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The Journal of Pediatric Academy is the official publication of the Kayseri Child Health Association.

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Manuscript Types

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Research Articles:

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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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Editorial comment	1500	No abstract	15	2	5
Original Article	3500	300	50	6	6
Invited Review	5000	350	100	6	10
Case Report	1500	200	15	2	5
Image corner	500	No abstract	5	-	3
Letter to the Editor	100	No abstract	5	1	1

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Cite unpublished data—such as papers submitted but not yet accepted for publication and personal communications, including e-mail communications—in parentheses in the text. If there are more than three authors, name only the first three authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names, or access the list at http://www.nlm.nih.gov/tsd/serials/lji.html. Sample references are given below:

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.

URL (Uniform Resource Locator)

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

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Retrospective Analysis of Transfusion-Related Adverse Reactions: A 15-Month Study of a Single Center's Experience

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Abstract

The aim of this study was to evaluate transfusion-related adverse reactions (TRARs). In this study, all adverse reactions (ARs) related to blood/blood product transfusions conducted between 01.01.2022 and 31.03.2023 at the Health Sciences University Türkiye, Adana City Training and Research Hospital were evaluated. In total, 97,926 records of blood and blood component transfusions were evaluated during the study period. The distribution of blood components used was as follows: 57,066 (58.2%) red blood cell concentrates, 27,345 (28%) fresh frozen plasma, 12,282 (12.5%) pooled platelet concentrates, 564 (0.6%) apheresis platelet concentrates, and 669 (0.7%) cryoprecipitates. In total, 40 AR reports were associated with transfusions. The probability levels of the relationship degrees of reactions for these 40 cases were as follows: 2 cases; not likely (5%); 32 cases; likely (80%); 2 cases; highly likely (5%); and 4 cases, unassessable (10%). All unwanted reactions were acute, and there were no delayed reactions. No transfusion reaction (TR) leading to death occurred. Of the patients who developed reactions, 60% (n=24) were female, and 40% (n=16) were male. The ages of patients with unwanted reactions ranged from 2 to 86 years, with a median age of 33. Among the cases with unwanted reactions, 8 were children (20%) and 32 were adults (80%). In our study, the frequency of allergic TR was 8.1 per 100,000 children and 32.6 per 100,000 adults. A statistically significant difference in the distribution of blood component types among cases based on the types of unwanted reaction was observed (p=0.003).

Keywords: Transfusion, hemovigilance, hemolysis

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Introduction

Red blood cell (RBC) transfusion is necessary to enhance a patient's oxygen-carrying capacity[1]. Transfusion reactions (TRs) are defined as adverse events associated with whole blood or its components transfusion. Their severity can range from minor to life-threatening[2]. According to the onset time, adverse reactions (ARs) of blood transfusion are classified as acute (occurring within the first 24 hours) and delayed (occurring after 24 hours). In cases of acute TR, prompt identification and immediate cessation of transfusion are critical. These reactions can typically occur immediately or within a few hours after transfusion, and their severity varies depending on the type of reaction, the patient's overall health condition, and promptness of treatment response. Occasionally, some patients may develop anaphylaxis or severe allergic reactions during or after transfusion, characterized by rapidly spreading skin rashes, respiratory distress, low blood pressure, and even shock. Acute hemolytic reactions occur as rapid and intense immune responses to blood cells. If an incorrect blood group is transfused or in cases of severe incompatibility, the patient's own blood cells can break down, leading to serious consequences, such as kidney damage and organ failure. Transfusion-related acute lung injury is a rare but serious condition characterized by acute respiratory failure and fluid accumulation shortly after transfusion, triggered by antibodies in the donor's blood reacting with the recipient's immune system. Transfusion-associated circulatory overload is associated with significant fluid overload and cardiovascular stress, particularly following high-volume transfusions, leading to septic symptoms[3,4]. Vigilance is necessary to differentiate delayed responses or reactions displaying non-specific signs and symptoms[5]. Transfusion-related ARs (TRAR) are classified according to the National Hemovigilance Guide version 2, March 2020[6]. Hemovigilance encompasses a set of monitoring procedures that involve collecting, evaluating, and preventing the recurrence of unwanted events and reactions related to the entire transfusion chain, from the collection and processing of blood and blood components to their transfusion and follow-up, aiming to gather information [7].

The present study aimed to determine the frequency of TRAR among patients receiving blood transfusions in our tertiary care hospital and contribute to the national hemovigilance data.

Highlights

- Frequency of transfusion-related adverse reactions (TRARs): TRARs are very rare. In this retrospective study conducted at a single center and based on 15 months of data, 97,926 blood component transfusions were performed, and the prevalence of TRARs was 40.8 per 100,000 blood components.
- Hemovigilance: Hemovigilance encompasses the reporting, monitoring, and analysis of adverse events with the inclusive goal of improving donor and patient safety throughout the transfusion process. The current study aimed to determine the frequency of TRARs in patients undergoing blood transfusion at our tertiary care hospital and contribute to national hemovigilance data.
- Evaluation of blood component types by adverse reaction types: In our study, statistically significant differences were found in the distribution of blood component types among cases according to the types of adverse reactions (p=0.003).

Material and Method

In this study, all ARs related to blood/blood product transfusions conducted at the Health Sciences University Türkiye, Adana Faculty of Medicine, Adana City Training and Research Hospital between January 01, 2022, and March 31, 2023, were evaluated. Transfusion monitoring forms specific to patients, suspected adverse reaction forms related to transfusion, investigation and treatment forms, rapid notification forms, and verification forms standardized in the NHG were retrospectively examined from the hospital's hemovigilance unit archive and Hospital Information Management System.

All TR reported to the hemovigilance unit were classified according to the degree of evidence-based relationship degree[6]. The severity of TRAR was graded according to the form specified in the NGH version 2, March 2020[6].

This study was approved by the Adana City Training and Research Hospital Clinical Research Ethics Committee (decision no: 2426, date: 06.04.2023).

Statistical Analysis

The statistical analysis of the study was conducted using the Statistical Package for the Social Sciences version 26 (IBM Corp., Armonk, NY, USA) software. The demographic data of the patients were presented using descriptive statistics. Categorical measurements were presented as counts and percentages, whereas numerical measurements were presented as means and standard deviations (or medians and interquartile ranges where necessary). The chi-square test was used to compare categorical measurements between groups, and the chi-square test for multiple proportions was employed for multi-category comparisons. A statistical significance level (p) of 0.05 was considered statistically significant in all analyses.

Results

In total, 97,926 records of blood and blood component transfusions were evaluated during the study period. The distribution of blood components used was as follows: 57,066 (58.2%) RBC concentrates, 27,345 (28%) fresh frozen plasma (FFP), 12,282 (12.5%) pooled platelet concentrates, 564 (0.6%) apheresis platelet concentrates, and 669 (0.7%) cryoprecipitates. In total, 40 TRARs were reported. The probability levels of the

relationship degrees (imputability) for the reactions of these 40 cases were as follows: 2 cases; not likely (5%); 32 cases; likely (80%); 2 cases; highly likely (5%); and 4 cases, unassessable (10%). All unwanted reactions were acute, and there were no delayed reactions. No TR leading to death occurred.

Among the patients who developed reactions, 60% (n=24) were female, and 40% (n=16) were male. The ages of patients with unwanted reactions ranged from 2 to 86 years, with a median age of 33. Among the cases with unwanted reactions, 8 were children (20%) and 32 were adults (80%). In our study, the frequency of allergic TR was 8.1 per 100,000 children and 32.6 per 100,000 adults. Among these patients, 16 were blood group A Rh-positive (40%), 1 was A Rh-negative (2.5%), 7 were B Rh-positive (17.5%), 14 were O Rh-positive (35%), and 2 were AB Rh-positive (5%). The most common symptom observed was itching, with a rate of 37.5% (n=15). The second most frequently observed symptom was fever, at a rate of 15% (n=6). Redness, shortness of breath, and rash were observed at a rate of 12.5% each (n1=5, n2=5, n3=5). Other observed symptoms included hypotension, headache, nausea, and tachycardia at a rate of 2.5% (n1=1, n2=1, n3=1, n4=1).

When unwanted reactions were evaluated, “mild allergic reaction” was observed in 26 patients (65%). “Febrile non-hemolytic transfusion reaction” (FNHTR) was observed in 6 patients (15%). “Acute undefined transfusion reaction” was observed in 3 patients (7.5%). “Transfusion-associated shortness of breath” was observed in 2 patients (5%), and “anaphylactic reaction” was observed in 2 patients (5%). Unwanted reactions related to transfusion were associated with 18 cases (45%) of RBC Suspension, 20 cases (50%) FFP, and 2 cases (5%) of pooled platelet suspension.

A statistically significant difference in the distribution of blood component types among cases based on the types of unwanted reaction was observed (p=0.003) (**Table 1**).

Discussion

Hemovigilance encompasses the reporting, monitoring, and analysis of adverse events with the inclusive goal of improving donor and patient safety throughout the process of transfusion from vein to vein[7]. In this study, conducted at a single center and retrospectively evaluating 15 months of data, a total of 97,926 blood component transfusions were performed, and the prevalence of TRAR was 40.8 per 100,000 blood components. Although blood transfusion is a life-saving treatment method, TRAR is associated with common complications that rarely result in death[8]. When the literature is reviewed, it provides significant insights into the frequency, diversity, and impact of TRs. Large-scale epidemiological studies have indicated that the most common ARs post-transfusion are febrile non-hemolytic TRs (FNHTR) and mild allergic reactions. FNHTR is characterized by symptoms such as high fever and chills, typically resulting from immune responses. Allergic reactions may present with mild symptoms like itching, redness, hives, or localized angioedema, and may occasionally escalate to serious conditions, such as anaphylaxis[9,10].

Despite the high safety of blood transfusion, adverse effects can still occur. Generally, unwanted reactions occur in approximately 1% of transfusions[11]. Allergic TR is mostly characterized by mild clinical symptoms, such as itching, redness, urticaria, or localized angioedema. Anaphylactic reactions, on the other hand, are severe allergic reactions accompanied by bronchospasm and hypotension[12]. In our study, when unwanted reactions were evaluated, “mild allergic reaction” was observed in 26 patients (65%). The second most frequent allergic reaction is FNHTR. These reactions are defined by the U.S. Centers for Disease Control and Prevention defined as an increase in body temperature to 38°C or higher, an increase of ≥1°C within 4 hours of transfusion, or the occurrence of chills and shivering[13]. In our study, “febrile non-hemolytic reaction” was observed in 6 patients (15%). Literature reports also highlight the occurrence of rare yet life-threatening reactions, such as acute hemolytic reactions. These reactions can occur due to factors like mismatched blood transfusions or pre-existing antibodies to transfused blood products, which significantly impact the patient’s health. Hemovigilance programs play a crucial role in the early identification and management of such serious reactions[14,15].

Hericks et al.[16] reported a case of acute hemolytic reaction in a neonate likely caused by transfusion of an FFP product containing autoantibodies. In our study, ARs were observed in 20 out of 40 patients receiving FFP who developed unwanted reactions. A comparison of TR rates between children and adults in a tertiary

Table 1.
Evaluation of blood component types according to unwanted reaction types

Transfusion reactions	Blood products			p-value
	Red blood cell suspension	Fresh frozen plasma	Platelet suspension	
	n (%)	n (%)	n (%)	
Mild allergic reaction	4 (%22.22)	20 (%100)	2 (%100)	
Febrile nonhematologic transfusion reaction	6 (%33.33)	0	0	
Hypertensive transfusion reaction	1 (%5.56)	0	0	
Acute undefined transfusion reaction	3 (%16.67)	0	0	**0.003**
Transfusion-related dyspnea	2 (%11.11)	0	0	
Anaphylactic reaction	2 (%11.11)	0	0	
Total	18 (%100)	20 (%100)	2 (%100)	

care institution in the United States was published by Oakley et al.[17] in 2015. During the 2-year study period, the incidence of allergic TR was 2.7 per 1000 individuals in children and 1.1 per 1000 adults[17]. Kracalik et al.[18] reported 18,308 TRARs among 8.34 million transfused blood components (220 per 100,000) from 2013 to 2018 in 201 facilities. In our study, the frequency of allergic TR was 8.1 per 100,000 children and 32.6 per 100,000 adults. Advancements in the management and prevention of TRs play a pivotal role in clinical practice and the formulation of transfusion policies. Recent research continuously enhances the knowledge and practices related to transfusion safety, thereby ensuring optimal patient outcomes from this critical medical intervention. In this context, hemovigilance is central to enhancing transfusion safety and minimizing potential risks[19,20].

Furthermore, our study found a statistically significant difference in the distribution of blood component types among cases based on the types of unwanted reaction (p=0.003).

Conclusion

The present study aimed to determine the frequency of TRAR among patients receiving blood transfusions in our tertiary care hospital and contribute to the national hemovigilance data. In this retrospective evaluation of 15 months of data from a single center, 97,926 blood component transfusions were performed, and the prevalence of TRAR was 40.8 per 100,000 blood components.

Ethics

Ethical Approval: This study was approved by the Adana City Training and Research Hospital Clinical Research Ethics Committee (decision no: 2426, date: 06.04.2023).

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

Acknowledgments

This article was presented as an oral presentation at the 22nd International Eastern Mediterranean Family Medicine Congress.

Footnotes

Author Contributions: Çalışkan Kamış Ş: Literature Search, Writing.; Tuncel DA: Data Collection or Processing.; Küpeli GB: Concept, Design, Data Collection or Processing, Analysis or Interpretation.

Conflict of Interest: The authors declare no conflicts of interest.

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

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Do Vitamin D Levels Play a Role in Urinary Tract Infection in Children?

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Abstract

Previous studies have shown the protective effects of vitamin D supplementation against urinary tract infection (UTI). However, there are a few contradictory studies on the negative effect of vitamin D supplementation on UTI. Our objective was to establish whether there existed a relationship between serum vitamin D levels and UTIs in children. This study compared the serum 25-hydroxy vitamin D [25(OH)D] levels of children diagnosed with UTIs with those of healthy children (control group). We found a high rate of 25(OH)D deficiency in both the control and case groups (90% and 66.7%, respectively). Therefore, we added the laboratory parameters calcium, phosphate, and parathormone (PTH) to our analysis. We assessed the medical records of 60 patients diagnosed with UTIs and 20 healthy controls. The mean serum 25(OH)D level and PTH level were significantly higher in the patient group than in the control group. The PTH level was significantly lower in the acute pyelonephritis (APN) group than in the control group (p=0.016). Phosphate levels in the APN group were significantly lower than those in the control and cystitis groups (p=0.04, p=0.006 respectively). Because there was no correlation between 25(OH)D level and UTI, we concluded that 25(OH)D had no effect on UTI.

Keywords: Urinary tract infection, children, vitamin D, parathormone, fibroblast growth factor 23

Introduction

Urinary tract infection (UTI) is a common infection in the pediatric age group.[1] The lifetime incidence is approximately 8% in girls and 2% in boys, and it is 3-5 times higher in girls than boys.[2] Recurrent UTI and acute pyelonephritis (APN) may cause renal scarring, hypertension, and ultimately chronic kidney disease.[3] 25-hydroxy vitamin D [25(OH)D] has an immunomodulatory effect through the synthesis and production of cytokines.[4] Some studies have shown its positive role in respiratory tract infections.[5,6] Additionally, studies have shown the protective effect of 25(OH)D in UTI.[7,8] However, there are a few contradictory studies about the negative effect of 25(OH)D on UTI, which have shown that the administration of 25(OH)D supplements increases the risk of UTI.[9,10] In this study, we aimed to evaluate vitamin D levels in children with upper and lower UTIs and compare them with healthy individuals, thus investigating whether there is a relationship between UTI and vitamin D. As is well known, 25(OH)D has a stimulatory effect on

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calcium and phosphate homeostasis and a negative feedback effect on parathormone (PTH).[11] Therefore, we also examined the relationship of these parameters (calcium, phosphate, PTH) with UTI.

