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Publisher Contact
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Phone: +90 (530) 177 30 97
E-mail: info@galenos.com.tr
yayin@galenos.com.tr
Web: www.galenos.com.tr
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The Journal of Pediatric Academy is the official publication of the Kayseri Child Health Association.

The Journal of Pediatric Academy which was established in 2020 is an international, unbiased double blinded peer-reviewed, open-access electronic and only-online published journal in the English language. The Journal of Pediatric Academy is published 4 times a year (March, June, September, December) and accepts original research articles, invited review articles, case reports and clinical images in all areas of pediatric research, which summarize recent developments about a particular subject based on standards of excellence and expertise.

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Kayseri Chamber of Medicine,
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Phone: +90.352.2076666 (int) 25373

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Manuscript Types

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Letters to the editor should pertain to articles published within the Journal of Pediatric Academy or highlight important new clinical or laboratory insights. The text should contain 1000 words or fewer.

Table 1.
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Original Article	3500	300	50	6	6
Invited Review	5000	350	100	6	10
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Journal Article:

1. Ang KK, Price RE, Stephens LC, et al. The tolerance of primate spinal cord to re-irradiation. *Int J Radiat Oncol Biol Phys*. 1993;25:459–464.

Journal Article published in non-English Languages:

2. Altuntaş N, Çelebi DT, Koçak M, Andıran N. Yenidoğan bebeklerde direkt coombs testi taraması ve pozitifliğinin morbidite üzerine, etkisi; tek merkezd eneyimi. *Pam Tıp Derg* 2015;8:39-44. (in Turkish)

Book Chapter:

3. Dimery IW. Chemotherapy in head and neck cancer. In: Myerhoff WI, Rice DH, eds. Otolaryngology: head and neck surgery, 2nd ed. Philadelphia: WB Saunders, 1992:1027–1045.

Entire Book:

4. Virchow R. Cellular Pathology. Philadelphia: JB Lippincott, 1863.

Software:

5. Epi Info [computer program]. Version 6. Atlanta, GA: Centers for Disease Control and Prevention; 1994.

Online Journals:

6. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database:

7. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

World Wide Web:

8. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS Web site]. June 1, 1996. Available at: http://www.ama-assn.org/special/hiv/ethics. Accessed June 26, 1997.

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9. (J. M. Kramer, K. Kramer [jmkramer@umich.edu], e-mail, March 6, 1996).

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Constipation in Children: An Example of A Conflict Situation

Author(s)

Derya Altay

Affiliation(s)

Erciyes University Faculty of Medicine, Department of Pediatric Gastroenterology, Kayseri, Turkey

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Abstract

Constipation is a prevalent issue in the pediatric population and is predominantly of functional origin. It often presents with symptoms such as abdominal pain, vomiting, and anorexia, making it a significant complaint among young patients. A comprehensive patient history and physical examination are typically sufficient for the diagnosis of functional constipation. Early intervention and patient and parent education are crucial for the success of treatment, which involves dietary adjustments, toilet training, and medical interventions. This review outlines an approach to managing constipation in children.

Keywords: Constipation, functional, pediatrics

Introduction

Constipation is a common public health concern worldwide, with its impact evident in both general pediatric outpatient visits (3%) and pediatric gastroenterology outpatient visits (30%).[1] Contrary to parental expectations, most constipated children have normal large intestines. Approximately 95% of constipation cases in childhood are of functional origin, with organic pathologies typically diagnosed during the early stages of life. The diagnosis of functional constipation is based on the Rome IV criteria (**Table 1**). The major causes of constipation in children are listed in **Table 2**, and the risk factors differentiating organic constipation from functional constipation are outlined in **Table 3**.[2,3]

Frequency of Defecation

The frequency of defecation varies among healthy infants, with exclusive breastfeeding often associated with more frequent bowel movements in the early weeks of life. Breastfed newborns may have as many as 1-8 defecations daily during the first weeks of life.[4,5] A study conducted in our country found that approximately one-fourth of infants fed both breast milk and formula defecate once a week in the second month.[6] Healthy children in our country defecate 3-4 times a day during the first 6 months, twice a day between 1 and 2 years, and once a day between 3 and 6 years.[7] Although there is variability in defecation frequency, normal stool consistency is the key distinguishing factor, and infrequent defecation

Correspondence: Derya Altay, Erciyes University Faculty of Medicine, Department of Pediatric Gastroenterology, Kayseri, Turkey
E-mail: dr.deryaaltay@gmail.com **ORCID:** 0000-0002-7415-1595

with normal stool consistency is not classified as constipation.

Constipation Can be Confused with Infant Dyschezia

Infant dyschezia is a functional gastrointestinal disorder in infants under 9 months of age and is characterized by the inability to relax pelvic floor muscles during defecation. Soft stool typically follows at least 10 min of straining, and this disorder typically resolves as infants grow.[8]

Main periods of stool withholding behavior patterns in childhood:[9,10]

1- Complementary feeding period in infancy due to the transition to solid foods

2- During toilet training by strict parents

3- Starting school because of fear or feeling uncomfortable using any toilet outside the home

Children may experience constipation during any of these periods or exhibit withholding behavior following painful and hard defecation for any reason.

Pathophysiology of Constipation

The majority of children with constipation experience functional constipation because of low-fiber diets and stool withholding behaviors without an organic cause.[11] Hard, painful defecation leads to the persistence of withholding behaviors. Children may assume back-arching positions or engage in specific behaviors such as standing on their toes, extending their legs, or rocking back and forth in response to hard, painful, and large defecation.[12] Chronic withholding behavior can lead to fecal impaction. Prolonged retention of stool in the large intestine and rectum results in stool hardening due to water absorption, causing liquid stool from the proximal colon to leak around the hard stool into the underwear, potentially leading to fecal incontinence without the patient's awareness. As withholding behavior continues, compliance increases in the rectum, which can result in megarectum development, with larger stool volumes remaining in the rectum and longer defecation intervals.

Slow transit constipation, which is categorized as a functional disorder, is caused by disturbances in the autonomic and enteric nervous systems. These disturbances result from an increased concentration of

Table 1.
Functional constipation diagnostic criteria (Rome IV criteria)

<4 years of age, at least two of the following for at least one month:
1- Two or fewer defecations per week
2- History of excessive stool accumulation
3- Painful and difficult defecation
4- Large-scale defecation
After acquiring toilet skills, it should includethe following:
1- History of fecal incontinence at least once a week
2- A history of large-scale defecation that may even clog the toilet

Table 2.
Etiology of constipation in children[2]

Functional	**Intestinal**	**Neurological**
	Hirschsprung disease	Spinal cord trauma, anomalies, and tumor
	Anorectal malformation	Neurofibromatosis
	Neuronal intestinal dysplasia	Cerebral palsy
	Celiac disease	Tethered cord
	Cow's milk protein allergy	
	Cystic fibrosis	
Metabolic	**Drugs**	**Others**
Hypothyroidism	Opioids	Anorexia nervosa
Diabetes mellitus	Anticholinergics	Sexual abuse
Hypercalcemia	Antidepressants	Scleroderma
Hypokalemia	Diuretics	Heavy metal ingestion (lead, mercury)
Vitamin D intoxication		

Table 3.
Risk factors for organic constipation[3]

Early-onset constipation (<1 month)	Anal position abnormality
Abdominal distension	Thyroid gland abnormalities
Delayed meconium passage (>48 h)	Megarectum
Ribbon stools	Gluteal cleft deviation
Visible or occult blood in stool	Absence of the anal reflex
Failure to thrive	Decreased lower extremity reflex
Fever	Spina bifida
Bilious vomiting	Perianal fistula/scars
Lack of a lumbosacral curve	Family history of Hirschsprung disease

colonic mast cells contributing to visceral hypersensitivity and a decrease in colonic Cajal cells, often referred to as pacemaker cells. Chronic withholding behavior can lead to slow transit constipation. Slow transit constipation also plays a role in constipation-predominant irritable bowel syndrome.[13]

Although approximately half of the children with functional constipation have a positive family history, no genetic mutation is typically identified. Family history assumes more significance when organic diseases, such as cystic fibrosis and Hirschsprung disease, are considered. Family history is also relevant to functional constipation because of shared social environments and nutritional habits within the family. Low dietary fiber intake, excessive consumption of junk food, and insufficient physical activity have been associated with constipation.[14]

Chronic constipation may lead to fecal incontinence (encopresis). Fecal incontinence can be categorized as retentive or non-retentive, with the former being more common. Non-retentive fecal incontinence is characterized by a lack of fecal accumulation in the rectum, normal fecal consistency, and fecal leakage due to inadequate toilet training, psychiatric issues, rectosigmoid colon surgery, or proctitis.[15]

Diagnosis of Constipation

The differentiation between functional and organic constipation was initially established through a comprehensive patient history and physical examination. The Rome IV criteria are valuable for diagnosing functional constipation. Laboratory assessments are essential, especially for children with risk factors for organic constipation. In cases of intractable constipation, it is advisable to evaluate serum calcium and phosphorus levels, thyroid function tests, and celiac serology.[16]

Signs such as delayed meconium passage, failure to thrive, and abdominal distension may suggest Hirschsprung disease or neuronal intestinal dysplasia. Although contrast enema can offer diagnostic clues for Hirschsprung disease, rectal biopsy is necessary to distinguish between these two conditions. Histopathologically, Hirschsprung disease is characterized by the absence of ganglion cells in the submucosal and myenteric plexus, whereas neuronal intestinal dysplasia shows hyperplasia in the submucosal nerve plexuses.[17]

Neurogenic bowel dysfunction results in chronic constipation, particularly in patients with spina bifida and spinal cord anomalies. The initial diagnostic assessment for these patients involves medical history and examination of the anal sphincter tone, with absent anal reflexes indicating spinal cord injury below the L1 vertebrae.[18]

Cow's milk protein allergy is the most common non-IgE-related food allergy, which affects gastrointestinal motility. This condition typically results in diarrhea, but constipation can also occur. Constipation may develop because of the early introduction of cow's milk after breastfeeding. Inflammation, stool-withholding behavior, and abnormal anal sphincter function contribute to food allergy-related constipation. Allergy tests are not diagnostic because the immune reaction is not IgE-related. Treatment typically involves an elimination diet.[19]

Routine use of abdominal radiography, transabdominal rectal ultrasonography, or colonic scintigraphy for the diagnosis of constipation is not recommended because of insufficient evidence. Similarly, spinal MRI is not recommended for children without neurological disorders.[11] Colon transit time, a method based on the passage time of radio-opaque plastic markers through the colon via X-ray imaging on the fourth day after ingestion, can be employed to differentiate functional constipation from functional non-retentive fecal incontinence. However, it is not routinely used for diagnosing functional constipation.[11] Anorectal manometry can measure sphincter function and anorectal coordination, and colonic manometry can predict the effectiveness of antegrade continence enema. However, due to their invasiveness and limited data in children, these tests are not typically included in the diagnosis of constipation. The wireless motility capsule is a non-invasive, non-radioactive method that can offer insights into colonic motility; however, research in children remains limited.[2]

Treatment Strategies for Constipation

Treating constipation requires addressing fecal impaction before proceeding to maintenance therapy. Patient education on daily defecation, prevention of fecal impaction, regular toilet use, and adherence to medical treatment is crucial. Explaining the constipation mechanism through illustrations to patients and their families can enhance treatment compliance. Patience is a fundamental aspect of treatment.

A low-fiber diet significantly contributes to functional constipation, underscoring the importance of ensuring sufficient daily fiber intake. A rule of thumb is to provide fiber intake equivalent to the child's age in years plus 5-10 g/day for children older than two years. **Table 4** illustrates the fiber content of the various foods. Adequate water intake is also essential for children.

Toilet training should be conducted with a supportive and positive approach between 18 and 24 months of age, avoiding insistence during constipation. Children should be encouraged to sit on the toilet 1-3 times a day for 5 min after meals. For children, using a step stool and decorating the toilet with their favorite cartoon characters can create a more welcoming environment. With increased dietary fiber intake and medical treatment, withholding behavior gradually diminishes as defecation becomes painless.

Although most patients experience functional constipation, organic diseases may be diagnosed in a small portion of cases and treated accordingly. In infants, the treatment of functional constipation involves ensuring adequate fiber intake and limiting excessive dairy consumption. Rectal stimulating objects are not recommended because of potential anal mucosal trauma. Mineral oil, bisacodyl, and enemas containing phosphate are also discouraged in infants, with lactulose

Table 4.
Dietary fiber contents of some foods (fiber g/100 g edible part of food)[20]

Food	Fiber	Food	Fiber	Food	Fiber
Wheat bran	33.75	Avocado	9.70	Flaxseed	35.06
Bread wheat	12.66	Pear	3.50	Black cumin	37.14
Ashura wheat	13.80	Carrot	2.58	Carob	25.83
Einkorn wheat	11.30	Apple	1.91	Sesame	19.88
White bread	4.32	Orange	1.89	Coconut (dry)	18.91
Gluten-free read	5.44	Peach	1.82	Pestil	3.06
Dry beans	32.17	Cherry	1.89	Garlic	2.64
Chickpea	23.03	Banana	1.69	Tahini	12.78
Lentils	25.99	Strawberry	1.98	Raisins	7.20
Bulgur	6.79	Apricot	1.24	Blueberries	2.73
Semolina	4.57	Pineapple	3.15	Leaf wrap	3.86
Rice	3.46	Watermelon	0.54	Prune (dry)	12.18
Apricot kernel	17.67	Artichoke	4.74		
Peanuts	12.54	Pumpkin	1.63		
Almond	12.00	Potato	1.54		
Nuts	11.54	Tomato	1.10		
Walnut	11.50	Green beans	2.08		
Fig	10.06	Spinach	2.27		
		Okra	3.36		
		Lettuce curly	2.09		

and glycerin suppositories serving as effective treatment options for this age group

Osmotic laxatives constitute the first-line treatment by increasing the osmotic load in the lumen and retaining water, thus softening stool consistency. Stimulant laxatives, considered second-line therapy, enhance intestinal motility and prevent epithelial water and electrolyte transport. It is important to note that laxatives may have side effects such as diarrhea and abdominal cramping, which can be mitigated through dose adjustments. **Table 5** lists the medical treatment options for constipation.

In cases with fecal impaction (hard feces accumulation in the rectum), fecal disimpaction should be performed before starting maintenance treatment to ensure the success of treatment and patient compliance. Polyethylene glycol is the preferred choice for disimpaction. Rectal applications for disimpaction should be avoided whenever possible because of their invasiveness and traumatic nature. For children with chronic intractable constipation or neurogenic bowel dysfunction, treatment options may include transanal irrigation, antegrade colonic enema, or surgical methods such as resection or ostomy.[21]

Medical treatment should be continued for a minimum of two months, with no complaints of constipation for one month before considering treatment reduction and discontinuation.[11] Non-pharmacological treatments, such as prebiotics, probiotics, synbiotics, biofeedback, abdominal massage therapy, and alternative medicine, are not recommended for treating functional constipation.[11] **Figure 1** shows the approach to constipation in children.

