmonary function and skin prick tests for asthmatic patients who met the study criteria.

Echocardiography

A Philips Affinity 50 echocardiography device (Philips Healthcare, Andover, Netherlands) with an S4-2 (3.4 MHz) transducer was used by the same pediatric cardiologist (R.Ö.) to perform all echocardiographic examinations. The methodology from previous guideline was applied to ensure the accuracy of all echocardiographic measurements[12]. TDI technique indices, including Em, Am, Em/Am, Sm, and myocardial performance index (MPI), were used to evaluate ventricular functions. A decreased Sm indicated impaired ventricular systolic function, while decreased Em and Em/Am ratios, as well as increased Am, indicated impaired diastolic function. An increased MPI index indicated impaired ventricular functions. We acquired suprasternal window images while the patients were lying in the supine position. We aligned a M-mode cursor with the flow direction in the aorta, using a Nyquist limit of 30-50 cm/s and an M-mode sweep rate of 200 mm/s. We adjusted the aliasing velocity to improve the velocity slope delineation, and divided the propagation slope distance by the velocity slope duration. The estimated value was defined as the APV (Figure 1).

Pulmonary Function Tests

Spirometric evaluations were done using Spirobank G USB spirometer (Rome, Italy). The patients were sitting and had a clip on their nasal airways during the tests, which were done at room temperature. The parameters of FEV1, FVC, FEV1/FVC ratio, PEF, and FEF 25-75 were used by experienced technicians to perform all pulmonary function tests as recommended[13].

Statistical Analysis

The study data were analyzed using the Statistical Package for the Social Sciences program (version 15.0, Chicago, Illinois, USA). The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test the normality of all data distributions. The variance homogeneity was determined using the Levene test. The group comparisons were evaluated using the Student t-test for parametric parameters and the Mann-Whitney U test for non-normally distributed data. The parametric parameters evaluated using the Student t-test were presented as meanstandard deviation, and the parameters evaluated using the Mann-Whitney U test were presented as median (interquartile range). The level of statistical significance for all data was p<0.05.

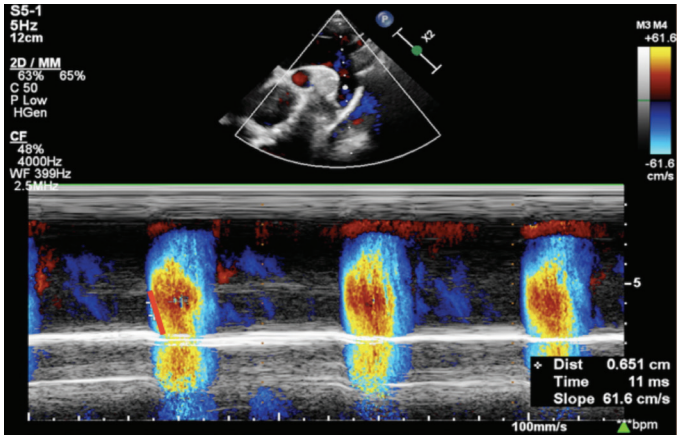

Figure 1. Aortic propagation velocity in a patient. The red line shows time slope

RESULTS

The study group had a mean age of 8.3±3.1 years, with no statistically significant difference compared to control group (p>0.05, Table 1). The mean Em/Am ratios of the mitral valve were 1.55±0.96 in the study group and 1.57±0.73 in the control group (p=0.72). The mean Em/Am ratios of the tricuspid valve were 1.25±0.43 in the study group and 1.13±0.33 in the control group (p=0.15). The mean mitral valve MPIs derived from tissue Doppler were 41±6 in the study group and 40±7 in the control group (p=0.91). The mean tricuspid valve MPIs derived from tissue Doppler were 45±9 in the study group and 44±6 in the control group (p=0.72). The mean Em velocities of the mitral valve were 0.15±0.03 m/s in the study group and 0.16±0.04 m/s in the control group (p=0.67). The mean Em velocities of the tricuspid valve were 0.15±0.032 m/s in the study group and 0.14±0.022 m/s in the control group (p=0.02) (Table 2). No statistically significant differences were detected between the groups for Am, Sm, Em, Em/Am ratio, and left heart MPI values obtained from the mitral lateral annulus in LV echocardiography (p=0.56, p=0.89, p=0.67, p=0.72, and p=0.91, respectively) (Table 2). Similarly, no statistically significant differences were seen between the groups for Am, Sm, Em/Am ratio, and right heart MPI parameters obtained from the tricuspid lateral annulus in RV echocardiography (p=0.95, p=0.8, p=0.15, and p=0.72, respectively). However, there was a statistically significant difference between the groups for measurements indicating the diastolic function of right cardiac performance, with tricuspid valve mean Em (m/s) being 0.15±0.032 in the study group and

Table 1. Characteristics of patients with asthma and spirometry findings

	Minimum	Maximum	Mean	SD
Age (year)	2.5	16	8.3	3.1
Weight (kg)	15	60	31.1	12.7
Height (cm)	95	160	129.4	16.9
FVC (cm^3)	52	123	82.2	13.5
FEV1 (L)	54	126	86.6	14.5
FEV1/FVC	87	117	104.1	8.3
PEF (L/min)	38	103	68.2	15.5
FEF 25/75	39	144	81.7	20.1

SD: Standard deviation, FVC: Forced vital capacity, FEV1: Forced expiratory volume in one second, PEF: Peak expiratory flow, FEF 25/5: Forced expiratory flow over the middle one-half of the FVC

0.14±0.022 in the control group (p=0.02). The mean APVs (cm/s) were 60±13.2 in the study group and 64±13 in the control group without any statistically significant intergroup difference (p=0.27). LV conventional M mode measurements did not differ statistically significantly between groups (Table 2).

DISCUSSION

Although publications have indicated cardiac involvement and impairment of ventricular function in bronchial asthma, the evaluation of aortic flow velocity using Doppler echocardiography have not been reported previously. In this study, we found that Em, a right ventricular diastolic function parameter, was affected in children with asthma. Myocardial performance indices of right and left ventricles were higher in asthmatic patients without any statistically significant inter-ventricular difference. The APV, which was investigated for the first time, was similar in both groups. Conflicting results have been shown by studies evaluating the right and LV functions in asthmatic patients, but it has been shown that RV function may be impaired in the early stage of the disease[14-17]. In the study of Abdalla and El Azeem[16], LV diastolic function was impaired in young adult asthmatic patients while RV diastolic function was unaffected. In the same study, it was found that LV MPI parameters impaired in study group, while RV MPI parameters did not demonstrate any significant intergroup difference. Similarly, in our study, RV MPI values did not show any deterioration. However, in our study, LV diastolic function was not statistically different between the asthmatic cases and the control group. In our study, only Em parameter of RV diastolic function was found to be affected in asthmatic children. In a study of Ozde et al.[18], the tricuspid Em was lower in the control group. However, Ozdemir et al.[14] showed that the tricuspid Em value was higher in the asthmatic children. We also found that Em was higher in asthmatic patients when compared to healthy subjects. These discrepancies may be explained by the younger age of our study participants and their response to treatment. Accordingly, our study participants were younger than those in the other two studies. In the study by Shedeed[4], RV diastolic functions worsened as the severity of asthma increased, and diastolic functions improved thanks to the reduction of RV afterload by treatment. As inflammation is involved in all stages of the atherosclerotic process[19], atherosclerosis is commonly recognized as a chronic

Table 2. Ventricular tissue Doppler and conventional echocardiographic measurements in patient and control groups

Variables	Patients (n=44)	Control subjects (n=40)	p-values
Mitral lateral annulus TDI			
Sm (m/s), median (IQR)	0.1 (0.02)	0.1 (0.03)	0.89
Em (m/s), mean ± SD	0.15±0.03	0.16±0.04	0.67
Am (m/s), median (IQR)	0.1 (0.03)	0.09 (0.03)	0.56
Em/Am, median (IQR)	1.55 (0.96)	1.57 (0.73)	0.72
LV MPI, mean ± SD	41±6	40±7	0.91
Tricuspid lateral annulus TDI			
Sm (m/s), mean ± SD	0.13±0.019	0.13±0.017	0.8
Em (m/s), mean ± SD	0.15±0.03	0.14±0.02	0.02
Am (m/s), median (IQR)	0.13 (0.06)	0.13 (0.06)	0.95
Em/Am, mean ± SD	1.25±0.43	1.13±0.33	0.15
RV MPI, mean ± SD	45±9	44±6	0.72
Aortic propagation velocity			
(cm/s), mean ± SD	60±13.2	64±13	0.27
M-mode parameters			
LVEDd (cm), mean ± SD	4.21±0.46	4.32±0.72	0.81
IVSDd (cm), mean ± SD	0.807±0.14	0.81±0.16	0.72
LVPWd (cm), mean ± SD	0.86±0.15	0.88±0.12	0.68
Fractional shortening (%), mean ± SD	40.1±6.2	42±3.8	0.59

IQR: Interquartile range, Sm: Systolic myocardial velocity, Em: Early diastolic myocardial velocity, Am: Late myocardial velocity, LV: Left ventricle, RV: Right ventricle, MPI: Myocardial performance index; LVEDd: Left ventricular end-diastolic diameter, IVSDd: Interventricular septal diameter in diastole, LVPWd: Left ventricular posterior wall thickness in diastole

inflammatory disease[20]. Inflammation serves as a shared underlying mechanism for the physiological and pathological modifications occurring during the onset and progression of atherosclerosis. It is well known that atherosclerosis affects both medium-sized and larger vessels, including the thoracic aorta.

Endothelial dysfunction serves as an early sign of vascular damage and subclinical atherosclerosis[21]. Moreover, endothelial damage causes vascular fibrosis in larger arteries, leading to reduced arterial elasticity[22]. Recently, the link between systemic inflammation and asthma has attracted increasing attention. A notable study by Wood et al.[1] demonstrated that a subset of asthmatic patients with airway inflammation had worsened systemic inflammation, as evidenced by elevated levels of IL-6 and high-sensitivity C-reactive protein. In addition, this systemic inflammation was associated with poorer clinical outcomes. Therefore, we aimed to evaluate subclinical atherosclerosis in asthmatic children using a novel method namely color M-mode-derived propagation velocity of the descending aorta (APV). The color M-mode propagation velocity is an effective tool for generating spatiotemporal maps of blood flow velocities within the arterial lumen which is currently used for noninvasive evaluation of aortic distensibility. Various studies have explored the correlation between APV and endothelial dysfunction and carotid intima-media thickness (CIMT) has been closely linked with APV[7,23]. Several studies have been conducted to examine the correlation between systemic inflammation and arterial stiffness[24,25]. One such study by Demiralp et al.[25] explored changes in aortic elasticity in patients with ankylosing spondylitis. The study showed that these patients had reduced aortic elasticity, irrespective of how long they had the disease, and their average aortic stiffness index was higher than the control group.