Material and Method

Children with UTI (case group) who presented to our pediatric nephrology clinic were compared with a randomly selected control group. A "true" UTI is defined as urinalysis positive for leukocytes ± nitrites, a urine culture consisting of >50,000 colony-forming units/milliliter (CFU/mL) from a urinary catheter, or >100,000 CFU/mL in a midstream urine sample.[12] We also compared the UTIs in 2 groups: APN and cystitis. Symptoms such as fever, chills, fatigue, flank pain, nausea/vomiting, and laboratory findings such as elevated white blood cell count, C-reactive protein, or erythrocyte sedimentation rate distinguish APN from cystitis.[2] As is well known, 25(OH)D has a stimulatory effect on calcium and phosphate homeostasis and a negative feedback effect on PTH.[11] Therefore, we added the laboratory parameters calcium, phosphate, and PTH to our analysis. We excluded patients younger than 2 years of age because most infants in our study population receive vitamin D supplementation until 1-2 years of age. Children with malnutrition, chronic diseases, or vitamin D supplementation were excluded. The inclusion criteria for the case group were patients diagnosed with UTI with pyuria and/or positive nitrite test and positive urine culture of a single pathogen.[11] The two groups were also compared in terms of age, gender, height, weight, and body mass index (BMI). After obtaining written consent from the parents, 3 mL of blood was collected from both the control and patient groups during outpatient clinic visits throughout the day to assess serum 25(OH)D levels. The blood samples were centrifuged to separate the serum, which was then stored at 20°C until the tests were conducted. Serum 25(OH)D levels were measured using the ELISA method.

Ultrasonography and voiding cystourethrogram were performed to rule out urinary system abnormalities and vesicoureteral reflux (VUR) in patients with atypical and recurrent UTIs. Labial adhesions and phimosis were excluded by clinical examination. Patients with 25(OH)D levels 20 ng/mL (<50nmol/L) were classified as having 25(OH)D deficiency.[9] Ethics committee approval of the study was obtained from the Kırıkkale University Non-Interventional Clinical Research Ethics Committee (decision no: 2022.04.25, date: 27.04.2022). The authors confirm that informed consent was obtained from the participants or their legal guardians, and all required ethical approvals were obtained for the study.

Highlights

- 25-hydroxy vitamin D [25(OH)D] exhibits immunomodulatory effects through the synthesis and generation of cytokines.
- Additionally, previous studies have demonstrated the protective impact of 25(OH)D against urinary tract infections (UTIs).
- Given the absence of a connection between 25(OH)D levels and UTI, we deduced that 25(OH)D does not influence UTI.
- Findings regarding parathormone (PTH), particularly in the acute pyelonephritis (APN) group, might be associated with fibroblast growth factor (FGF)-23; decreased PTH in the APN group could result from elevated FGF-23 levels in these individuals.

Statistical Analysis

The normality of data distribution was examined using the Kolmogorov-Smirnov test. All the parameters under investigation were normally distributed. Differences between the groups in terms of continuous variables in the two and three groups were evaluated using the Student's t-test, and when appropriate, ANOVA was employed. Differences in proportion were evaluated using the chi-square test. Correlations between parameters were assessed using Pearson's and Spearman's correlation tests. Regression analysis was used to assess the relationship of 25(OH)D, PTH, calcium, and phosphate with outcome parameters, including UTI and APN.

Results

We evaluated the medical records of 60 patients with UTI and 20 healthy controls. The mean age of the UTI patients was 87.7±43.1 months and 55 (91.7%) were women. The control group was composed of 10 (50%) women, and the mean age of 103.2±35.4 months. There was a significantly higher proportion of women in the UTI group than in the control group ($p<0.001$). No significant difference was found between the two groups in terms of age, height, weight, and BMI ($p>0.05$). We found a high rate of 25(OH)D deficiency in both the control and case groups (90% and 66.7%, respectively) (**Table 1**). The mean serum 25(OH)D level was significantly higher in the patient group than in the control group (18.1±8.1ng/mL vs. 12.7±5.6 ng/mL, $p=0.002$). Although there were no significant differences in serum calcium and phosphate levels ($p=0.055$, $p=0.386$, respectively), the PTH level was significantly lower in the patient group than in the control group (27.7±13.6 pg/mL, vs. 36.7±14.6 pg/mL, $p=0.044$). The proportion of individuals with 25(OH)D deficiency was significantly higher in the control group than in the UTI group ($p=0.043$) (**Table 1**).

In the UTI group, 41 (68.3%) patients were diagnosed with cystitis and 19 (31.7%) with APN. The most common causative agent was *E. coli* (81.67%), followed by *Klebsiella* spp. (6.67%), *Proteus* spp. (3.33%), *Pseudomonas* spp. (3.33%), *Citrobacter* spp. (3.33%) and *Enterobacter* (1.67%). Of the 49 patients with *E. coli* growth, 9 (18.4%) were extended-spectrum beta-lactamase (ESBL) positive. In addition, 27 (45%) patients were nitrite-positive. In terms of concomitant urinary disorders, VUR and nephrolithiasis were present in 3 (5%) patients. Eight patients experienced recurrent UTI.

There was no significant difference in mean age between the control, cystitis, and APN groups ($p=0.113$). Similarly, no significant difference was found between the groups in terms of BMI ($p=0.736$) (**Table 2**). The proportion of

girls in the APN and cystitis groups were significantly higher than that in the control group (p<0.001). However, there was no significant difference between the cystitis and APN groups in terms of gender (p=0.558). There were significant differences between the groups in terms of mean 25(OH)D, PTH, calcium, and phosphate levels (p=0.003, p=0.020, p=0.006, p=0.007 respectively). The 25(OH)D level was significantly higher in the cystitis group than in the control group (p=0.002); there was no significant difference between the APN and control groups (p=0.361). The proportion of individuals with 25(OH)D deficiency was significantly different between groups (p=0.045); the proportion was significantly higher in the control group than in the cystitis group (p=0.020). The PTH level was significantly lower in the APN group than in the control group; however, there was no significant difference between the cystitis and control groups or between the APN and cystitis groups (p=0.016, p=0.901, p=0.160, respectively). The calcium level in the APN group was significantly lower than that in the control and cystitis groups (p=0.011, p=0.016, respectively). There was no significant difference between the cystitis and control groups in terms of the mean calcium level (p=0.874). The phosphate level in the APN group was significantly lower than that in the control and cystitis groups (p=0.040, p=0.006, respectively). There was no significant difference between the cystitis and control groups in mean phosphate level (p=0.967).

25(OH)D levels were significantly lower in patients with positive urinary nitrite than in those with negative urinary nitrite (15.9±6.1 vs. 20.0±9.1; p=0.047). There was no significant difference in mean 25(OH)D levels between patients with *E. coli* growth and those without (17.6±7.9 vs. 19.5±8.9; p=0.464). In addition, there was no significant difference in the mean 25(OH)D level between patients with and without ESBL-positive urine culture versus those without (17.3±10.0 vs. 18.3±7.8; p=0.783, respectively).

Table 1.
Comparison of variables between case and control groups

Variable	Case group (n=60)	Control group (n=20)	P-value
Gender (female) n (%)	55 (91.7)	10 (50)	**<0.001***
Mean age (month)	87.7±43.1	103.2±35.4	0.118
Height (cm)	124±21	135±23	0.102
Weight (kg)	29.2±14.4	32.6±14.8	0.398
Body mass index	17.9±4.2	17.0±4.0	0.426
Serum 25(OH)D (ng/mL)	18.1±8.1	12.7±5.6	**0.002***
25(OH)D deficiency n (%)	40 (66.7)	18 (90)	**0.043***
Serum calcium (mg/dL)	9.8±0.5	10.1±0.3	0.055
Serum phosphate (mg/dL)	4.5±1.1	4.7±0.7	0.386
PTH (pg/mL)	27.7±13.6	36.7±14.6	**0.044***

*;p<0.05, 25(OH)D; 25-hydroxy vitamin D

Table 2.
Comparison of demographic and laboratory data between control, cystitis and acute pylenephritis groups (ANOVA test)

Variable	Control (n=20)	Cytitis (n=41)	APN (n=19)	P-value	Post-hoc analysis A=between control and cystitis B=between control and APN C=between APN and cystitis
Gender (female) n (%)	10 (50)	37 (90.2)	18 (94.7)	**<0.001***	**A=<0.001*** **B=<0.001*** C=0.558
Mean age (month)	103.2±35.4	83.7±43.2	96.3±42.7	0.113	
Body mass index	17.0±4.0	17.9±4.2	17.9±4.1	0.736	
Serum 25(OH) D (ng/mL)[1]	12.7±5.6	19.4±8.8	15.4±5.6	**0.003***	**A=0.002*** B=0.361 C=0.107
25(OH)D deficiency n (%)	18 (90)	25 (61)	15 (78.9)	**0.045***	**A=0.020*** B=0.339 C=0.170
Serum PTH (pg/mL)	36.7±14.6	31.8±14.4	20.0±7.7	**0.020***	A=0.901 **B=0.016*** C=0.160
Serum calcium (mg/dL)	10.1±0.3	10.0±0.5	9.6±0.5	**0.006***	A=0.874 **B=0.011*** **C=0.016***
Serum phosphate (mg/dL)	4.7±0.7	4.8±1.0	3.9±1.2	**0.007***	A=0.967 **B=0.040*** **C=0.006***

*;p<0.05, 25(OH)D; 25-hydroxy vitamin D, PHT; Parathormone, ANOVA; One-way analysis of variance

There was a negative correlation between age and 25(OH)D level (r=-0.267, p=0.039) in the patient group. In contrast, there was a positive correlation between age and PTH level (r=0.421, p=0.045). There was no correlation between 25(OH)D level and PTH level (r=-0.274, p=0.206).

Female gender and a high 25(OH)D level were found to be risk factors for UTI, although only female gender was found to be an independent risk factor in multiple regression analysis [odds ratio (OR)=0.058, 95% confidence interval (CI) 0.006-0.549; p=0.013*] (**Tables 3 and 4**).

Low calcium and phosphate levels were identified as risk factors for APN. However, multiple regression analysis showed that these variables were not independent risk factors (for calcium: OR=0.308, 95% CI 0.029-3.287; p=0.329; for phosphate: OR=0.547, 95% CI 0.161-1.862; p=0.334) (**Tables 5 and 6**).

Discussion

In the present study, we found a significantly higher 25(OH)D level and lower PTH level in patients with UTI. In addition, although there was no significant difference with respect to mean 25(OH)D and PTH levels, we found lower calcium and phosphate levels in APN than in cystitis.

In our study, we found a female sex preponderance in UTI, consistent with previous studies.[1] UTIs are more common in females than in males because of anatomic and physiologic factors.[1] In recent years, UTI caused by ESBL-positive microorganisms has not only been encountered in hospitals but also spread widely in the community.[13] Two studies from Thailand and Korea reported ESBL UTI rates of approximately 20%, which are comparable to the rate reported in our study.[14,15] VUR is found in 1-3% of the general population, whereas its prevalence is 30-45% in children with UTI.[16] Our patients had a relatively low VUR prevalence (5%) because our

Table 3.
Univariate regression analysis of variables in terms of risk for urinary tract infection

Variables	Univariate regression analysis
Gender (female)	OR=11.0, 95% CI=3.097-39.070; **p=<0.001***
Serum 25(OH)D (ng/mL)[1]	OR=1.135, 95% CI=1.029-1.252; **p=0.011***
Serum calcium (mg/dL)	OR=0.422, 95% CI=0.138-1.289; **p=0.130****
Serum phosphate (mg/dL)	OR=0.842, 95% CI=0.519-1.364; **p=0.484**
PTH (pg/mL)	OR=0.951, 95% CI=0.904-1.001; **p=0.057****

*; p<0.05, **; p<0.200, 25(OH)D; 25-hydroxy vitamin D, OR; Odds ratio, CI; Confidence interval, PTH; Parathormone

Table 4.
Multivariate regression analysis of variables in terms of risk for urinary tract infection

Variables	Multivariate regression analysis
Gender (female)	OR=0.058, 95% CI=0.006-0.549; **p=0.013***
Serum 25(OH)D (ng/mL)[1]	OR=1.097, 95% CI= 0.946-1.271; p=0.220
Serum calcium (mg/dL)	OR=0.373, 95% CI=0.063-0.549; p=0.278
PTH (pg/mL)	OR=0.940, 95% CI=0.0877-1.008; p=0.084

*; p<0.05, 25(OH)D; 25-hydroxy vitamin D, OR; Odds ratio, CI; Confidence interval, PTH; Parathormone

Table 5.
Univariate regression analysis of variables in terms of risk for pyelonephritis

Variables	Univariate regression analysis
Gender	OR=0.514, 95% CI=0.053-4.937; p=0.564
Serum 25(OH)D (ng/mL)[1]	OR=0.930, 95% CI=0.857-1.009; **p=0.082***
Serum calcium (mg/dL)	OR=0.220, 95% CI=0.062-0.778; **p=0.019***
Serum phosphate (mg/dL)	OR=0.432, 95% CI=0.229-0.814; **p=0.009****
PTH (pg/mL)	OR=0.915, 95% CI=0.833-1.006; **p=0.066***

*; p<0.05, **; p<0.200, 25(OH)D; 25-hydroxy vitamin D, OR; Odds ratio, CI; Confidence interval, PTH; Parathormone

Table 6.
Multivariate regression analysis of variables in terms of risk for pyelonephritis

Variables	Multivariate regression analysis
Serum 25(OH)D (ng/mL)[1]	OR=0.805, 95% CI=0.600-1.081; p=0.150
Serum calcium (mg/dL)	OR=0.308, 95% CI=0.029-3.287; p=0.329
Serum phosphate (mg/dL)	OR=0.547, 95% CI=0.161-1.862; p=0.334
PTH (pg/mL)	OR=0.884, 95% CI=0.766-1.020; p=0.091

25(OH)D; 25-hydroxy vitamin D, OR; Odds ratio, CI; Confidence interval, PTH; Parathormone

study population was mostly composed of patients with first-time UTI; thus, the indication for voiding cystography had not yet been established, potentially missing some VUR cases.

Previous studies have indicated that 25(OH)D deficiency is a risk factor for recurrent UTI.[17,18] Tekin et al.[8] showed that the serum 25(OH)D levels of 82 pediatric patients with UTI were lower than those of healthy children. It has been shown that 25(OH)D increases the secretion of the antimicrobial peptide cathelicidin by bladder epithelial cells, which protect against bacterial infections.[19-21] However, our results were contradictory, as 25(OH)D levels were higher in the UTI group than in the control group. Likewise, Katikaneni's study[9] with 315 infants aged 3 months demonstrated that 25(OH)D supplementation increases the risk of UTI in formula-fed infants by up to 76%. Mahyar et al.[10] from Qazvin, Iran enrolled children aged 1 month to 12 years who presented with UTI. Similar to our study, Mahyar et al.[10] found a significantly higher mean serum 25(OH)D level in children with UTI than in the control group.

Some hypotheses have been put forth in the literature in relation to this subject; Katikaneni et al.[9] proposed a slight nephrocalcinosis effect of 25(OH)D administration because nephrocalcinosis is an excellent context for bacterial growth. Second, the selective immunosuppressive role of 25(OH)D has been demonstrated in animal models of autoimmune disease; thus, it is known that 25(OH)D has immunosuppressive properties in autoimmune diseases.[22] 25(OH)D induces the production of transforming growth factor beta 1 and interleukin 4, potentially dampening inflammatory T-cell activity.[23] Another hypothesis suggests that 25(OH)D acts as an antagonist to 1,25-dihydroxy vitamin D3 [1,25(OH)2D3] at the vitamin D receptor (VDR) level, possibly leading to a dysregulated hyperactive immune response against infection in cases of elevated 25(OH)D levels.[9] We think that the answer to the contradictory effects of vitamin D, protective versus predisposing, against UTI may lie in the VDR. The direct effect of 1,25(OH)2D3 is variable, which may be due to the concentration of VDR associated with T lymphocyte activation.[24,25] Genetic and epigenetic factors are known to affect VDR concentration.[26] This may cause differences between populations. While studies focusing on the protective effects of vitamin D are abundant in the literature, research on its adverse effects is limited.