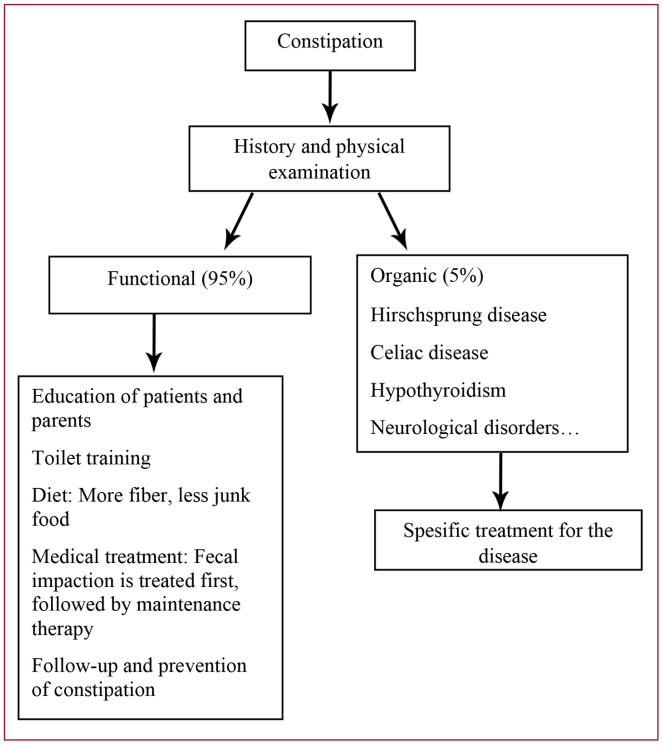

Figure 1. Approach to constipation in children

Children with Neurological Disorders: Patients Prone to Constipation

Constipation is a frequent issue among children with neurological disorders, particularly in tube-fed patients. Prolonged immobility, inadequate fiber intake, and antiepileptic medications contribute to constipation. Diagnosis in these cases involves a comprehensive history, abdominal and perineal examination, evaluation

Table 5.
Medical treatment options for constipation[2]

Osmotic laxatives	Stimulant laxatives	Rectal enemas
Lactulose (any age) 1-2 mL/kg/day	Senna (>2 years) 7.5-15 mg/kg/day	Sodium phosphate (>1 year) 2.5 mg/kg
PEG (any age) 0.4-0.8 g/kg/day; 1-1.5 g/kg/day for fecal impaction	Bisacodyl (>2 years) 5-10 mg/day	Bisacodyl 5 mg/day for 2-12 years; 5-10 mg/day for more than 12 years
Magnesium hydroxide (>2 years) 1-3 mL/kg/day	Sodium picosulfate 3 mg/day for 4-5 years; 4-6 mg/day for more than 6 years	Saline enema 5 mL for <1 kg, 10 mL for more than 1 kg; 6 mL/kg/day for more than 1 year
	Glycerine suppository (<1 yr) half of the pediatric form/day	

PEG; Polyethylene glycol

of anal reflex, and assessment of the density of feces in the rectum through rectal touch. In cases where the diagnosis is uncertain, plain abdominal radiography may be performed. Colon transit time testing can provide quantitative evaluation, with the delay in colonic transit time correlating with the severity of neurological impairment. Treatment typically involves enemas followed by laxatives, although these may be less effective in patients not receiving sufficient fluids and fiber. It is essential to consider the risk of aspiration pneumonia when using laxatives, particularly in patients with neurological disorders. For cases that are unresponsive to standard medical therapy, antegrade continence enema may be a viable option.[22]

In conclusion, constipation is a common symptom in children and is mainly of functional origin. Preventing constipation, which is typically functional, should be a priority. Ensuring adequate fiber and fluid intake and conducting sensitive toilet training for children are critical in this regard. In cases of constipation occurring before six months of age, healthcare professionals should evaluate potential organic causes such as Hirschsprung disease, anatomical anomalies, metabolic disorders, cow's milk protein allergy, and neurological issues. A thorough patient history and physical examination are generally sufficient to distinguish between functional and organic pathologies. Informing patients and their families about the nature of constipation plays a vital role in treatment compliance.

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Microbiologically Documented Infection-related Mortality in Children with Acute Leukemia: A Single-center Experience

Author(s)

Şerife Öztekin Güntaş[1], Volkan Köse[1], Ayça Koca Yozgat[1], Vildan Çulha[1], Namık Yaşar Özbek[1], Aslınur Parlakay[2], Neşe Yaralı[1]

Affiliation(s)

[1]Ankara Children's Hematology Oncology Training and Research Hospital, Clinic of Pediatric Hematology and Oncology, Ankara, Turkey

[2]Ankara Children's Hematology Oncology Training and Research Hospital, Clinic of Pediatric Infection, Ankara, Turkey

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Abstract

Infections are a significant cause of morbidity and mortality of chemotherapy-induced neutropenia in children with acute leukemia. The aim of this study was to evaluate microbiologically documented infections (MDIs) during febrile neutropenia (FN) episodes and their relation to mortality. Four hundred eighty-seven FN episodes of 140 children were enrolled in this single-center study, and MDI in those FN episodes was retrospectively examined. Eighty-four patients (60%) had at least one positive peripheral blood, central line, or urine culture. MDIs were detected in 163 of 487 (33.4%) FN episodes of 84 children with leukemia. Gram-negative bacteria, Gram-positive bacteria, and fungal agents were isolated in 52.7%, 40.4%, and 6.9% of whole episodes. Coagulase-negative *Staphylococci* and *Enterococci* were the most detected Gram-positive bacteria. *Klebsiella* spp. and *Escherichia coli* were the most common Gram-negative bacteria isolated in the entire cohort. A central line was present in 145 MDI episodes, and catheter removal was required in 35 cases (17.7%) due to infection with Gram-negative bacteria, Gram-positive bacteria 43%, 28.5%, and fungus 28.5%, respectively. MDI-related mortality was 9.8%. The highest mortality rate (16.7%) was observed in Gram-negative bacteria and patients with relapsed and resistant leukemia. The most common infectious agent related to mortality was *Klebsiella* (31%). Resistance to third- or fourth-generation cephalosporins in Gram-negative bacteria was found to be over 50% of our cohort uri. Empirical antibiotic therapy at the onset of FN in neutropenic patients is crucial; therefore, the institution's predominant pathogens and resistance patterns should guide the choice of empirical antimicrobials. To reduce mortality and morbidity, each center should know its local epidemiological data and antibiotic susceptibility.

Keywords: Acute leukemia, febrile neutropenia, microbiologically documented infection

Correspondence: Ayça Koca Yozgat, Ankara Children's Hematology Oncology Training and Research Hospital, Clinic of Pediatric Hematology and Oncology, Ankara, Turkey
E-mail: draycayozgat@yahoo.com **ORCID:** 0000-0001-6690-721X

Introduction

Febrile neutropenia (FN) is a severe complication of chemotherapy, classified into three groups such as *microbiologically documented infections (MDIs)*, clinically documented infections, and fever of unknown origin, and their incidences were reported as 30-40%, 20%, and 30-40%, respectively.[1-3] Bacteriemia and central line-associated bloodstream infections are the most common complications in patients receiving intensive therapy via a central venous catheter. Although mortality was reduced when using empirical broad-spectrum antibiotics, it has contributed to the evolution of resistant microbiological flora. Resistance to antibiotics is a widespread global problem with a high prevalence of mortality, and the resistance pattern varies between centers and countries.[4] Rapid clinical and microbiological assessments and appropriate antibiotic treatment initiation are the most important steps for these patients. The objective of this retrospective *study* was to appraise the etiology and frequency of *MDIs* during FN episodes in children with leukemia to determine our institutional microbiological *status,* susceptibility, and resistance patterns and to determine MDI-related mortality.

Material and Method

One hundred forty-eight pediatric patients with acute lymphoblastic leukemia (ALL) and 42 pediatric patients with acute myeloid leukemia (AML) were treated in Ankara Children's Health Hematology and Oncology Hospital between January 2012 and December 2014. In total, 487 FN episodes were detected in 140 patients (110 ALL, 30 AML), and MDIs in those FN episodes were retrospectively examined. FN episodes that occurred after hematopoietic stem cell transplantation were excluded. Patients' demographic characteristics, leukemia type, remission/relapse status, treatment protocol and treatment phase, and clinical findings were recorded from electronic files. Peripheral blood culture, port catheter and/or central venous catheter culture, urine culture results, susceptibility and resistance patterns of microorganisms, post-treatment clinical improvement, or death were documented. Analysis was conducted at both patient and episode levels. Routine antibacterial and antifungal prophylaxis except trimethoprim-sulfamethoxazole for *Pneumocystis jiroveci pneumonia* was not used.

FN is described as a single temperature of ≥38.3 °C or a temperature ≥38 °C for 1 h continually, neutropenia is described as an absolute neutrophil count (ANC) of <500 cells/mm³ or ANC expected to decline to <500 cells/mm³ during the next 48 hour.[2] MDIs were defined as positive blood, central line or urine cultures. Each new event in a prior febrile patient with documented MDI was noted as a new episode. Bacteremia was described as the presence of viable bacteria in the bloodstream that indicated a positive blood culture. The same bacterial strain was isolated in both peripheral and central-line cultures collected at the same time, and central-line culture positivity was within 2 h before blood culture positivity was defined as central-line-associated bloodstream infections. For skin flora contaminants such as *Coagulase-negative staphylococci (CoNS)*, *Corynebacterium*, or *Streptococcus* spp., clinical signs of sepsis with two positive blood cultures were considered significant.[3] Polymicrobial bacteremia is defined as more than one microorganism isolated from blood culture within 24 h of the first positive blood culture specimen.

The respiratory tract infection is described as any infectious disease of the upper or lower respiratory tract. Upper and lower respiratory tract infections include pharyngitis/tonsillitis, the common cold, acute rhinosinusitis, laryngitis, and acute otitis media and acute bronchitis, bronchiolitis, pneumonia, and tracheitis, respectively. Gastroenteritis is *inflammation of the lining of the stomach and intestines*. Urinary system (the kidneys, ureters, bladder and urethra) infection is defined as the urinary *tract infection*. Urosepsis is a term used to *describe a type of sepsis that can result from an infection in the urinary tract*.

All patients were admitted for FN episodes and remained hospitalized until antibiotic therapy was completed and neutrophil count recovered. Cultures of both peripheral blood and central line (if present) and urine cultures were collected before the start of antibiotics. Cultures from other sites, e.g., pus swabs and stool cultures, were also performed if any symptoms existed. Cefepime, piperacillin/tazobactam, and cefoperazone/sulbactam were used for the initial treatment of fever and neutropenia. Routine galactomannan antigen and beta-glucan tests for fungal infections were not performed, and empirical antifungal therapy was not administered. If the fever persisted, repeat cultures were collected and antibiotic modification was done, and/or antifungal therapy was started.

The study was approved by the Ethics Committee of Ankara Bilkent City Hospital (decision no: E2-23-5322, date: 25.10.2023).

Statistical Analysis

The IBM SPSS for Windows Version 22.0 package program was used for statistical analyses. Numerical variables were determined by mean ± standard deviation or median (minimum-maximum) values. Categorical variables were demonstrated by number and percentage. Whether there was any difference in categorical variables between the groups was investigated by the chi-square test. The Mann-Whitney U test analyzed the differences between two independent groups in terms of numerical variables. Kruskal-Wallis test was performed for the comparison of more than one independent group. The significance level was taken as $p<0.05$.

Results

Four hundred eighty-seven FN episodes of 140 children with acute leukemia (110 ALL, 30 AML) were studied. *MDIs* were detected in 163 FN episodes (33.4%) in 84 patients. Fifty-eight (69.1%) were ALL, and 26 patients (30.9%) were AML. Most of these patients were in remission (75%); 15% had relapsed or resistant disease. The mean age was 7.9±5.3 years (median:

6.6 years; 6 months-18 years). Fifty-one percent (n=43) of 84 patients with MDI were female. Forty (47.6%) of 84 patients had one MDI episode, 18 patients (21.4%) had two MDI episodes, 19 patients (22.6%) had three episodes, and seven patients (8.4%) had four or more MDI episodes during the study period. A port catheter was in 145 of the 163 MDI episodes.

Peripheral blood, central line, and urine cultures positivity was 20.5% (n=100), 25.2% (n=123), and 6.9% (n=34), respectively. 42.3% (n=69) of episodes had both positive peripheral blood and central line cultures.

Highlights

- Empirical antibiotic therapy at the onset of febrile neutropenia in neutropenic patients is crucial; therefore, the institution's predominant pathogens and resistance patterns should guide the choice of empirical antimicrobials.

Respiratory system infections (28.2%) and gastroenteritis (28.2%) were the most common clinical infection sites through MDIs. Urinary tract infection was detected in 34 cases (20.8%), and five of them had urosepsis. Thirty-three cases had mccositis, and two patients had sinusitis. Infective endocarditis, vulvovaginal abscess, staphylococcal toxic shock syndrome, and cholecystitis were detected in one. Sepsis and septic shock were noted in 13.5% and 3.7%, respectively. No clinical signs were detected in 38% of MDIs.

Gram-negative bacteria, Gram-positive bacteria, and fungal agents were isolated in 52.7% (n=138), 40.4% (n=106), and 6.9% (n=18) of whole episodes, respectively. Peripheral blood or central-line Gram-positive, Gram-negative bacteria and fungus isolations were noted in 48%, 44%, and 8% of events, respectively. The frequency of isolated microorganisms in peripheral blood or central-line cultures is shown in **Table 1**. *CoNS* and *Enterococci* were the most detected Gram-positive bacteria. *Klebsiella* spp. and *Escherichia coli (E. coli)* were the most common Gram-negative bacteria isolated in the entire cohort. Polymicrobial growth was present in 12.8% of episodes. The most frequent organisms isolated from urine culture were extended-spectrum beta-lactamases (ESBL) positive *Klebsiella* (6.7%) and ESBL-negative *E. Coli* (4.3%) and *Pseudomonas aeruginosa* (4.3%) were the other common microorganisms. The other isolated organisms in the centralline were noted as ESBL (+) *E. Coli* (2.5%), *Serratia marcescens* (1.2%), ESBL (-) *Klebsiella* (1.2%), and *Stenotrophomonas maltophilia* (0.6%).

Penicillin resistance was detected in 81.2%, and oxacillin resistance was detected in 66.7% of the Gram-positive bacteria. No glycopeptide or linezolid resistance was observed. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* were not noted. Resistance to third- or fourth-generation cephalosporins was detected in 53.7% and 51.2% of Gram-negative bacteria, respectively. Carbapenem resistance was 21.5% and quinolone resistance was 38.2%. Colistin resistance was observed in one patient. *Candida* spp. susceptibility and resistance did not analyze in our study. Antibiotic susceptibility rates of microorganisms are shown in **Table 2**.

Catheter removal for catheter-related systemic infections (the presence of bacteremia originating from an intravenous catheter) was required in 35 cases (17.7%) due to infection with Gram-negative bacteria, Gram-positive bacteria, and fungi 43%, 28.5%, respectively).

Complete recovery was observed in 77.9%, and breakthrough infection (*the development of an infection with an organism resistant to prophylaxis)* was observed in 12.3% of MDIs. *MDI*-related mortality was 9.8%. The highest mortality rate (16.7%) was observed with Gram-negative bacteria, mostly *Klebsiella pneumonia*, although it was not statistically significant (p>0.1). Mortality rates were 8.3%, both in Gram-positive and fungal infections. The characteristics of mortality associated with MDIs are shown in **Table 3**. Eighty percent of patients who had mortality had relapsed or refractory disease, and most of them had gastrointestinal infections such as gastroenteritis and thyphilitis.

Discussion

In this single-center retrospective study of MDIs in pediatric patients with leukemia, we demonstrated that 60% of the children with acute leukemia developed at least one MDI during chemotherapy. *MDI* was encountered in 33.4% of FN episodes, and most microorganisms were Gram-negative agents. Although Gram-negative bacteria were more common in the whole cohort, 49.5% of central-line culture isolates were Gram-positive organisms, mostly skin flora bacteria. *Candida* spp. was detected in 15.5% of peripheral and

Table 1.
Microbiologically documented infection agents (blood cultures)

Bacteria	Number	Frequency (%)
Gram-positive	**72**	**48**
Coagulase-negative Staphylococcus	51	34
Staphylococcus aureus	1	0.6
Streptococcus spp.	5	3.3
Enterococcus spp.	10	6.7
Micrococcus	1	0.6
Bacillus licheniformis	1	0.6
Corynebacterium spp.	3	2
Gram-negative	**66**	**44**
Escherichia coli	15	10
Klebsiella spp.	29	19.3
Pseudomonas aeruginosa	9	6
Acinetobacter baumannii	3	2
Enterobacter cloacae	3	2
Moraxella spp	1	0.6
Pantoea agglomerans	1	0.6
Burkholderia cepacia	1	0.6
Fungus	**12**	**8**
Candida albicans	5	3.3
Candida non-albicans	6	4
Trichosporon asahii	1	0.6
Total	**150**	**100**

Table 2.
Antibiotic susceptibility of microorganisms

	Gram (+) n=72		Gram (-) n=95	
	Susceptible	Resistant	Susceptible	Resistant
	n (%)	n (%)	n (%)	n (%)
Penicillin	13 (18.8)	56 (82.1)	-	-
Oxacillin	6 (33.3)	12 (66.7)	-	-
Glycopeptide	71 (100)	0	-	-
Linezolid	71 (100)	0	-	-
Aminoglycosides	-		35 (83.4)	7 (16.7)
Cefotaxime	-		20 (48.8)	21 (51.2)
Ceftazidime	-		25 (46.3)	29 (53.7)
Cephaperasone	-		12 (52.2)	11 (47.8)
Cefepime	-		27 (49.1)	28 (50.9)
Piperacillin	-		32 (55.2)	26 (44.8)
Meropenem	-		51 (78.5)	14 (21.5)
Ciprofloxacin	-		34 (61.8)	21 (38.2)
Colistin	-		14 (93.3)	1 (6.7)

catheter blood cultures, and the most common agent was nonalbicans *Candida* spp.