The development of atherosclerosis is thought to mediate these arterial wall changes, which can be explained by the adverse effects of inflammation. A study conducted by Karaman et al.[24] observed that patients diagnosed with Familial Mediterranean Fever (FMF) had lower APV values compared to control subjects. Moreover, a correlation was found between APV and mean CIMT values, with APV being identified as an independent predictor of FMF. The researchers proposed that the lower APV values may be linked to endothelial dysfunction and may indicate the presence of subclinical inflammation in FMF patients who had not cardiovascular involvement. Our study has revealed that asthmatic children had lower APV values compared to the control group. However, there was no significant statistical difference between both groups. Previous studies have explored the relationship between asthma and CIMT in both children and adults[26,27]. Cakmak et al.[26] demonstrated that CIMT values were significantly higher in asthmatic children compared to the control group. This increase is known to be correlated with the progression of atherosclerosis seen in adult patients with inflammation. Furthermore, the patient group had a significant increase in oxidative stress, which was positively correlated with CIMT. The findings of the current study do not support the results of Cakmak et al.'s [26] study. This discrepancy might be explained by our younger study population, different treatment strategies used and severity of asthma. One potential explanation could be linked to the diverse range of pathophysiological mechanisms associated with asthma. Liang et al.[28] found higher levels of systemic inflammation markers including leptin and vascular endothelial growth factor in asthmatic subjects. An adult study found that women with adult-onset asthma had thicker far (deeper) wall intima-media thickness, indicating carotid atherosclerosis, compared to non-asthmatic counterparts[27]. This difference in CIMT values was also detected between smoking and non-smoking women in the cohort which was partially explained by smoking, physical activity, pulmonary function, and other confounding factors. There was no significant difference in APV values among male and female asthmatic patients, according to our study. Childhood-onset asthma and asthma in men were not associated with carotid atherosclerosis[28].

Study Limitations

First limitation that deserve attention is the absence of data regarding the occurrence of systemic inflammation in the population under investigation. Another study limitation is that the color M-mode echocardiography method used for the assessment of APV based on aortic flow propagation velocity may be subject to errors. Careful attention is required in identifying the starting and ending points of the propagation slope, as even small placement errors can result in significant calculation errors. Secondly, the statistical power of the study may have been compromised due to the limited number of patients included in the analysis. Although our study provides valuable insights, since it was conducted at a single center, our findings require validation through prospective, multi center studies with long-term follow-up.

CONCLUSION

Assessment of APV for the first time in children with asthma have shown that asthma in small children is not significantly associated with arterial stiffness or subclinical atherosclerosis. This research study has also shown that children with asthma have RV diastolic dysfunction. Further research studies with control groups should be conducted to elucidate the association between asthma and subclinical atherosclerosis in asthmatic children.

Ethics

Ethics Committee Approval: The Kütahya Health Sciences University Ethics Committee approved the study (decision no: 2018/14-6, date: 14.11.2018).

Informed Consent: The parents provided the informed consent.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Concept: R.Ö., A.T, Design: R.Ö., B.G., Data Collection and/or Processing: H.B.İ, D.G., Analysis and/ or Interpretation: B.G, D.G, Literature Search: R.Ö., B.G., H.B.İ., D.G., Writing: R.Ö., B.G.

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Original Article

Molecular Heterogeneity in Neuroblastoma and Its Clinical Significance

Nöroblastomda Moleküler Heterojenite ve Klinik Önemi

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ABSTRACT

Objective: Tumor heterogeneity describes the differences between cancer cells in the same tumor sample. Neuroblastoma (NB) is a type of cancer where tumor heterogeneity complicates its treatment. This study aims to explore the role of molecular heterogeneity detected by routine molecular tests in NB.

Method: Seventy-one patients were included in the study. NB samples were chosen among 1,300 NB samples that were evaluated using molecular tests between 2012-2020 according to the guidelines of Turkish Pediatric Oncology Group Protocol. Molecular investigations were performed (total 142 samples) obtained from two different areas of the tumor (synchronous) or at two different times (metachronous). Heterogeneity was questioned for five tests: MYCN amplification, 1p36LOH, 11q23 deletion and 17q25 gain (identified with real-time polymerase chain reaction) and DNA ploidy (identified with flow cytometry).

Results: Heterogeneity was observed for MYCN in 22.53%, for 1p36LOH in 36.62%, for 11q23del in 29.58%, and for 17q25 gain in 40.85% of cases, while DNA ploidy was heterogeneous in 36.4% of cases. Molecular heterogeneity did not show statistical difference among metachronous and synchronous cases. High-risk cases more frequently displayed molecular heterogeneity without any statistically significant difference between both groups.

Conclusions: Our findings support the fact that molecular heterogeneity either exists in different areas of a tumor or seen in the same tumor at different times. It will be beneficial to perform more than one molecular analysis on the tumor tissue specimens. In addition, recurrences or re-biopsy specimens from metachronous metastases shall be re-evaluated using molecular tests in cases of NB.

Keywords: Neuroblastoma, MYCN, molecular heterogeneity

ÖZ

Amaç: Tümör heterojenitesi, aynı tümör numunesindeki kanser hücreleri arasındaki farklılıkları tanımlar. Nöroblastom (NB), tümör heterojenitesinin tedavisini zorlaştırdığı bir kanser türüdür. Bu çalışma, NB'de rutin moleküler testlerle tespit edilen moleküler heterojenliğin rolünü araştırmayı amaçlamaktadır.

Yöntem: Çalışmaya 71 hasta dahil edildi. NB örnekleri 2012-2020 yılları arasında moleküler testlerle değerlendirilen 1.300 NB örneği arasından Türk Pediatrik Onkoloji Grubu Protokolü yönergelerine göre seçildi. Tümörün iki farklı bölgesinden (senkron) veya iki farklı zamanda (metakron) alınan moleküler araştırmalar (toplam 142 örnek) yapıldı. Heterojenite beş test için sorgulandı: MYCN amplifikasyonu, 1p36LOH, 11q23 silme ve 17q25 kazancı (gerçek zamanlı PCR ile tanımlandı) ve DNA ploidi (akış sitometrisi ile tanımlandı).

Bulgular: MYCN için %22,53, 11q23del için %29,58, 1p36LOH için %36,62 ve 17q25 kazancı için %40,85 oranında heterojenite gözlenirken, DNA ploidisi %36,4 oranında heterojendi. Moleküler heterojenite, metakron ve senkron olgular arasında istatistiksel olarak farklılık göstermedi. Yüksek riskli olgularda her iki grup arasında anlamlı bir fark olmaksızın daha sık moleküler heterojenite sergilemiştir.

Sonuç: Bulgularımız, moleküler heterojenitenin bir tümörün farklı bölgelerinde var olduğunu veya aynı tümörde farklı zamanlarda görüldüğünü desteklemektedir. Tümör doku örneklerinde birden fazla moleküler analiz yapılması faydalı olacaktır. Ek olarak, metakron metastazlardan nüksler veya yeniden biyopsi örnekleri, NB olgularında moleküler testler kullanılarak yeniden değerlendirilmelidir.

Anahtar kelimeler: Nöroblastom, MYCN, moleküler heterojenite

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INTRODUCTION

Cancer heterogeneity refers to the presence of distinct biological features in various sections of the tumor, attributed to tumor cells exhibiting diverse characteristics[1-3]. In metachronous heterogeneity samples from the same patient at different times display different biological features. The simplest example of this is the disappearance of treatment response of a tumor due to survival of resistant cells after chemotherapy. In synchronous heterogeneity, samples taken from different regions of the same tumor display different biological features[4]. Treatment targeting specific molecules to reduce side effects in current medical approaches has gained importance with the identification and interpretation of heterogeneity[5]. The main mechanism in resistance against molecular targeted therapies is considered to be biological heterogeneity[6,7]. As a result, identification of different clones in targeted treatment and mapping these clones has gained importance in molecular treatment.

Neuroblastoma (NB) shows pronounced heterogeneity[8] which complicates the treatment of NB[9]. Contrary to good survival in the low and moderate risk groups, treatment efficacy is limited in the high-risk group. In Turkey, survival rates for NB are 100%, 75.8%, 34.1%, 6.5% and 59.4% for stage 1, 2, 3, 4 and 4S, respectively[10,11]. Turkish Pediatric Oncology Group (TPOG) NB data from 2009 shows that in the low-risk group the three-year overall survival (OS), and event free survival (EFS) rates were 98%, and 90%, respectively. In the moderate risk favourable histology group, three-year OS, and EFS were 100% and 93%, respectively. In the moderate risk unfavourable histology group, three-year OS, and EFS were 90% and 76%, respectively. In the high-risk group, conventional CT branch had three-year OS of 53% and EFS of 37%, whereas the high-dose computed tomography (CT) branch had OS of 59% and EFS of 33%[12]. Though NB contains a variety of molecular targets like ALK and GD-2[13,14], efficacy of molecular treatment due to pronounced heterogeneity is a topic of current research[8,15-17].

Studies related to molecular heterogeneity are based on investigation of pathologic sections and determination of cell features. As chromosomal aberrations are frequently observed in NB, FISH and qPCR methods are commonly used in heterogeneity studies. Currently, guidelines recommend performing two molecular studies in at least two areas of the tumor, if macroscopic areas from NB display different colors[18]. Though the presence of *MYCN* with molecular heterogeneity is known for a long time, clinical implications of this heterogeneity has not been clarified yet[16].

In our study, molecular investigations were performed prospectively in NB cases on tumor tissue samples obtained from two different areas at diagnosis (synchronous) or at two different time points (metachronous). Heterogeneity was examined with *MYCN* amplification, 1pLOH, 11q deletion and 17q gain identified with real-time polymerase chain reaction (PCR) and DNA ploidy identified with flow cytometry. The correlation between presence of heterogeneity with clinical findings was investigated with the aim of revealing the effect of molecular heterogeneity in NB on clinical risk classification.

MATERIALS and METHODS

Patient and Samples

Our research was approved by the Ethics Committee of Dokuz Eylül University Non-Interventional Research Ethics Committee (decision no: 2018/05-21, date: 15.02.2018) and patient relatives signed an informed consent form. The universe of the study comprised 1,300 fresh or paraffin-embedded tumor tissue samples sent to us between 2012-2020 for molecular evaluation according to the TPOG 2009 NB protocol. Two samples taken from the same patient for molecular investigation were included in two groups. The metachronous group (n=37) comprised all patients who gave more than one sample at least two months apart (a total of 1,300 samples, and 1,263 cases). The synchronous group (n=34) comprised randomized patients with enough tissue on surgical specimens retrieved from different areas of differentiation. Samples were chosen from differently colored macroscopic areas of the tumor. For the synchronous group, the first sample was taken from less differentiated dark-colored areas, the most undifferentiated blastic area (sample A), while the second sample was taken from the most differentiated area, which matches to light-colored areas in paraffin-embedded blocks (sample B). The clinical data for 71 patients included in the study (37 double sample metachronous group, 34 double sample synchronous group) were recorded from the patient medical files.

Molecular Investigations

Real time PCR and DNA ploidy method have been described in our previous studies[19].