Interestingly, although serum 25(OH)D was significantly higher in the cystitis group than in the control group, there was no significant difference between the APN and control groups. Similar to our study, Yang et al.[7] concluded in their study that patients with lower UTI had higher vitamin D levels than those with APN. Most previous studies have focused on the protective effects of vitamin D, but there is very limited research on its negative effects. We believe that our study is important in this regard. Through our research, we can shed light on the fact that vitamin D is not as harmless as is often assumed to be.

We found lower PTH levels in the UTI and APN groups than in the control group; however, there was no significant difference between the cystitis and control groups in terms of mean PTH levels. This finding does not align with the previous study by Shalaby et al.[27] found higher PTH levels in UTIs in a relatively younger group of patients (0.98±1.15 year). They also found a significantly lower 25(OH)D level in the APN group ($p<0.001$) than in the cystitis group. The results related to PTH, especially in the APN group, may be related with fibroblast growth factor 23 (FGF-23): A lower PTH level in the APN group may have been caused by a high FGF-23 level in these patients. In our opinion, these findings point to a possible FGF-23 effect, which has not been demonstrated previously.

The relatively low levels of phosphate in our APN group support this hypothesis. FGF-23 decreases *PTH* gene expression and secretion from the parathyroid gland and acts directly on renal proximal tubules to induce phosphaturia.[28,29] We believe that infection of the renal parenchyma creates resistance to FGF-23. We speculate that this occurs through epithelial damage in the proximal tubule, followed by a decrease in FGF-23 activity. To our knowledge, no previous study has examined this relationship. When such a study is conducted, high levels of FGF-23 can serve as a marker for APN damage and scar formation.

A positive nitrite test result is specific for UTI, mainly due to urease-positive organisms.[30] Our results indicate a similar rate of nitrite positivity as that reported in previous studies (45% vs. 43.1%).[31] Herein, 25(OH)D levels of patients with positive urinary nitrite were significantly lower than those with negative urinary nitrite levels. We hypothesized that 25(OH)D deficiency could lead to the colonization of bacteria with the capability of producing urease. However, we recommend further investigations in this area to validate this hypothesis. We found a negative correlation between age and 25(OH)D levels, similar to previous studies.[32] Regression analysis showed that high 25(OH)D level is a risk factor for UTI, but not an independent risk factor. Female gender was a risk factor, as mentioned before.

Study Limitations

In terms of limitations, the sample size was relatively small, and this was a cross-sectional study. Second, the gender ratios were not equalized in both the control and study groups although vitamin D levels were not greatly affected by gender and age interval sex ratios. In contrast to many studies on the protective effects of vitamin D against infectious diseases, our findings provide a new perspective, which is a significant strength of our study.

Conclusion

Because there was no correlation between 25(OH)D level and UTI, we concluded that 25(OH)D had no effect on UTI. The results related to PTH, particularly in the APN group, may be related to FGF-23; lower PTH in the APN group may be due to the high FGF-23 levels in these patients. However, we believe that the effects of FGF-23 on APN should be thoroughly investigated. More studies are needed to validate these findings.

Ethics

Ethical Approval: Ethics committee approval of the study was obtained from the Kırıkkale University Non-Interventional Clinical Research Ethics Committee (decision no: 2022.04.25, date: 27.04.2022) the study was conducted according to the principles of the Declaration of Helsinki.

Informed Consent: The authors confirm that informed consent was obtained from the participants or their legal guardians, and all required ethical approvals were obtained for the study.

Footnotes

Author Contributions: Mergan Çetiner G: Surgical and Medical Practices, Concept, Design, Analysis or Interpretation, Literature Search.; Kandur Y: Surgical and Medical Practices, Concept, Design, Data Collection or Processing, Analysis or Interpretation, Literature Search, Writing.

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

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Detection of Neurological Complications by Cranial Ultrasound in Neonatal Sepsis: A Cross-sectional Study

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Abstract

Neonates with sepsis are at risk of neurological complications. Cranial ultrasound (CUS) has been widely used in neonates for early detection of these intra-cranial abnormalities. It is a convenient, non-invasive, safe with no radiation exposure and quick imaging technique to visualize the neonatal brain parenchyma and ventricular system. To detect neurological complications in neonates with sepsis by CUS. This cross-sectional study was conducted in Department of Neonatology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from June 2019 to September 2020. Inborn neonates with sepsis and inborn preterm neonates without sepsis satisfying the inclusion and exclusion criteria who were admitted into neonatal intensive care unit (NICU), BSMMU during study period were the study population. Among 110 neonates, 75 neonates with sepsis and 35 pre-term neonates without sepsis were assessed for eligibility. Among these 75 septic neonates 19 were excluded. Finally,56 septic neonates were analyzed. Out of them 21 (37.5%) neonates had abnormal neurological findings in CUS. Distribution of abnormal CUS findings were intraventricular hemorrhage (33%), features of meningitis (24%), hydrocephalus (14%), prominent lateral and third ventricles (10%), intracerebral hemorrhage (5%), features of ventriculitis (5%). Among 35 non-septic pre-term neonates, 5 were excluded due to guardians did not give consent to perform CUS. So, 30 non-septic pre-term neonates were analyzed, among them, 2 neonates (7%) had feature of intraventricular hemorrhage in CUS. Septic neonates with abnormal CUS findings, most of the neonates were male (71%), gestational age (34.57±3.043) weeks and birth weight (1985.2±684.132) gm. In babies with abnormal CUS, convulsion

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was significantly associated (p value 0.01). This study showed proven sepsis was significantly associated with abnormal CUS findings. The abnormal CUS findings in newborn with sepsis was 37.5%. So CUS may be an important investigatory modality in NICU for early detection of intracranial complications.

Keywords: Neonatal sepsis, cranial ultrasound, neurological complications

Introduction

Sepsis is the most common cause of neonatal mortality that causes 30-50% of all neonatal deaths annually in developing countries.[1] It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis-related causes.[2] The most commonly implicated bacteria are *Staphylococcus aureus*, coagulase negative staphylococci (CONS), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* species, and Group B *Streptococcus*.[3] In the early-onset type, the patient may have a history of fetal distress, including fetal tachycardia during the peripartum period. Soon after delivery, other clinical signs, such as meconium-stained amniotic fluid and low Apgar scores, may be present. Vital sign derangements include both hypothermia and fever, tachycardia or bradycardia, signs of poor perfusion, and signs of respiratory distress, which are common in neonatal sepsis. Gastrointestinal signs include decreased feeding, vomiting, jaundice, abdominal distension, and hepatosplenomegaly, while neurological signs include lethargy, seizures, irregular respiration, and high-pitched cry, which may present in sepsis. Sepsis often presents as neonatal meningitis, which is inflammatory response of cerebrospinal fluid (CSF) and pia-arachnoid infection, and other neurological complications, including intraventricular hemorrhage, hydrocephalus, encephalomalacia, cerebral infarction, subdural empyema, ventriculitis, and abscess. The outcome of sepsis with central nervous system involvement should be considered. Various cranial sonographic findings, including echogenic and widened sulci, ventriculomegaly, ventriculitis, hydrocephalus, extra-axial fluid collections, cerebritis, and brain abscesses, may be observed in sepsis.[4]

Cranial ultrasound (CUS) has become an essential diagnostic tool in modern neonatology for observing normal anatomy and pathological changes in neonates. In the neonate, many sutures and fontanels are still open, and these can be used as acoustic windows to "look" into the brain.[5]

In Bangladesh, the neonatal health status is still not satisfactory, despite recent advances in biophysical and biochemical monitoring of the fetus during labor and delivery. One of the major causes of neonatal death is sepsis. Sepsis with abnormal ultrasonographic findings is associated with an increased risk of death and neuro-developmental disability in the future.

This objective CUS was performed to assess intracranial abnormalities in newborn babies with sepsis, and prompt action can be taken in time as it is a non-invasive, portable, real multi-planner imaging modality.

Highlights

- Sepsis is the commonest cause of neonatal mortality and is responsible for 30-50% of total neonatal deaths each year in developing countries.
- It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis.
- The most commonly implicated bacteria include *Staphylococcus aureus*, coagulase negative staphylococci, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* species, and Group B *Streptococcus*.
- Cranial ultrasound has become an essential diagnostic tool in modern neonatology for depicting normal anatomy and pathological changes in neonatal brain.
- It can easily be performed, cost-effective, no risk of radiation exposure, it can be repeated whenever needed, enabling visualization of ongoing brain maturation and the evolution of lesions and it can be performed without sedation.

Material and Method

This cross-sectional study was conducted at the Department of Neonatology Bangabandhu Sheikh Mujib Medical University (BSMMU) from June 2019 to September 2020. Inborn neonates with sepsis and inborn pre-term neonates without sepsis satisfying the inclusion and exclusion criteria who were admitted to the neonatal intensive care unit (NICU), BSMMU during the study period were the study population. Thorough histories of these newborns, including demographic and socioeconomic information, clinical features, sepsis workup including blood culture, and CSF study reports, were enrolled in the data sheet. After obtaining written informed consent from the parents/guardians, CUS was performed in all enrolled neonates. CUS was performed using a Philips Affiniti 30 ultrasound machine (manufactured in USA). Sonologists performed a 2D ultrasonogram of the brain, real time B mode gray scale brain scan was performed through anterior fontanels (both sagittal and coronal view) with 3.5 to 12 MHz transducer. Normal blood flow within the brain parenchyma was seen by color doppler application. All findings were included in the data sheet.

Inclusion Criteria

- Inborn neonates with probable or proven sepsis.
- Inborn pre-term neonates without sepsis.

Exclusion Criteria

- Known case of congenital anomaly or infection.
- Unwillingness to participate the study.

Ethical Consideration

Minimum physical, psychological, social, and legal risk during history, physical examination and investigations. Proper safety measures were taken in every step of the study. Only the researcher was allowed to access the collected data. Ethical clearance was obtained from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (decision no: BSMMU/2019/7052, date: 25.06.2019) for the current study. According to the Declaration of Helsinki 1964, parents of all neonates were informed about the study design. The underlying hypothesis and the right of the participants to withdraw from the study at any time and for any reason. Informed written consent was obtained from parents or guardians who voluntarily provided consent to participate in this study.

The Following Ethical Issues were Addressed

- Strict confidentiality and security of patient data were maintained.
- The presentation of data and information related to patient were documented anonymously.
- The data analysis was completed for subjects who completed the study according to the protocol after recruitment of subjects with valid informed consent.
- There were no additional risks or safety concerns due to the research process for either the patient or researcher.
- There were no potential conflicts of interest in this study and the entire academic research project.
- Financial issues related to this research were managed by the principal investigator when parents did not make an effort.

Statistical Analysis

After collection, data were entered into a computer and edited, analyzed, and plotted in graphs and tables. Data were analyzed using the Statistical Package for Social Sciences version 20. All data were calculated and presented as proportions. All quantitative variables (between septic neonates with abnormal and normal CUS findings) were compared using the independent t-test, and categorical variables were compared using the chi-squared test. A p value <0.05 was considered significant.

Results

During the study period, 110 neonates were assessed, among whom 75 neonates were septic and 35 preterm neonates were non-septic. Of the 75 septic neonates, 19 were excluded, so 56 neonates were analyzed. Out of them 21 (37.5%) neonates had abnormal neurological findings in CUS. Among 56 neonates, 42 were preterm with sepsis. Of 35 nonseptic pre-term neonates, 5 were excluded because the guardians did not give consent to perform CUS; thus, 30 nonseptic preterm neonates were analyzed.

The baseline characteristics of the infants are presented in **Table 1**. According to gestational age range 28-<34 weeks reflected (52%) more predominance, gender distribution reflected male predominance (63%) and common mode of delivery was lower segment cesarean section (80%) (**Table 1**).

Data are presented as number (percentage) unless otherwise indicated. Among 56 neonates with sepsis, the common maternal characteristics were irregular antenatal care 36 (64%), pregnancy induced hypertension 32 (57%).

Among 56 neonates, regarding age at onset of sepsis, early onset sepsis 42 (75%) and late onset sepsis 14 (25%) (**Figure 1**). Among 56 neonates with sepsis, 22 (39%) had proven sepsis and 34 (61%) had proven sepsis and probable sepsis (**Figure 2**). Among 22 cases of proven sepsis, 12 neonates had early-onset neonatal sepsis (EONS) (**Figure 3**). Regarding the septic screening result, total count of white blood counts was <5000/cumm in 10 (18%) neonates and >25000/cumm in 14 (25%) neonates. Twelve (21%) neonates had an absolute neutrophil count <1500/cumm, IT ratio was >0.2 in 40 (71%) neonates, positive C-reactive protein (CRP) was found in 39 (70%) and platelet count <150000 in 42 (75%) neonates.

Table 1.
Baseline characteristics of the study group, n=56

Characteristics	Frequency	Percentage
Gestational age		
28-<34 weeks	29	52
34-<37 weeks	13	23
≥37 weeks	14	25
Sex		
Male	35	63
Female	21	37
Mode of delivery		
LUCS	45	80
NVD	11	20

Data are presented as number (percentage) unless otherwise indicated, LUCS; Lower segment caesarean section, NVD; Normal vaginal delivery

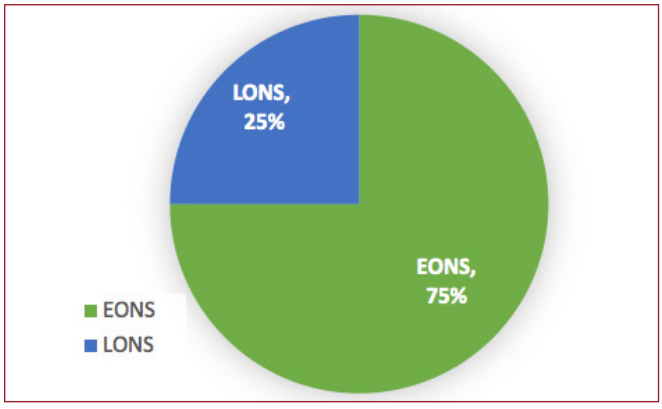

Figure 1. Distribution of age of onset of sepsis among study group, n=56
EONS; Early onset neonatal sepsis, LONS; Late onset neonatal sepsis

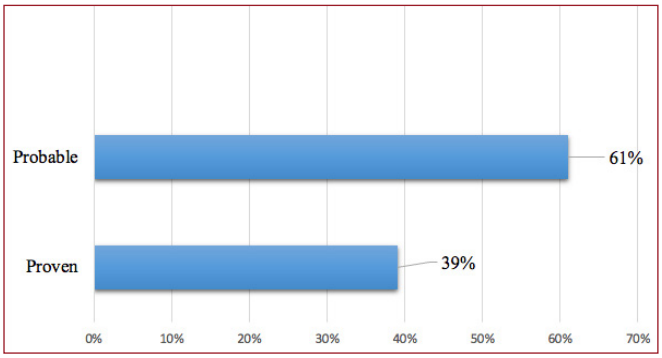

Figure 2. Types of sepsis of study group according to culture, n=56

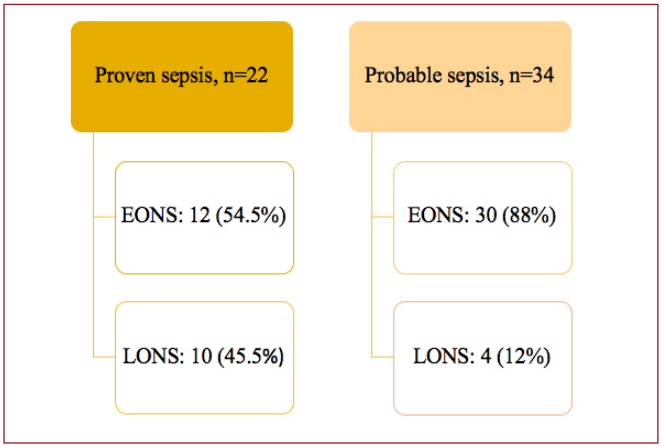

Figure 3. Flow chart of EONS and LONS between proven and probable sepsis, n=56

EONS; Early onset neonatal sepsis, LONS; Late onset neonatal sepsis

Among the 22 culture-positive neonates, *Acinetobacter* was found in 10 (45.5%) cases, *Klebsiella* in 7 (31.8%) neonates, *Salmonella* in 2 (9.1%), *Escherichia coli* in 2 (9.1%), and *Pseudomonas* in 1 (4.5%).