Studies have emphasized that there is a shift in the prevalence of microbiological infections from Gram-positive bacteria to Gram-negative bacteria, and antimicrobial-resistant strains commonly occur among Gram-negative bacteria isolated from blood.[5] Febrile neutropenic episodes due to Gram-negative organisms are still more common in centers where quinolone prophylaxis and intravenous catheterization are less.[6] Although routine quinolone prophylaxis is not used in our clinic and most of our patients had a central line, Gram-negative microorganisms were noted more frequently (52.7%) in the whole cohort, and the most common microorganisms were *Klebsiella* species (43.9%). The majority of MDIs were bacteria mostly seen in the oral cavity and gastrointestinal tract. The presence of mucositis and gastroenteritis was associated with damaged mucosal barrier integrity and a risk factor for Gram-negative bacteriemia due to the translocation of bacteria across the mucosal barriers.[7] Infectious agents vary from center to center due to the variability of microbial flora in hospitals and communities. *E. coli* and *P. aeruginosa* represent the most common species among the Gram-negatives, and an increasing frequency of *Acinetobacter* spp. and *Stenotrophomonas maltophilia* was also reported.[8]

Despite discontinuation of quinolone-based antibacterial prophylaxis, it was emphasized that the rates of multidrug-resistant Gram-negative strains increased among *Enterobacteriaceae* and nonfermenting Gram-negative rods. In addition, antimicrobial resistance and/or failure of empirical antibiotic therapy have often been associated with a poor outcome in cancer patients with bloodstream infections caused by Gram-negative isolates.[5] Resistance to third- or fourth-generation cephalosporins in Gram-negative bacteria was found to be over 50% of our cohort. Carbapenem resistance was noted in 21.5% and quinolone resistance in 38.2% of MDIs. Carbapenems are therapeutic options often used in clinically unstable patients, so it is important to monitor the colonization of these resistant pathogens. Carbapenem-producing *Klebsiella pneumoniae* is an important cause of hospital-acquired infections, which is the most common and has high mortality among carbapenemase-producing *Enterobacterales*, and this agent represents a fast-growing global threat. The use of carbapenems in patients colonized with such genotypes results in increased carbapenemase-producing *Enterobacteria* in the gastrointestinal tract, resulting in a fourfold increased risk of bloodstream infections.[9] Colistin resistance was observed in one patient. Most of resistant infections were detected in relapsed/refractory or high-risk leukemia patients who received intensive chemotherapy with prolonged neutropenia.

Untreated FN and bacteremia can be lethal within hours after the onset of fever, and fever *should* be considered infectious *until proven otherwise.*[10] Early initiation of broad-spectrum antibiotics in hospitalization and aggressive management of patients with close monitoring have reduced the mortality rates due to FN. *Treatment within the first few hours the following fever will affect success rates.*[11]

Conclusion

Empirical antibiotic therapy is crucial in neutropenic patients when fever first starts; therefore, the *institution's predominant pathogens* and their *resistance patterns should* guide the choice of *empirical* antimicrobials. Thus, patients who do not respond to initial therapy may have the chance to modify their initial empirical treatment. To reduce mortality and morbidity, each center should know its local epidemiological data and antibiotic susceptibility.

Table 3.
Microbiologically documented infections related mortality

No	Age (years)	Diagnosis/risk classification	Remission status	ANC (/mm^3)	Neutropenia duration (day)	Day of hospitalization	Sepsis/septic shock	Clinical manifestation	Microorganism	Resistance
1	5.3	Pre B ALL/ HRG	Relapse	0	3	158	Sepsis	Mucositis	*CoNS* and *Candida albicans*	
2	15.3	Pre B ALL/ HRG	Relapse	100	4	33	Septic shock	*Pneumonia* Typhlitis	*E.coli* ESBL (+)	Cephalosporin, ciprofloxacin resistant
3	0.5	Infant AML/ HRG	Non-remission	400	4	10	-	*Pneumonia* Gastroenteritis	*Micrococcus*	
4	6.7	AML/HRG	Relapse refractory	0	0	23	-	Typhlitis Gastroenteritis	*K. pneumonia* ESBL (+)	Cephalosporin, piperacillin resistan
5	2.8	Pre B ALL/ MRG	Remission	350	17	16	-	*Pneumonia* Intracranial fungal abscess	*Acinetobacter*	Cephalosporin, piperacillin, meropenem resistan
6	12.3	T-ALL/MRG	Relapse refractory	0	17	33	Septic shock	Typhlitis	*K. pneumonia* ESBL (+)	-
7	3.8	Pre B ALL/SRG	Remission	0	25	58	-	*Pneumonia* Gastroenteritis cellulitis	*S. epidermidis*	*Penicillin resistan*
8	14.5	Pre B ALL/ HRG	Relapse refractory	100	64	30	Sepsis	*Pneumonia*	*CoNS*	-
9	18	T-ALL/MRG	Remission	100	4	0	Sepsis	*Pneumonia* Gastroenteritis	*E. coli* ESBL (-)	Ciprofloxacin resistant
10	16	Pre B ALL/ HRG	Relapse refractory	0	9	92	Septic shock	*Pneumonia* Typhlitis	*Acinetobacter* + ESBL (-) *E. coli* + *Enterococcus*	Cephalosporin, piperacillin, meropenem, ciprofloxacin resistan
11	16.5	AMLal	Relapse refractory	200	142	93	-	Fungal Sinusitis *Pneumonia* Gastroenteritis	*K. pneumonia* ESBL (+)	Cephalosporin, piperacillin, meropenem, ciprofloxacin resistan
12	2.5	AML	Relapse refractory	0	7	41	Sepsis	*Pneumonia* Gastroenteritis	*S. epidermidis*	-
13	17.5	AML	Relapse	0	3	43	Septic shock	Vulvovaginal Abscess Gastroenteritis	*P. aeruginosa*	Cephalosporin, meropenem, ciprofloxacin resistan
14	10.5	AML	Non-remission	500	14	34	Septic shock	*Pneumonia* Typhlitis	*K. pneumonia* ESBL (+)	Cephalosporin, piperacillin, meropenem, ciprofloxacin resistan
15	3.5	AML	Relapse refractory	0	17	30	Sepsis	Perianal Abscess Mucositis Gastroenteritis	*K. pneumonia* ESBL (+)	Cephalosporin, piperacillin, meropenem, ciprofloxacin resistan
16	16	AML	Relapse refractory	0	32	17	-	Mucositis *Pneumonia* Gastroenteritis	*K. pneumonia* ESBL (-)	-

ANC; Absolute neutrophil count, SRG; Standard risk group, MRG; Medium risk group, HRG; High-risk group, ALL; Acute lymphoblastic leukemia, AML; Acute myeloid leukemia, CoNS; *Coagulase-negative staphylococci*, ESBL; Extended-spectrum beta-lactamases

Ethical Approval: This retrospective study was approved by the Ethics Committee of Ankara Bilkent City Hospital (decision no: E2-23-5322, date: 25.10.2023).

Informed Consent: Not necessary. Because the study was designed retrospectively no written informed consent form was obtained from the patients.

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Demographic, Clinical, and Laboratory Characteristics of Children with Renal Tubular Acidosis

Author(s)

Aybüke Yazıcı[1], Nilgün Çakar[2]

Affiliation(s)

[1]Muş State Hospital, Clinic of Neonatology, Muş, Turkey

[2]Ankara University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Rheumatology and Nephrology, Ankara, Turkey

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Abstract

This study included patients followed up for primary renal tubular acidosis (RTA) between 1991 and 2012. Clinical characteristics at presentation, physical examination findings, laboratory test results, and treatments were recorded. The patients' laboratory results, drug doses, height, and weight were recorded every 3 months for the first year of follow-up. Standard deviation scores (Z-scores) of height and weight for age were determined and the patients' growth rates were evaluated. Of 50 patients followed up for primary RTA, 31 (62%) had distal RTA and 19 (38%) had proximal RTA. The median age at diagnosis was 3 months (range, 1-174 months) for patients with distal RTA and 10 months (range, 2-33 months) for patients with proximal RTA. The median follow-up times in these two groups were 96 months (range, 6-204 months) and 89 months (range, 6-180 months), respectively. Family history of RTA was more common among patients with distal RTA than those with proximal RTA (p=0.013). Nephrocalcinosis and deafness were detected more frequently in the distal RTA group (p=0.001), while ocular pathologies were more common in the proximal RTA group (p<0.001). In patients with distal RTA, older age at diagnosis was associated with lower weight and height Z-scores (p<0.05). Early diagnosis had a positive effect on the growth of patients with primary RTA.

Keywords: Renal tubular acidosis, pediatric, growth, nephrocalcinosis, deafness

Introduction

Renal tubular acidosis (RTA) is characterized by normal anion gap metabolic acidosis resulting from reduced bicarbonate absorption by the tubules or impaired hydrogen ion excretion without impaired glomerular filtration.[1] RTA is classified as distal (type I), proximal (type II), and hyperkalemic (type IV), according to the nephron segment in which renal tubular dysfunction occurs. Type III RTA, which is associated with hereditary carbonic anhydrase enzyme deficiency and has some characteristics of type I and type II RTA, has also been defined. RTA can be inherited or may

Correspondence: Aybüke Yazıcı, Muş State Hospital, Clinic of Neonatology, Muş, Turkey
E-mail: aybukeyzc07@gmail.com **ORCID:** 0000-0001-9387-0029
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develop due to toxic substances or diseases affecting the renal tubules.[2]

In children, 2-3 mmol/kg of hydrogen ions (H^+) are produced per day from the diet, and hydroxyapatites are released from growing bones.[3] Net acid excretion from the body is achieved through the prevention of the loss of bicarbonate (HCO_3^-) ions and excretion of ammonium ions and titratable acids. Approximately 85% of the filtered HCO_3^- ions are reabsorbed through the proximal tubules.[4] Transport in the proximal tubule occurs via the sodium (Na^+)-dependent transport system and megalin/cubilin-mediated endocytosis. Impaired HCO_3^- absorption causes low intracellular Na^+ concentration, disrupting the absorption of other solutes via the Na^+-dependent transport system. This general dysfunction of the proximal tubule is called Fanconi syndrome (FS). Proximal RTA can occur as isolated proximal RTA or as FS.[4,5] Congenital causes of FS in children include galactosemia, Lowe syndrome, cystinosis, mitochondrial diseases, Dent disease, hereditary fructose intolerance, Fanconi-Bickel syndrome, glycogen storage disease type 1, Wilson disease, and tyrosinemia type 1.[5,6] In distal RTA, H^+ secretion into the tubular lumen is impaired.[7] Distal RTA may be congenital or acquired. Congenital distal RTA shows autosomal dominant (type 1a) or autosomal recessive (type 1b, type 1c) inheritance.[8]

Highlights

- Early diagnosis of patients with RTA positively affected their growth
- Early diagnosis should reduce exposure to malnutrition, hypokalemia, hypophosphatemia, and metabolic acidosis and prevent potential adverse consequences by enabling the provision of necessary treatment.
- Early diagnosis was found to have a positive effect on the growth of patients with primary RTA.

In the distal RTA, metabolic abnormalities cause complications and failure to thrive. Blood test results in patients with distal RTA indicate normal anion gap metabolic acidosis, often accompanied by hypokalemia. Children with distal RTA have acidic blood pH but alkaline urine. Hypercalciuria, nephrocalcinosis, and nephrolithiasis are frequently associated with distal RTA. Autosomal-recessive inherited forms of distal RTA result from mutations in *ATP6V1B1* or *ATP6V0A4*, genes that encode the apical H1-ATPase, which is also found in some structures of the ear. Therefore, these mutations also lead to sensorineural hearing loss.[1,2,8]

In proximal RTA, defective proximal tubular HCO_3^- reabsorption leads to normal anion gap metabolic acidosis due to loss of HCO_3^- in the urine. As with distal RTA, proximal RTA may also be accompanied by hypokalemia. Unlike in distal RTA, however, in proximal RTA, the distal nephrons retain their capacity to acidify the urine; therefore, proximal RTA patients have a urine pH of 5.5 or lower when plasma HCO_3^- concentrations are below the tubular reabsorption threshold.[1] Compared with patients with distal RTA, patients with isolated proximal RTA are less likely to develop nephrocalcinosis and nephrolithiasis because the alkaline luminal pH inhibits proximal citrate reabsorption, ensuring adequate citrate excretion in the urine.[1,4,5] Nevertheless, nephrocalcinosis can be observed in some subgroups of proximal RTA patients who develop hypercalciuria, such as patients with Dent's disease.[9] The increased urinary calcium excretion caused by both acute and chronic metabolic acidosis directly impacts bone growth.[1,4,5]

The treatments for this patient group aim to ensure adequate growth as well as prevent bone abnormalities, nephrocalcinosis, and nephrolithiasis.[1] Proximal RTA patients require alkali therapy more than distal RTA patients, given their limited capacity for proximal HCO_3^- reabsorption, and increasing the filtered load of HCO_3^- will cause the increased urinary loss.[10]

Typical complaints of patients with RTA at admission include growth retardation and episodes of recurrent vomiting and dehydration. Growth retardation is a result of malnutrition, hypokalemia, hypophosphatemia, and metabolic acidosis.[11] In light of this information, this study was carried out to evaluate the demographic, clinical, and laboratory characteristics of children followed up with the diagnosis of RTA in our hospital between 1991 and 2012.

Material and Method

The population of this retrospective study consisted of children followed up with the diagnosis of RTA in the nephrology outpatient clinic of Ankara Children's Hematology Oncology Training and Research Hospital between 1991 and 2012. Patients with RTA secondary to diseases such as vesicoureteral reflux, nephrolithiasis, and medullary sponge kidney were excluded from the study. In the end, 50 children with primary RTA were included in the study sample. The patients' height and weight data, laboratory results, and drug doses were recorded every three months during the first year of follow-up. Based on these data, patients' standard deviation scores (SDS) (Z-scores) of height- and weight-for-age and growth rates were calculated. Height and weight data were expressed as SDS in line with National Center for Health Statistics standards. Values between +2 SD and -2 SD were accepted as normal lower and upper limits. In addition, delta SDS values were calculated by comparing the weight and height SDS of the patients at admission with their last measured weight and height SDS.[12] Z-scores were calculated as below:

Z = (subject's height-mean height)/standard deviation of mean

Urinalysis was performed using an Iris Q 200 device. Based on urinalysis results, patients' urine pH, protein and calcium levels, and glucose presence were recorded. A Roche Hitachi P800 device was used to study patients' biochemistry. Potassium levels of <3.5 mmol/L (normal range 3.5-5.5 mmol/L), phosphorus levels of <2.7 mg/dL (normal range 2.7-6 mg/dL), and calcium levels of <8.5 mg/dL (normal range 8.5-10.5 mg/dL) were deemed to indicate hypokalemia, hypophosphatemia, and hypocalcemia, respectively. Blood gas analysis was performed using an ABL 735 radiometer. Low

serum HCO_3^- concentrations (<18 mEq/L) and pH values <7.35 were deemed to indicate metabolic acidosis.[13] RTA types were determined based on clinical assessment and laboratory data, i.e., blood and urine pH values, blood and urine electrolyte levels, metabolic assessment, and kidney imaging findings. Accordingly, hypokalemic hyperchloremic metabolic acidosis, hypercalciuria, nephrocalcinosis, and spontaneous acidemia with inability to lower urine pH below 5.5 were deemed to indicate distal RTA, whereas normal to mildly low serum potassium levels and hyperchloremic metabolic acidosis with spontaneous acidemia in which urine pH can be lowered below 5.5 were deemed to indicate isolated proximal RTA.[4,14] Additionally, proximal RTA accompanied by the urinary loss of glucose, protein, phosphate, and amino acids was deemed to indicate renal FS.[15] Renal FS accompanied by corneal cystine crystal accumulation and/or elevated leukocyte cystine levels was deemed to indicate cystinosis,[16] renal FS accompanied by ocular anomalies (congenital cataracts) and central nervous system anomalies was considered to indicate Lowe syndrome.[17] Furthermore, renal FS accompanied by developmental delay, baby face appearance, hepatomegaly, nephromegaly, severe rickets, hypoglycemia, and galactose tolerance disorder was deemed to indicate Fanconi-Bickel syndrome, whereas renal FS accompanied by symptomatic hypoglycemia and vomiting after fructose, sucrose, or sorbitol intake, growth retardation, hepatomegaly, jaundice, hepatic cirrhosis, and nephrocalcinosis with prolonged exposure was deemed to indicate hereditary fructose intolerance.[15] Lastly, RTA associated with osteopetrosis and intracranial calcification was deemed to indicate marble brain disease.[18]

Clinical findings and laboratory results including serum and urine pH, serum biochemistry, serum HCO_3^-, urinalysis, urine protein, urinary creatinine excretion, urine calcium, tubular phosphate reabsorption, metabolic tests and also imaging findings, and follow-up data were obtained from patient files and electronic records. The study protocol was approved by the Ankara Children's Hematology Oncology Training and Research Hospital Ethics Committee (document no: 135-12/2012).