DNA Isolation

DNA isolation was performed using a PCR (Roche® High Pure PCR Template Preparation Kit) kit according to manufacturer's guidelines. Fresh tumor tissue samples were imprinted on a slide, stained with toluidine blue and examined under a light microscope. Tumor tissue samples each 3 mm in diameter were used. After mechanical disintegration of tissue samples, they were left in proteinase K and lysis buffer for 2 hours at 55 °C. Paraffin-embedded samples were left in proteinase K and tissue lysis buffer overnight at 37 °C. The DNA samples were isolated according to manufacturer's guidelines. Quantification was assessed with Qubit® fluorometer.

Real-Time PCR

DNA obtained from tumor tissues were assessed in terms of *MYCN* amplification, 11q23 deletion, 1p36 LOH, and 17q25 gain with real-time PCR tests. Identification of abberations in these regions were assessed using labeled probes designed for these regions. The properties of genes are listed in Table 1.

The primer pairs, TaqMan probe, enzyme mixture were used for Real time PCR tests. As control DNA normal DNA not containing these aberrations (custom standard DNA) was used. Results were calculated as target/control proportional values and delta Ct method was used to assess rates of gene expressions according to cut-off values. For *MYCN* amplification, 10 times positive amplification was assessed. For 1p36 LOH and 11q23 deletion <0.5 value was accepted as positive, while for 17q25 gain >1.3 value was taken as cut-off value and results were recorded as positive or negative.

DNA Ploidy

The transfer solution (containing tumor cells after tissue was removed) was centrifuged at 2,000 rpm for 7 minutes, then tissue medium cells obtained were suspended in freeze media (95% RPMI complete solution (88% RPMI+10% Fetal Bovine Serum +1% L-glutamine+1% penicillin-streptomycin) +5% DMSO) for assessment of DNA index. These cells were stored at -20 °C until DNA index analysis. DNA index was identified with BD Accuri™ C6 cytometer using cell cycle kit. Flow cytometry analyzes light radiated at 564 nm and 606 nm by cells with stained routinely with propidium

Table 1. The Properties of real time PCR design

Gene	MYCN	GNB1 (guanine nucleotide binding protein (G protein))	ARCN1 (archain 1)	Survivin
Location	2p24.3	1p36.33	11q23.3	17q25
Mutation, $2^{-\Delta\Delta Ct}$	Amplification, >10	Deletion, <0.5	Deletion, <0.5	Gain, >1.3
Primer Pair	5'GTGCTCTCCAATTCTCGCCT-3' 5'-GATGGCCTAGAGGAGGGCT-3'	5'-AGCCAGTGGCAAATGCATT-3' 5'TCTCTGCAGCCCTACCATTGA-3'	5'- ATCTGGAGGCAGCACAGCT-3' 5'- TACACTGGATTATACCTGGCTGG-3'	5'-GGGCTGCCACGTCCAC-3' 5'-GTCGTCATCTGGCTCCCA-3'
Taqman Probe	'5'FAM-CACTAAAGTTCCTT CCACCCTCTCCT-TAMRA-3'	'5'-FAMAGCAAATCAAGACA TCATGTAAACGCTCA- BBQ(tamra)'	'5'-FAM- CCATGATCACAG AGACCATCATTGAAA-BBQ(tamra)	'5'FAM-TTCATCCACTGCCC CACTGAGAACGA-TAMRA-3'
Reference Gene, Location	NAGK (n-acetylglucosamine kinase) 2p13.3	NGFB (nerve growth factor (beta polypeptide) 1p13.1	MYBPC3(myosin binding protein C cardiac) 11p11.2	TP53 17p13.1
Reference Gene Primer Pair	5'-TGGGCAGACACATCGTAGCA-3' 5'-CACCTTCACTCCCACCTCAAC-3'	5'- TTCTATCCTGGCCACACTGAG-3' 5'- TTCTGACTTGCCCTCTCAGGT-3'	5'-TGGTGTACGAGATGCGCGTC-3' 5'TCACCGATAGGCATGAAGGG-3'	5'TGTCCTTCCTGGAGCGATCT-3' 5'-CAAACCCCTGGTTTAGCACTTC -3'
Reference Gene Taqman Probe	'5'-VIC-TGTTGCCCGAGAT TGACCCCGT-TAMRA-3' '	5'-FAM(VIC)- TTG CCA AGG TCC TCC CTC TGC AGC T-BBQ (tamra)	'5'-FAM(VIC)- TCAACGCCATC GGCATGTCCAGG-BBQ(tamra)	'5'-(FAM) Yakima yellow-CAGC CCCCGGCTCCGCTAGA-TAMRA-3'

iodide. The fluorescent histograms obtained are assessed for the presence of DNA aneuploidy using normal cells from peripheral blood mononuclear cells (PBMN). DNA index is calculated by dividing relative DNA content mode in the sample G0/G1 population by relative DNA content in the control G0/G1 population. Additionally, variation coefficients are given for each G0/G1 peak.

Statistical Analysis

Findings were analyzed using SPSS 22.0. After descriptive statistics, cases were assessed for the presence of heterogeneity. The parameters were tested with Kolmogorov-Smirnov test under normality plots. Chi-square or Fisher's exact test were done as needed to the smallest groups and two-element parameters. Age was in normal Q-Q plot with p=0.0001. After normal distribution tests non-parametric (chi-square, Mann-Whitney U tests) or parametric tests (t-test) (for normal distribution) were used for statistical analysis.

RESULTS

In total, 71 double samples (142 samples from 71 patients) were obtained in this study from 71 cases. The metachronous group comprised 37 cases, while synchronous group comprised 34 cases. The cases comprised 35 girls and 36 boys. The metachronous group comprised 6 low, 5 intermediate and 26 high-risk cases, while the synchronous group comprised 14 low, 7 intermediate and 13 high-risk cases. The clinical and molecular features are present in Table 2.

Heterogeneities regarding MYCN (22.53%), 1pLOH (36.62%), 11qdel (29.58%), 17qGain (40.85%) were detected at indicated rates. 36.4% of the cases was heterogeneous on DNA ploidy. Based on patients' clinical data, 58.5% of cases had metastatic disease and 32.7% of them had relapse. Among patients with available survival information, 67.3% (33/49) had disease-free survival, 8.5% had survival with disease and 22.4% died. In total, 6 cases had risk difference between samples. Two of these 6 cases were diagnosed with Stage 4S at the time of diagnosis, which was later corrected to stage 4. One case was refractory to treatment so transferred from the moderate-risk unfavorable histology group to high-risk group. Three cases had variations in risk groups due to heterogeneity. All these cases were negative for *MYCN* with risk class variations due to heterogeneity observed in terms of 1pLOH, 11qdel and 17qGain. All cases with *MYCN* heterogeneity were in the high-risk group (Table 3).

In our study, the case coded 18 had a unique characteristic and so it is appropriate to present relevant findings independently. The female patient aged 12 months and 1 week was assessed in the low-risk group due to being stage 2B at the time of diagnosis but she was later included in the refractory treatment group due to lack of response to treatment. The patient died at 30 months and 2 weeks of age (survival time: 18 months 1 week). Two tissue samples were assessed with Shimada classification as unfavourable histology. Firstly tru-cut (NB407) biopsy samples, and 3-days later resection

Table 2. Distribution of metachronous and synchronous cases according to molecular heterogeneity. Molecular heterogeneity did not show statistical difference among metachronous and synchronous cases. In both groups high-risk cases more often showed molecular heterogeneity

	MYCN heteroGENEITY	**1pLOH heteroGENEITY**	**11q Del heteroGENEITY**	**17q 25 Gain heteroGENEITY**
MetaCHRONous	27.02% (10/37) Low risk: 0/6 Intermediate risk: 0/5 High risk: 10/26	40.54% (15/37) Low risk: 1/6 Intermediate risk: 3/5 High risk: 10/26	21.62% (8/37) Low risk: 0/6 Intermediate risk: 1/5 High risk: 7/26	45.95% (17/37) Low risk: 1/6 Intermediate risk: 2/5 High risk: 17/26
sYNCHRONous	17.65% (6/34) Low risk: 1/14 Intermediate risk: 0/7 High risk: 5/13	35.29% (13/34) Low risk: 6/14 Intermediate risk: 1/7 High risk: 3/13	38.23% (13/34) Low risk: 6/14 Intermediate risk: 1/7 High risk: 6/13	32.35% (11/34) Low risk: 4/14 Intermediate risk: 1/7 High risk: 4/13
Total	22.53% (16/71) Low risk: 1/20 Intermediate risk: 0/12 High risk: 15/39	36.62% (26/71) Low risk: 7/20 Intermediate risk: 4/12 High risk: 13/39	29.58% (21/71) Low risk: 6/20 Intermediate risk: 2/12 High risk: 13/39	40.85% (29/71) Low risk: 5/20 Intermediate risk: 3/12 High risk: 21/39
P-value chi-square	0.7	0.07	0.54	0.277

material (NB413) were sent to us. Samples were assessed in the synchronous group, and any differences in risk groups could not be found in samples coded NB407 and NB413 as for gene expressions studied [*MYCN*: negative (<5), 1pLOH: positive, 11qdel: negative, 17qGain: negative]. Later, a second sample was taken from NB413 for better compatibility with the synchronous group (NB413-B). This sample had *MYCN* amplification (139 times) with 11qdel- negativity, 17qGain- negativity and 1pLOH could not be studied. This patient would be assessed as low-risk group according to TPOG-2009 NB protocol (19); however, the *MYCN* amplification in the third tissue sample obtained from the patient leads to consideration of different genetic structuring in this region.

The chi-square test showed no correlation between gender and heterogeneity, risk differences and frequencies of metastases. Chi-square test could not reveal any correlation between metachronous-synchronous groups and heterogeneity, risk differences and frequencies of metastases. Though high-risk cases more frequently showed molecular heterogeneities, the Fisher exact test could detect any statistically significance difference between both groups (p=0.190).

DISCUSSION

In this study, molecular investigation was performed in tumor tissue samples taken from two different areas of tumor samples were examined prospectively at time of diagnosis (synchronous) or at two different time points (metachronous) to investigate heterogeneity in different samples of NB cases. Our study used PCR for molecular investigation instead of the FISH method used in the literature and rates of heterogeneity were compared with RT-PCR findings. Though the presence of *MYCN* heterogeneous cases is known in NB[17,20,21], studies about other genetic markers are limited in number. In our study, 1pLOH, 11qdel and 17qGain heterogeneity was observed with high frequency and this heterogeneity may affect clinical decision-making process.

In the literature, the percentage of heterogeneity for *MYCN* amplification identified with the FISH method in NB patients is above 40%[22]. In our study, we used PCR method instead of FISH method for identification of heterogeneity and our lower heterogeneity rates might be due to our preference for PCR.