Out of 56, 46 neonates underwent lumber puncture among them 14 (30%) had positive CSF findings.

Among the 56 neonates, 21 (37.50%) had abnormal ultrasound findings (**Figure 4**). Among the abnormal findings of CUS, intraventricular hemorrhage (IVH) 7 (33%), most common (**Table 2**). Among 56 neonates with sepsis, the most common abnormal CUS finding was IVH in pre-term than term neonates (**Table 3**).

Among 42 pre-term neonates with sepsis, 6 (14%) and 30 pre-term neonates without sepsis, 2 (6.7%) had IVH in CUS finding. Among 42 pre-term neonates with sepsis, 1 (2.4%) had periventricular leukomalacia (PVL), whereas 30 preterm neonates without sepsis did not have PVL on CUS. Among the 21 neonates with abnormal CUS

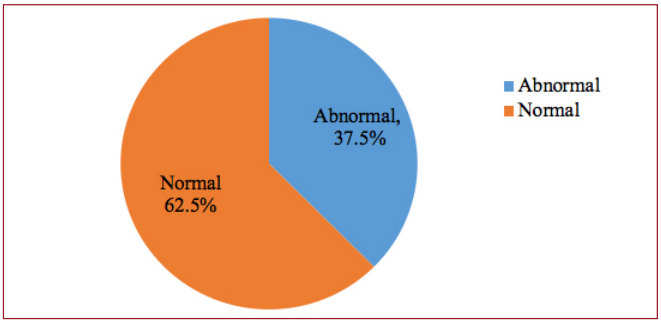

Figure 4. Distribution of CUS results among study neonates, n=56

CUS; Cranial ultrasound

Table 2.
Abnormal CUS findings, n=21

Abnormal ultrasound findings	Frequency	Percentage
Prominent echogenic sulci and gyri with increased vascularity of brain parenchyma-suggestive meningitis	5	24
Echogenic area with ventricular dilatation-suggestive of IVH	7	33
Hydrocephalus	3	14
Intracerebral hemorrhage	1	5
Hyperechoic periventricular area suggestive PVL	1	5
Increased periventricular echogenicity with irregular margin suggestive ventriculitis	1	5
Persistent cavum septum pellucidum	1	5
Prominent both lateral and third ventricles	2	10

IVH; Intraventricular hemorrhage, PVL; Periventricular leukomalacia, CUS; Cranial ultrasound

Table 3.
Comparison of abnormal CUS findings between pre-term and term neonates with sepsis, n=56

Abnormal CUS findings	Pre-term neonates with sepsis, n=42	Term neonates with sepsis, n=14	P value
IVH	6 (14%)	1 (7.1%)	0.484
Features of meningitis	2 (4.8%)	3 (21.4%)	0.058
Hydrocephalus	1 (2.4%)	2 (14.2%)	0.732
Intracerebral hemorrhage	1 (2.4%)	0	0.560
PVL	1 (2.4%)	0	0.560
Prominent ventricles	2 (4.8%)	0	0.406
Ventriculitis	1 (2.4%)	0	0.08
Persistent cavum septum pellucidum	1 (2.4%)	0	0.560

Statistical test; Chi-square test, IVH; Intraventricular hemorrhage, PVL; Periventricular leukomalacia, CUS; Cranial ultrasound

Table 4.
Maternal and neonatal characteristics in septic neonates with abnormal and normal CUS findings, n=56

Characteristics	Sepsis with abnormal CUS, n=21	Sepsis with normal CUS, n=35	P value
Maternal			
HTN	15 (71%)	21 (60%)	0.39
GDM	4 (19%)	3 (8.6%)	0.25
PROM	7 (33%)	19 (54.3%)	0.13
Neonatal			
Lethargy	12 (57%)	22 (63%)	0.67
Respiratory distress	15 (71%)	18 (51.4%)	0.14
Convulsion	8 (38%)	3 (8.6%)	**0.01**
Temperature instability	4 (19%)	8 (23%)	0.74
Feeding problem	6 (28.6%)	6 (17%)	0.31

Statistical test; Chi-square test, CUS; Cranial ultrasound, HTN; Hypertension, GDM; Gestational diabetes mellitus, PROM; Prelabor rupture of membranes

Table 5.
Abnormal CUS findings among neonates with probable and proven sepsis, n=21

Abnormal CUS findings	Probable sepsis, n=7	Proven sepsis, n=14	P value
IVH	1 (14.3%)	6 (43%)	**<0.001**
Features of meningitis	2 (28.5%)	3 (21.4%)	0.051
Hydrocephalus	2 (28.5%)	1 (7.1%)	0.828
Intracerebral hemorrhage	1 (14.3%)	0	0.417
Prominent ventricles	1 (14.3%)	1 (7.1%)	0.752
Ventriculitis	0	1 (7.1%)	0.210
Periventricular leukomalacia	1 (14.3%)	0	0.417
Persistent cavum septum pellucidum	1 (14.3%)	0	0.417

Statistical test; Chi-square test, CUS; Cranial ultrasound, IVH; Intraventricular hemorrhage

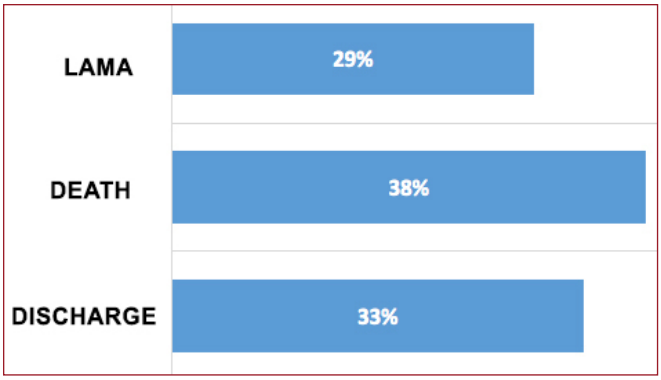

Figure 5. Outcome of the neonates with abnormal CUS, n=21
CUS; Cranial ultrasound, LAMA; Leave against medical advice

findings, those with low birth weight (1500-2499) gm and low gestational age (28-<34) weeks were more.

When comparing babies with abnormal CUS findings with septic babies without abnormalities, birth weight and gestational age were not significantly different. Comparison of septic neonates with abnormal and normal CUS findings showed that proven sepsis was found more frequently in septic neonates with abnormal CUS findings, which was statistically significant. The number of male babies, EONS, and negative CSF were higher in the abnormal CUS group, but the difference was not statistically significant. Comparison of septic neonates with abnormal and normal CUS findings and maternal conditions [hypertension, gestational diabetes mellitus (GDM), pre-labor rupture of membranes] were not statistically significant between the two groups. In terms of neonatal characteristics, convulsions were found more frequently in septic neonates with abnormal CUS, which was statistically significant (**Table 4**).

Abnormal CUS findings among neonates with probable and proven sepsis, IVH was more common in proven sepsis which was statistically significant (p<0.001) (**Table 5**). Among 21 neonates with abnormal CUS findings, 11 neonates (61%) stayed in hospital for (7-21) days. Among the 21 neonates with abnormal ultrasound 8 neonates (38%) were died, 6 neonates (29%) were taken leave against medical advice (LAMA) and 7 neonates (33%) were discharged with advice (**Figure 5**).

Discussion

Sepsis is a clinical syndrome of multiple involvement. Sepsis with neurological involvement is associated with increased morbidity and mortality. Probable sepsis as well as proven both can cause neurological complications. CUS is an important tool for early detection of intracranial complications and neurological outcomes in newborns with sepsis.

This cross-sectional study was conducted with the objective of identifying neurological complications of neonatal sepsis detected by CUS. In this study, 56 neonates with sepsis were analyzed, among whom abnormal CUS findings (approximately 37.5%). In one study, abnormal CUS findings were observed in approximately 38%.[6] In this study, premature babies mostly (28-<34) weeks and low birth weight babies

(1500-2499) gm were more in abnormal CUS group. Birth weight and gestational age between septic neonates with abnormal and normal CUS findings were approximately similar, p value not significant.

According to a previous report,[7] neurological complications on CUS had a high percentage (63.9%) in late pre-term (33-36) weeks. Among 56 neonates with sepsis, the most common abnormal CUS finding was IVH in pre-term than term neonates, but the p value was not significant (**Table 3**).

Among 42 pre-term neonates with sepsis, 6 (14%) and 30 pre-term neonates without sepsis, 2 (6.7%) had IVH in CUS finding. Among 42 pre-term neonates with sepsis, 1 (2.4%) had PVL, whereas 30 preterm neonates without sepsis did not. No neonate had PVL in CUS, but the p value was not significant. In one study, late-onset sepsis was more than early onset sepsis in terms of the cause of abnormal CUS.[8] In the present study, early-onset sepsis was more common in septic neonates with abnormal CUS, but the difference was not statistically significant.

In this study, proven sepsis was significantly more common in septic neonates with abnormal CUS, but a positive CSF study was not significant. In, proven sepsis was reported at approximately 73%, but in this study probable sepsis were more around 67%.[4] According to, newborn infants affected by group-B *Streptococcus*, *E. coli*, *Klebsiella*, *Listeria*, and *Citrobacter* have more devastating consequences in septic newborns.[9] In, *Staphylococcus aureus* and *E. coli* were shown to be coagulase-negative *Staphylococcus* responsible for abnormal neurosonogram findings in newborns with sepsis.[4] In, group B-*Streptococcus*, *E. coli*, *Klebsiella*, *Salmonella*, *Listeria* monocytogenes, and pseudomonas were common. In this study, *Acinetobacter*, *Klebsiella*, *Salmonella*, *E. coli*, and *Pseudomonas* were detected in blood cultures.[7]

This study showed that maternal hypertension, GDM, and premature rupture of membrane >18 h were common maternal characteristics in both septic neonates with abnormal and normal CUS groups (**Table 4**).

According to, maternal pre-eclampsia, premature prolonged rupture of membrane, maternal chorioamnionitis were common maternal characteristics.[4]

In this study, lethargy, signs of respiratory distress, convulsions, temperature instability, and poor feeding were common clinical presentations of septic neonates, and among these, 8 septic neonates with abnormal CUS had convulsions rather than 3 neonates with normal CUS, which was statistically significant (**Table 4**). In, respiratory distress, disseminated intravascular coagulation, coagulopathy, and gastrointestinal bleeding were common clinical manifestations of sepsis in newborns.[7]

In, a significant correlation was observed between a positive CRP level and a low platelet count with abnormal CUS findings. In this study, positive CRP levels, low platelet counts, and significant IT ratio were more common in septic neonates with abnormal CUS.[6]

In this study, abnormal CUS findings were increased periventricular echogenicity (ventriculitis), increased vascularity of brain parenchyma with prominent echogenic sulci and gyri (meningitis), intraventricular hemorrhage, prominent ventricles or ventricular dilatation (hydrocephalus), and hyperechoic areas in a distinctive fashion in the periventriculararea (periventricular leukomalacia) (**Table 2**). In pre-term babies, intraventricular hemorrhage and increased vascularity of brain parenchyma suggestive of meningitis were more common findings (**Table 3**).Features of meningitis and IVH in CUS findings were more common in proven sepsis, and IVH was significantly more common in proven sepsis (**Table 5**). In one study, IVH was a more common CUS finding in preterm babies.[4]

Among 21 neonates with abnormal CUS findings, 61% had a hospital stay (7-21) days, 28% had >28 days. Regarding the outcome of these babies with abnormal CUS findings, 38% died, 29% took LAMA, and 33% were discharged with advice.

Sepsis is a more common condition in the NICU, and sepsis-related neurological complications may occur in patients with probable and proven sepsis. Undetected and untreated neurological complications may cause neurodevelopmental sequelae. CUS can detect intracranial lesions earlier. After the detection of abnormalities, appropriate treatment duration and prompt management can reduce neurodevelopmental deficit in septic neonates.

Study Limitation

It was not possible to perform all CUS by the same radiologist. Unavailability of the bed side CUS in NICU, BSMMU. Scarcity of appropriate probe for newborn CUS in radiology department in BSMMU.

Conclusion

Abnormal CUS findings like IVH, features of meningitis, ventriculitis, hydrocephalus, and PVL were found in both preterm and term septic neonates. IVH was significantly more common in patients with proven sepsis.

Ethics

Ethical Approval: Ethical clearance was obtained from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (decision no: BSMMU/2019/7052, date: 25.06.2019) for the current study.

Informed Consent: Informed written consent was obtained from parents or guardians who voluntarily provided consent to participate in this study.

Footnotes

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Variations of 25-Hydroxyvitamin D Levels During COVID-19 Pandemic and its Relation to Season, Sex and Age in Children

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Abstract

We aimed to investigate variations in vitamin D levels (VDLs) during the coronavirus disease-19 pandemic and their relationship to season, sex, and age in otherwise healthy children and adolescents. We conducted a retrospective cross-sectional study at an outpatient pediatric clinic, which included 4,262 children aged 1-18 years. The study cohort was divided into three groups: Group 1 (pre-pandemic), Group 2 (pandemic), and Group 3 (post-pandemic). Vitamin D deficiency (VDD) was defined as a level below 12 ng/mL, insufficiency as a level between 12 and 20 ng/mL, and sufficiency as a serum level above 20 ng/mL. The pandemic cohort exhibited significantly lower VDLs compared with both the pre-pandemic and post-pandemic cohorts. Females had significantly lower VDLs than males. The prevalence of VDD was highest among adolescents. A significantly higher rate of VDD was observed in the pandemic group among the 6-11 and 12-18 age groups compared with both the pre-pandemic and post-pandemic groups. VDLs were significantly lower in spring and winter than in summer and autumn. Additionally, an inverse relationship was observed between age and VDLs. Our study revealed a significant prevalence of VDD in school-aged children and adolescents, with a notable decrease in VDLs observed during the pandemic compared with other time periods. Furthermore, our study highlighted the increased vulnerability of female adolescents to VDD.

Keywords: Adolescent, children, COVID-19, pandemic, vitamin D deficiency

Introduction

Vitamin D plays a critical role in regulating calcium and phosphate metabolism and exerts various effects on peripheral organs, tissues, and immune system components, demonstrating anti-inflammatory and immunomodulatory properties.[1] While it can be acquired from dietary sources, its main source is endogenously produced in the skin through ultraviolet-B exposure. Several factors, such as skin pigmentation, race, season, body mass index, and nutrition, can influence vitamin D levels (VDLs).[2,3] Beyond its traditional association with bone health, vitamin D regulates multiple organ systems; thus, identifying and addressing deficiencies in vitamin D are crucial. Previously linked to rickets in children, vitamin D deficiency (VDD) is now associated with significant extra-skeletal conditions like atopic and autoimmune disorders. Managing VDD

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through preventive measures and supplementation can alleviate these disorders. Hence, early detection of asymptomatic individuals who may appear healthy but have VDD is vital in preventing insufficiency.[4]

Although children generally experience mild symptoms from coronavirus disease-19 (COVID-19), the disease has led to severe complications and significant morbidity and mortality in adults.[5] Consequently, many countries have enforced measures like social distancing, stay-at-home orders, and curfews to manage the outbreak.[6] After the first COVID-19 case in the country, online education was switched to schools. To mitigate the spread of the virus, the Turkish government initially imposed a curfew for individuals aged 65 years and older and later extended this restriction to include those aged 20 years and older. Throughout the pandemic, various restrictions were gradually introduced and modified, culminating in the lifting of the curfew on July 1, 2021, as part of Türkiye's comprehensive pandemic control strategy. The implementation of various measures and widespread vaccination efforts have gradually diminished the spread and incidence of COVID-19. Nevertheless, the restrictions imposed during the pandemic have adversely affected children in both the short- and long-term.[7-9] The pandemic-induced curfew has led to increased sedentary behavior and reduced sunlight exposure among children.[10] There is a lack of studies investigating fluctuations in of VDLs in the COVID-19 pandemic periods and the interplay of sex, age, and season in pediatric populations. Hence, we aimed to investigate the variations of VDLs in COVID-19 pandemic period and its relation to season, sex, and age in otherwise healthy children and adolescents.