Statistical Analysis

Data interpretation was conducted using the SPSS 11.5 software (SPSS Inc., Chicago, IL, US, 2002 for Windows). The Shapiro-Wilk test was employed to assess the normal distribution of continuous variables. Descriptive statistics for the data were presented as mean and standard deviation for continuous variables with a normal distribution, median with minimum-maximum range for those without a normal distribution, and as count (n) and percentage (%) for categorical variables. The student's t-test for variables with a normal distribution, the Mann-Whitney U test for those without a normal distribution, and Fisher's exact test for categorical variables were used to determine the significance of differences between groups. Spearman's correlation analysis was utilized to test the relationship between continuous variables. To assess the significant variation in clinical measurements' average values at the end of the 12-month follow-up compared to the initial values, the dependent samples t-test was applied. Moreover, the Wilcoxon signed-rank test was used to evaluate any significant shifts in the median values of clinical measurements after 12 months compared to the initial measurements. A p-value less than 0.05 was considered statistically significant.

Results

In this study, there was 50 children followed up with the diagnosis of primary RTA. Of these children, 31 (62%) had distal RTA, and 19 (38%) had proximal RTA. The median age of the children with distal and proximal RTA at diagnosis was 3 months (range, 1-74 months) and 10 months (range, 2-33 months), respectively. In the proximal RTA group, the median age of the children with cystinosis and Lowe syndrome at diagnosis was 9 months (range, 7-12 months) and 23 months (range, 18-30 months), respectively. The median follow-up time of the children with distal and proximal RTA was 96 months (range, 6-204 months) and 89 months (range, 6-180 months), respectively. The rate of children with a familial history of RTA was significantly higher among the children with distal RTA compared to the children with proximal RTA (p=0.013) (**Table 1**).

Of the 19 children diagnosed with proximal RTA, 8 had cystinosis, 5 had isolated proximal RTA, 3 had Lowe syndrome, and 1 patient each had Fanconi-Bickel syndrome, hereditary fructose intolerance, and marble brain disease.

Nephrocalcinosis, deafness, and ocular findings are important in the differential diagnosis of patients with RTA. As expected, nephrocalcinosis and deafness were significantly more common in the distal RTA group than in the proximal RTA group (p=0.001), whereas ocular findings were more significantly common in the proximal RTA group, where patients with cystinosis predominated patients with other subdiagnoses than in the distal RTA group (p<0.001). There were no significant differences between the patient groups and subgroups in other clinical findings (**Table 2**). RTA was associated with hypotonicity and cognitive delay in two of the three patients diagnosed with Lowe syndrome.

Comparison of the patients with distal and proximal RTA in terms of laboratory measurements revealed

Table 1. Demographic characteristics of the patients by group

	Distal RTA (n=31)	Proximal RTA (n=19)	Total (n=50)	p
Gender, male, n (%)	15 (48.4)	12 (66.7)	27 (55.1)	0.24
Consanguineous marriage, n (%)	20 (71.4)	13 (72.2)	33 (71.7)	0.95
Family history, n (%)	8 (28.6)	0 (0)	8 (17.4)	**0.013**

*Median (minimum-maximum), RTA; Renal tubular acidosis

significantly higher blood pH and lower blood sodium, phosphorus, and urine pH levels at admission in the proximal RTA group than in the distal RTA group (p=0.027, p=0.014, p=0.042, and p=0.010, respectively). There was no significant difference between the distal and proximal RTA groups in other blood or urine parameters (p>0.05) (**Table 3**).

In patients with distal RTA, there was a significant increase in body weight (ΔZ-score) at the 12th month of follow-up compared to before the treatment (p<0.05), but no significant change in height (p>0.05) (**Table 4**). Delayed diagnosis was associated with worse Z-scores of height-for-age and weight-for-age in patients with distal RTA. There were significant correlations between age at diagnosis and Z-scores of weight-for-age (r=-0.618 and p<0.001) and Z-scores of height-for-age (r=-0.648 and p<0.001) in the negative direction. On the other hand, there were no significant correlations between Z-scores of height-for-age and weight-for-age and HCO_3^-, potassium, or phosphorus levels at admission (p>0.05) (**Table 5**).

The median Z-scores of weight-for-age and height-for-age of five patients with isolated proximal RTA at admission were -3.89 (range, -6.36 to -1.57) and -3.37 (range, -6.97 to 0.04), respectively. The median Z-scores of weight-for-age and height-for-age of five patients with isolated proximal RTA at the 12th month of follow-up were -2.49 (range, -2.83 to -1.81) and -2.1 (range, -3.03 to -0.6), respectively. These results indicated an improvement in patients' clinical conditions; however, a statistical

Table 2. Patients' hospital admission complaints and clinical findings by group

	Distal RTA (n=31)	Proximal RTA (n=19)	Total (n=50)	p
Growth retardation	5 (16.1)	7 (38.9)	12 (24.5)	0.13
Anorexia	2 (6.5)	3 (16.7)	5 (10.2)	0.31
Polyuria	3 (9.7)	2 (11.1)	5 (10.2)	0.54
Polydipsia	3 (9.7)	2 (11.1)	5 (10.2)	0.54
Rickets	4 (12.9)	5 (27.8)	9 (18.4)	0.26
Bone fractures	0 (0)	1 (5.6)	1 (2)	0.23
Nephrocalcinosis	23 (74.2)	3 (16.7)	26 (53.1)	**0.001**
Deafness	7 (22.6)	0 (0)	7 (14.3)	**0.001**
Ocular pathologies	0 (0)	8 (44.4)	8 (16.3)	**<0.001**
Weight loss	3 (9.7)	2 (11.1)	5 (10.2)	0.54
Failure to gain weight	8 (25.8)	1 (5.6)	9 (18.4)	0.09
Fever	8 (25.8)	3 (16.7)	11 (22.4)	0.37
Respiratory distress	3 (9.7)	0 (0)	3 (6.1)	0.19
Abdominal distention	3 (9.7)	1 (5.6)	4 (8.2)	0.46
Diarrhea	3 (9.7)	2 (11.1)	5 (10.2)	0.54
Constipation	3 (9.7)	3 (16.7)	6 (12.2)	0.44
Agitation	3 (9.7)	3 (16.7)	6 (12.2)	0.44
Malaise	6 (19.4)	3 (16.7)	9 (18.4)	0.51
Vomiting	9 (29)	8 (44.4)	17 (34.7)	0.35

Data expressed in n (%). RTA; Renal tubular acidosis. Statistically significant results (p<0.05) shown in bold

Table 3. *Patients' laboratory data at hospital admission by group*

	Distal RTA (n=31)	Proximal RTA (n=19)	p
HCO_3	**10.9 (3.4-20.4)**	11.8 (6.1-20)	0.26
Blood pH	7.28 (6.95-7.38)	7.34 (7.11-7.6)	**0.027**
Na	138 (125-157)	135 (121-153)	0.014
Cl	110 (95-126)	104 (98-140)	0.19
K	3.1 (1.7-6.2)	2.9 (1.4-5.5)	0.78
P	4.8 (1.6-7.7)	3 (0.3-10.1)	0.04
Ca	9.4 (6.1-13.8)	9.3 (8.5-11.3)	0.96
Urea	27.5 (8-178)	19.5 (9.4-96)	0.24
Creatine	0.4 (0.19-1)	0.43 (0.2-0.94)	0.78
Alkaline phosphatase	296 (9-3,363)	542 (222-2,420)	0.11
Urine density	1,010 (1,001-1,024)	1,010 (1,003-1,030)	0.75
Urine pH	7 (6-8)	5.5 (5-8)	**0.01**
Spot urine Ca/Cr	0.41 (0.01-4.4)	0.41 (0.04-1.5)	0.91

Data expressed as median (min-max). RTA; Renal tubular acidosis, Ca/Cr; Calcium/creatinine ratio, statistically significant results (p<0.05) shown in bold

Table 4.
Z-scores (presenting and ΔZ-score) for weight and height at presentation and after 12 months of treatment

Variable	Presenting Z-score	Month-12 Z-score	ΔZ-score	p
Weight	-2.90±1.10	-1.39±1.41	1.51±1.38	**<0.001**
Height	-1.70±1.38	-1.75±1.24	-0.05±1.44	0.899

Statistically significant results (p<0.05) shown in bold

Table 5.
Relationship between weight-for-age and height-for-age Z-scores and age at diagnosis, HCO_3, potassium, and phosphorus in distal RTA

	Weight-for-age Z-score		Height-for-age Z-score	
	Correlation coefficient	p	Correlation coefficient	p
Age at diagnosis	-0.618	**<0.001**	-0.648	**<0.001**
HCO_3	-0.047	0.762	-0.076	0.626
K	0.140	0.352	0.084	0.585
P	0.053	0.740	0.106	0.508

Statistically significant results (p<0.05) shown in bold, RTA; Renal tubular acidosis

evaluation could not be made due to the insufficient number of patients in the respective subgroups. The median Z-scores of weight-for-age and height-for-age of three patients with Lowe syndrome at admission were -3.83 (range, -4.98 to -2.68), respectively. The median Z-scores of weight-for-age and height-for-age of three patients with Lowe syndrome at the 12th month of follow-up were -3.94 (range, -5.56 to -2.32) and -4.83 (range, -5.2 to -4.47) at 12 months, respectively. Additionally, the median Z-scores of weight-for-age and height-for-age of three patients with cystinosis at admission were -2.89 (range, -5.53 to -1.6) and -3.08 (range, -5.63 to -0.04), respectively. Two of these patients developed kidney failure during follow-up. The median Z-scores of height-for-age of five patients at the last follow-up visit before they developed renal failure were -4.1 (range, -4.99 to -2.85).

The mean HCO_3^- dose required to achieve HCO_3^- >20 mEq/L was 3.39 mEq/kg/day in the distal RTA group and 12.4 mEq/kg/day in the proximal RTA group. Required alkali doses could not be statistically compared between the two groups due to the varying follow-up durations of the patients.

Nephrocalcinosis was detected in 23 of the 31 patients with distal RTA (74.2%) at admission compared to 3 of the 19 patients with proximal RTA (16.7%). Nephrocalcinosis resolved during the follow-up period in 6 patients with distal RTA. On the other hand, one of the two patients without nephrocalcinosis at admission developed nephrocalcinosis during the follow-up period. Nephrocalcinosis persisted in other patients with distal RTA. Nephrocalcinosis regressed during the follow-up period in all affected proximal RTA patients. The relationship between the resolution of nephrocalcinosis and alkali therapy could not be statistically evaluated due to the varying follow-up durations of the patients.

Discussion

RTA is a disorder resulting from impaired bicarbonate absorption or urinary hydrogen ion excretion without impairment of glomerular filtration. Early diagnosis of RTA, effective treatment of acidosis, and electrolyte balancing with supportive therapies positively affect the growth and development of patients with RTA.[19-21] This study was carried out to investigate the demographic, clinical, and laboratory characteristics of children followed up with the diagnosis of RTA, including subdiagnoses, treatments received, and growth development. The median age at diagnosis of our patients [3 months, range: 1-174 months in the distal RTA group (n=31); and 10 months, range: 2-33 months in the proximal RTA group (n=18)] was relatively younger compared to that of the patients investigated by Bajpai et al.[22] [1.8 years, range: 3 months-7.5 years (n=18)]. Bajpai et al.[22] emphasized that earlier diagnosis of RTA, i.e., within the first two years of life, which is a phase of growth acquisition, led to better height and weight gains during the follow-up period.

In our study, the rate of children with a familial history of RTA was significantly higher among the children with distal RTA compared to the children with proximal RTA, with no significant difference between the genders. In comparison, Caldas et al.[23] reported that 10 of the 28 patients with primary distal RTA, of whom 15 were male, had a familial history of RTA.

Mutations in *ATP6V1B1*, *ATP6VOA4*, and the newly identified *FOXII* gene reportedly cause neurosensory deafness in autosomal recessive distal RTA patients.[7,8,11] In comparison, in our study, nephrocalcinosis and deafness were more frequent in the distal RTA group than in the proximal RTA group. Caldas et al.[23] reported deafness and nephrocalcinosis in 14 of the 28 patients with distal RTA but did not report mutations in deaf patients. Additionally, Bajpai et al.[22] reported nephrocalcinosis in 8 of 18 patients with distal RTA.

Ocular pathologies associated with RTA can be observed in patients with proximal RTA. One example is cystinosis, where cystine accumulation can lead to photophobia, corneal ulceration, and even blindness if left untreated. Another example is galactosemia, an ophthalmic pathology associated with cataracts in Wilson disease and congenital cataracts in Lowe syndrome.[5,6,11] In our study, ocular pathologies were significantly more common in the proximal RTA group than in the distal

RTA group. Of the eight patients followed up with ocular pathologies (n=8, 44.4%), six had cystinosis, and one each had Lowe syndrome and isolated proximal RTA.

Nephrocalcinosis resolved during the follow-up period in 33% of the affected patients in the distal RTA group and all of the affected patients in the proximal RTA group. Soriano et al.[24] reported nephrocalcinosis in three of the five patients with distal RTA. They emphasized that nephrocalcinosis is a severe complication of distal RTA and that timely correction of hypercalciuria with early alkali therapy is needed to prevent kidney damage.

Malnutrition, hypokalemia, hypophosphatemia, metabolic acidosis, and delayed diagnosis are causes of growth retardation associated with RTA. Hypokalemia reduces levels of growth hormone (GH) and insulin-like growth factor-1 (IGF-1).[9,22] Appetite suppression and decreased extracellular volume also occur because of hypokalemia. Metabolic acidosis suppresses GH secretion, IGF-1, and GH receptors[25,26] and inhibits osteoblastic activity while increasing osteoclastic activity.[19] The release of Ca^{+2}, as well as Na^{+}, K^{+}, and $CO3^{-2}$ from the soft tissues and bones to buffer acidosis, reduces bone mineral density.[27] Early diagnosis, effective treatment of acidosis, and electrolyte balancing with supportive therapy reportedly improve growth and development in RTA patients.[20-22]

In our study, while Z-scores of weight-for-age showed a significant improvement at the 12th month of follow-up compared to before the treatment, the Z-scores of height-for-age did not show a significant improvement. The lack of a significant improvement in the Z-scores of height-for-age may be due to the relatively short follow-up period. Bajpai et al.[22] reported the median Z-score of height-for-age of 18 patients with distal RTA as -5.2 (range, -7.5 to -0.4) at the time of diagnosis and -2.7 (range, -4.8 to -1.1) at the last follow-up visit. Compared to our study, the patients in the study by Bajpai et al.[22] were diagnosed late and had worse Z-scores of height-for-age at the time of diagnosis. Bajpai et al.[22] 22 also noted that poor Z-scores at the time of diagnosis attenuated the improvement that could otherwise be observed in Z-scores of height-for-age during the follow-up period. They emphasized that concomitant bone deformity, rickets, and genetic potential also influence growth. Caldas et al.[23] divided 28 patients with distal RTA into two different groups according to their time of diagnosis and observed that patients who were diagnosed early had better Z-scores of height-for-age at the time of diagnosis and during the follow-up period compared to those of distal RTA patients who were diagnosed late. Similarly, in our study, Z-scores of weight-for-age and height-for-age of distal RTA patients who were diagnosed early were better than those of distal RTA patients who were diagnosed late. In our study, bicarbonate, potassium, and phosphorus levels at admission were not found to be associated with Z-scores of weight-for-age and height-for-age. In contrast, Caldas et al.[23] reported a negative correlation between serum HCO_3^- level and Z-scores of height-for-age at the time of diagnosis. Soriano et al.[24] reported that early diagnosis of primary RTA and early initiation of alkali therapy to treat primary RTA had a positive effect on the growth of the affected patients. Hsu et al.[28] evaluated the growth of 21 patients with proximal RTA (n=15) or FS (n=6) and found that treating metabolic acidosis significantly contributed to the growth in the positive direction in patients with proximal RTA, but did not produce a significant impact on the growth of patients with FS. It has been reported that early diagnosis and early initiation of treatment in patients with FS, especially early treatment of hypophosphatemia, positively affects growth. Haffner et al.[29] did not find any significant correlation between the Z-score of height-for-age and serum potassium and phosphorus levels in 9 patients with FS. In comparison, in our study, we could not separately analyze the impact of potassium, bicarbonate, and phosphorus levels on growth due to the insufficient number of patients in the subgroups of the proximal RTA group.