Marrano et al.[17] reported a series of 30 cases where 102 tumor tissue specimens were examined for *MYCN* amplification to reveal heterogeneity. They studied metachronous tissue samples for comparison, evaluated *MYCN* status before and after treatment, and showed changes in *MYCN* expression in 20 cases. *MYCN* copy number was reduced in nine cases. Focal MYCN amplification was observed in five cases that had initially shown diffuse MYCN amplification. Conversely, two cases initially exhibiting focal MYCN amplification transitioned to diffuse MYCN amplification. Furthermore, one case underwent a change from diffuse MYCN amplification to MYCN gain. Additionally, three cases initially demonstrating focal amplification in the first specimen later tested negative for MYCN amplification. When we compare their findings with ours, although we used a different method for evaluation of our data, greater number of cases included in our study, and investigation of both synchronous and metachronous cases constitute strengths of our study We also studied

Table 3. Clinical properties of the cases. The cases with double samples in both metachronous and synchronous groups are similar in distribution

	n	Mean age months	Sex M: n F: n	MycN amp	1p LOH	11q del	17q gain	Risk class
Metachronous	37	38.81±40.34 (0-192) Median: 30	M: 17 F: 20	7 18.9%	14 37.8%	10 27%	15 40.5%	Low risk: 6 Intermediate risk: 5 High risk: 26
Synchronous	34	38.09±44.86 (2-192) Median: 18	M: 19 F: 15	5 14.7%	14 41.2%	10 29.4%	17 50%	Low risk: 14 Intermediate risk: 7 High risk: 13
Total	71	38.47±42.26 (0-192) Median: 24	M: 36 F: 35	12 16.9%	28 39.4%	20 28.2%	32 45.1%	Low risk: 20 Intermediate risk: 12 High risk: 39

M: Male, F: Female

molecular heterogeneity in other parameters including 1p36LOH, 11q23 deletion, 17q25 gain and DNA ploidy.

Study Limitations

Heterogeneity in metachronous cases of NB should be determined. This point of view may be helpful in approaching to molecular evaluation of NB from a different perspective. The main cause of samples sent from clinics being recollected and resent is sample insufficiency, which is the main limitation due to very scarce amount of data for dependent group assessment of patients included in our study. In clinical practice, oncology patients are not always available for rebiopsy at relapse and/or treatment-refractory conditions.

Investigation of specific genes in chromosomal regions using more specific genetic markers for heterogeneity in NB may ensure more effective research about the significance of heterogeneity in NB especially for next-generation sequencing. In this way, an effective increase in rates of clinical prediction and more successful treatment planning for high-risk NB cases may be achieved[23-25].

CONCLUSION

Based on our results we have concluded that there is molecular heterogeneity for *MYCN* amplification, 1p36LOH, 11q23 deletion, 17q25 gain and DNA ploidy in NB. It will be beneficial to perform molecular studies more than once on several tissues in NB cases. Our findings suggest that it will be beneficial to perform molecular analyses by sampling as much tumor as possible in cases where full response could not be achieved from the treatment of recurrence(s) or treatments applied at different time periods during the clinical course of NB. As number of studies on liquid biopsy or bone marrow aspiration increase, more clarity will be gained about whether these applications can take the place of tumor tissue biopsy.

Ethics

Ethics Committee Approval: Our research was approved by the Ethics Committee of Dokuz Eylül University Non-Interventional Research Ethics Committee (decision no: 2018/05-21, date: 15.02.2018).

Informed Consent: Patient relatives signed informed consent forms.

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Author Contributions

Surgical and Medical Practices: T.Ç.A., S.A., Concept: T.Ç.A., S.A., Design: T.Ç.A., S.A., P.E., N.O., Data Collection or Processing: T.Ç.A., S.A., E.Ö.S., P.E., M.A., Ö.E.G., A.E., Analysis or Interpretation: T.Ç.A., Literature Search: T.Ç.A., S.A., E.Ö.S., Z.A., N.O., Writing: T.Ç.A., S.A., E.Ö.S., Z.A.

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Original Article

A Practical Approach to Super Refractory Status Epilepticus in Pediatric Intensive Care Unit

Çocuk Yoğun Bakım Ünitesinde Süper Refrakter Status Epileptikus'a Pratik Yaklaşım

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ABSTRACT

Objective: In this study, we aimed to evaluate the demographic, clinical features, long-term electroencephalography (EEG) findings and treatment modalities of pediatric patients with super refractory status epilepticus (SRSE).

Method: A retrospective, observational study was conducted in patients diagnosed as SRSE between 1 June 2018 and 30 May 2021 in the pediatric intensive care unit. Patients with SRSE between 1 month and 18 years of age who underwent continuous electroencephalogram (cEEG) monitoring were included in the study. Demographic data, clinical, and electroencephalographic characteristics were collected.

Results: A total of 11 patients were included in the study. The median age of the patients was 31 months (IQR 8-72 months). Nine (81.8%) patients had symptomatic etiology. Of the symptomatic etiologies, 4 (36.3%) patients had acute symptomatic, 3 (27.2%) patients had remote symptomatic and 2 (18.2%) patients had progressive etiology. The most common etiology was immune-related. The median cEEG duration of the patients was 60 hours (IQR 52-72 hours). Midazolam infusion was given to 11 (100%) patients, ketamine infusion was given to 9 (81.8%) patients, thiopental infusion was given to 6 (54.5%) patients, and propofol infusion was given to 2 (18.1%) patients as coma induction treatment. Intravenous immunoglobulin, corticosteroid and plasmapheresis were administered to 3 (27.2%) patients with immune etiology. The overall mortality was 18.1%.

Conclusion: SRSE is a neurological emergency with high mortality and morbidity. cEEG monitoring is very important in diagnosis and treatment. Immune etiology should be considered in long-lasting seizures, especially if they are resistant to anesthetics. The immunomodulatory therapy should be started.

Keywords: Super refractory status epilepticus, continuous electroencephalographic monitoring, non-convulsive status epilepticus, febrile infection-related epilepsy syndrome, pediatric intensive care unit

ÖZ

Amaç: Bu çalışmada, süper refrakter status epileptikus'lu (SRSE) çocuk hastaların demografik, klinik özellikleri, uzun dönem elektroensefalografi (EEG) bulguları ve tedavi modalitelerini değerlendirmeyi amaçladık.

Yöntem: Bir Haziran 2018-30 Mayıs 2021 tarihleri arasında çocuk yoğun bakım ünitesinde SRSE tanısı konulan hastalarda retrospektif, gözlemsel bir çalışma yapıldı. Çalışmaya 1 ay-18 yaş arasında sürekli elektroensefalogram (cEEG) takibi yapılan SRSE'li hastalar dahil edildi. Demografik veriler, klinik ve el EEG özellikler toplandı.

Bulgular: Çalışmaya toplam 11 hasta dahil edildi. Hastaların medyan yaşı 31 aydı (IQR 8-72 ay). Dokuz (%81,8) hastada semptomatik etiyoloji mevcuttu. Semptomatik etiyolojilerden 4 (%36,3) hastada akut semptomatik, 3 (%27,2) hastada remote semptomatik ve 2 (%18,2) hastada progresif etiyoloji vardı. En sık etyoloji immün nedenli idi. Hastaların medyan cEEG süresi 60 saat (IQR 52-72 saat) idi. On bir (%100) hastaya midazolam infüzyonu, 9 (%81,8) hastaya ketamin infüzyonu, 6 (%54,5) hastaya tiyopental infüzyonu ve 2 (18,8) hastaya koma indüksiyon tedavisi olarak propofol infüzyonu verildi. İmmün etiyolojisi olan 3 (%27,2) hastaya intravenöz immünoglobulin, kortikosteroid ve plazmaferez uygulandı. Genel mortalite %18,1 idi.

Sonuç: SRSE yüksek mortalite ve morbiditeye neden olan nörolojik bir acildir. Sürekli elektroensefalogram takibi tanı ve tedavide çok önemlidir. Uzun süreli ve özellikle anesteziklere dirençli nöbetlerde immün etiyoloji düşünülmelidir. İmmünomodülatör tedavi başlatılmalıdır.

Anahtar kelimeler: Süper refrakter status epilepticus, sürekli elektroensefalogram izlemi, non-konvülzif status epilepticus, febril ilişkili epilepsi sendromu, çocuk yoğun bakım ünitesi

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INTRODUCTION

Status epilepticus (SE) is defined as a seizure lasting longer than 5 minutes, or 2 or more consecutive seizures without consciousness. Refractory status epilepticus (RSE) is defined as SE that does not respond to an adequate dose of benzodiazepines and another appropriately selected second-line antiepileptic therapy. Super refractory status epilepticus (SRSE) is defined as seizure activity lasting more than 24 hours despite the anesthetic drugs given and recurrent seizures during reduction of anesthetic drugs[1,2]. There are few epidemiological studies on SRSE in the pediatric age group; In a retrospective study, SRSE was reported to occur in 7.14% of 602 cases with SE[3]. The most commonly reported etiologies are acute symptomatic causes (infectious or immune-mediated encephalitis, central nervous system infections, traumatic brain injury, brain ischemia); remote symptomatic causes (e.g., lymphoproliferative disease, human immunodeficiency virus infection, hypoxic-ischemic encephalopathy, developmental delay, epilepsy), progressive encephalopathies (metabolic diseases, epileptic encephalopathies), and cryptogenic etiologies[1,3,4]. Low serum antiepileptic drug (AED) levels in children with epilepsy, acute symptomatic etiologies, and neurodegenerative diseases are common risk factors for SRSE[5-8].

SRSE mortality has been reported to be 15.4-39.9% in adult patients and 5-20% in pediatric patients. An irreversible serious neurological deficit may develop in the majority of survivors[3,7,9-11]. The most commonly used drugs for treatment in SRSE are anesthetics such as midazolam, barbiturates, ketamine, and propofol[12]. The response of patients with immune etiologies to anesthetics are poor and they respond better to immunomodulatory treatment [steroid, intravenous immunoglobulin (IVIG), plasma exchange][13]. However, to date, there is still no evidence of optimal treatment for the individual pediatric patient. Difficulties in treatment arise because the underlying etiologies are not always immediately recognized and treatment options are limited by prolonged seizures. Treatment decisions are mainly based on case series or expert opinions. The comparative efficacy of different treatment strategies has not been evaluated in large prospective serie or randomized clinical trials[2,11-13].

Our study aims to evaluate the demographic, clinical features, long-term EEG findings, and treatment modalities of patients with SRSE.

MATERIALS and METHODS

A retrospective, observational study was conducted at the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, in pediatric intensive care unit (PICU) from June 1, 2018, and May 30, 2021, in patients diagnosed with SRSE.

Inclusion criteria;

One month to 18 years old with SRSE,

Patients with SRSE undergoing continuous electroencephalogram (cEEG) monitoring

Exclusion criteria;

All patients aged 1 month ≤ >18 years,

Patients whose cEEG monitoring cannot be performed or whose data cannot be accessed for technical reasons, patients with deficiencies in demographic and clinical data.