Highlights

- 25-hydroxyvitamin D levels were lower in the pandemic group than in the pre-pandemic and post-pandemic groups.
- The current study highlighted the risk of 25-hydroxyvitamin D deficiency among school-aged children and adolescents, particularly female students.
- Vitamin D levels were significantly lower in spring and winter than in summer and autumn.

Material and Method

Approval was granted by the KTO Karatay University Faculty of Medicine, Pharmaceutical and Non-Medical Device Research Ethics Committee (meeting date: 17.11.2023, decision no: 2023/021). The present study was conducted at the outpatient pediatric clinic of the University of Health Sciences Türkiye, Konya Beyhekim Training and Research Hospital from March 2018 to October 2023. The study included children whose VDLs were measured during the study period and who were between the ages of 1 and 18. Information on the dates of application, sex, age, and blood results of the participants was obtained from the hospital's computerized database. In order to measure VDLs prior to, during, and following the COVID-19 pandemic as well as to assess the effect of the pandemic's restriction measures on VDLs, the study cohort was stratified into three distinct groups: Group 1 (pre-pandemic, March 2018 to March 2020), Group 2 (pandemic, April 2020 to June 2021), and Group 3 (post-pandemic, July 2021 to October 2023). The study exclusively utilized the first recorded VDL of participants who underwent multiple measurements. The study excluded children younger than 1 year old, those with a medical history of metabolic disorders, and individuals with conditions known to adversely affect vitamin D metabolism and levels, including chronic kidney disease, liver disease, celiac disease, and malabsorption syndromes, those receiving corticosteroid therapy or antiepileptic medications, neurological patients who were bedridden for an extended period, and individuals diagnosed with type 1 or type 2 diabetes mellitus, as well as oncology and transplantation patients.

The participants were divided into three age groups [1-5 years old (pre-schoolers), 6-11 years old (school children), and 12-18 years old (adolescents)] to allow for comparison of VDLs. To further explore the impact of seasons on VDLs, the application seasons were categorized based on the dates that the participants submitted their applications. Serum 25 (OH) D was measured using a fully automated immunoassay method (ADVIA Centaur XP®, Siemens, Munich, Germany). VDD was defined as a serum level below 12 ng/mL, vitamin D insufficiency (VDI) as a range between 12 and 20 ng/mL, and vitamin D sufficiency (VDS) as a serum level above 20 ng/mL.[11]

Statistical Analysis

Categorical variables are presented as n (%). Because of their non-normal distribution, all data are presented as median (IQR). For group comparisons, the Mann-Whitney U test was used, the chi-square test or Fisher's exact test was used, and Spearman's test was used to assess correlations. Group comparisons were performed using the Kruskal-Wallis test, and multiple comparisons were adjusted using the Bonferroni corrected Mann-Whitney U test. Using stepwise multivariate linear regression analysis, it was discovered that independent determinants significantly ($p<0.05$) explained the variance of the dependent variable. The analysis was performed using SPSS software version 21.0 for Windows, with a significance level of $p<0.05$. GraphPad Prism 9.0 was used to create the figures.

Results

Demographic Characteristics and VDLs of the Study Groups

The study included 4262 children aged 1-18 years. The cohort's median age was 7.5 years (IQR: 8.2 years), with 2028 (47.6%) males and 2234 (52.4%) females. The median serum VDL level was 15.13 ng/mL (IQR: 10.33 ng/mL). The sex distribution was similar between the three study groups, ($p>0.05$). In addition to having a lower median VDL than the other two groups, the pandemic group also had a younger median age (**Table 1**, **Figure 1**).

Only 28.4% of the participants had adequate VDLs compared with 32.1% who had VDD and 39.5% who

Table 1.
Demographic characteristics and 25(OH)D levels

Characteristics	Pre-pandemic group (n=2111)	Pandemic group (n=179)	Post-pandemic group (n=1972)	P value*	P value§	P value€	P value€	P value€
Sex								
(Males/females)	989/1112	83/96	956/1016	0.550				
(%)	(46.8/53.2)	(46.4/53.6)	(48.5/51.5)					
Age (years)	7.3 (8.4)	5.5 (8.4)	8.1 (7.90)		**<0.0001**	**#0.004**	**&0.004**	**¥<0.0001**
25(OH)D level (ng/mL)	15.01 (10.14)	14.12 (11)	15.33 (10)		**0.003**	#0.170	**&0.006**	**¥0.010**

Quantitative variables are presented as the median (interquartile range). Qualitative variables are expressed as numbers and percentages. Results were compared using the Kruskal-Wallis test followed by the Bonferroni-corrected Mann-Whitney U test. Significant differences were determined by p<0.05 for the Kruskal-Wallis test and p<0.016 (p=0.05/3) for the Bonferroni correction. P values are indicated in bold. The chi-square test was performed to compare categorical variables
§; Kruskal-Wallis test, €; Mann-Whitney U test, *; Chi-square test, #; Pre-pandemic group versus pandemic group, &; Pre-pandemic group versus post-pandemic group , ¥; Pandemic group versus post-pandemic group, 25(OH)D; 25-hydroxyvitamin D

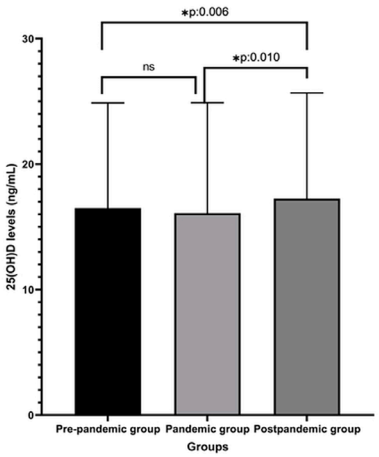

Figure 1. 25(OH)D values in the study groups

Table 2.
Rates of vitamin D deficiency, insufficiency, and sufficiency according to age group

	25(OH)D level (ng/mL)				
Age groups	**<12 (n, %)**	**12-20 (n, %)**	**>20 (n, %)**	**Total (n, %)**	**P value***
1-5 years	373[a] (22.3)	656[b] (39.1)	647[c] (38.6)	1676 (39.3)	**<0.0001**
6-11 years	461[a] (31)	664[b] (44.7)	362[a] (24.3)	1487 (34.9)	
12-18 years	532[a] (48.4)	365[b] (33.2)	202[c] (18.4)	1099 (25.8)	
Total	1366 (32.1)	1685 (39.5)	1211 (28.4)	4262	

Qualitative variables are expressed as numbers and percentages. P values are highlighted in bold
*; Chi-square test, 25(OH)D; 25-hydroxyvitamin D

had VDI. A comparison of the median ages of individuals with VDD (9.9 years), VDI (7.4 years), and VDS (5.3 years) revealed a significant difference in the median age of those with VDD, with individuals in this group being notably older (p<0.0001). Among all study participants, females exhibited a significantly lower median VDL than males (14 ng/mL versus 16.56 ng/mL). A significant disparity in the rate of VDD was noted when comparing age groups within the entire study cohort, with the highest occurrence observed among adolescents (**Table 2**, **Figure 2**).

Comparison of VDLs During the COVID-19 Pandemic According to Sex and Age

When comparing sex, regardless of age group, females had lower median VDLs than males in all three groups. Females aged 6-11 and 12-18 years showed significantly lower VDLs than males in both the pre-pandemic and post-pandemic groups. Females aged 12-18 years in the pandemic group had significantly lower VDLs than males (**Table 3**).

Comparison of VDLs During the COVID-19 Pandemic According to Age Groups

There was no obvious difference between the three groups' VDLs for children aged 1-5, whereas there was a discernible difference between the groups' median VDLs for children 6-11 and 12-18. The pandemic group showed considerably lower VDLs in these two age groups than the post-pandemic group (**Table 4**).

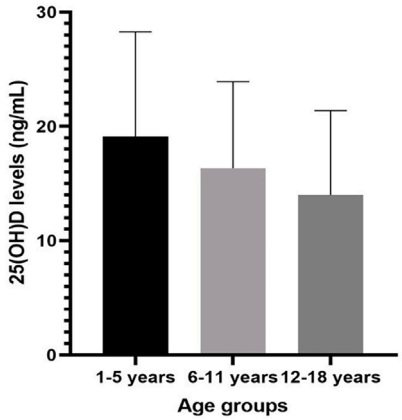

Figure 2. 25(OH)D levels in the study population by age group

During the COVID-19 Pandemic, the Rates of VDD, VDI, and VDS by Age Group

When comparing the three groups based on age groups, a greater rate of VDD was observed in the pandemic group in the 6-11 and 12-18 age groups than in the other groups (**Table 5**).

VDLs According to Seasons

VDLs were obtained from 1149 (27%) participants in the spring, 1068 (25.1%) in the summer, 989 (23.2%) in the fall, and 1056 (24.8%) in the winter. The median VDLs were 18.97 ng/mL in summer, 17.50 ng/mL in autumn,

Table 3.
25-hydroxyvitamin D levels during the COVID-19 pandemic according to age and sex

Characteristics	Pre-pandemic group			Pandemic group			Post-pandemic group		
	Males	Females	P value€	Males	Females	P value€	Males	Females	P value€
1-5 years (n, %)	474 (53.9)	404 (46.1)		50 (51.5)	47 (48.4)		374 (53.3)	327 (46.6)	
25(OH)D level (ng/mL)	17.66 (10.93)	16.08 (10.53)	0.068	18.99 (10)	16.59 (11)	0.691	19.62 (10.6)	18.68 (12.68)	0.252
6-11 years (n, %)	339 (48.8)	355 (51.1)		19 (43.1)	25 (56.8)		381 (50.8)	368 (49.1)	
25(OH)D level (ng/mL)	15.71 (8.80)	14.11 (9.07)	**0.002**	12.78 (13)	10.60 (10)	0.538	16.82 (9.89)	15.19 (8.36)	**0.001**
12-18 years (n, %)	176 (32.6)	363 (67.3)		14 (36.8)	24 (63.1)		201 (38.5)	321 (61.4)	
25(OH)D level (ng/mL)	14.41 (9.58)	10.89 (7.99)	**<0.0001**	11.38 (4)	8.47 (5)	**0.019**	16 (8.83)	11.2 (7)	**<0.0001**
Total number of participants (%)	989 (46.8)	1122 (53.1)		83 (46.3)	96 (53.6)		956 (48.4)	1016 (51.5)	
25(OH)D level (ng/mL)	16.46 (10.22)	13.82 (9.9)	**<0.0001**	15.12 (12)	12.29 (9)	**0.042**	16.85 (10)	14.30 (10)	**<0.0001**

Quantitative variables are presented as the median (interquartile range). Qualitative variables are expressed as numbers and percentages. P values are indicated in bold
€; Mann-Whitney U test, COVID-19; Coronavirus disease-19, 25(OH)D; 25-hydroxyvitamin D

Table 4.
25-hydroxyvitamin D levels by age group during the COVID-19 pandemic

Characteristics	Pre-pandemic group	Pandemic group	Post-pandemic group	P value§	P value€		
1-5 years (n)	878	97	701				
25 (OH) D level	16.82 (11.22)	17.32 (10.70)	18.20 (10.90)	0.064			
6-11 years (n)	694	44	749				
25 (OH) D level	14.92 (9.01)	11.15 (10.90)	15.08 (8.73)	**0.006**	#0.038	&0.066	¥**0.003**
12-18 years (n)	539	38	522				
25 (OH) D level	12.20 (8.73)	9.65 (5.05)	12.79 (9.03)	**0.001**	#**0.009**	&0.026	¥**<0.0001**

Quantitative variables are presented as the median (interquartile range). Qualitative variables are expressed as numbers. Results were compared using the Kruskal-Wallis test followed by the Bonferroni-corrected Mann-Whitney U test. Significant differences were determined by p<0.05 for the Kruskal-Wallis test and p<0.016 (p=0.05/3) for the Bonferroni correction. P values are indicated in bold
§; Kruskal-Wallis test, €; Mann-Whitney U test, #; Pre-pandemic group versus pandemic group, &; Pre-pandemic group versus post-pandemic group, ¥; Pandemic group versus post-pandemic group, COVID-19; Coronavirus disease-19, 25(OH)D; 25-hydroxyvitamin D

Table 5.
Deficiency, insufficiency, and sufficiency rates of 25(OH)D in different age groups during the COVID-19 pandemic

Age groups	25(OH)D level (ng/mL)	Pre-pandemic group (n, %)	Pandemic group (n, %)	Post-pandemic group (n, %)	P value*
1-5 years	<12	204[a] (23.2)	22[a] (22.7)	147[a] (21)	0.309
	12-20	354[a] (40.3)	40[a] (38.4)	262[a] (37.6)	
	>20	320[a] (36.4)	35[a] (36.1)	292[a] (41.7)	
6-11 years	<12	225[a] (32.4)	23[b] (52.3)	213[a] (28.4)	**0.012**
	12-20	308[a,b] (44.4)	12[b] (27.3)	344[a] (45.9)	
	>20	161[a] (23.2)	9[a] (20.5)	192[a] (25.6)	
12-18 years	<12	265[a] (49.2)	29[b] (76.3)	238[a] (45.6)	**0.007**
	12-20	173[a] (32.1)	7[a] (18.4)	185[a] (35.4)	
	>20	101[a] (18.7)	2[a] (5.3)	99[a] (19)	

Qualitative variables are expressed as numbers and percentages. P values are highlighted in bold
*; Chi-square test, 25(OH)D; 25-hydroxyvitamin D

12.89 ng/mL in spring, and 12.45 ng/mL in winter. Notably, compared with summer and autumn, the median VDLs were lower in spring and winter (**Figure 3**).

Independent Variables for VDD in All Study Groups

Age and VDLs were negatively correlated (r=-0.269, p<0.0001) in the study cohort. Furthermore, all three groups exhibited age-related correlations with VDLs, with corresponding correlation coefficients of r=-0.288 (p<0.0001), r=-0.500 (p<0.0001), and r=-0.290 (p<0.0001). A multivariate regression analysis revealed that the significant independent variables for the dependent variable of VDD were winter season [odds ratio (OR): 2.725, confidence interval (CI) % 2.343-3.170, p<0.0001], adolescent age group (OR: 2.520, CI% 2.173-2.923, p<0.0001), pandemic group (OR: 2.170, CI% 1.579-2.983, p<0.0001), and female sex (OR: 0.560, CI% 0.487-0.643, p<0.0001).

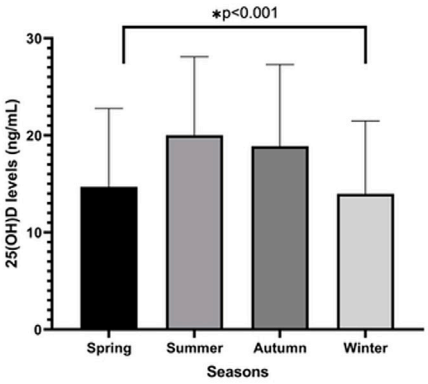

Figure 3. Season-specific 25(OH)D levels in the study populat

Discussion

This study highlights the variation in VDLs among children during different phases of the COVID-19 pandemic by providing a thorough analysis of VDLs in a pediatric cohort. Moreover, it provides insightful information about vitamin D status and VDD in relation to age, sex, and seasonal variations.

VDI and VDD are major public health issues in developing countries. This is significant because VDD is more common in the pediatric population and it has an impact on bone health during the growth period.[12] Studies continue to focus on the growing body of evidence linking VDD to non-skeletal disorders, such as inflammatory bowel disease, asthma, atopic dermatitis, type 1 diabetes mellitus, and others. Although routine vitamin D measurement is not recommended in clinical practice, it is helpful for children who may be at risk of VDD. This study assessed a pediatric cohort's vitamin D status at different points during the COVID-19 pandemic. This study clarifies how children's VDL fluctuate during these periods.