The treatment methods to be used in the treatment of RTA are determined according to the type of RTA and the respective etiology. That being said, HCO_3^--replacement is the foundation of treatment in all types of RTA.[30,31] High-dose alkali therapy (5-15 mEq/kg/day) is needed to treat proximal RTA, whereas low-dose alkali therapy (2-4 mEq/kg/day) is sufficient to treat distal RTA. In our study, the bicarbonate dose required to achieve HCO_3^- >20 mEq/L was 3.39 mEq/kg/day in the distal RTA group and 12.4 mEq/kg/day in the proximal RTA group.

Study Limitations

Notwithstanding the study's strengths, including its relatively large sample size considering the rarity of this patient group, there were also some limitations to this study. Its retrospective and single-center design was the primary limitation. The fact that patients' long-term growth outcomes were not presented may be considered another limitation of the study.

Conclusion

This study's findings indicated that early diagnosis of primary RTA in children positively affected their growth compared to late diagnosis. As a reason, early diagnosis reduces exposure to malnutrition, hypokalemia, hypophosphatemia, and metabolic acidosis and prevents potentially adverse consequences that might otherwise occur by ensuring the timely application of the necessary treatments.

Ethical Approval: The study was approved by the Ankara Children's Hematology Oncology Training and Research Hospital Ethics Committee (document no: 135-12/2012).

Informed Consent: Retrospective study.

Author Contributions: Concept: A.Y., N.Ç., Design: A.Y., N.Ç., Data Collection or Processing: A.Y., N.Ç., Analysis or Interpretation: A.Y., N.Ç., Literature Search: A.Y., Writing: A.Y., N.Ç.

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Evaluation of Transfusion-Related Infections in Patients with Beta Thalassemia Major in Southeast Turkey

Author(s)

Esra Pekpak Şahinoğlu[1], Miray Karakoyun[2]

Affiliation(s)

[1]Gaziantep University Faculty of Medicine, Department of Pediatric Hematology, Gaziantep, Turkey
[2]Ege University Faculty of Medicine, Department of Pediatric Gastroenterology, İzmir, Turkey

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Abstract

Thalassemia is the most common monogenic disorder and the only curative treatment is stem cell transplantation. Patients must have a regular blood transfusion to maintain life. Multi-transfusion is a risk factor for transfusion-transmitted infections (TTIs). This study aims to assess the TTIs in pediatric thalassemia patients. This retrospective study was conducted between April 2015 and December 2016. In this study, 240 Beta-thalassemia children were enrolled. Enzyme-Linked Immunosorbent Assays test results for hepatitis B, C, human immunodeficiency virus (HIV) and reverse transcriptase-polymerase chain reaction results, hepatitis C virus (HCV) genotype results, serum ferritin and transaminase levels were obtained from medical records. The findings obtained in this study showed that the prevalence of HCV infection and hepatitis B virus infection was 5.4% and 0.8%, respectively, and there were no patients with HIV infection. The serum transaminase levels were higher in the patients with HCV infection. There was no difference in serum ferritin levels between hepatitis or non-hepatitis patients. The development of blood screening systems for TTIs is important for blood safety. Especially the patients, who live in places that have poor quality screening systems, are at high risk of TTIs.

Keywords: Beta Thalassemia, children, hepatitis, blood transfusions

Introduction

Thalassemia is the most common monogenic disorder.[1] It is related to mutations that may affect the synthesis of hemoglobin. Normal hemoglobins are tetramers of two alpha (α) and two beta (β) globin polypeptides. The down-regulation of β globin results in an increase at α globin chains that leads to hemolytic anemia. Beta thalassemia major is the most severe form of the disease.[2]

The only curative treatment of beta-thalassemia is stem cell transplantation. Until that time, the patients must have regular blood transfusions for survival. The aim of blood transfusion is to correct the anemia and prevent ineffective

Correspondence: Esra Pekpak Şahinoğlu, Gaziantep University Faculty of Medicine, Department of Pediatric Hematology, Gaziantep, Turkey
E-mail: mdesrapekpak@yahoo.com **ORCID:** 0000-0003-2143-1435

erythropoiesis. These multi-transfused patients may expose to various transfusion-related complications, such as infections and iron overload.[3,4] The main reason for mortality and morbidity in these patients are transfusion-related complications and iron overload.

Transfusion-related infections can be reduced by safe donor selection by reliable screening methods. This study was designed to evaluate the incidence of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections in thalassemia patients in our center.

Material and Method

A retrospective study was conducted from April 2015 to December 2016. In this study, 240 beta-thalassemia patients who had regular transfusion at Gaziantep Cengiz Gokcek Maternity and Children Hospital, Division of Pediatric Hematology, were included. Enzyme-Linked Immunosorbent Assays (ELISA) were performed for routine detection of hepatitis which detects circulating antibodies against HCV and HBV.

When a positive screening test was found, it was confirmed by reverse transcription-polymerase chain reaction (PCR). The COBAS® TaqMan® was used to measure PCR product accumulation through dual-labeled fluorogenic TaqMan® probes. HBV DNA was studied for hepatitis B and HCV-RNA was carried out for hepatitis C infection. The HCV genotype analysis was done for HCV RNA positive patients.

The serum ferritin and transaminase levels and, history of splenectomy were recorded for the patients who had positive serologic test results for hepatitis.

All of these findings were gathered retrospectively from the medical records of the patients. The ethics committee of Gaziantep University approved this study (approval number: 2018/161, date: 04.07.2018).

Statistical Analysis

Descriptive statistics for the continuous variables were presented as mean, standard deviation, minimum and maximum values while count and percentages for categorical variables. Mann-Whitney U test was used to compare two groups. Statistical significance level was considered as 5% and SPSS (ver: 13) statistical program was used for all statistical computations.

Results

In this study, 240 patients with beta-thalassemia were enrolled. One hundred and eighteen (49.2%) patients were female and 122 (50.8%) patients were male. Male to female ratio was 1.03. One hundred and forty-six (60.8%) of patients with beta-thalassemia were refugees from Syria and Iraq. The mean age at the time of this study was 74.6 months (range: 7 months - 18 years) (**Table 1**).

Table 1. *The characteristics of beta-thalassemia patients with anti HCV (+)*

Patient No	Gender	Age (Month)	HBsAg	Anti- HCV	Anti- HIV	HBV DNA (copy/ml)	HCV RNA (IU/ml)
1	M	107	+	+	-	102569526	-
2	F	143	-	+	-	-	-
3	M	119	-	+	-	-	1411965
4	M	96	-	+	-	-	233097
5	M	192	-	+	-	-	-
6	M	121	-	+	-	-	-
7	M	41	-	+	-	-	-
8	F	179	-	+	-	-	1988192
9	M	119	-	+	-	-	8794834
10	F	204	-	+	-	-	-
11	F	180	-	+	-	-	-
12	F	216	-	+	-	-	-
13	F	83	+	+	-	1092	-
14	M	71	-	+	-	-	207458
15	M	197	-	+	-	-	2269469
16	F	150	-	+	-	-	250575
17	M	167	-	+	-	-	11403120
18	F	202	-	+	-	-	-
19	F	192	-	+	-	-	-
20	M	203	-	+	-	-	-
21	F	120	-	+	-	-	7310281
22	F	208	-	+	-	-	166825
23	F	155	-	+	-	-	1701014
24	M	171	-	+	-	-	-
25	F	180	-	+	-	-	-
26	M	61	-	+	-	-	4444
27	F	48	-	+	-	-	-
28	M	119	-	+	-	-	4544644

HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antigen, HIV: Human immunodeficiency virus, HCV: Hepatitis C virus

At our center, we routinely screen patients with beta-thalassemia for hepatitis at admission and then every other three months. According to the medical records, 28 (11.7%) patients were anti-HCV positive. Male to female ratio was 1.0. HCV RNA was studied and, 13 (46.4%) of these patients were positive. Prevalence of hepatitis C infection was 5.4% among patients with beta-thalassemia at our center. HCV genotyping was performed and genotype 4 was determined as the most common type (46.2%). Genotype 4 and genotype 1 were determined at six (46.2%) and three patients (23.1%), respectively. We could not determine the HCV genotype in four patients (30.7%), who were anti HCV positive, despite recurrent studies with new blood samples. Two (0.8%) patients also had a positive test result for HBsAg and hepatitis B infection was confirmed by HBV DNA in these patients. One of these patients with hepatitis B infection, recovered from hepatitis C one year ago. All of these patients were refugees from Syria and Iraq. There were no cases that were positive for anti-HIV type 1 or type 2 (**Table 1**).

Highlights

- Transfusion transmitted infections are one of the reasons of morbidity and mortality in beta thalassemia patients.
- Hepatitis C infection is still a problem for thalassemia patients despite blood screening methods.
- A positive Anti-HCV result is not sufficient to determine HCV infection. PCR should be used for definitive diagnosis.

Serum ferritin levels and transaminase levels were evaluated for the patients who had positive HCV RNA test results. The mean serum ferritin, alanine aminotransferase and aspartate transaminase levels was 6.838 ng/mL (minimum: 3.688 ng/mL-maximum: 12.177 ng/mL), 296 U/L (minimum: 46 U/L-maximum: 1.032 U/L), and 227 U/L (minimum: 88 U/L-maximum: 705 U/L), respectively. The serum transaminase levels were higher in patients with hepatitis C infection. There was no difference in serum ferritin levels between the patients who had hepatitis or not (p>0.05) (**Table 2**).

Fourteen (50%) patients who were Anti-HCV positive had a history of splenectomy. Despite these patients had positive anti-HCV serology, only eight (28.5%) of them had positive HCV RNA test results. Depending on their past medical history, all of these patients had Hepatitis C infection before the surgery for splenectomy.

Discussion

Patients with beta-thalassemia need a regular blood transfusion to maintain life. Recurrent blood transfusions increase the risk of transfusion-transmitted infections (TTIs). Hepatitis C, B, and HIV-I/II are the main reasons for TTIs.[5] Especially in developing countries, TTIs are still high, as a result of poor screening programs.

Despite blood screening programs, hepatitis C infection is still a problem in patients with transfusion-dependent thalassemia. The prevalence of HCV among multi-transfused patients differs from region to region. The highest prevalence reported in Egypt, in which 75% of patients with β-thalassemia infected with HCV infection.[6-8] The prevalence of HCV infection was reported between 11-40.5% in different studies.[9] In our country, Canatan[10] found HCV prevalence 18%, and Ocak et al.[11] found 4.5%. In this study, we found the prevalence of HCV infection by 5.4% in patients with thalassemia that were confirmed by HCV RNA. Only 46.4% of the anti-HCV positive patients were HCV RNA positive in this study. We realized that most of the studies performed only anti-HCV to determine the HCV infection. Thus, the findings suggest that unless HCV RNA is performed; patients can be overdiagnosed with HCV infection.

The prevalence of HBV infection has been reduced by the effects of vaccination programs worldwide, so hepatitis B infection is less common than HCV in patients with thalassemia. Mirmomen et al.[12] and Vidja et al.[13] found the prevalence of hepatitis B among patients with beta-thalassemia at 1.5% and 2.0%, respectively. The prevalence of hepatitis B in this current study was 0. 8%. In our country, Ocak et al.[11] found this prevalence at 0.75%. The literature data support our finding.

The prevalence of HBV and HCV infections among refugee patients were 0.8% and 5.4% respectively. Yazal Erdem et al.[14] found hepatitis B antigenemia 0.6% and antihepatitis C 5.3% among 299 refugee patients. This data is in accordance with our immigrant patients.

Human immunodeficiency virus infections can be related to drug abuse, contact between broken skin, wounds, or mucous membranes and HIV-infected blood or blood-contaminated body fluids. The prevalence of HIV among blood donors is different in various parts of the world. In patients with beta-thalassemia, the prevalence of HIV infection is generally found negative in studies.[15,16] On the other hand, Manisha et al.[17] and Vidja et al.[13] found HIV prevalence 1.5%, 3.0%, respectively. In our study, none of the patients were diagnosed with HIV.

Table 2. *The comparision of serum AST, ALT and ferritin levels among anti-HCV (+) patients*

	HCV RNA (+) (n=13)			HCV RNA (-) (n=15)			P values
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
Serum ferritin (ng/mL)	6839±2225.5	3.688	12.177	6.502±2994.3	1.310	10.931	p=0.742
AST (U/L)	Median 164	Min 88	Max 705	Median 68	Min 20	Max 179	p=0.000
ALT (U/L)	194	46	1.032	54	20	157	p=0.000

SD: Standart deviation, AST: Aspartate transferase, ALT: Alanine aminotransferase, HCV: Hepatitis C virus

In patients with thalassemia, measuring serum ferritin level is not the gold standard procedure to point iron overload, but it is a practical predictor for this. We try to maintain serum ferritin level below 1000 ng/mL in patients with thalassemia to minimalize the risk of iron toxicity. In our study, we found high ferritin levels (1.310 ng/mL-12.177 ng/mL) in patients with beta-thalassemia. We compared the serum ferritin levels between the patients with or without hepatitis and did not find a significant difference. Ameli et al.[18] found the mean serum iron level higher in anti-HCV positive versus negative patients.

The liver is one of the targeted organs for iron toxicity in patients with thalassemia. Both irregular use of oral iron chelation treatment and the presence of hepatitis can give damage to the liver. Elevated serum transaminase levels are related to liver damage. In our study, we determined high transaminase levels in patients with hepatitis. This finding is similar to the literature. Salama et al.[19] and Ameli et al.[18] found higher serum transaminase levels in patients with hepatitis.

Conclusion

In conclusion, in this study we found the prevelance of HCV and HBV infection 5.4% and 0.8%, respectively, and there were no cases of HIV infection. Sometimes ELISA cannot be enough to determine the infection, in this condition PCR should be used to confirm the exact diagnosis unless the infections can be overdiagnosed.

Ethical Approval: The ethics committee of Gaziantep University approved this study (approval number: 2018/161, date: 04.07.2018).

Informed Consent: Because the study was designed retrospectively no written informed consent form was obtained from the patients.

Author Contributions: Pekpak Şahinoğlu E: Surgical and Medical Practices, Concept, Design, Data Collection or Processing, Literature Search, Writing.; Karakoyun M: Analysis or Interpretation, Writing.