SRSE was defined as seizure activity for more than 24 hours despite administration of anesthetic drugs or recurrent seizure during tapering of the anesthetic drug. In the follow-up of all patients; EEG monitoring lasting at least 24 hours was used. It was done using the Nihon Kohden (Neurofax EEG-1200) EEG system. EEG interpretation was done by a pediatric neurologist. Patients with electrographic findings without clinical seizures (without motor symptoms) were defined as non-convulsive SE, and patients with both clinical seizures (with motor symptoms) and electrographic seizures were defined as convulsive SE. cEEG findings are divided into 4 different localizations: lateralized epileptic activity, bilateral independent epileptic activity, multifocal epileptic activity, and generalized epileptic activity[14]. Age, gender, previous neurological diseases, other etiologies, length of hospital stay, number of AEDs for prophylaxis, irregular use of AED, mechanical ventilator support, positive microbial cultures (blood, urine, and cerebrospinal fluid (CSF), bronchoalveolar lavage, nasopharyngeal aspirate), magnetic resonance findings, the pediatric risk score of mortality (PRISM III) and mortality were recorded. Treatments were administered and recorded within the framework of the protocol of our PICU (Table 1).

The ethics committee approval for the study was obtained from Ethics Committee of the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital (decision no: 2019/356, date: 02.01.2020).

Statistical Analysis

Descriptive statistics were given as mean ± standard deviation for normal distribution, medians and interquartile ranges (IQR) for abnormally distributed parameters, and numbers and percentages for categorical variables. Statistical analysis was performed using SPSS statistical software (version 22; SPSS, Chicago, IL, USA).

RESULTS

A total of 11 patients, 4 (36.4%) boys, and 7 (63.6%) girls were included in the study. The median age of the patients was 31 months (IQR 8-72 months) and 5 patients were under 2 years of age. Nine (81.8%) patients had symptomatic etiology (Table 2). Of the symptomatic etiologies, 4 (36.3%) patients had acute symptomatic, 3 (27.2%) patients had remote symptomatic and 2 (18.2%) patients had progressive etiology. Immune etiology was the most common among symptomatic etiologies. Two (18.2%) patients were diagnosed as non-convulsive SE after cEEG activity was detected in the comatose state without clinical seizures. Four of the patients were hospitalized with the diagnosis of febrile SE. Blood, urine, and CSF, bronchoalveolar lavage, nasopharyngeal aspirate cultures were taken for the etiology of fever. In one patient with a diagnosis of hypoxic-ischemic encephalopathy, *Klebsiella pneumonia* was grown in blood culture and it was defined as SRSE triggered by infection with the clinic of sepsis. Herpes virus was detected in CSF viral polymerase chain reaction of 1 patient followed up for encephalitis and herpes encephalitis was diagnosed. The cultures of the patients who were given SRSE treatment, who had complaints of high fever and impaired consciousness, were found to be negative in terms of infectious. For etiology and differential diagnosis, viral antibody tests in serum (toxoplasma, rubella, cytomegalovirus, and herpes simplex), antinuclear antibody, thyroid antibody tests, oncological evaluation, limbic encephalitis panel, methyl-d-aspartate (NMDA) receptor antibodies in CSF, and serum and neuroradiological imaging were performed and NMDA encephalitis was diagnosed in 1 patient. Cranial MR imaging was found to be abnormal in 3 of 4 patients diagnosed with encephalitis, while it was found to be normal in a 6.5-year-old patient with a diagnosis of FIRES. Mechanical ventilator support was given to all patients. While 4 patients were intubated with respiratory failure in the course of refractory SE,

Table 1. Status epilepticus protocol of our pediatric intensive care unit

0-5 minutes Emergency stabilitizion Airway protection Breathing support Monitoring Check glukose and electrolytes	**Midazolam IV:** 0.1- 0.2 mg/kg/dose (max 5 mg) **Midazolam IM:** 0.1- 0.2 mg/kg/dose (If vascular access no available)
5-15 minutes	**Midazolam IV/IO:** 0.2 mg/kg/dose (may repeat if seizures persist) If vascular access no available, use intraosseous vascular access
15-30 minutes	**Phenytoin IV:** 20 mg/kg/dose (max 1,000 mg) **Sodium valproate IV:** 20 mg/kg/dose (If >2 years, max 3,000 mg) **Levetiracetam IV:** 30 mg/kg/dose (max 4,500 mg) **Phenobarbital IV:** 15-20 mg/kg/dose (max 1,000 mg) If there are antiepileptics used by the patients, a loading dose is given, and if necessary, the drug that the patient does not use one of the AED selected according to according individual characteristics of the patient as a second-line treatment
30-60 minutes	**Midazolam:** After 0.2 mg/kg bolus, 0.1 mg/kg/h infusion is started. An additional bolus of 0.2 mg/kg is given every 10-15 minutes and titrated by increasing the infusion rate by 1 mg/kg/h EEG monitoring is started
Refractory SE & Super refractory SE	**Ketamine IV infusion:** 1 mg/kg IV loading dose, after 1-6 mg/kg/h continuous IV infusion **Thiopental sodium IV infusion:** 3 mg/kg IV loading dose, after 1-6 mg/kg/h continuous IV infusion. Titrate by 1 mg/kg/hour every 10 minutes **Propofol IV infusion:** 1-2 mg/kg loading dose, 1-12mg/kg/h continuous IV infusion **Pyridoxine:** <3 years patients 100 mg/dose IV

AED: Antiepileptic drugs, SE: Status epilepticus

7 patients progressed to SRSE and were intubated electively with titration of anesthetic drugs.

Benzodiazepines (IV 2 doses of midazolam) were initially given to all of our patients as first-line therapy except for patients with FIRES, herpes encephalitis, and NMDA encephalitis, all patients had a previous diagnosis of epilepsy and had a history of multiple AED use. In patients diagnosed with epilepsy, a loading dose was applied to the drugs used as second-line therapy, and/or another AED was added to the treatment. As second-line therapy, phenytoin 5 (45.5%), levetiracetam 8 (72.7%), phenobarbital 7 (63.6%), sodium valproate 3 (27.3%), topiramate 2 (18.2%), clonazepam 2 (18.2%) and clobazam 2 (18.2%) patients were given. cEEG monitoring was started in refractory and super-refractory SE patients with ongoing seizures. Midazolam infusion was given to 11 (100%) patients, ketamine infusion was given to 9 (81.8%) patients, thiopental infusion was given to 6 (54.5%) patients, and propofol infusion was given to 2 (18.1%) patients as coma induction treatment. IVIG, high-dose corticosteroids and were administered to 3 (27.2%) patients with immune etiology. Antiviral agents were started in patients with encephalitis. In addition, intravenous pyridoxine was given to 5 (45.4%) patients and magnesium sulfate infusion to 9 (81.8%) patients. It was not given because the other 2 patients had high serum magnesium levels (>3 mg/dL). After clinical seizure or electrographic seizure was controlled for at least 24 hours (by providing burst suppression), anesthetics were started to be decreased every 4-6 hours according to cEEG monitoring and increased again in those who developed seizure activity again. The median cEEG duration of the patients was 60 hours (IQR 52-72 hours). When we evaluate the cEEG data; 2 (18.1%) had lateralized, 3 (27.2%) multifocal, and 5 (45.5%) generalized epileptic activity. The median time to burst suppression after induction of anesthetic was 4 hours (IQR 2-7 hours). All patients developed medical complications during their intensive care stay. Inotropic therapy was initiated as a result of pneumonia in 3 patients, urosepsis in 1 patient, electrolyte disturbance in all patients, and cardiovascular dysfunction in 4 patients with prolonged seizure duration. In 1 patient; mechanical ventilator-associated lung injury and severe ARDS developed (Table 3). Two patients died after multiorgan dysfunction and the overall mortality was 18.1%. One patient could not tolerate weaning from the mechanical ventilator for a long time and was discharged with a tracheostomy. Th median length of stay in the PICU was 13 days (8-20 days), and the median PRISM score was 10[6-17].

Table 2. Etiological classification of case

Etiologies	n (%)
Structural disorders	1 (9.1%)
Hypoxic-ischemic encephalopathy	1 (9.1%)
Genetic epilepsy syndromes	2 (18.2%)
West syndrome	1 (9.1%)
Dravet syndrome	1 (9.1%)
Metabolic disorders	2 (18.2%)
Pyruvate dehydrogenase deficiency	1 (9.1%)
GLUT 1 deficiency	1 (9.1%)
Immunologic etiology	3 (27.3%)
Anti-N-methyl-d-aspartate receptor encephalitis	1 (9.1%)
FIRES	2 (18.2%)
Infectious etiology	1 (9.1%)
Herpes encephalitis	1 (9.1%)
Unknown (cryptogenic)	2 (18.2%)
Idiopathic generalized epilepsies	2 (18.2%)
Structural disorders	1 (9.1%)
Hypoxic-ischemic encephalopathy	1 (9.1%)
Genetic epilepsy syndromes	2 (18.2%)
West syndrome	1 (9.1%)
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Unknown (cryptogenic)	2 (18.2%)
Idiopathic generalized epilepsies	2 (18.2%)

GLUT: Glucose transporter, FIRES: Febrile infection-related epilepsy syndrome

DISCUSSION

Super refractory status epilepticus (SRSE) is a neurological emergency with high mortality and morbidity. Studies for SRSE are limited and generally based on case reports or small series[2-4]. SRSE presents in different age groups according to the etiology. In a study conducted in the PICU, the mean age was 5.4 years[15], and in another study, the median age was 7 years[11]. In our study, we found a lower median age (31 months) compared to other studies, and 4 (36.3%) patients were under the age of 2 years. Common risk factors are that the dose of

Table 3. Clinical characteristics of all patients

Patient no/ gender/age (month)	Etiology	MR findings	AED(s)	Convulsive/ non-convulsive	Complication	cEEG findings before treatment	Mortality	Treatments (induction and immunomodulatory)
1/F/6	Hypoxic-ischemic encephalopathy	Diffuse hypomyelinated areas in the cerebral hemispheres	3	Convulsive	Urosepsis, multiple organ dysfunction syndrome, electrolyte disturbance	Sharp-wave discharges in fronto centro temporal regions	Non-survivor	Midazolam Ketamine Pyridoxine
2/F/8	West syndrome	Areas of hyperintense gliosis in T2A-FLAIR in the basal ganglionic localization in the parietooccipital region	3	Convulsive	ARDS, electrolyte disturbance	Active multifocal epileptic activity	Survivor	Midazolam Ketamine Thiopental Pyridoxine
3/F/72	Dravet syndrome	Atrophy of cerebral sulci, leukoaraiosis in cerebral white matter	3	Convulsive	Electrolyte disturbance	Active generalized epileptic disorder	Survivor	Midazolam Ketamine Thiopental Propofol
4/M/10	Pyruvate dehydrogenase deficiency	In white matter, T2A is high at basal ganglionic levels, T1A isointense pathological signal intensity foci	3	Convulsive	Electrolyte disturbance, pneumonia	Focal epileptic activity in the bilateral frontotemporal region	Survivor	Midazolam Ketamine Thiopental Propofol Pyridoxine
5/M/31	Glucose transporter protein type 1 deficiency	Diffuse atrophy of cerebral cortical sulci	3	Non-convulsive	Multiple organ dysfunction syndrome, electrolyte disturbance	Generalized spike-wave bursts in the temporo-parieto-occipital regions	Non-survivor	Midazolam Ketamine Thiopental Pyridoxine
6/F/164	Anti-N-methyl-d-aspartate receptor encephalitis	Right posterior parietal region T2-FLAIR hyperintense focal focus. diffusion bilateral thalamic region acute ischemia	0	Non-convulsive	Cardiovascular dysfunction, electrolyte disturbance	Sharp spike-wave discharges in bilateral temporooccipital regions	Survivor	Midazolam Ketamine Corticosteroids IVIG Plasmapheresis