Measuring serum VDLs is necessary to determine vitamin D status because of its long half-life, which makes it unaffected by changes in children's parathyroid hormone levels.[4] International guidelines offer varying reported reference ranges for VDD and VDI, and there is no set cut-off serum VDL that indicates VDD.[12] The outcomes of clinical research may be impacted by these differences in reference ranges. As a result, we used recently suggested consensus reference values for children in our study.[11]

A study involving children aged 3-17 found a prevalence of 80.3% for VDD using a cut-off value of <15 ng/mL.[13] Another study including children aged 1-18, reported a prevalence of 34.1% for VDD and 27.5% for VDI.[14] Our study revealed a prevalence of 32.1% for VDD and 39.5% for VDI. These variations underscore the potential for differences in VDD prevalence not only between countries but also within different geographical regions of the same country. Even though it was conducted in a specific region, the results of our study reflecting the situation in Türkiye are quite strong because it was conducted with a significant sample size. However, the potential for limited generalizability of the results to different settings or population warrants further investigation. Exploring how regional disparities might influence the extrapolation of these findings to other groups would be beneficial.

According to some theories, females are more likely to suffer from VDD because they spend less time outside, cover up more, and receive less sunlight exposure.[15] In a study involving 331 Saudi children aged 6 to 17, it was found that girls' VDLs were substantially lower in girls than in boys. Female sex was shown to be an independent risk factor for VDD in Chinese children in another extensive study conducted by Hu et al.[16] In all three COVID-19 pandemic periods, we found that females had lower VDLs than males. This finding supports the findings of previous research and highlights the significance of female sex in VDD, independent of the pandemic period. Our research revealed that female sex was an independent variable of VDD in our study groups.

According to recent studies, teenage girls are more likely than boys of the same age to have VDD and frequently have lower VDLs.[14] It has been proposed that the sex effect, which is associated with variations in the levels of sex steroids released during puberty, contributes to VDD in adolescence.[17] A multi-center study reported prevalence rates of 13.7% in pre-school-aged children, 18.2% in school-aged children, and 23.9% in adolescents, defined as VDD 12 ng/mL. The prevalence of VDD among teenagers was found to be 23.46% in another study.[18] These rates were found to be 17.8%, 24.9%, and 42.6%, respectively, in a recent study.[14] In these age groups, the prevalence of VDD was found to be, respectively, 22.3%, 31%, and 48.4% in our study. In all three periods, school-aged and adolescent females had significantly lower VDLs than males, according to our study, which also found a similar sex distribution among all the children. In addition, compared with the other age groups, adolescents had a noticeably higher prevalence of VDD. These results support those of earlier clinical studies.[14] Our research indicates that children and adolescents with VDD have a higher prevalence. According to our research, adolescence is one of the independent variables associated with VDD, and the risk of the condition is roughly 2.5 times higher in this age group. This suggests that teenagers are especially susceptible to low VDLs. According to a recent study[6], the data thus imply that vitamin D supplementation may be necessary in this age group.

According to recent research, there is a significant negative correlation between children's age and their VDL.[19,20] According to our research, VDLs fall with advancing age. In the pre-pandemic, pandemic, and post-pandemic periods, we also discovered a negative correlation between age and VDLs.

A study conducted in Italy[21] involved the examination of 491 children. The average VDLs were found to be lower in the post-pandemic group than in the other groups in the study. The VDLs of children in our study prior to the pandemic were comparable to those observed during the pandemic, as reported in Italy.[21] However, our findings indicate that during the pandemic, VDLs in children were lower than those documented in earlier periods. The reduction in sun exposure and outdoor activities could be attributed to pandemic-related measures,

such as stay-at-home orders and school closures. In contrast to the other two times, children's VDLs were significantly higher in our study following the pandemic. Our research revealed that the pandemic group was an independent variable associated with VDD in our study groups. The detrimental effects of COVID-19 restrictions and extended stays home on VDLs are highlighted by these findings. Extended isolation caused by COVID-19 is probably associated with less time spent in the sun, which lowers the rate at which vitamin D is synthesized cutaneously.[9]

In a study, the impact of COVID-19 precautions on VDLs in 3600 children aged 0-6 years was examined.[9] A comparison of 2020 with previous years demonstrated that home confinement in children aged 3-6 years not only reduced their VDLs but also increased the prevalence of VDD. Furthermore, the study found that VDLs were lower in children aged 3-6 compared to those under 3 years before and after pandemic-related home confinement, indicating an age-related decline in VDLs. In comparison with other age groups, the 1-5 age group had a lower prevalence of VDD (22.3%), according to our study. When comparing the pandemic periods, we were unable to find a significant difference in VDLs in the 1-5 age group, which may have been caused by the fact that children in this age range were exempt from pandemic restrictions. The differences in prevalence rates observed between Yu et al.[9] study and our own study raise the possibility that different vitamin D supplementation strategies used in various nations may have an effect.

According to a recent study, year and season significantly contribute to variation in VDLs when analyzed using linear regression analysis.[21] Regardless of the study period, we observed seasonal variations in VDLs, with winter and spring showing noticeably lower VDLs than other seasons. Our results support earlier research[21] and show that sun exposure is essential for vitamin synthesis. All three study periods showed the expected seasonal variation, with lower VDLs in spring and winter and higher VDLs in summer. Our research also revealed that winter season was the most effective independent variable for predicting VDD in our study groups.

Study Limitations

Due to the retrospective cross-sectional study design, we were unable to access records related to factors that could influence VDLs, such as dietary intake and vitamin D supplementation. Additionally, we lack information on the exact amount of sun exposure for children and adolescents. Nevertheless, one strength of our study is the inclusion of a large number of children and adolescents.

Conclusion

Our study revealed a significant prevalence of VDD in school-aged children and adolescents, with a notable decrease in VDLs observed during the pandemic compared with other time periods. Furthermore, our study highlighted the increased vulnerability of female adolescents to VDD. We delineated winter season, adolescence, pandemic circumstances, and female sex as significant determinants affecting VDLs independently.

Ethics

Ethical Approval: Approval was granted by the KTO Karatay University Faculty of Medicine, Pharmaceutical and Non-Medical Device Research Ethics Committee (meeting date: 17.11.2023, decision no: 2023/021).

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

Footnotes

Author Contributions: Sert S: Surgical and Medical Practices, Concept, Design, Data Collection or Processing, Analysis or Interpretation, Literature Search, Writing.; Taner A: Surgical and Medical Practices, Concept, Design, Data Collection or Processing, Literature Search, Writing.

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Can Mean Platelet Volume be an Inflammatory Marker in Pediatric Diabetic Ketoacidosis?

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Abstract

This study explores the association between mean platelet volume (MPV) and other hematological parameters in children with diabetic ketoacidosis (DKA), aiming to identify hematological changes and their implications for management and treatment strategies in pediatric type 1 diabetes mellitus (T1DM). In a retrospective, two-center analysis of 323 children, participants were categorized into three groups: DKA, T1DM without ketoacidosis, and healthy controls (95). Hematological parameters and HbA1c levels were collected. Blood pH levels classified DKA severity, and statistical analyses included One-way ANOVA, correlation tests, receiver operating characteristic curve analysis, and logistic regression to assess the predictive value of hematological parameters for DKA. No significant demographic differences were noted among the groups. Patients with DKA exhibited significantly lower MPV and higher neutrophil-to-lymphocyte ratio (NLR) compared with both patients with T1DM without ketoacidosis and healthy controls. Logistic regression showed MPV ≤9.35 and NLR ≥2.73 significantly increased DKA risk. This study demonstrated a significant relationship between DKA and altered hematological parameters (MPV and NLR) in pediatric patients, highlighting their potential as markers for early detection and risk assessment of DKA.

Keywords: Diabetes mellitus, mean platelet volume, diabetic ketoacidosis

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Introduction

Diabetic ketoacidosis (DKA) is a critical acute complication of type 1 diabetes mellitus (T1DM). Ketoacidosis emerges as the primary indicator of T1DM and manifests when insulin demand escalates due to ailments, stressors, or a reduction in insulin administration.[1] Diabetes is an increasing health problem in children; thus, the risk factors, causes, and complications of DKA should be known.[2]

The mean platelet volume (MPV) is a parameter measured during the automatic blood count that increases in cases of increased thrombopoiesis. These young, large platelets that increase in circulation are also known as stress platelets and are more metabolically and functionally active than other platelets.[3] MPV is associated with systemic inflammation and prothrombotic states in various conditions, including cardiovascular and metabolic disorders.[4] In the context of DKA, elevated MPV levels may reflect a hypercoagulable state and a heightened inflammatory response during acute metabolic crises.[5]

Highlights

- Diabetic ketoacidosis (DKA) significantly lowers mean platelet volume (MPV) and elevates neutrophil-to-lymphocyte ratio (NLR) in pediatric patients.
- An MPV value ≤9.35 and an NLR value ≥2.73 can serve as predictive markers for DKA, with NLR offering higher specificity.
- MPV and NLR are accessible, cost-effective parameters useful in diagnosing DKA in children, particularly in settings lacking advanced diagnostics.
- Logistic regression analysis revealed that NLR ≥2.73 increases DKA risk by 28-fold, while MPV ≤9.35 increases it by 3.7-fold.

The neutrophil-to-lymphocyte ratio (NLR) is an indicator of inflammation and immune response. High NLR values may indicate increased inflammation and activation of the immune system.[6-9] Elevated NLR levels have been identified as a marker of systemic inflammation and oxidative stress in metabolic and inflammatory diseases.[10] In DKA, NLR can potentially serve as a prognostic marker correlated with inflammation severity and the risk of complications.[11]

This article explores the association between MPV and NLR in pediatric patients with DKA. The results of this study may reveal the DKA effect on children's hematological parameters of children, helping us better understand this condition's importance in pediatric health and its potential effects on treatment strategies.

Material and Method

Study Design

This retrospective, two-center study included 323 children aged 1-18. This group included 139 individuals diagnosed with DKA, 89 with type 1 diabetes but without ketoacidosis, and 95 healthy, non-diabetic children. Given its retrospective nature, explicit patient consent was deemed unnecessary. Hatay Mustafa Kemal University Ethics Committee gave ethical permission for the research, and the research complied with the principles stated in the Declaration of Helsinki (decision no: 06, date:25/12/2023). The authors did not conduct any experiments involving human participants or animals. The screening period spanned from November 2021 to January 2023, during which time children admitted to the Pediatric Endocrinology services at Hatay Mustafa Kemal University and Mersin City Hospital with DKA were identified through the system. Patients with concurrent health issues, infections, or medication use were excluded from the study.

The MPV, platelet distribution width (PDW) and NLRs of patients with average platelet counts (PLT) from their complete blood counts were recorded. Additionally, the HbA1c values at the time of the blood test were recorded for patients with diabetes. The height and weight of the patients were plotted on percentile curves specific to Turkish children.

Patients with DKA were categorized into three groups based on their pH values: mild, moderate, and severe. Those with a blood gas pH below 7.1 were classified as severe, those with pH values between 7.1 and 7.2 were considered moderate, and those with pH values between 7.2 and 7.3 were classified as mild ketoacidosis. The relationship between the severity of ketoacidosis and MPV values and NLR was assessed by comparing these parameters among the three groups. The study also examined the association between HbA1c levels and MPV and NLR in these patients. Additionally, the hematology profiles of patients with DKA were compared with those of patients with T1DM attending outpatient pediatric endocrinology clinics for routine checkups and healthy children undergoing routine checkups for child health and diseases.

Statistical Analysis

SPSS version 22 for Windows (SPSS Inc., Chicago) was used for all statistical analyses. Statistical data were presented as percentages, whereas continuous data were presented as mean ± standard deviation. A One-way ANOVA and Post-hoc Scheffe alpha tests were employed to assess the mean value differences in a dependent variable across two independent groups. Correlation analysis was performed to determine the magnitude and direction of the association between the two quantitative variables. The Pearson correlation coefficient was applied to data exhibiting a normal distribution, whereas the Spearman rank correlation coefficient was utilized for data not following a normal distribution. To identify the ideal cut-off value along with the sensitivity and specificity of MPV and NLR for predicting DKA, an analysis of the receiver operating characteristic (ROC) curve was conducted. For each testing technique, a p value of 0.05 indicated statistical significance. Logistic regression analysis was performed to examine whether the MPV and NLR thresholds, as determined through ROC curve analysis, indicate a risk of DKA.

Results

The study incorporated a total of 323 participants, distributed among three distinct groups: the DKA group comprised 139 individuals, representing 43% of the total; the T1DM group included 90 participants, accounting for 27.9%; and the healthy control group comprised 94 individuals, making up 29.1% of the study population. The mean age of the participants was 129.39 (range: 2.5-213) ±55.80 months. With p values of 0.143 and 0.998, respectively, statistical analysis revealed no discernible differences in age or sex across the groups.

There were no significant variations in the height, weight, or age of the groups, as indicated by the analysis of the demographic and laboratory data (p values of 0.069, 0.473, and 0.143, respectively. However, the DKA (DKA) group exhibited significantly higher values for PLT, PDW, neutrophil count, and NLR compared with the other groups, all with p values below 0.001. In contrast, the MPV was significantly lower in the DKA group (p<0.001). No significant differences in lymphocyte counts were observed between the groups (p=0.666) (**Table 1**).

In the diabetic patient group, hematological parameters revealed a moderate positive correlation between the severity of DKA and neutrophil counts, as well as a weak positive correlation with the NLR (correlation coefficients r=0.471, p<0.001 and r=0.235, p=0.007, respectively). Additionally, a moderate positive correlation was observed between HbA1c levels and PDW, while a weak positive correlation was noted with neutrophil counts (r=0.402, p<0.001 and r=0.252, p<0.001, respectively). No significant correlations were found between MPV, PLT, and DKA severity or HbA1c levels (**Table 2**).

In patients with diabetes, ROC curve analysis was performed to establish the optimal cut-off values for MPV and NLR for predicting DKA. The results indicated that an MPV value of ≤9.35 predicted DKA with 72% sensitivity and 56% specificity. Additionally, an NLR value of ≥2.73 predicted DKA with 74% sensitivity and 91% specificity (**Table 3**).

Subsequent risk analysis utilizing logistic regression based on these cut-off points indicated that an MPV value ≤9.35 increases the risk of developing DKA by 3.7-fold, whereas an NLR value ≥2.73 elevates the risk of DKA development by 28-fold (**Table 4**).