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Clinical and Electrophysiological Evaluation of Neonatal Seizures

Author(s)

Mustafa Alper Aykanat[1], Ünal Akça[2], Gülfer Akça[3], Hasibe Canan Seren[4], Hamit Özyürek[5]

Affiliation(s)

[1]Ondokuz Mayıs University Faculty of Medicine, Department of Pediatrics, Samsun, Turkey
[2]Samsun University Faculty of Medicine, Department of Pediatric Neurology, Samsun, Turkey
[3]Samsun University Faculty of Medicine, Department of Pediatrics, Samsun, Turkey
[4]Ondokuz Mayıs University Faculty of Medicine, Department of Neonatology, Samsun, Turkey
[5]Ondokuz Mayıs University Faculty of Medicine, Department of Pediatric Neurology, Samsun, Turkey

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Abstract

Neonatal seizures are the most prevalent and distinctive sign of neurologic dysfunction in early-life. In spite the recent advances in medical care and technology in newborn intensive care units (NICU), it remains an important clinical issue of diagnosis, treatment, and prognosis. This was a retrospective, observational cohort study of neonates with seizures treated in the Ondokuz Mayıs University Faculty of Medicine NICU. Demographics of the babies, risk factors and etiology of seizure, type of clinical seizure, electroencephalographic and radiological findings, and anti-seizure treatments were recorded. The incidence of neonatal seizures was 4.5% in NICU admissions. Seventy-two babies with seizures included, 69,4% were diagnosed with electroclinical seizures. The most common seizure types were clonic (35.8%) and motor automatisms (32.8%). Perinatal asphyxia/hypoxic ischemic encephalopathy (HIE) (29.2%) was the most common etiological factor, whereas hypoglycemia was the most common metabolic problem (15.3%). Eighty-one percent of seizures due to HIE were observed in the first 48 h. Hyperbilirubinemia (kern icterus), hypocalcemia, and idiopathic neonatal convulsions were observed after the first 48 h. Abnormal findings were detected in 76.4% of electroencephalographies obtained during the neonatal period. Phenobarbital was the first-line therapy in 98.6% of babies, and 83.3% of the infants were seizure-free with phenobarbital. Seizures are common in the neonatal period and may be associated with significant brain damage. Seizures appear as an important symptom of the underlying pathology and not as a disease.

Keywords: Neonatal seizures, electroencephalography, motor automatism, perinatal asphyxia, phenobarbital

Correspondence: Gülfer Akça, Samsun University Faculty of Medicine, Department of Pediatrics, Samsun, Turkey
E-mail: gulfer.akca@samsun.edu.tr **ORCID:** 0000-0002-7139-3521

Introduction

Although neonatal seizures are one of the most common reasons for admission to neonatal intensive care units (NICU), there are still controversies regarding their management. The main reason for this is the inconsistency between clinical and electroencephalographic (EEG) findings. While some motor phenomena that are accepted as seizures by clinical observation are not accompanied by EEG changes (clinical seizure), it is also possible to observe unexpected and frequent EEG changes (electrographic seizure) in long-term video EEG recordings of high-risk newborns without any motor phenomenon. However, seizures with correlated clinical and EEG findings (electroclinical seizures) can also be observe in newborns.[1] According to motor findings, seizures were generally defined as focal clonic, multifocal clonic, generalized tonic, myoclonic, and motor automatisms. Motor automatisms (subtle seizures), the most common seizure form, are usually without an electrographic correlate, can be triggered by stimuli, and present as abnormal eye movements, mouth smacking, swimming, or pedaling movements that do not conform to common semiology. Non-epileptic paroxysmal events are also common in this age group and may be difficult to distinguish from seizures at times.[2,3]

Almost 85% of seizures are symptomatic, and early and accurate diagnosis of the specific underlying etiology is critical. Most seizures have an acute symptomatic etiology such as hypoxic ischemic encephalopathy (HIE), vascular causes, acute metabolic disorders, and central nervous system (CNS) infections, and these seizures are usually self-limited and treatable. Some neonatal seizures are due to genetic origin, congenital viral infections, and brain malformations, and seizures originating from indirect symptomatology are more resistant and require long-term treatment.[4]

The aim of this study was to retrospectively evaluate the clinical, electrophysiological, and imaging results of newborns hospitalized in the NICU for seizures or who had seizures during their NICU stay and to determine the relationships between etiology, physical examination, and treatment.

Materials and Methods

This was a retrospective study of newborns admitted, evaluated, and treated in Ondokuz Mayıs University Medical Faculty Children's Hospital NICU due to seizures or had seizures during their stay between June 1, 2013 and March 31, 2015. The babies were evaluated metabolically and by EEG, cranial magnetic resonance imaging (MRI), and transfontanel ultrasonography (TFUS).

Patients were classified after birth by calculating their corrected age at 37 weeks for preterm births and within the first month for term births. Therefore, patients whose EEG was not performed within the first 30 days (in the neonatal period) after 37 weeks according to their term or adjusted age were excluded from the study.

Neonatal EEGs were evaluated in terms of basic activity, taking into account the conceptional age. Accordingly, EEG findings were classified as normal, slightly increased sharp wave discharges, increased sharp wave discharges and immaturation, burst suppression, and low amplitude.

Information about the first seizure time and the clinical type of seizure was recorded from patient files. Patients were grouped as babies who had seizures in the first 48 h after birth and those who had seizures after 48 h. Seizures were classified according to Volpe, which is based on clinical features, as motor automatism (subtle), clonic, tonic, and myoclonic seizures, during the study period.[5] Serum glucose levels below 47 mg/dL were defined as hypoglycemia, and serum total calcium concentrations below 8 mg/dL in term infants and below 7 mg/dL in preterm infants were defined as hypocalcemia.[6]

Perinatal asphyxia/HIE was evaluated according to the American College of Obstetricians and Gynecologists (ACOG) criteria: Cord blood pH <7, base deficit >16 mmol/L, neonatal encephalopathy and seizures, and 5th minute Apgar score <3 was considered as severe fetal asphyxia.[7] Mild, moderate and severe HIE diagnoses were evaluated according to the Thompson scoring system. Those with a Thompson score of ten or less were classified as mild, those with 11-14 points as moderate, and those with higher were classified as severe HIE.[8] The Ethics Committee of Ondokuz Mayıs University Non-Interventional Clinical Research approved the study (no: 2015/173, date: 27.03.2015).

Statistical Analysis

In the analysis of the data, in addition to descriptive statistics, chi-square and Fischer exact tests for group comparisons of categorical variables were used. The results were evaluated with a 95% confidence interval and significance level of $p<0.05$. The IBM SPSS Statistics Version 22.0 package program was used for statistical analysis of the data.

Results

One thousand six hundred four babies were admitted to the NICU during the study period, and data of 96 patients with suspected seizures were retrospectively analyzed. Sixteen babies (16.6%) were hospitalized for investigating the seizure etiology, and 80 (83.4%) were hospitalized for other reasons but had seizures during their NICU stay. Twenty-four patients were excluded from the study because of the diagnosis of benign non-epileptic movements, mainly benign sleep myoclonus, and 72 babies were eventually included in the study.

The incidence of neonatal seizures was 4.5% in NICU admissions. 43 (59.7%) of the patients were boys, 45 (62.5%) were term infants, and 56 (77.8%) were born by cesarean section. The average birth weight was 2791 (840-4700) grams, and the average gestational age was 36.5±3.36 ($27^{6/7}$-42) weeks.

Sixty-seven patients had clinical seizures and five patients had epileptiform EEG changes without clinical correlation (electrographic seizures). No EEG abnormality was detected in 17 (25.4%) patients who had clinical seizures and were classified as clinical only

seizures, whereas the remaining 50 (74.6%) patients had coexistence of seizures and EEG abnormalities and were classified as electroclinical seizures.

When 67 patients with clinical seizures were classified according to the time of seizure onset, 23 (34.7%) infants presented with seizures in the first 48 h and 44 (65.3%) after 48 h (**Table 1**). Treatment was started in five patients without clinical seizures because they had EEG findings.

According to the gestational age, 23 patients had clinical seizures before 37 weeks, and the most common seizure type was clonic seizures in 11 (47.9%), motor automatisms in 7 (30.4%), tonic type in 2 (8.7%), tonic+clonic type in 2 (8.7%) and tonic+myoclonic type in 1 (4.3%) patient. According to the gestational age, in 44 patients who had clinical seizures after 37 weeks, the most common seizure type was motor automatisms in 15 (34.2%), whereas clonic type was observed in 13 (29.5%), tonic type in 8 (18.2%), tonic+clonic type in 6 (13.6%), and tonic+myoclonic type in 2 (4.5%) patients (**Table 2**).

In 60 (83.3%) babies, phenobarbital alone was the first-line anticonvulsive treatment, and seizure control was achieved. In eleven patients, levetiracetam and/or valproic acid and vigabatrin were added to phenobarbital treatment to control resistant seizures (**Table 3**).

TFUS was performed in 69 (95.8%) patients, and 49 (71%) patients were evaluated as normal. In 20 (29%) babie, abnormalities were found in TFUS, including intracranial hemorrhage (ICH) in 9 (13%), hydrocephalus in 6 (8.7%), cystic encephalomalacic changes in 3 (4.3%), meningitis in 1 (1.4%), and sinus vein thrombosis in 1 (1.4%).

54 (75%) patients underwent cranial MRI, of which 19 (35.2%) were reported as normal. Abnormalities were found in the cranial MRIs of the remaining 35 (64.8%) babies. These were: Intracranial hemorrhage in 8 (14.8%) patients,increase in extra-axial CSF distance in 6 (11.1%), cystic encephalomalacic changes in 6 (11.1%), hydrocephalus in 5 (9.3%), basal ganglia involvement in 2 (3.7%), cerebral dysgenesis in 2 (3.7%), meningitis in 1 (1.9%), sinus vein thrombosis in 1 (1.9%), tuberous sclerosis in 1 (1.9%), microcephaly in 1 (1.9%), polymicrogyria in 1 (1.9%), and myelination disorder in 1 (1.9%) patient.

When etiologies for neonatal seizure were considered, perinatal asphyxia/HIE was the most common factor (29.2%). Transient metabolic disorders were found in 20.8% of the patients, cerebral developmental abnormalities in 16.8%, intracranial hemorrhage, hematoma, and thrombosis in 12.5%, and sepsis/meningitis in 12.5%. Five patients had bilirubin-induced encephalopathy (BIE). Etiology could not be uncovered in one patient (1.4%) despite all investigations (**Table 4**).

When 67 patients with clinical seizures were classified according to the etiology of the seizure type, 2 of 8 patients with metabolic disease had concomitant tonic myoclonic seizures, two had tonic-clonic seizures, two had clonic seizures, 1 had subtle seizures, and 1 had

Highlights

- Neonatal seizures may be associated with significant brain damage, appear as an important symptom of the underlying pathology, not as a disease.
- Motor automatism type seizures were the most frequent type of seizures in hypoxic ischemic encephalopathy.
- The efficacy of phenobarbital is much higher than levetiracetam.
- The findings of cranial magnetic resonance imaging and transfontanel ultrasound were highly overlapped.
- When myoclonic seizures are observed, the clinical course will be more severe and there may be resistance to treatment.

Table 1. *Clinical characteristics of the patients (n=72)*

	Number of patients (n)	Percentage (%)
Gender		
Boy	43	59.7
Girl	29	40.3
Time of delivery		
Term	45	62.5
Premature	27	37.5
Mode of delivery		
Cesarean	56	77.8
NSVD	16	22.2
Birth weight (grams)		
>2.500 g	47	65.3
1.500-2.499 g	15	20.8
<1.500 g	10	13.9
Onset of first seizure (n=67)		
First 48 h	23	34.3
After 48 h	44	65.7

NSVD: Normal spontaneous vaginal delivery

Table 2. *Distribution of clinical seizure types by time of birth (n=67)*

Seizure types	Time of birth			
	Premature		Term	
	Patients (n=23)	(%)	Patients (n=44)	(%)
Motor automatism	7	30.4	15	34.2
Clonic	11	47.9	13	29.5
Tonic	2	8.7	8	18.2
Tonic+clonic	2	8.7	6	13.6
Tonic+myoclonic	1	4.3	2	4.5

Table 3. *Distribution of patients according to the anti-seizure drugs used*

Drugs used in the treatment	Number of patients (n)	Percentage (%)
Phenobarbital	60	83.3
Levetiracetam	1	1.4
Phenobarbital + levetiracetam	9	12.5
Phenobarbital + levetiracetam + vigabatrin	1	1.4
Phenobarbital + levetiracetam + valproic acid	1	1.4
Total	72	100

tonic seizures. Seizures in the form of motor automatism were observed more frequently in patients with idiopathic neonatal seizures and perinatal asphyxia, clonic seizures were observed more frequently in patients with hypoglycemia or sepsis, and tonic seizures were observed more frequently in patients with hypocalcemia.

When patients were classified according to the time of seizure, BIE, hypocalcemia, idiopathic neonatal convulsion, and sepsis-related seizures were observed after the first 48 h, whereas most seizures due to HIE were observed in the first 48 h. Most patients with hypoglycemia, ICH, and cerebral developmental abnormalities also had seizures after the first 48 h (**Table 5**).

Table 4. *Distribution of neonatal seizures according to the etiological causes*

Etiological causes	Number of patients (n)	Percentage (%)
Perinatal asphyxia/HIE	21	29.2
Transient metabolic disorders	15	20.8
Hypoglycemia	11	15.3
Hypocalcemia	4	5.5
Cerebral developmental abnormalities	12	16.7
Cerebral dysgenesis	4	5.5
Hydrocephalus	3	4.2
Microcephaly	2	2.8
Agenesis of the corpus callosum	1	1.4
Tuberous sclerosis	1	1.4
Polymicrogyria/pachygyria	1	1.4
Intracranial hemorrhage/hematoma/thrombosis	9	12.5
ICH	6	8.3
Subdural hematoma	2	2.8
Sinus vein thrombosis	1	1.4
Hyperbilirubinemia/kernicterus	5	6.9
Sepsis/meningitis	9	12.5
Unknown cause	1	1.4
Idiopathic neonatal convulsion	1	1.4
Total	72	100

HIE: Hypoxic ischemic encephalopathy

When the patients with seizures were evaluated according to EEG findings: In 22 babies with subtle seizures, EEG was normal in 22.7%, slightly increased sharp wave discharges were observed in 45.5%, and increased sharp wave discharges and immaturation were found in 31.8%. In 24 patients with clonic seizures, normal EEG findings were observed in 37.5%, slightly increased sharp wave discharges were observed in 25%, and increased sharp wave discharges and immaturation findings were observed in 37.5%. In 10 patients with tonic-type seizures, normal EEG findings were observed in one (10%) and increased sharp wave discharges were observed in the rest (90%). Burst suppression and low amplitude findings in EEG were observed only in patients with tonic and myoclonic seizures, and normal EEG findings were not observed in patients with this type of seizure (**Table 6**).

There was no significant association between gender, term/preterm birth, mode of delivery, birth weight, seizure type, EEG findings, and seizure etiology ($p>0.05$). Compared with infants with metabolic and other etiological causes, the incidence of seizures in the first 24 h was significantly higher in the perinatal asphyxia group ($p<0.01$).

Discussion

Because of the high morbidity and mortality of neonatal seizures, it is necessary to conduct studies in this area. Early recognition of newborn seizures and performing emergency interventions, identification of valuable prognostic factors in long-term follow-up by determination of prenatal, natal, and postnatal risk factors, and evaluation of treatment methods and duration are the main objectives.

In our study, seizures occurred after 48 h of life in 65.3% of the patients, regardless of the etiology. In many studies, it has been emphasized that seizures are mostly of the subtle type and occur in the first 12-24 h in patients with HIE.[5,9] In our series, seizures were observed within the first 48 h in 81% of the babies with HIE, and motor automatism was the most common seizure type.