Table 3. Continued

Patient no/ gender/age (month)	Etiology	MR findings	AED(s)	Convulsive/ non-convulsive	Complication	cEEG findings before treatment	Mortality	Treatments (induction and immunomodulatory)
7/F/60	Febrile infection-related epilepsy syndrome	Mild leukomalacia changes in white matter	0	Convulsive	Electrolyte disturbance, pneumonia	Significant generalized spike-wave discharges in bilateral temporo-parietal regions	Survivor	Midazolam Ketamine Corticosteroids IVIG Plasmapheresis
8/M/80	Febrile infection-related epilepsy syndrome	Normal	0	Convulsive	Electrolyte disturbance,	Pronounced generalized spike-wave discharges in bilateral temporo parieto areas	Survivor	Midazolam Ketamine Corticosteroids IVIG Plasmapheresis
9/F/80	Herpes encephalitis	Hyperintense signals in the left frontotemporal region on T2 images	2	Convulsive	Electrolyte disturbance,	Slow-wave activity in the left hemisphere	Survivor	Midazolam Ketamine
10/M/13	Idiopathic generalized epilepsy	Atrophy of cerebral sulci Chronic leukomalacia changes in periventricular white matter	3	Convulsive	Electrolyte disturbance, pneumonia	Very active generalized epileptic activity	Survivor	Midazolam Ketamine Thiopental Pyridoxine
11/M/60	Idiopathic generalized epilepsy	Normal	2	Convulsive	Cardiovascular dysfunction, electrolyte disturbance	Sharp spike-wave activity in the right hemisphere fronto sentro temporoparietal region	Survivor	Midazolam Thiopental

AED used in patients with epilepsy is not given at an appropriate dose, that the dose is reduced, and that the serum AED level is low[6,8]. In our study, serum AED levels of 4 patients were significantly lower than the therapeutic range. The etiologies of SRSE patients differs in many studies, and the most common acute symptomatic causes (infectious or immune-mediated encephalitis, traumatic brain injury, brain ischemia) are observed[4]. In another study, the most common etiology was progressive encephalopathy[3]. In our study, immune etiologies were found to be the most common acute symptomatic etiologies. Among them, 2 patients were diagnosed with FIRES and 1 patient was diagnosed with anti-NMDA-receptor encephalitis. FIRES is a variant of NORSE that previously affected completely healthy children and was thought to be immune-mediated, but the pathogenesis was not known clearly[13-16]. It is typical for a febrile episode to occur between 24 hours and 2 weeks before the onset of the refractory seizure. Fever was described a few days ago in 2 patients aged 5 years and 6.5 years without any known disease. Another important disease among immune etiologies is anti-NMDA-receptor encephalitis. Patients clinically; may present with fever, headache, behavioral disorder, and RSE/SRSE. Immunomodulation therapy (steroid, intravenous immunoglobulin, plasma exchange) was also applied in anti-NMDA-receptor encephalitis[17]. EEG monitoring has a very important place in the diagnosis of SRSE and the evaluation of treatment. Non-convulsive SE, characterized by electrical activity without motor findings, can be seen in 15% of comatose patients[18]. In our study, two (18.1%) patients were diagnosed with nonconvulsive SE in EEG monitoring performed by presenting in a comatose condition. EEG monitoring also plays an important role in the titration of anesthetic infusions. In our practice, we dose anesthetic infusions to induce burst suppression in cEEG. In a multicenter study, the median cEEG duration was 30 hours[19], and in a study with a high number of FIRES patients, the median cEEG duration was 9 days[11]. In our study, the median cEEG duration was 60 hours. In general, we perform cEEG monitoring until the anesthetics are discontinued after creating burst suppression and following at least 24 hours without seizures. The treatment approach in SRSE aims to prevent neuronal excitotoxicity, neuroprotection and prevent systemic complications, and to provide seizure control[2]. Although coma induction is the most common treatment, there are no randomized controlled studies to guide clinical practice[11,20]. Our general approach for coma induction; under the guidance of cEEG monitoring, anesthetics are started to be reduced in the follow-up of seizure-free monitoring for at least 24 hours. If electrographic or clinical seizures are observed again during reduction, anesthetics are increased again and cEEG monitoring is continued. Benzodiazepines are used as first-line therapy in SRSE. In our study, intravenous midazolam was given to all patients as first-line treatment. Second-line treatment selection was planned following our intensive care protocol. It was determined according to the age of the patient, the antiepileptic he used, and the seizure type. For example we didn't prefer valproate under 2 years of age. Among this group of drugs, we used levetiracetam, phenobarbital, phenytoin, and sodium valproate, respectively. In the literature, no superiority has been shown in terms of the effectiveness of second-line treatments[21]. The main treatment in SRSE is coma induction and immunomodulatory (corticosteroids, intravenous immunoglobulin, plasmapheresis) treatments. Among the anesthetics inducing a coma, midazolam is the most commonly used, followed by barbiturates, ketamine, and propofol[12,13]. In some studies, it has been shown that SRSE can be controlled with a ketogenic diet[15]. In our study, midazolam infusion was applied to all patients by starting cEEG monitoring. After then ketamine infusion was given to 81.8% of the patients. Thiopental was used in 54.5% and propofol in 18.1% of patients. In our study, midazolam infusion was found to be the most common agent, similar to the literature[22]. In some studies, the most commonly used agents after midazolam failure were found to be barbiturates[11,23]. In our study, we detected more frequently ketamine, which also acts on the NMDA receptor and is frequently used as a sedo-analgesia in our intensive care unit. In our practice, we see that the side effect profile is lower than other coma-inducing agents, apart from seizure control. It is used with increasing frequency in the treatment of SE, especially in pediatric intensive care units[24,25]. We think that it will be included in the guidelines for earlier step treatments with future studies. All of the anesthetics used for coma induction have serious side effects. With the induction of midazolam, hemodynamic instability and suppression of the respiratory system are common[12,24]. Barbiturates may cause stronger cardiorespiratory suppression, arrhythmia, immunosuppression, renal failure, and multiple organ failure[24]. Ketamine may cause cardiac side effects, increased secretion, and signs

of laryngospasm[25]. Propofol, on the other hand, may cause propofol infusion syndrome characterized by heart failure, rhabdomyolysis, metabolic acidosis, and renal failure[26,27]. Complications may occur with the prolongation of the SRSE period, apart from the side effects of anesthetic drugs. In our study, it was observed that inotropic therapy was initiated in all patients as a result of electrolyte disturbances and cardiovascular dysfunction in 4 patients, severe ARDS developed in 1 patient, and 2 patients with multiple organ dysfunction syndrome (MODS) were found to have died. Immunological treatments such as IVIG, corticosteroid, and plasmapheresis have been reported in many studies. However, there are no randomized studies in the literature, but small series and case reports[28,29]. It is used especially in autoimmune encephalitis because it is held responsible for seizures[30]. In our study, IVIG, steroids, and plasmapheresis were applied to 2 patients diagnosed with FIRES and 1 patient diagnosed with NMDA encephalitis due to immune etiology. All patients received IVIG 1 gr/kg for 2 days, methylprednisolone 3 days pulse 30 mg/kg, then maintenance 2 mg/kg and was discontinued depending on the clinic. Plasmapheresis treatment was applied to all patients for 10 days. Patients with immune etiology had longer seizure control and hospital stay. However, mortality did not develop in the patients, except for comorbidity. The mortality rate of SRSE varies between 5-20% in pediatric patients[3,9,11]. In our study, the mortality rate was found to be 18.1%. When the patients who died were evaluated, it was observed that although seizure control was achieved, the patients died due to sepsis and subsequent MODS.

Study Limitations

The most important limitation of the study is the small number of cases and its retrospective design. Since the data in our study were analyzed retrospectively, long-term neurological outcomes were not included.

CONCLUSION

In our study, we evaluated the clinical features of patients diagnosed with SRSE. Since there are no studies in the literature to guide treatment management, we planned the treatments within the framework of our intensive care protocol. cEEG monitoring should be used in the recognition of seizures and titration of anesthetic drugs. Immune etiologies should be considered and immunomodulatory treatment should not be delayed, especially in SRSE patients with history, clinical course, cEEG findings, and seizures resistant to anesthetics.

Ethics

Ethics Committee Approval: The ethics committee approval for the study was obtained from Ethics Committee of the University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital (decision no: 2019/356, date: 02.01.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer reviewed.

Author Contributions

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

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Case Report

A Case of Waardenburg Syndrome Type 1 with Maturity-onset Diabetes of The Young Type 2

MODY Tip 2'nin Eşlik Ettiği Waardenburg Sendromu Tip 1 Olgusu

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ABSTRACT

Waardenburg syndrome (WS) is known as a group of genetic conditions associated with hearing problems and pigmentary abnormalities of the hair, skin, and eyes. The association between WS and maturity-onset diabetes of the young (MODY) is rarely reported. Herein we present a 9-year-old male patient with MODY type 2 and WS whose genetic analysis revealed a known pathogenic variant i.e. c.143G>A (p.Gly48Asp)(c.1603+2T>C) in paired box gene 3.

Keywords: Waardenburg syndrome, glucokinase gene mutation, diabetes mellitus

ÖZ

Waardenburg sendromu (WS), işitme kaybı ile saç, deri ve gözlerdeki pigment anormallikleri ile ilişkili bir grup genetik hastalık olarak bilinir. WS ile gençlerin olgunluk başlangıçlı diyabeti (MODY) arasındaki ilişki nadiren bildirilmektedir. Bu çalışmada, genetik analizi sonucu paired box gene 3'te patojenik varyantı c.143G>A'yı (p.Gly48Asp)(c.1603+2T>C) saptanan MODY tip 2'nin eşlik ettigi WS'li 9 yaşında bir erkek hasta sunulmaktadır.

Anahtar kelimeler: Waardenburg sendromu, glukokinaz gen mutasyonu, diabetes mellitus

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INTRODUCTION

Waardenburg syndrome (WS) is known as an inherited disorder associated with sensorineural deafness and pigmentary abnormalities, affecting the skin, hair, and eyes. The incidence of this autosomal dominant disorder is estimated to be roughly 2/100,000 worldwide. This syndrome manifests itself with sensorineural deafness; pigmentation defects of the skin, hair, and iris; and various defects of neural crest-derived tissues. Paired box gene 3 (PAX3) mutations are responsible for most cases of WS type 1 cases[1]. To date, only one case of WS associated with diabetes mellitus (DM) has been reported in the literature[2].

We report a 9-year-old male patient with MODY type 2 and WS whose genetic analysis revealed a known pathogenic variant i.e. c.143G>A (p.Gly48Asp) (c.1603+2T>C) in PAX3 in this study.