Discussion

Our research indicates that this is among the first studies to investigate the relationship between MPV, NLR, and DKA. Previous studies have shown a relationship between MPV readings of patients with diabetes and metabolic management. The depletion of circulating platelets may occur in individuals with diabetes due to platelet activation and aggregation triggered by an augmented response to endogenous stimuli. This

Table 1.
Comparison of group-to-group variations in laboratory and demographic data

	Control	Type 1 DM	DKA	P value
Age (months)	125.86±53.38	122.32±54	136.25±54.76	0.143
Height Z score	0.117±1.04	-0.121±1.17	-0.359±1.14	0.069
Weight Z score	0.036±2.53	-0.271±1.341	-0.309±1.37	0.473
HbA1c	-	10.64±2.92[a]	12.44±2.36[b]	**<0.001**
MPV	10.31±1.28[a]	10.05±1.30[a]	9.12±1.27[b]	**<0.001**
PLT	313.712±81.825[a]	328.670±96.215[a]	387.171±114.865[b]	**<0.001**
PDW	15.12±1.49[a]	14.99±1.73[a]	35.32±13.31[b]	**<0.001**
Neutrophil	4.32±1.98[a]	3.90±2.23[a]	12.33±7.39[b]	**<0.001**
Lymphocyte	2.83±1.28	3.05±1.74	2.88±2.05	0.666
NLR	2.01±2.63[a]	1.48±0.90[a]	5.98±6.08[b]	**<0.001**

The difference in mean values between groups a and b was considered statistically significant (p<0.05).
MPV; Mean platelet volume, PLT; Platelet counts, PDW; Platelet distribution width, NLR; Neutrophil-to-lymphocyte ratio, DKA; Diabetic ketoacidosis

Table 2.
DKA severity and the relationship between Hba1c and hematological parameters

		DKA severity	HbA1c
MPV	Pearson correlation	0.039	-0.092
	Sig. (2-tailed)	0.660	0.187
PLT	Pearson correlation	0.108	0.013
	Sig. (2-tailed)	0.231	0.856
PDW	Pearson correlation	-0.004	0.402**
	Sig. (2-tailed)	0.964	0.000
Neutrophil	Pearson correlation	0.471**	0.252**
	Sig. (2-tailed)	0.000	0.000
NLR	Pearson correlation	0.235**	0.100
	Sig. (2-tailed)	0.007	0.150

**; Correlation is significant at the 0.01 level (2-tailed)
MPV; Mean platelet volume, PLT; Platelet counts, PDW; Platelet distribution width, NLR; Neutrophil-to-lymphocyte ratio, DKA; Diabetic ketoacidosis

Table 3.
Identification of optimal cut-off points for predicting diabetic ketoacidosis

	Optimal threshold for diabetic ketoacidosis	AUC	Sensitivity	Specificity	95% CI	P value
MPV	≤9.35	0.704	0.72	0.56	0.634-0.773	<0.001
NLR	≥2.73	0.871	0.74	0.91	0.825-0.918	<0.001

MPV; Mean platelet volume, NLR; Neutrophil-to-lymphocyte ratio, CI; Confidence interval, AUC; Area under the curve

Table 4.
Risk analysis for the development of diabetic ketoacidosis

	OR	95% CI	P value
MPV (≤9.35)	3.7	2.075-6.591	<0.001
NLR (≥2.73)	28	12.325-63.889	<0.001

MPV; Mean platelet volume, NLR; Neutrophil-to-lymphocyte ratio, CI; Confidence interval, OR; Odds ratio

phenomenon may also be associated with variations in platelet production and lifespan observed in patients with diabetes.[12,13] In a study by Pirgon et al.[12] children with type 1 diabetes were compared with healthy controls. The results showed that children with T1DM had significantly higher MPV levels than the controls. In individuals with type 1 diabetes, elevated platelet activity is associated with a higher risk of microvascular and macrovascular complications. Nevertheless, no correlation was observed between MPV and HbA1c levels.

In contrast, a study conducted by Sobü et al.[13] found no difference in MPV values between children with T1DM and robust controls. They also found that MPV levels increased with longer illness duration and higher HbA1c levels in those with poorly managed diabetes, and that these individuals' MPV levels were significantly greater than those of healthy controls. The MPV did not statistically differ significantly in this study between the group of healthy control children and the children with diabetes. In contrast to the diabetic and control groups, MPV was significantly lower in patients with DKA. This finding is consistent with the hypothesis that DKA induces severe inflammation, leading to the consumption and destruction of younger, larger platelets, thereby resulting in lower MPV levels. The inflammatory cascade in DKA is likely mediated by increased oxidative stress, cytokine release, and coagulation pathway activation, all of which contribute to platelet consumption and turnover. From this theory, it can be concluded that DKA induces severe inflammation in the body.[14-16] Furthermore, other studies have suggested that low MPV in acute inflammatory states may indicate a compensatory mechanism in response to heightened platelet consumption or sequestration.[16] This contrasts with chronic inflammatory conditions, in which the MPV is typically elevated, underscoring the dynamic and context-dependent role of the MPV as an inflammatory marker. Comparing these findings with similar acute inflammatory states, such as sepsis and acute pancreatitis, may help delineate the specific pathways influencing MPV in DKA.[17,18] The lack of a significant difference in MPV between children with T1DM without ketoacidosis and healthy controls, in line with studies suggesting an increase in MPV with longer disease duration and elevated HbA1c levels, may be attributed to the young age of the patients and the relatively shorter duration of their condition.[12,13]

In a study evaluating the connection between the NLR and DKA, patients with DKA were compared with those without ketoacidosis, revealing that the NLR was significantly elevated in cases of DKA. Additionally, the severity of ketoacidosis was correlated with an increase in NLR.[19] Another study in adults demonstrated that NLR could be used to predict systemic inflammatory response in the presence of DKA. Similar to our research, children with T1DM without ketoacidosis were compared with healthy controls, and it was found that the NLR was significantly higher in children with T1DM than in the control group, with this ratio further increasing in the presence of ketoacidosis.[11] This study found no significant difference in the NLR between children with T1DM and healthy controls. This result may be associated with the younger patient population and the shorter duration of diabetes. However, in patients with DKA, a significant increase in NLR was observed compared with that in patients with type 1 diabetes without ketoacidosis and healthy controls. Furthermore, an increase in the severity of DKA was associated with an increase in NLR. This result indicates that the presence and severity of DKA are correlated with an increase in inflammation intensity. Based on our findings, when assessing the predictive value of MPV and NLR for DKA, we found that an MPV ≤9.35 predicted DKA with 72% sensitivity and 56% specificity. Likewise, an NLR ≥2.73 demonstrated a sensitivity of 74% and specificity of 91% for predicting DKA. In a study by Scutca et al.[19] an NLR threshold of 1.84 was identified, with a reported sensitivity of 80.2% and specificity of 80% for predicting the onset of DKA. Furthermore, this study's risk analysis showed that a 3.7-fold higher chance of developing DKA was linked to an MPV value of ≤9.35. Moreover, when the NLR is ≥2.73, the risk of DKA increases by 28-fold. Considering the importance of early diagnosis in pediatric patients presenting with high blood sugar levels to emergency departments, especially in healthcare facilities lacking the capability to perform blood gas analysis, MPV and NLR serve as simple, economical, and accessible parameters for predicting DKA.

This retrospective study design limits the ability to capture certain clinical details that could be obtained in a prospective setting. Additionally, the study population was drawn from only two centers, which may limit the generalizability of the findings. The inflammatory markers used, specifically MPV and NLR, are not entirely specific to DKA and may be influenced by other systemic inflammatory conditions. Furthermore, the prognostic value of MPV and NLR for diagnosing DKA should be validated in larger multicenter studies to confirm their broader applicability.

Conclusion

The hematological effects of DKA in pediatric patients provide significant insights into the relationship between systemic inflammation and platelet dynamics in the context of T1DM. Our results highlight a strong association between DKA and specific hematological changes, including reduced MPV and increased NLR, thereby providing a deeper understanding of the complex mechanisms underlying acute diabetic complications.

This study unequivocally demonstrated that the hematological parameters of MPV and NLR can serve as pivotal indicators of DKA, offering a window into the systemic inflammatory state and platelet functionality in pediatric patients. The predictive value of MPV ≤9.35 and NLR ≥2.73 for DKA heralds a significant advancement in our ability to preemptively identify and stratify the risk of DKA in children with T1D. This, in turn, paves the way for timely intervention strategies, potentially mitigating the severity of DKA presentations and improving patient outcomes.

In clinical settings, MPV and NLR are accessible and cost-effective parameters that could be routinely incorporated into initial evaluations for pediatric patients presenting with hyperglycemia or suspected DKA. For example, these markers could help stratify risk in emergency departments, especially in settings where advanced diagnostic tools, such as blood gas analyzers, are not readily available. Furthermore, the dynamic characteristics of MPV and NLR can provide insights into disease progression and the effectiveness of long-term therapeutic interventions.

However, it is important to note the potential limitations of these markers. MPV and NLR are not specific to DKA and may be influenced by other systemic inflammatory or hematological conditions, potentially leading to diagnostic ambiguity. Thus, their use should complement, rather than replace, standard diagnostic approaches. Future multicenter studies with larger sample sizes are necessary to validate these findings and refine their clinical application in diverse healthcare settings.

Ethics

Ethical Approval: Hatay Mustafa Kemal University Ethics Committee gave ethical permission for the research, and the research complied with the principles stated in the Declaration of Helsinki (decision no: 06, date:25/12/2023).

Informed Consent: Given its retrospective nature, explicit patient consent was deemed unnecessary.

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Footnotes

Author Contributions: Trabzon G: Concept, Design, Data Collection or Processing, Analysis or Interpretation, Literature Search, Writing.; Çiçek D: Design, Data Collection or Processing, Literature Search.; Güngör Ş: Design, Analysis or Interpretation, Literature Search.; Demiray Güllü Ş: Design, Data Collection or Processing, Literature Search.; Yazarlı E: Concept, Design, Data Collection or Processing, Literature Search.; Güllü UU: Concept, Design, Literature Search.; El Ç: Design, Data Collection or Processing, Literature Search.

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Hirschsprung Disease in a Female Infant: A Case Report

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Abstract

Hirschsprung's disease (HD) is a congenital disease characterized by the absence of ganglion cells in the intestinal muscularis nerve plexus. The segment most affected was the rectosigmoid colon (80%). The clinical manifestations are non-specific; the common signs include vomiting, abdominal distention, and defecation alterations in early-life another feasible alteration is anemia. Anorectal manometry and contrast enemas are also highly useful. We present the case of a 9-month-old female with refractory constipation who was diagnosed with HD. Some theories explain that there is dysregulation of the microecological balance and intestinal mucosa. Imaging diagnostic methods are useful tools for screening HD. The mortality rate of these conditions is between 2% and 5%; therefore, a group of qualified professionals is necessary for treatment and postsurgical care.

Keywords: Hirschsprung disease, constipation, children

Introduction

Hirschsprung's disease (HD) is a congenital disease characterized by the absence of ganglion cells (GC) in the nerve plexus of the intestinal muscularis.[1] The prevalence of this condition ranges from 1 to 1.63 per 10,000 births, with a higher occurrence in males than in females at a ratio of 5 to 1.[2] The most affected segment is the rectosigmoid colon (80%), followed by the sigmoid colon and the entire colon (15% and 5%, respectively).[1]

The clinical manifestations are nonspecific, making diagnosis challenging. Common signs of defecation include vomiting, abdominal distention, and alterations in defecation in early life.[3] Anorectal manometry and contrast enemas are highly useful diagnostic tools, avoiding the necessity of more invasive methods, such as biopsy. Histopathological examinations of rectal biopsies provide a definitive diagnosis, but are not indicated for all patients.[1]

Resection of the abnormal intestinal segment is the preferred treatment; endorectal pull-through and Duhamel pull-through are the most common techniques performed.[4] We describe the case of a 9-month-old female patient with refractory constipation who was diagnosed with HD.

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Case Report

A 9-month-old female with a history of hip dysplasia consulted the emergency room with her mother for 15 days because of absence of stool, vomiting on two occasions, and subjective fever. The mother administered laxatives to her, but they did not produce the desired effects. On physical examination, her vital signs were as follows: heart rate, 159 bpm; respiratory rate: 24 bpm; temperature: 38.5°C; oxygen saturation: 95%; weight: 10 kg; height: 69 cm. Additionally, the abdomen had intestinal sounds, a palpable mass on the hypogastric and anal regions without lesions, and abundant dry depositions.

Initial labs reported mild microcytic hypochromic anemia [hemoglobin 10.3 g/dL, normal range (NR): 11.3-14.1, hematocrit: 32.2%, NR: 31-41%, mean corpuscular volume: 73.3 um^3, NR: 71-88 um^3, mean corpuscular hemoglobin: 23.5 pg, NR: 24-30 pg]; the rest of the laboratories were within NR. Imaging studies revealed simple abdominal radiography with abundant fecal matter and no air-fluid levels (**Figure 1A**). Abdominal ultrasound showing distended intestinal loops with liquid fluid in the lower abdomen.

Pediatricians initially considered fecal impaction, and the patient was treated with enemas and oral laxatives. Her first stool was three days after admission; it was abundant, liquid, and fetid, without blood. Control of simple abdominal radiography using fecal matter in smaller quantities without air-fluid levels **(Figures 1B and 1C)**. The patient continued with refractory constipation due to amplified laboratory findings on suspicion of aganglionic colon; thyroid function was normal, and a barium enema revealed sigmoid elongation with a difference in the maximum transverse diameter recto-sigmoid of 3 cm and slight distal haustration (**Figure 2**). The patient was transferred to another hospital to continue his in-hospital stay at a high-level complex institution. Consent from the patient's mother was obtained before the inclusion of the child for reporting the case in accordance with the institutional ethics guidelines.

Discussion

The first documented cases of these intestinal conditions appeared in the Hindu literature; it was many years until 1691, when Frederick Ruysch reported the first case of congenital megacolon. However, an extensive disease description was realized 200 years later by Harald Hirschprung.[2,5] Since then, many theories have been developed regarding the etiology and physiopathological mechanisms of these uncommon diseases. Okamoto and Ueda in 1967 proposed a theory regarding alterations in the migration of cranial-caudal intestinal GCs, and it was not until the 1990s that the genes involved began to be studied.[5]

Clinical manifestations vary according to the aganglionic segment and age at presentation. In the case of newborns, there are predominantly symptoms that mimic an intestinal obstruction; later, in infants, children, and adults, the symptoms become more unspecific, including vomiting, abdominal distension, and refractory constipation to medications and rectal therapies.[6] Furthermore, the absence of glucocorticoid causes intestinal overactivity with an elevated production of acetylcholine because the affected colon segment has a persistent contraction with a subsequently progressive to secondary dilatation of the non-affected proximal colon.[1]

Anemia is another possible complication of HD. Some theories explain that there is dysregulation in the microecology balance and intestinal mucosa; these microbes regulate the expression of iron transporter genes and stimulate hepcidin, which inhibits the release of iron into plasma.[3] Our patient had a clinical manifestation suggesting constipation although normal treatment was ineffective, which raised suspicion of an aganglionic intestine. Additionally, she experienced mild, low-volume anemia characteristic of iron deficiency anemia. Although it was not possible to determine the complete iron deficiency profile, this alterations may have been associated with her colon involvement.