When the seizure types of our patients were examined, mostly clonic (35.8%) and subtle type (32.8%) seizures were observed. These were followed by multiple seizure

Table 5. *Distribution of etiology of seizures according to seizure type and time*

	Seizure type					Seizure time	
						First 48 h	After 48 h
Etiology of seizure	Subtle n (%)	Clonic n (%)	Tonic n (%)	T+C n (%)	T+M n (%)	n (%)	n (%)
Idiopathic neonatal convulsion	1 (100)	0 (0)	0 (0)	0 (0)	0 (%0)	0 (%0)	1 (100)
ICH	1 (16.7)	2 (33.3)	1 (16.7)	2 (33.3)	0 (0)	2 (33.3)	4 (66.7)
Perinatal asphyxia/HIE	11 (52.4)	2 (9.5)	2 (9.5)	5 (23.8)	1 (4.8)	17 (81)	4 (19)
Hypocalcemia	0 (0)	1 (25)	3 (75)	0 (0)	0 (0)	0 (0)	4 (100)
Hypoglycemia	3 (27.3)	6 (54.5)	0 (0)	0 (0)	2 (8.2)	3 (27.3)	8 (72.7)
Hyperbilirubinemia/kernicterus	1 (20)	2 (40)	2 (40)	0 (0)	0 (0)	0 (%0)	5 (100)
Cerebral developmental abnormalities	2 (25)	3 (37.5)	2 (25)	1 (12.5)	0 (0)	2 (25)	6 (75)
Subdural hematoma	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
Sepsis/meningitis	3 (33.3)	6 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	9 (100)

HIE: Hypoxic ischemic encephalopathy, ICH: Intracranial hemorrhage, T+C: Tonic+clonic, T+M: Tonic+myoclonic

Table 6. *Evaluation of EEG findings according to seizure type*

	Seizure type				
EEG findings	**Subtle**	**Clonic**	**Tonic**	**Tonic+clonic**	**Tonic+myoclonic**
Normal	5 (22.7)	9 (37.5)	1 (10.0)	2 (25.0)	0 (0.0)
Slightly increased sharp wave discharges	10 (45.5)	6 (25.0)	8 (80.0)	1 (12.5)	1 (33.3)
Increased sharp wave discharges and immaturation	7 (31.8)	9 (37.5)	1 (10.0)	5 (62.5)	1 (33.3)
Burst suppression and low amplitude	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)

EEG: Electroencephalographic

types (16.4%) and tonic seizures (14.9%). The type of seizure observed is related to the etiological cause. Tonic seizures are more common in patients with severe cerebral dysfunction and hypocalcemia.[1] In our series, 66.7% of the seizures caused by infection were of the clonic type. Studies have reported different results for the frequency of seizure types. Mizrahi and Clancy[10] reported that subtle seizures were more common than other seizure types. Similarly, Scher[11] evaluated 62 term and 30 preterm infants in their study and reported that subtle seizures were the most common type of seizure in both groups, and the distribution of clonic, myoclonic, and tonic seizures was almost equal. Tekgul et al.[12] reported that clonic (64%) seizures were observed most frequently, followed by tonic (19%), subtle (13%), and myoclonic (7%) seizures. Pisani et al.[4] grouped the cases as those with one type of seizure and those with more than one type of seizure, and reported that the number of patients in the second group doubled the number of patients in the first group. Similarly, Ronen et al.[13] also found that cases experiencing more than one type of seizure were the most common. In our study, we observed that one-third of the patients had clonic seizures. Although this result is compatible with literature data, the diagnosis of almost all seizures in preterm and term newborns only by clinical observation in NICUs and the possibility of different interpretations of clinical seizure types by physicians who are following the patients suggest that more reliable data can be obtained with long-term video-EEG monitoring.

Perinatal asphyxia/HIE is the most common clinical condition known to cause acute neurological disorders and seizures in the neonatal period.[14,15] The incidence of perinatal asphyxia causing seizures was around 30% in the late 1960s, and then increased to current rates, which is 40-45%.[16] Although only patients with EEG recordings were included in our study, perinatal asphyxia/HIE was found to be the most common cause of neonatal seizures (30.5%), which was compatible with the literature. Intracranial hemorrhage is responsible for 12.5% of etiological causes. The most common cause of intracranial hemorrhage is prematurity.

In our study, the incidence of transient metabolic disorders in seizures was 20.8%. The most common metabolic disorder that can cause severe sequelae in the neonatal period is hypoglycemia. In particular, babies with low birth weight and low gestational age and infants of diabetic mothers are at high risk. Volpe reported the frequency of seizures due to isolated hypoglycemia without any other metabolic defect to be 9%.[5] In the study by Kumar et al., the frequency was 11.1% and the median time of seizures was 63.5 h.[17] Similarly, in our study, seizures were observed after the first 48 h in 72.7% of the hypoglycemic cases. Studies showing EEG findings in hypoglycemic seizures are limited. However, the rate of abnormal EEG accompanying hypoglycemic seizures has been reported in a very wide range in studies on neonatal seizures. While this rate was 42.9% in the study by Arhan et al.[18] reported this rate as 88.8%. In this study, abnormal EEG findings were observed in 7 of 11 patients (63.6%) with hypoglycemic seizures.

Hypocalcemia was the etiology of seizures in four patients (5.5%s) and BIE was the etiology of seizures in five patients (6.9%). Of these five babies with severe hyperbilirubinemia, two were treated only with phototherapy and three had exchange transfusion followed by phototherapy. The first neurological manifestations of BIE are poor sucking, lethargy, opisthotonus, and high-pitched cry. Early encephalopathy findings may be confused with sepsis, asphyxia, and hypoglycemia. The development of neurological complications in the early period indicates a poor prognosis.[19] In our study, hypotonia, poor sucking, lethargy, and seizures were the major abnormal neurological findings in patients with BIE.

Abnormal findings were detected in 76.4% of EEGs taken during the neonatal period. Slightly increased sharp wave discharges were observed in 31 (43.1%) patients, increased spike wave discharges and immaturation in 23 (31.9%) patients, and burst suppression and low amplitude findings were observed in 1 patient (1.4%). When these EEGs were evaluated according to the seizure type, we observed that myoclonic seizures did not have normal EEG findings and progressed with severe clinical and EEG findings (burst suppression), whereas subtle, tonic, clonic, and tonic/clonic seizures had a milder clinical course and EEG findings. In addition, we argue that continuous EEG recording is necessary in suspicious cases because there may be electrographic seizures only that can be diagnosed electrographically.

We also evaluated cranial imaging findings based on TFUS and cranial MRI. Intracranial hemorrhage was detected in 9 of 69 patients who underwent TFUS and in 8 of 54 patients who underwent cranial MRI. The advantages of TFUS such as easy accessibility, ease of application at the bedside, and non-invasiveness have been proven in many studies.[20] Therefore, TFUS is the first-line imaging study for neonatal seizures in the NICU. Leijser et al.[21] compared the cranial US findings of babies born before 32 weeks gestational age that were obtained in the neonatal period with the MRI images taken after the 40th gestational week and showed that the US findings overlapped with MRI findings, especially in recognizing periventricular, intraventricular, and intraparenchymal lesions. In our study, it was also

observed that the findings of cranial MRI and TFUS were highly overlapping. Wang et al.[22] evaluated the cranial MRI findings of 24 infants (two preterm and 22 term) aged 6-18 days with hyperbilirubinemia and kernicterus symptoms and reported that an increase in signal intensity in the basal ganglia and thalamus is rare in T2-weighted MRI images taken during the acute phase of kernicterus. All five cases in our study were term newborns, and an increase in signal intensity was observed in the basal ganglia and thalamus on T2-weighted images in the acute period in two of them.

The decision about the duration of treatment in cases with neonatal seizures should be made by considering the risk of recurrence after discontinuation of anti-seizure treatment and the side effects of the current treatment.[23,24] To date, no satisfactory study has been conducted regarding the appropriate duration of use and withdrawal of drugs, and specific principles have not been established.[10,16] Although a very common problem, there are few randomized controlled trials with anti-seizure drugs such as phenobarbital, phenytoin, benzodiazepines, and levetiracetam, which limits the treatment options in neonatal seizures.[1,25,26] Sharpe et al.[27] stated that the efficacy of phenobarbital is much higher than that of levetiracetam, which has a better side effect profile and is being used frequently. In our study, seizures were controlled with phenobarbital in 83.3% of infants. Similarly, in the study by Pisani et al.[4], 53.7% of the patients had a rapid response to treatment. Regarding response to antiepileptic medications, 84.7% of the study group achieved antiepileptic treatment and seizure control, whereas 14.3% did not and were switched to combined antiepileptic treatment.

Study Limitations

One of the important limitations of our study is the inability to conduct genetic studies for etiology, especially for channelopathies, in cases of unknown cause or in those with resistant seizures. Another limitation is that the study was conducted before therapeutic hypothermia was available in the NICU. The lack of long-term follow-up is another limitation. Therefore, no clear conclusion can be drawn regarding the course and prognosis of these cases.

Conclusion

Seizures, which are common in the neonatal period and may be associated with significant brain damage, appear as an important symptom of the underlying pathology and not as a disease. HIE is the most common cause of neonatal seizures. Although the incidence of HIE has decreased due to advances in obstetrics and fetal monitoring in recent years, it still holds in the first place. Subtle-type seizures were the most frequent type of seizures in babies with HIE. Therefore, it appears that perinatal asphyxia/HIE and metabolic disease should be considered in the etiology of seizures observed in the first 2 days of life, while CNS developmental disorders, infections, and transient metabolic disorders should be investigated in seizures that occur after the first 2 days. When the EEGs were evaluated according to the type of seizure, it was found that myoclonic-type seizures did not have normal EEG findings and progressed with severe clinical and EEG findings (burst suppression), whereas subtle, tonic, clonic, and tonic/clonic seizures had a milder clinical course and EEG findings. Therefore, it should be kept in mind that when myoclonic seizures are observed, the clinical course will be more severe and there may be resistance to treatment.

Ethical Approval: The Ethics Committee of Ondokuz Mayıs University Non-Interventional Clinical Research approved the study (no: 2015/173, date: 27.03.2015).

Informed Consent: Written informed consent was obtained from the patients/ guardians.

Author Contributions: Aykanat MA: Surgical and Medical Practices, Design, Data Collection or Processing, Literature Search, Writing, Akça Ü: Concept, Design, Analysis or Interpretation, Literature Search, Writing, Akça G: Design, Analysis or Interpretation, Seren HC: Surgical and Medical Practices, Data Collection or Processing, Özyürek H: Concept.

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Evaluation of Lupus Cases Related to TNF Inhibitors in Children

Author(s)

Şeyma Türkmen[1], Nelgin Gerenli[2], Betül Sözeri[1]

Affiliation(s)

[1]University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Pediatric Rheumatology, İstanbul, Turkey

[2]University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Pediatric Gastroenterology, İstanbul, Turkey

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Abstract

Systemic lupus erythematosus (SLE) due to anti-tumor necrosis factor (TNF) agents is a rare entity. We reported three cases who developed lupus-like syndrome while receiving infliximab therapy for various reasons. All cases demonstrated clinical and laboratory findings of SLE. And all of them needed treatment. We would like to emphasize that the risk of anti-TNF-alpha-induced lupus should be kept in mind in patients receiving anti-TNF therapy for any reason.

Keywords: Child, infliximab, systemic lupus erythematosus

Introduction

Drug-induced lupus (DIL) usually presents with a clinical pattern similar to systemic lupus erythematosus (SLE); however, typical SLE complications are not observed. Classical DIL is characterized by anti-nuclear antibody (ANA) and anti-histone antibody positivity, accompanied by symptoms such as fever, arthralgia, myalgia, and atypical skin rashes. It is believed that drug metabolites can induce T-cell response and autoantibody production, leading to this clinical presentation.[1]

The most implicated agents are procainamide and hydralazine, but various antibiotics, antiarrhythmics, antihypertensives, antiepileptics, and biological treatments, such as anti-tumor necrosis factor alpha (anti-TNF-α) agents, can also trigger lupus.[2]

The mechanism by which biologic treatments cause lupus differs from other drugs, as they directly affect the immune response and resemble idiopathic SLE rather than DIL. This may result in clinical findings characterized by hypocomplementemia, low anti-histone antibodies, high anti-double stranded DNA (anti-dsDNA) antibodies, and a typical SLE eruption. Since low TNF levels play a role in the pathogenesis of SLE, the reduction of TNF levels, apoptosis of cytotoxic T-cells, and induction of B-cell activation with anti-TNF-α treatments are possible mechanisms that increase the susceptibility to SLE.[3] However, anti-

Correspondence: Şeyma Türkmen, University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Pediatric Rheumatology, İstanbul, Turkey
E-mail: drpieni@hotmail.com **ORCID:** 0000-0002-2318-0361

TNF-α-induced lupus (ATIL) is rare.[4] Here, we report three cases that developed lupus-like syndrome while receiving infliximab therapy for various reasons.

Case Reports

Case-1

A 16-year-old girl, who had been receiving infliximab treatment for ulcerative colitis for eight months, was referred to us because of joint pain, morning stiffness lasting half an hour, hair loss, malar rash, recurrent oral aphthae, and Raynaud's phenomenon. In laboratory examination, ANA was positive (1/160 titer in nucleolar pattern), both complement 3 (C3) and complement 4 (C4) were lower [0.85 g/L (0.9-1.8) and 0.06 g/L (0.1-0.4), respectively], though anti-dsDNA and anti-histone antibody were negative. The patient was evaluated as ATIL because of new onset clinical findings under infliximab treatment and at least one laboratory finding. Infliximab treatment was discontinued. In the follow-up, low-dose corticosteroid and hydroxychloroquine treatment was started. Clinical and laboratory findings were recovered in the second month of follow-up.

Case-2

A 16-year-old girl with a diagnosis of juvenile idiopathic arthritis was diagnosed with ATIL at the tenth month of treatment after receiving infliximab for nine months for uveitis. The patient had a malar rash, anemia, and persistent hypocomplementemia. ANA, anti-dsDNA, and anti-histone antibody were found positive. Infliximab was discontinued. Therefore, the patient had refractory uveitis, adalimumab treatment was started. Low-dose corticosteroid and hydroxychloroquine were added to her treatment. During the follow-up, mycophenolate mofetil (MMF) treatment was started as a steroid-sparing treatment. Her clinical findings regressed in the sixth month of her follow-up.

Case-3

A 14-year-old male patient had been receiving infliximab treatment for 20 months for uveitis and was diagnosed with ATIL in the 21st month of treatment. In the examinations of the patient due to weight loss and recurrent oral aphthae, ANA was found 2 positive (a granular pattern at a titer of 1/320), and anti-dsDNA: 177.96 IU/mL (+). The complement levels of the patient were normal, anti-histone antibody was negative. Infliximab treatment was discontinued in the patient whose uveitis was under control. Hydroxychloroquine was added to the treatment of the patient whose symptoms continued. While the patient was being followed up in remission, in the examinations performed due to recurrence of oral aphthae six months later, ANA was found to be (+) in a homogeneous pattern at a titer of 1/160. Since there was no response to the hydroxychloroquine treatment, it was discontinued and MMF was started as a steroid-sparing treatment. At the follow-up one month later, the patient's ANA positivity continued, but his symptoms regressed. Adalimumab treatment was started in the patient who had an attack of uveitis in the follow-up. However, the patient's SLE findings did not recur. The clinical features of three patients diagnosed with ATIL are summarized in **Table 1**.

Discussion

DIL is an autoimmune condition in which certain drugs can induce clinical features resembling SLE. Typical complications of SLE are not observed in DIL.[2,5]

Anti-TNF treatments are among the causes of DIL. Most cases of ATIL occur due to infliximab therapy because infliximab is the most immunogenic anti-TNF agent due to its chimeric structure and ability to reach high tissue concentrations.[5]

ATIL findings were observed in these cases, and in two of them, adalimumab treatment was initiated following the development of uveitis after discontinuing infliximab, without recurrence of ATIL. Although all anti-TNF agents can lead to autoantibody production, the development of SLE is rare.[6] The precise incidence of ATIL is not well-known due to its relatively recent recognition, and few studies have been conducted on this topic.[7]

Some prospective studies have reported variable frequencies of ANA and anti-dsDNA positivity related to infliximab treatment.[8-10] The concomitant use of methotrexate with anti-TNF treatments may suppress autoantibody development and reduce the incidence of

Table 1. *Clinical characteristics of patients with anti-TNF-α therapy-induced lupus*

Parameters	Case-1	Case-2	Case-3
The age of diagnosis (years)	16	16	14
ATIL symptoms	- Malar rash, - Joint pain, - Morning stiffness - Hair loss, - Recurrent oral aphthae, - Raynaud's phenomenon	- Malar rash	- Weight loss, - Recurrent oral aphthae
Laboratory findings of ATIL			
ANA (titer and pattern)	1/160, nucleolar	1/1000, homogeneous	1/320, granular
Complement 3 (g/L)	0.85	0.75	1.22
Complement 4 (g/L)	0.06	0.05	0.15
Anti-dsDNA Ab (IU/mL)	<10	106.48	177.96
Anti-histon Ab	Negative	Positive	Negative

ATIL: Anti-TNF-α-induced lupus, ANA: Anti-nuclear antibody, Anti-dsDNA: anti-double stranded DNA, Ab: Antibody, TNF:Tumor necrosis factor

ATIL.[10,11] In our second and third cases, methotrexate was used for uveitis before biologic therapy. However, infliximab treatment was started in our cases because of severe methotrexate intolerance and refractory uveitis. MMF was added to adalimumab treatment after the development of ATIL due to methotrexate intolerance.