CASE REPORT

A 9-year-old boy of a consanguineous family was admitted to the pediatric endocrine department because of fasting and postprandial hyperglycemia. The patient had no hypoglycemic events in postnatal history. His medical history revealed that his aunt and uncle had fasting and postprandial hyperglycemia. His auxologic measurements were as follows: height: 131.5 cm (10-25 p), height standard deviation score (SDS): -0.76, weight: 28.9 kg (25-50p), weight SDS: -0.29, body mass index:

16.72 kg/m^2, and body mass index SDS: 0.16. Physical examination revealed a brilliant blue iris with dystopia canthorum, skin hypopigmentation, synophrys, broad nasal root, hypoplasia of the alae nasi, and mild sensorineural hearing loss (Figure 1). Based on diagnostic clinical criteria of Waardenburg Consortium (1992) diagnosis of WS was made[1]. A known missense pathogenic variant i.e. c.143G>A (p.Gly48Asp) in exon 2 of PAX3 was found in the proband and this variant had been transmitted from his mother. Levels of some of his remarkable laboratory parameters were as follows: blood glucose: 148 mg/dL (75-100 mg/dL), insulin: 19.1 IU/mL (2-18 IU/mL), serum C-peptide l: 3.72 ng/mL (1.1-4.4 ng/mL). HbA1c: 6.2%, anti-insulin antibody: 0.01 U/mL (0-0.5 U/mL), anti-GAD: <1U/mL (<1U/mL), and islet cell antibody was negative. Abnormal fasting and postprandial blood glucose levels were found when blood glucose levels of our patient were monitored for 5 days (Figure 2). When our patient was evaluated for maturity-onset diabetes of the young (MODY), MODY type 2 was diagnosed with heterozygote c.313delC (p.H105TfsX11) variant in the glucokinase gene. Repaglinide (0.3 mg/kg/d po bid) was started for glycemic control (Figure 2). Glycemic control was achieved, and fasting (130-140 mg/dL), and postprandial (150-180 mg/dL) blood glucose levels (150-180 mg/dL) were lowered to their normal levels (80, and 138 mg/dL, respectively) after repaglinide treatment. The participant' parent gave written, informed consent.

DISCUSSION

High fasting and abnormal postprandial levels of glycemia detected during the follow-up should suggest the presence of type 1 or 2 DM, MODY, mitochondrial diabetes, and Wolfram syndrome[3]. The work-up should include measurements of serum glucose, insulin, C-peptide, HbA1c levels, and diabetes insulin autoantibodies. In our patient mitochondrial diabetes, type 1 DM should be considered due to congenital rubella infection, and Wolfram syndrome due to the discordance between the serum glucose and insulin levels, lower C-peptide levels accompanied with family history, and hearing loss[3]. The immunodiagnostic autoantibodies in the present case were not detected during the period when non-immune diabetes was diagnosed. Our patient, did not present with diabetic ketoacidosis, low insulin, and C-peptide levels compatible with his blood glucose levels, thus type 1 DM was not considered. This study aimed to explain diabetes and hearing loss and to present signs under a single entity and WS that was rarely reported in association with DM, and different disease states that were not initially considered in the literature. The patient's hearing loss and diabetes and family history suggested Wolfram syndrome or mitochondrial diabetes. However, these diseases were excluded for the following reasons: lack of family history suggesting hereditary mitochondrial diabetes, normal optic and retinal examination, and absence of clinical findings suggestive of endocrine dysfunction and neuromuscular diseases. His physical examination revealed a brilliant blue iris with dystopia canthorum, skin hypopigmentation, synophrys, broad nasal root, hypoplasia of the alae nasi, and mild sensorineural hearing loss, thus WS was diagnosed associated with c.143G>A (p.Gly48Asp) pathogenic variant in exon 2 of PAX3 gene. With only one case report of WS with DM in the literature, concomitant presence of two different diseases were considered and MODY type 2 was diagnosed with heterozygote c.313delC (p.H105TfsX11) variant in the glucokinase gene.

Dystopia canthorum, brilliant blue iris, and synophrys were present in our patient, which are the most distinguishing diagnostic features of WS1[4]. Two or one major and two minor criteria must be present to

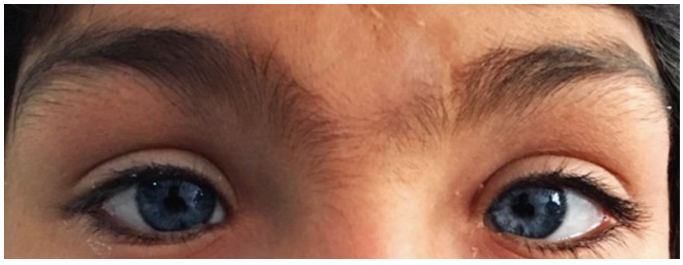

Figure 1. Dysmorphic features of the patient with Waardenburg syndrome

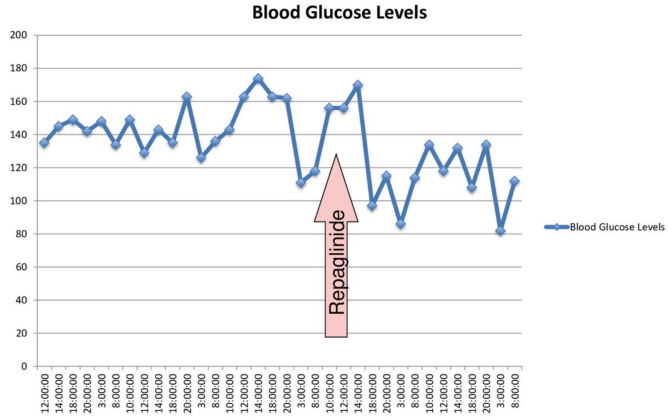

Figure 2. Decreasing trend in blood glucose levels after treatment with repaglinide

diagnose WS according to diagnostic criteria proposed by the Waardenburg consortium[1]. Evaluation of our patient based on these criteria revealed WS type 1 with four major and four minor diagnostic criteria. Galler et al.[5] reported the presence of MODY in 2.4% of their patients with newly diagnosed DM. Additionally, the Search for Diabetes in Youth study investigated MODY genes in 586 patients according to the MODY diagnostic criteria and found glucokinase gene mutation in 2.3% of their study participants. This study also reported the presence of MODY patients with an incidence rate of 8.0% in their cohort[6]. Herein we present a case of WS accompanied by glucokinase gene mutation, which was believed to be the first reported WS associated with this concomitant disorder. According to literature data, Kashima et al.[2] reported WS with diabetic retinopathy. A 30-year-old female patient presented with physical examination findings of vitreous hemorrhage and hypochromic iris, and hypopigmentation of the fundus. The *PAX3* gene homeobox domain mutation was revealed because of hypopigmentation of the fundus associated with the diagnosis of WS type 1. This patient presented with a microvascular complication of type 2 DM, such as diabetic retinopathy. After initiation of treatment with repaglinide, normal fasting and postprandial blood glucose levels were achieved and the risk of microvascular complications of DM was reduced. Kashima et al.[2] reported the association between the severity of diabetic retinopathy and the degree of hypopigmentation in the posterior fundus. The authors speculated that the hypopigmentation of the fundus in WS induced the aggravation of diabetic retinopathy. No relationship was found between WS and MODY in the literature. To our knowledge, this is the first report of a case of WS with concomitant MODY in the literature. Further observations are needed to disclose the association between DM and WS.

Ethics

Informed Consent: The participant' parent gave written, informed consent.

Peer-review: Externally peer reviewed.

Author Contributions

Surgical and Medical Practices: H.A.K., B.Ö., Concept: H.A.K., B.Ö., Design: H.A.K., B.Ö., Data Collection or Processing: L.Ö., Analysis or Interpretation: L.Ö., Literature Search: H.A.K., B.Ö., Writing: H.A.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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Case Report

A Unique Case with Tracheal Atresia Among Published Literature on TACRD and VACTERL Associations

TACRD ve VACTERL Birliktelikleri Hakkında Yayınlanmış Literatürler Arasında Trakeal Atrezili Özgün Bir Olgu

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ABSTRACT

Tracheal atresia/agenesis (TA) is associated with other congenital anomalies (TACRD and/or VACTERL). In this study, a female newborn with TA who had all the elements associated with TACRD and VACTERL was reported. The patient with TA, tracheo-esophageal fistula (TEF) connecting to the esophagus at the level of the carina, persistent left superior vena cava, duodenal atresia, vertebral and limb defects, bilateral hydronephrosis, and persistent cloaca was operated on postnatal 5th day. Band ligation to the abdominal esophagus, gastrostomy, duodenal atresia repair and diverting colostomy due to persistent cloaca were performed. The lungs were bilaterally expanded in the first postnatal, preoperative and postoperative chest radiographs. Oxygen saturation remained above 90% until the patient died suddenly on the postnatal 8th day. TA is a very rare congenital anomaly that causes postnatal respiratory distress. This case is unique among the literature on TACRD and VACTERL associations for many reasons. Esophageal band ligation in TA cases with TEF is a method that keeps the pressure in the esophagus at an appropriate level and provides air passage to the trachea via fistula.

Keywords: Duodenal atresia, newborn, TACRD, tracheal atresia/agenesis, VACTERL

ÖZ

Trakeal atrezi/agenezi (TA) diğer konjenital anomalilerle (TACRD ve/veya VACTERL) ilişkilidir. Bu çalışmada, TACRD ve VACTERL ile ilişkili tüm özelliklere sahip TA'lı bir kız yenidoğan raporlandı. TA, özofagusa karina seviyesinde bağlanan trakeo-özofageal fistül (TEF), persistan sol superior vena kava, duodenal atrezi, vertebra ve ekstremite defektleri, bilateral hidronefroz ve persistan kloaka tanılı hasta postnatal 5. günde opere edildi. Abdominal özofagusa bant ligasyonu, gastrostomi, duodenal atrezi onarımı ve persistan kloaka nedeniyle diverjan kolostomi uygulandı. Postnatal ilk, preoperatif ve postoperatif çekilen grafilerde akciğerler bilateral ekspanseydi. Hastanın oksijen satürasyonu, postnatal 8. gündeki ani ölümüne kadar, %90'ın üzerinde kaldı. TA, postnatal solunum sıkıntısına neden olan, çok nadir görülen bir konjenital anomalidir. Bu olgu, birçok nedenden dolayı, TACRD ve VACTERL birliktelikleri ile ilgili literatür arasında benzersizdir. TEF'li TA olgularında özofagus bant ligasyonu özofagustaki basıncı uygun seviyede tutan ve fistül yoluyla trakeaya hava geçişini sağlayan bir yöntemdir.

Anahtar kelimeler: Duodenal atrezi, yenidoğan, TACRD, trakeal atrezi/agenezi, VACTERL

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INTRODUCTION

Tracheal atresia/agenesis (TA) is a very rare congenital anomaly that causes postpartum respiratory distress and subsequent difficulties with endotracheal intubation[1]. The trachea below the larynx is completely or partially absent, and tracheo-esophageal or broncho-esophageal fistulas of varying shapes are usually seen.