Imaging diagnostic methods are useful tools and work as screening tests in HD. There are two imaging options

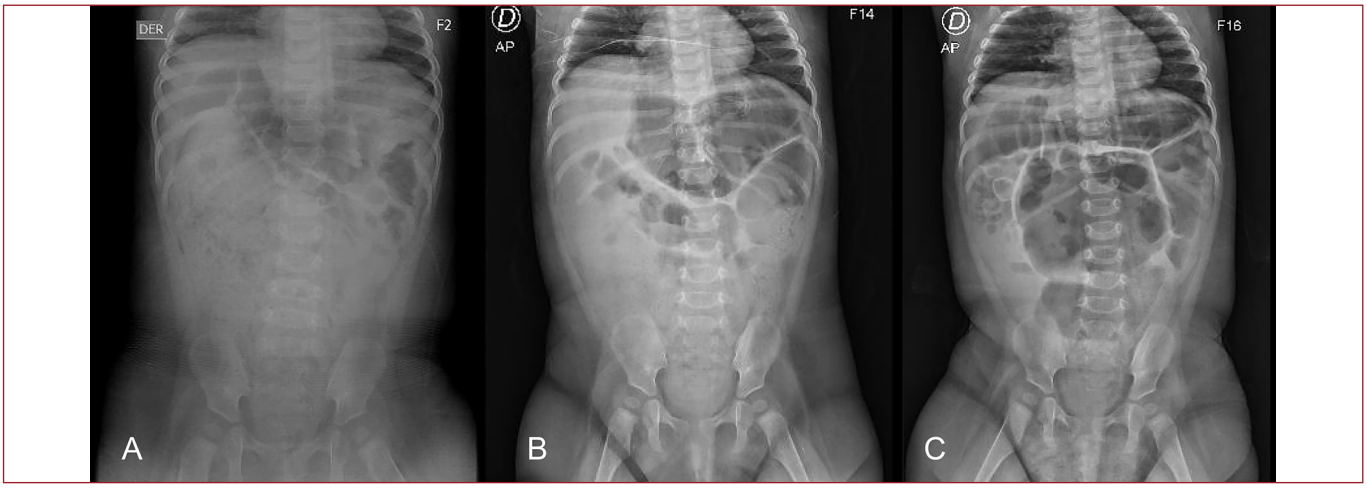

Figure 1. Simple abdominal radiography: A) abundant fecal matter and no air-fluid levels; B and C) control at days three and four showing fecal matter in smaller quantities and without air-fluid levels

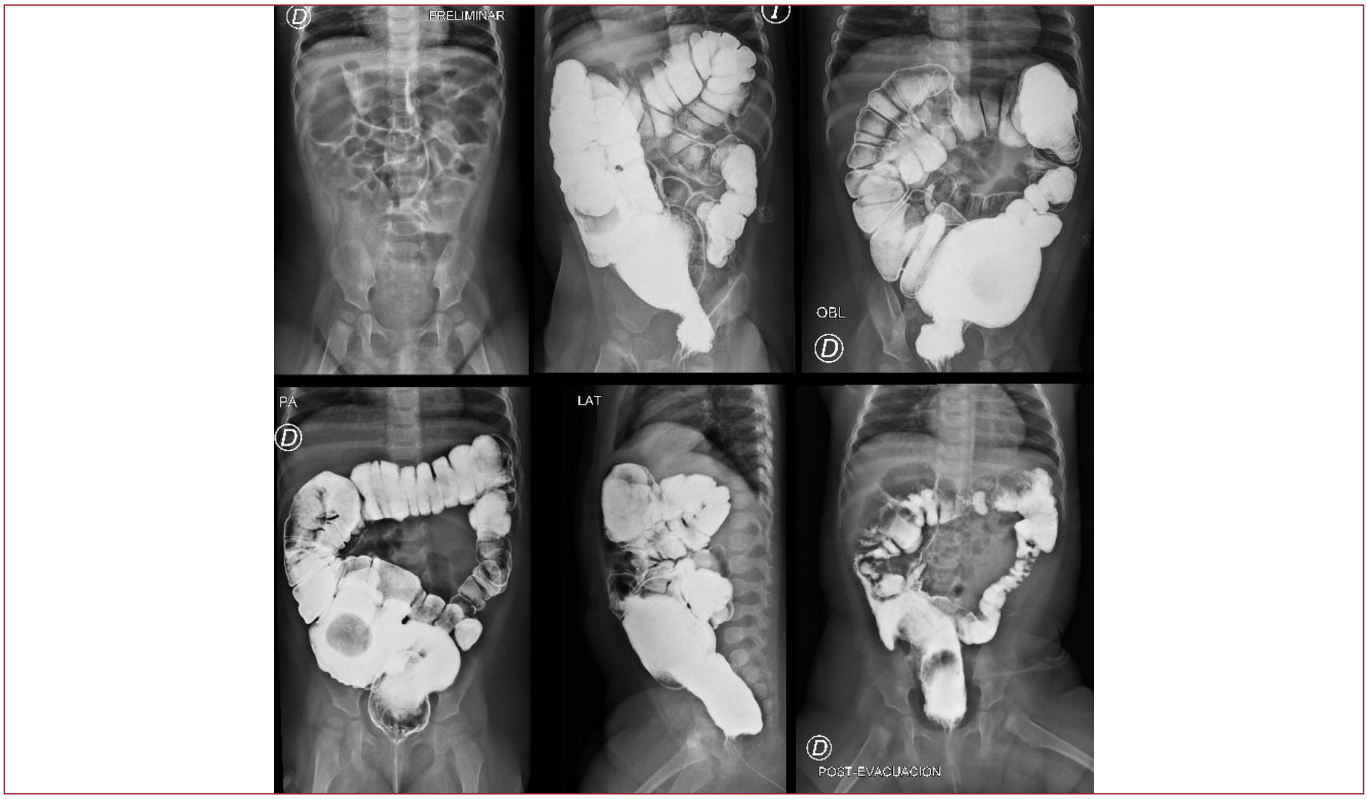

Figure 2. Enema contrast test showing sigmoid elongation with a difference in the maximum transverse diameter recto-sigmoid of 3 cm and slight distal haustration

that are especially useful in HD: Contrast enema and anorectal manometry.[6,7] The contrast enema is a widely available test in which a water-soluble contrast is administered into the colon by a catheter placed inside the anus. After contrast instillation, live images using fluoroscopy are taken.[6] Contrast enema is pathological when it reports a radiographic transition zone with a dilated proximal bowel, contrast retention, irregular mucosa and bowel, and reverse rectosigmoid ratio.[6,7] Notably, normally, the rectum is larger than the sigmoid colon because it is a reservoir and the rectosigmoid ratio is >1. When HD is present, the ratio is inverted because of the impossibility of the rectum to distend it; in contrast, the sigmoid colon starts to distend proximally to store the stool.[7]

Anorectal manometry is the second choice of test for imaging diagnosis of HD; it evaluates different measures of anorectal function and voluntary and involuntary anorectal properties. The procedure is performed using a flexible catheter with sensors introduced into the rectum to allow continuous measurement of pressures.[7] It is considered indicative of HD when the recto anal inhibitory reflex is absent.[6] According to Meinds et al.[8] anorectal manometry is a useful tool for excluding HD diagnosis and avoiding unnecessary rectal biopsy procedures.

Other imaging methods, such as hydrocolonic sonography, can be valuable tools with high sensitivity and specificity for HD diagnosis. This type of study allows for a morphological and vascular assessment of the colon.[9] In addition, histopathological studies using rectal biopsies, when available, are especially useful for definitive diagnosis in cases in which imaging methods are not conclusive or in cases in which there is a specific necessity to perform an invasive procedure such as rectal biopsy. In order to obtain a good specimen for studies, the biopsy should be taken 2 cm above the dentate line and should have a minimum of 3 cm of specimen, of which one-third should be submucosa.[4]

Currently, there are approximately 24 genes associated with this entity; however, the rearranged during transfection gene is the main one involved.[1,2] Approximately 30% of children with HD have other chromosomal and/or congenital anomalies, with Down syndrome being the most commonly associated. However, it has been studied for malformations in different systems, including limbs.[6] The patient's case has a history of hip dysplasia, an entity that has been correlated with multiple gene alteration[10], even though there are no studies that precise a correlation between these entities. Despite advances in medicine, the mortality rate of these conditions is between 2 to 5%.[6] For this reason, a group of qualified professionals is necessary for the treatment and postsurgical care of these patients to obtain better results and prevent complications.[1,4]

Conclusion

HD is a complex entity that has a lower incidence and is predominant in males. This approach is a clinical challenge due to clinical variability, although it should be considered in children with constipation refractory to treatment. Imaging tests are crucial for diagnosis, while histopathological studies provide diagnostic confirmation when available.

Ethics

Informed Consent: Consent from the patient's mother was obtained before the inclusion of the child for reporting the case in accordance with the institutional ethics guidelines.

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Footnotes

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First Cases of Transplacental Transmission of *Anaplasma* spp. in Gabon: Cases Reports and Brief Review of the Literature

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Abstract

Anaplasma spp. is an emerging human zoonosis, usually transmitted by tick bites. Human-to-human transmission is rare, and transplacental transmission is even less common. No cases of transplacental transmission have been previously reported. We report the cases of patients who were followed up for febrile syndrome diagnosed with maternal-fetal infection and treated with antibiotic therapy and hemodynamic correction. Investigation of the etiology revealed *Anaplasma* spp. in a neonatal infection. These cases prove that transplacental transmission of *Anaplasma* spp. is possible and that surveillance is important.

Keywords: *Anaplasma* spp., transplacental transmission, Gabon

Introduction

Originally considered for their importance in veterinary medicine, infections caused by *Anaplasmataceae* are becoming increasingly apparent in humans and are sometimes involved in the emergence of species that are not yet fully characterized.[1,2]

The emergence of these species as pathogenic agents, including *Anaplasma* spp., has had a remarkable impact on the health of humans and animals. This is due to the inversion of habitats resulting from the development of human activity, which brings humans closer to animal reservoirs, and to the domestication of animals, which facilitates the transmission of pathogens.[1,3,4]

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Several studies have reported the transmission of *Anaplasma* spp. to humans (accidental hosts) by tick bites, but only a few cases have demonstrated transmission from human to human. However, after a thorough literature review, no previously reported case in Gabon involved transplacental transmission. This study aimed to demonstrate that *Anaplasma* spp. is also involved as an emerging pathogen in febrile syndrome in neonatology and that it can be transmitted transplacentally. We report a case of anaplasmosis in two newborns consulted for febrile syndrome.

Case Reports

Neonates were enrolled in the febrile syndrome study after obtaining informed and signed consent from two of their parents. In a context of high malaria endemicity, blood samples were taken to investigate the different pathogens of bacterial origin involved in pediatric and especially neonatal febrile syndrome. We followed two newborns, one with a late maternal-fetal infection and the other with an early infection (neonatal infection); their clinical characteristics were similar to those of the syndromic diagnosis, comparable to those of the pathogens (group B *Streptococcus*, *Escherichia coli*, *Listeria monocytogenes*) most frequently encountered in this context, where routine antibiotic therapy was initiated, proportional to the regional pathogens according to the therapeutic algorithm. We noted good progress in all these patients. The first was discharged on the third day of hospitalization and the second on the fifth. After the sample collection campaign, we proceeded to the molecular analysis in the search for zoonotic bacteria that occur in humans, of which *Anaplasma* spp. was screened and found in these two newborns. They are listed below with clinical and paraclinical data (**Table 1**).

Case 1: On 23 February 2023, a newborn baby in his second day of life after a pregnancy of 37 weeks' amenorrhoea and 2 days, male, weighing 3200 g. He was admitted with a fever that had been present for 1 day without treatment, but the maternal history suggested a urogenital infection with no documented therapeutic initiation but domestication of dogs and cats has been reported. A fever of 39.1°C was recorded on admission, with a heart rate of 166 beats per minute and a respiratory rate of 68 cycles per minute. The newborn presented with dyspnoea but had good skin coloration and a neurological examination marked by the weak presence of archaic reflexes. Biological tests showed the hemoglobin level to be within norms at 20.8 mg/dL and the hematocrit at 45%. White blood cells were normal at 9200 cells/mm^3 and platelets at 167,000 cells/mm^3, and C-reactive protein (CRP) was positive as part of the inflammatory work-up. Antibiotic therapy (ampicillin and gentamicin) was given based on a probable neonatal infection.

Case 2: On 20 March 2023, he was on his 28th day of life after a pregnancy of 38 weeks amenorrhoea and 5 days, male, weighing 4500 g. He was brought to the clinic with a fever that had been present for 2 days and was treated at home with paracetamol. It should be noted that the mother had had a urogenital infection during the last trimester, with no evidence of recovery and no notion of domestication of animals. On admission, his temperature was 40°C, and her heart and respiratory rates were 178 beats per minute and 56 cycles per minute respectively. The general condition was altered by fever, dehydration, mucocutaneous pallor, and a neurological examination marked by a drop in muscle tone and weakness of archaic reflexes. Biological investigations showed anemia with hemoglobin at 7.8 mg/dL and hematocrit at 23%. With a hyperleukocytosis of 27600 cells/mm^3 and a hypoplakettosis of 137000 cells/mm^3, the CRP was negative as part of the inflammatory work-up. Hemodynamic correction by transfusion of iso group blood and antibiotic therapy (ceftriaxone) for late maternal-fetal infection complicated by severe sepsis in septic shock.

Whole blood samples (5 mL) from the study, including those from the two neonates, were centrifuged to separate the erythrocyte pellet from the plasma so that the pellet could be stored below 20°C; the pellet was then used for molecular analysis. Following the manufacturer's protocol, DNA extraction was performed using the Minikit whole blood genomic DNA purification kit (Thermo Scientific).

This was followed by polymerase chain reaction (PCR) amplification of *Anaplasmataceae* family genes encoding 23S rRNA and *Anaplasma* spp. genus DNA encoding rpoB (**Table 2**); these amplifications were performed in a Bio Rad® thermocycler (Mercure de Coanette, France). [5-7] This *in vitro* replication technique used specific primers (**Table 2**) for amplification of extracted DNA fragments for the detection of *Anaplasma* spp. Amplified DNA fragments were analyzed by electrophoresis on 2% agarose gel and visualized by ultraviolet light (520 nm) using Vilber E-Box (Grosseron, France).

Discussion

Anaplasmosis, ehrlichiosis, and neo-rickettsiosis are a group of intracellular bacterial infections belonging to the *Anaplasmataceae* family, which are equally specialized in their life cycle and have the particularity of infecting a

Table 1.
Clinic and biological parameters

	Clinical parameters					Biological parameters					
	Age (days)	Gender	Weights (g)	T° (°C)	HF (b/m)	BF (c/m)	Hb (mg/dL)	Hc (%)	WBC (cells/mm^3)	Plt (cells/mm^3)	CRP
Patient 1	2	M	3200	39.1	166	68	20.8	45	9200	167000	Positive
Patient 2	28	M	4500	40	178	56	7.8	23	27600	137000	Negative

CRP: C-reactive protein (reference <5 mg/L= negative); WBC: White blood cells (reference 10-20 × 10^3 cells/mm^3); Hb: Hemoglobin (reference18-23 mg/dL); Plt: Platelets (reference 150-450 × 10^3/mm^3); Hc: Haematocrit (reference 35-50%); HF: Heart frequency (reference 120-160 b/m); BF: Breath frequency (reference 30-50 c/m); T°: Temperature (reference 36.5-37.5°C)

Table 2.
Primers for PCR

Species	Target gene	Primers	Sequences (5'-3')	T° (°C)	Expected sizes	References
Anaplasmataceae	23S rRNA	TtAna-F	TGACAGCGTACCTTTTGCAT	54	191 bp	7
		TtAna-R	GTAACAGGTTCGGTCCTCCA			
Anaplasma spp.	rpoB	Ana-rpoBF	GCTGTTCCTAGGCTYTCTTACGCGA	51	525 bp	7
		Ana-rpoBR	AATCRAGCCAVGAGCCCCTRTAWGG			

PCR: Polymerase chain reaction; T°: Temperature

variety of hosts, reservoirs, and vectors. *Anaplasma* are vector-borne bacteria associated with ticks of the order Ixodida.[2]

Anaplasmosis is an emerging zoonotic infection that is becoming increasingly prevalent in our communities, where it is probably under-estimated due to a lack of investigative tools, given the high seroprevalence figures observed in the United States (11-15%) and Europe (2-28%).[6,8] But even more, the study carried out in Taiwan, showed 31.8% (87/274) of *Anaplasma phagocytophilum*.[4] The availability of diagnostic tools and various initiatives to investigate this emerging pathogen have led to it being found in newborn infants. The proximity of the forest and the promiscuity of animals (domestication) as an intermediate host are thought to be factors in this emerging infection.[4]

The detection of *Anaplsama* spp. in newborns raises the possibility of vertical transmission between vertebrates, something that has never been documented in our environment, let alone researched.

Bearing in mind that the probability of transmission of bacteria from ticks to humans or other vertebrates, in the event of a bite, requires a significant period of almost 36 hours.[9] Exposure of a newborn to such a sting does not seem to be obvious, but one of the newborns was less than 48 hours old. However, in the case of our two patients, we doubt the possibility of a bite (tick) due to the hygienic conditions (baths) requiring multiple changes of clothing and checks on their packaging (cradles).[10]

We believe that the presence of this germ in the newborn would suggest the possibility of definite vertical transmission. Although transmission from one vertebrate host to another is rare, transmission during blood transfusions in humans has been observed,[11,12] and even more so intrauterine transmission between cows and calves in veterinary medicine.[5]

Conclusions

These are the first cases of human-to-human transplacental transmission of *Anaplasma* spp. in Gabon. In a context where this infection has been associated with transplacental transmission (mother to child) during the intrauterine life of newborns, several arguments have been put forward to understand neonatal transmission of *Anaplasma* spp.

We urge clinicians to be aware that anaplasmosis, one of the emerging zoonosis, has the potential to be transmitted transplacentally and may be considered in the differential diagnosis of febrile syndromes in neonates when the environmental context of maternal life suggests proximity to a reservoir (dog, etc).

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Ethics

Informed Consent: Informed consent was obtained from the parents of the patients.

Footnotes

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