Although ATIL is considered one of the DIL forms; it differs in pathophysiology, clinical and laboratory findings. In 2018, Shovman et al.[4] have described ATIL cases with various clinical manifestations, such as thrombocytopenia, polyarthritis with lymphopenia, and severe serositis with pancytopenia, along with positive ANA and anti-dsDNA antibodies. The first of these was a patient with thrombocytopenia, which resolved after discontinuation of infliximab. The second patient had polyarthritis accompanied by lymphopenia after infliximab treatment. In the third case, severe serositis findings accompanied by ascites, pleural and pericardial effusion were present with pancytopenia. While ANA and anti-dsDNA positivity were detected in all three patients, anti-histone antibodies were positive only in the second case. Similarly, in 2022, Stranks and Chapman[6] described a case of infliximab-associated lupus in a patient receiving infliximab therapy for sarcoidosis. The patient had newly developed weakness, migratory joint pain, and positive serum autoantibodies. In 2008, Costa et al.[12] found that in a study, anti-dsDNA positivity, hypocomplementemia, rash and kidney disease more frequently in ATIL cases compared to DIL due to other drugs. While all of our cases had ANA positivity, two had anti-dsDNA positivity and two had low complement; anti-histone antibody positivity was detected in only one patient. All patients had at least one of the clinical findings of SLE. As seen in our patients, anti-TNF-associated DIL is reminiscent of idiopathic SLE; typical SLE rash, hypocomplementemia, low anti-histone and high anti-dsDNA antibodies may be found.[7]

There are no specific diagnostic criteria for the diagnosis of ATIL. However, a common approach used for diagnosis is to consider the presence of at least one clinical and serological criterion from the American College of Rheumatology criteria for SLE, along with the onset of symptoms after anti-TNF therapy and regression upon discontinuation of the treatment.[4] It should be noted that different criteria may be used in different studies.

The most important aspect of treatment is the withdrawal of the responsible agent. In general, clinical manifestations of ATIL tend to regress within the first six months, although autoantibodies may remain positive for an extended period.[5] Some patients may require corticosteroids and immunosuppressive agents.[4]

In mild cases, as observed in our patients, another anti-TNF agent may be considered. In a study by Ramos-Casals et al.[13] in 2007, most cases of ATIL showed regression of lupus-like symptoms after discontinuation of anti-TNF therapy. However, it should be noted that 40% of patients required corticosteroid treatment and 12% needed additional immunosuppressive therapy. Similarly, our cases required various immunosuppressive treatments in addition to discontinuation of infliximab therapy.

Conclusion

When patients receive anti-TNF therapy for any indication, the possibility of ATIL should be considered in the presence of a compatible medical history, clinical findings, and autoantibodies. Prompt recognition and appropriate management are crucial in optimizing patient outcomes.

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Case Report

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A Rare Cause of Hypotonia: 49,XXXXX (Pentasomy X)

Author(s)

Kübra Aydoğan[1], Selcan Öztürk[2], Munis Dündar[3], Hakan Gümüş[2], Çetin Saatçi[3], Hüseyin Per[2]

Affiliation(s)

[1]Recep Tayyip Erdoğan Training and Research Hospital, Clinic of Pediatrics, Rize, Turkey

[2]Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Kayseri, Turkey

[3]Erciyes University Faculty of Medicine, Department of Genetics, Kayseri, Turkey

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Abstract

Pentasomy X syndrome is a very rare sex chromosome numerical anomaly of unknown frequency. The karyotype consists of 49,XXXXX. Musculoskeletal, craniofacial, cardiac, and kidney anomalies accompany psychomotor developmental delays. This report describes, a 16-month-old girl who presented to the pediatric neurology outpatient clinic with complaints of joint laxity and inability to hold her head upright from the age of 3-4 months. The patient exhibited dysmorphic facial features and hand-foot deformities. Genetic consultation was requested, and cytogenetic examination revealed a 49,XXXXX chromosomal anomaly. The most prominent clinical feature of 49,XXXXX patients with pentasomy is severe hypotonia. This article emphasizes the importance of cytogenetic analysis in the evaluation of hypotonicity.

Keywords: 49,XXXXX, cytogenetic analysis, development delay, hypotonia

Introduction

Pentasomy X syndrome is a rare chromosomal disorder involving three additional X chromosomes (49,XXXXX rather than 46,XX).[1] It is characterized by severe hypotonia, microcephaly, craniofacial anomalies, bone, and joint abnormalities, heart and/or kidney defects, and mental disability.[1] The incidence is not known precisely although approximately 40 cases have been reported in the literature to date.[2,3] It is important to consider chromosomal diseases when physical dysmorphism and psychomotor developmental retardation are detected in the evaluation of hypotonic infants.

Case Report

This case report describes a 16-month-old girl with no consanguinity between her parents who was referred to a pediatric neurology clinic due to joint laxity compared to her peers. Her mother was a healthy 29-year-old, and this had been her first pregnancy. The baby was born at term, weighing 2500 grams. When the infant was 3-4

Correspondence: Hüseyin Per, Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Kayseri, Turkey
E-mail: huseyinper@yahoo.com **ORCID:** 0000-0001-9904-6479

months old, the parents noticed that she was unable to hold her head erect. At the physical examination, the patient weighed 11 kg (75p), with a length of 80 cm (75-90p), and a head circumference of 46 cm (25-50p). The patient exhibited various developmental abnormalities, including micrognathia, a short neck with a low hairline, posterior positional plagiocephaly with epicanthus, and hypertelorism (**Figure 1**). Additionally, she demonstrated hypotonia, motor delays, pes equinovarus, and decreased deep tendon reflexes (**Figure 2**). She could not touch the soles of her feet at axillary suspension, and the traction test was incompatible with her age. Comprehensive clinical evaluations, including laboratory tests [complete blood count, biochemistry values, and metabolic tests (ammonia, lactate, pyruvate, plasma amino acid, urinary amino acid, urine organic acid, and tandem mass spectrometry)], imaging, and genetic analysis, were performed to identify the underlying cause. Magnetic resonance imaging of the brain revealed an "expansion in central and peripheral cerebrospinal fluid distances, and bilateral mastoid effusion, other areas being within normal limits". There were no obvious abnormalities or pathologies detected in hearing tests, abdominal ultrasonography, and babygram. Cardiac echocardiography was normal. SMN gene analysis for spinal muscular atrophy was also normal. Chromosome analysis using the peripheral blood culture method was consistent with '49,XXXXX' (**Figure 3**). The investigations revealed a diagnosis of pentasomy X syndrome, a rare chromosomal disorder. This case report highlights the importance of considering chromosomal abnormalities in the differential diagnosis of developmental delays and joint laxity in pediatric patients.

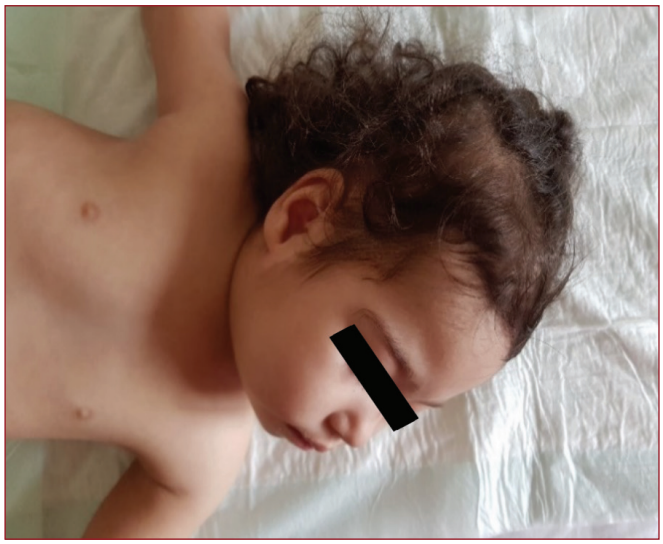

Figure 1. Facial appearance of the patient.

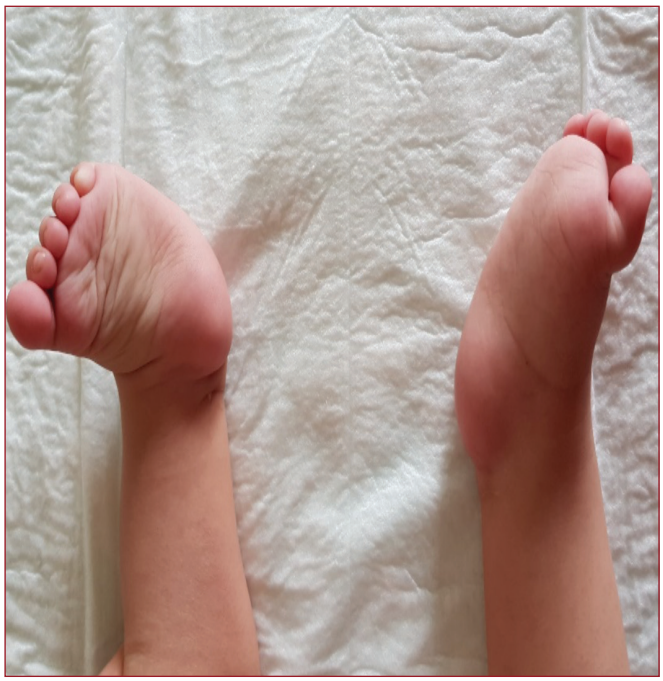

Figure 2. Pes equinovarus of the patient's lower extremity.

Discussion

Central nervous system disorders, cerebral malformations, Zellweger syndrome, lipid storage diseases, hypoxia, hemorrhage, infection, trauma, muscle diseases, muscle neuron junction diseases, motor neuron damage, spinal cord trauma, mitochondrial diseases, glycogen storage diseases, congenital disorders of glycosylation, and peripheral nerve diseases are some of the principal causes of hypotonia.[4-6] Some of these were investigated in the present case, and the chromosomal disorder pentasomy X was identified as the etiology. Pentasomy X is a very rare cause of hypotonia.

The first case of pentasomy X in the literature was reported by Kesaree and Wooley in 1963.[7] While sex chromosome numerical anomalies such as 45,X, 47,XXX, 47,XXY, 47,XYY, and 48,XXXX are seen in approximately one in 400 births, pentasomy X is a rare sex chromosome numerical anomaly of unknown frequency.[2] Approximately 40 cases with 49,XXXXX, and only five with an intrauterine diagnosis, have been reported worldwide. The condition has also been reported to occur in 1 in 85,000-250,000 females.[8-10]

Females affected by pentasomy X exhibit dysmorphic craniofacial anomalies such as microcephaly, a round face, a flattened nasal root, ear anomalies, pre-auricular skin tag, low hairline, ptosis, micrognathia, a high palate, cleft palate, thick lips, and irregular teeth. Ocular abnormalities may also be present, such as iris colobomas, hypertelorism, epicanthal folds, and upslanting palpebral fissures.[1,9] Musculoskeletal defects such as radioulnar synostosis, camptodactyly, clinodactyly, small hands and feet, thenar atrophy,

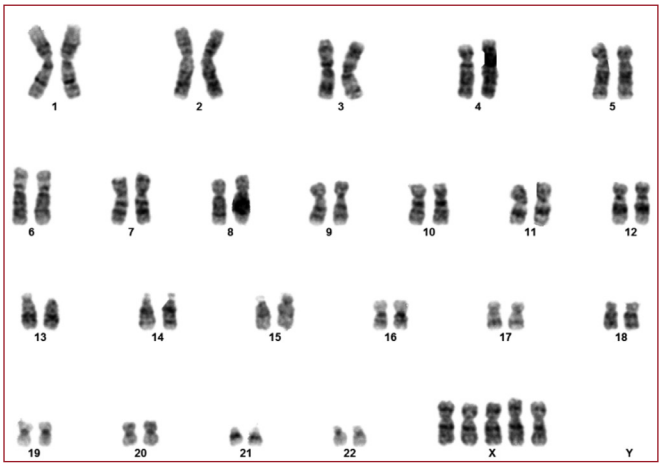

Figure 3. Pentasomy X appearance in the patient's chromosome analysis.

joint subluxation, hyporeflexia, hyperlaxity of joints, and hip dysplasia are also common in pentasomy X syndrome.[1] Our patient exhibited joint laxity, the elbow being particularly affected. Radioulnar synostosis, hydrocephalus, Dandy-Walker malformation, polyhydramnios, pleural effusion, and subcutaneous edema may be detected at prenatal ultrasonography.[8,9,11]

In some cases, pentasomy X syndrome may be accompanied by cardiac and genitourinary abnormalities such as ventricular septal defect and/or patent ductus arteriosus, horseshoe kidney, renal dysplasia, and a small uterus.[10] Although no external genital anomalies are usually detected, gonadal dysfunction and clinical infertility have been reported. Consistent with the literature, the genital examination in this study was also unremarkable. It is recommended that patients undergo pelvic/renal ultrasonography and echocardiographic examination.[1,8,9]

Some cases in the literature have been followed up with a diagnosis of Down syndrome due to clinical similarities between the two entities.[2] Cytogenetic analysis is therefore of great importance in the differential diagnosis.[3] In pentasomy, one X chromosome comes from the father and four from the mother because of non-splitting in the metaphase of meiosis.[1] According to previous studies, pentasomy is caused by a pathological X gene of maternal origin.[2] No stimulant-specific risk factor has been determined in the literature that might prompt intrauterine genetic counseling or a fetal karyotype study. Previous studies have also shown that maternal age is not a risk factor for pentasomy X.[8]

The absence of an identifiable risk factor for pentasomy X makes diagnosis difficult. The only known definite risk factor for pentasomy X is the female gender.[5] The literature reports consistent mental and growth delay in patients with pentasomy.[2,9] We applied the Denver Developmental Screening test to our patient and determined that she lagged behind her peers. Although a manifestation of immunodeficiency secondary to immunoglobulin disorder may be expected, our patient did not exhibit a history of frequent infections at clinical follow-up, and in contrast to the literature, no immunodeficiency was detected.[8,12]

Individual cases of pentasomy X may exhibit all the symptoms discussed above, and the condition can affect numerous systems. Treatment should be directed toward the specific symptoms in affected individuals. External ear anomalies can cause hearing impairment, and regular hearing screening is therefore recommended.[13] Loose joints and decreased muscle tone affect the posture of girls with pentasomy X; in a standing position, the feet are inclined inward, and ankle support is therefore needed before walking. If the patient has congenital heart disease, medical treatment or, surgical intervention may be required.[1,13] Patients should be given adequate developmental therapy, speech therapy, special education, and genetic counseling.

Conclusion

In conclusion, severe hypotonia is the most prominent clinical feature of patients with 49,XXXXX. There are thought to be more patients with pentasomy X, but that diagnosis is missed in many cases. More detailed studies and data are needed in order to identify these patients at an earlier stage. This case is therefore presented to emphasize the need for cytogenetic analysis, especially in female patients investigated for hypotonia.

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Ahmet Mithat Elmacı
Ahmet Özdemir
Ahmet Sami Güven
Aycan Ünalp
Ayşegül Bükülmez
Ayşegül Yılmaz
Betül Sözeri
Buket Kara
Burcu Güven
Can Acıpayam
Ceren Çetin
Demet Kartal
Eda Karadağ Öncel
Eda Kepenekli
Edanur Yeşil
Emel Kabakoğlu Ünsür
Esra Türe
Esranur Çiğ
Fatih Battal
Fatih Kardaş
Fatma Hancı
Fatma Nur Öz
Filiz Tubaş
Hacer Aktürk
İbrahim Gökçe
İsmail Dursun
Kenan Yılmaz
Maruf Göğebakan
Meda Kondolot
Mehmet Köse
Melih Hangül
Nagehan Emiralioğlu
Nagihan Erdoğ Şahin
Nihal Hatipoğlu
Nihal Şahin
Nurullah Çelik
Okan Akacı
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Ömür Parkan
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Selcan Demir
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Sinan Akbayram
Şefika Akyol
Taylan Çelik
Ülkü Gül Siraz
Ümit Altuğ
Veysel Garani Soylu
Yavuz Köksal
Zahide Ekici Tekin