Despite advances in surgical treatment methods, the mortality rate in TA is high, and about 85% of children with TA die within two days after birth[2]. The diagnosis is often made at post-mortem examination. Concomitant tracheo-esophageal or broncho-esophageal fistulas allow ventilation and resuscitation after esophageal intubation. After birth, a timely diagnosis and effective airway treatment are essential for patients' survival[3].

Although the cause of TA is still unknown, it is often associated with the components of TACRD (tracheal agenesis, cardiac, renal and duodenal malformations) and VACTERL (vertebral anomalies, anal atresia, cardiovascular anomalies, trachea-esophageal fistula (TEF), esophageal atresia, renal/radial anomalies, limb defects) associations[4]. In this study, a female newborn

with TA who had all the elements associated with TACRD and VACTERL was reported.

CASE REPORT

A female baby weighing 1,800 grams, born from a 27-year-old mother at the 34th gestational week, who was followed up with the diagnoses of Down syndrome, duodenal atresia and anomaly of great vessels in the antenatal period, was emergently consulted to the Department of Pediatric Surgery due to respiratory and endotracheal intubation difficulties. Abdominal "double bubble" appearance and left persistent superior vena cava were reported in the prenatal ultrasound. In the laryngoscopy performed during the initial evaluation, it was observed that the tracheal lumen was closed just distal to the vocal cords. Good ventilated lungs after esophageal intubation suggested the diagnosis of TA with TEF. Extremity anomalies and persistent cloaca were also detected in the patient. After the patient was stabilized, the lungs were bilaterally expanded in the X-ray, and also the diagnosis of duodenal atresia was confirmed with the abdominal "double bubble" appearance (Figure 1).

During the follow-up period in the neonatal intensive care unit, the patient underwent thoracic computed tomography on the postnatal 3rd day. The diagnosis of Floyd type II TA was confirmed by computed tomography. It was observed that the tracheal segment distal to the vocal cords was agenetic, and the main bronchi were connected to the esophagus with a fistula at the level of the carina (Figure 2). Thus, the patient had all the components of TACRD and VACTERL associations due to the diagnosis of other diseases such as persistent left superior vena cava, duodenal atresia, vertebral and limb defects, bilateral hydronephrosis, and persistent cloaca accompanying TA with TEF.

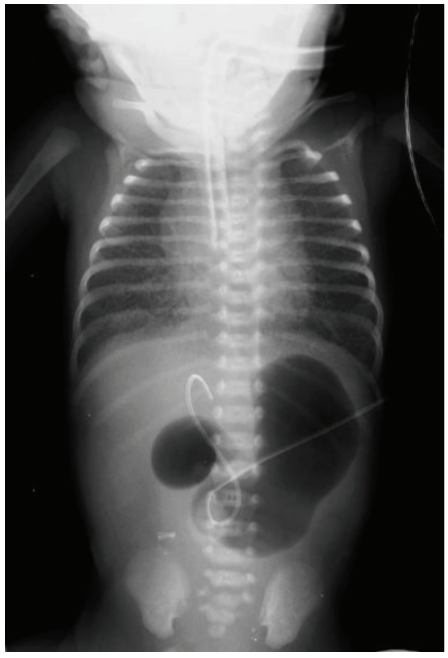

Figure 1. Postnatal first chest and abdominal X-ray

The patient, whose oxygen saturation was above 90% and who was clinically stable during the follow-up, was operated on the postnatal 5th day when the deteriorated coagulation parameters returned to the normal range. The lungs were bilaterally expanded in the preoperative chest X-ray (Figure 3). The patient underwent esophageal band ligation, which was expected to create intraluminal ventilation pressure provided by duodenal atresia in the preoperative period, gastrostomy, duodenal atresia repair, and colostomy due to persistent cloaca. The lungs were also bilaterally expanded in the postoperative chest X-ray (Figure 3), and no early complications were observed. The patient died on the postnatal 8th day due to sudden worsening of clinical condition and cardiac arrest.

During the hospital stay, informed consent was obtained from the patient's family that scientific studies could be conducted in the future.

DISCUSSION

The estimated incidence of tracheal abnormalities is two per 100,000 live births including severe congenital stenosis and atresia. Fewer than 100 cases have been recorded in the medical literature to date, and Payne reported the first case identified in 1900[5]. Floyd et al.[6] divided TA into three groups according to its severity. In type 1 TA (13%), the distal trachea is connected to the esophagus with a fistula. The carina opens directly into the esophagus in type 2 TA (65%). In type 3 TA (22%), both main bronchi open into the esophagus separately (Figure 2)[1,6].

The endoderm of the proximal foregut produces the trachea as a ventral protrusion. The original ventral respiratory diverticulum descends to create the trachea, links with the sixth aortic arch, and splits distally. It is encircled by angiogenic mesenchyme. Total to partial foregut cleavage failure is thought to cause TA[7].

Although most researchers claim the presence of at least three components for diagnosis, there is still no consensus on definitive diagnostic criteria for the VACTERL association. The exact etiology of the

VACTERL association is unknown. Its pathophysiology may include faulty mesodermal development during embryogenesis leading to overlapping symptoms. Possible causes include maternal diabetes, teratogenic medications, physical stress, uterine vascular disease, infertility treatment, and the use of oral contraceptives in the first trimester[8]. Furthermore, some authors believe that TA is not part of the VACTERL association, but that TA is one of the malformations in a different association pattern known as TACRD, which includes complex congenital heart anomalies, renal anomalies, and duodenal atresia[9].

In the delivery room, during the first postnatal examination, TA should be included in the differential diagnosis in newborns who do not cry and have findings such as apnea and difficulty in intubation[10]. Respiratory decompensation due to gastric decompression should primarily suggest an unusual airway anatomy. Gastric decompression can reduce esophageal pressure and airflow to the lungs due to high resistance TEF in patients with TA. Normally, removing extra air from the stomach improves ventilation by reducing pressure on the diaphragm. The bradycardia attacks occurring in the case with duodenal atresia in this study may be related to pressure changes due to gastric decompression. In addition, it can be thought that duodenal atresia provides the appropriate pressure in the preoperative period, enabling the lungs to be ventilated from the TEF without any problems. Prevention of pneumonia caused by reflux of gastric fluids or saliva may be the long-term benefit of decompression[10].

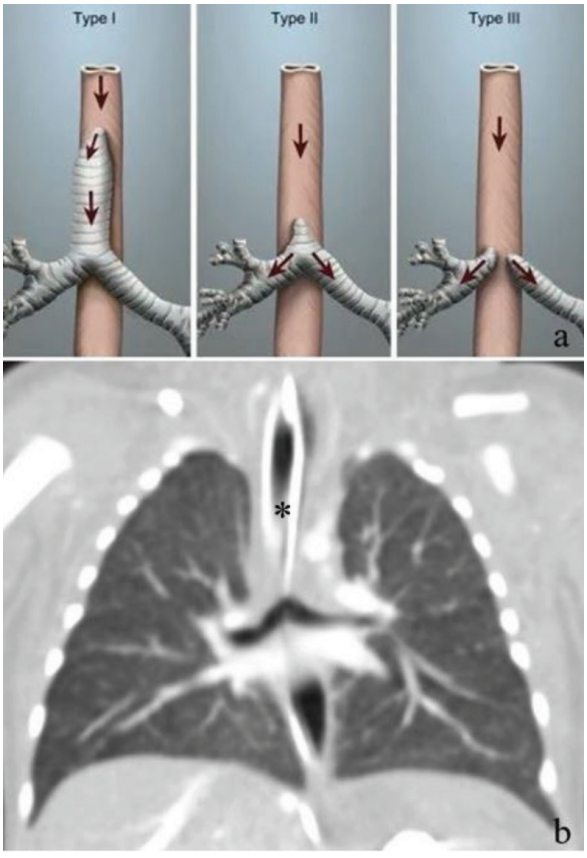

Figure 2. Floyd's TA classification **(a)** and type II TA **(b)** with TEF (Asterisk: Endotracheal tube inserted esophagus. **(a)** is from https://entokey.com/congenital-tracheal-anomalies/#fig1)

The diagnosis of airway obstruction in the prenatal period requires some precautions to be taken. In this condition, the most commonly used and most effective method is the EXIT (ex utero intrapartum treatment) procedure. In this method, the infant born by cesarean section is evaluated for possible airway obstruction before the umbilical cord is clamped (while fetal circulation continues) and tracheal intubation is provided if possible. When intubation is not possible, airway safety is ensured by emergency tracheotomy[11]. In this study, the EXIT procedure was not performed in the case who had no prenatal diagnosis, and lung ventilation was provided with an intubation tube placed in the esophagus of the patient who did not have the possibility of intubation or tracheotomy due to TA.

This recent case is very unique among the literature on TACRD and VACTERL associations for four reasons, although the patient was lost as a result of sudden cardiac arrest on the 3rd postoperative day. First of all, TA was confirmed by initial postnatal examination and subsequent imaging. Secondly, our patient was a

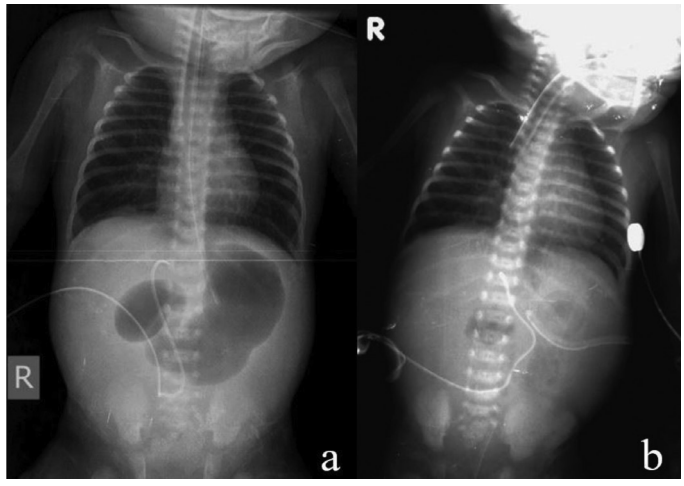

Figure 3. Preoperative **(a)** and postoperative **(b)** chest X-rays

case with multiple organ anomalies consistent with all TACRD and VACTERL associations. Third, despite having TA, the patient's lungs were expanded and oxygen saturation remained above 90% after esophageal intubation due to duodenal atresia. Fourth, abdominal esophageal band ligation performed before duodenal atresia repair ensured the infant's survival for three more postoperative days.

In conclusion, TA is a very rare congenital anomaly that causes postnatal respiratory distress and makes intubation impossible or complicated. This case is unique in that it includes all components of the TACRD and VACTERL associations. Detection of tracheal occlusion in the prenatal period may prevent the possible bad consequences of hypo-oxygenation in the infant. Esophageal band ligation is a viable method that provides lung ventilation from TEF by keeping the pressure in the gastrointestinal tract at an appropriate level.

Ethics

Informed Consent: During the hospital stay, informed consent was obtained from the patient's family that scientific studies could be conducted in the future.

Peer-review: Externally peer reviewed.

Author Contributions

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

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

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