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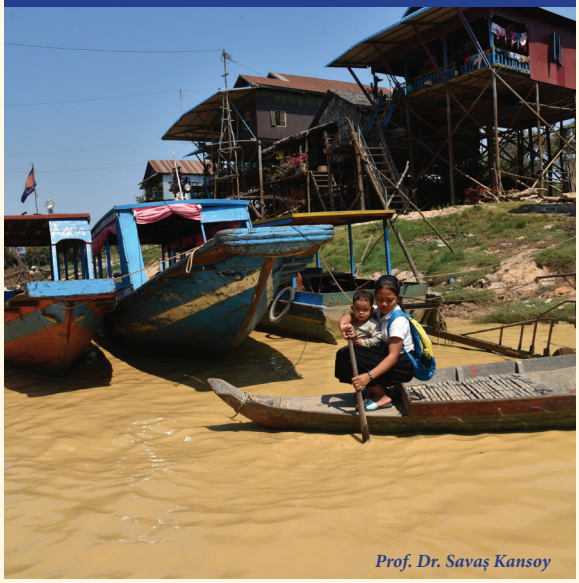

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PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/);

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al., for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/);

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All results of data and analysis should be presented in the Results section as tables, figures and graphics; biostatistical methods used and application details should be presented in the Materials and Methods section or under a separate title.

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Results: The results of the study should be stated, with tables/figures given in numerical order; the results should be evaluated according to the statistical analysis methods applied. See General Guidelines for details about the preparation of visual material.

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Editorial

Dear Readers,

We are pleased to announce the first issue of "The Journal of Pediatric Research" in 2019 has been published. We hope this year brings happiness, justice and peace worldwide.

We present you with 17 articles including 10 research articles, 1 review article, 5 case reports and 1 letter to the editor. In one of these studies, the authors found that game-based nutrition education improved the knowledge levels of students which can draw the interest of all Pediatricians. Another research article evaluated preseptal and orbital cellulitis and determined that conjunctivitis was the most common etiologic factor. The case reports are Diamond-Gardner Syndrome which is rare in childhood, an adolescent boy with steroid-responsive ophthalmologic migraine and a rare association of diabetic ketoacidosis and myasthenia gravis. We hope that these articles will provide unique clinical perspectives for our readers.

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We want to acknowledge the editorial team, the reviewers, authors and Galenos Publishing House for their magnificent support in preparing the first issue of 2019. And also it is a great honor to be a part of this editorial team. We look forward to your scientific contributions in our future issues.

Best wishes

March, 2019

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Review

The Quality of Life and Mental Health in Children with Primary Immunodeficiency

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ABSTRACT

Primary immunodeficiency disorders (PIDs) are characterized by recurrent and numerous infections, autoimmune disorders, and malignancies. These diseases are a heterogeneous group that contains many disorders caused by the disruption of the immune system. Despite being seen rarely, PIDs lead to serious morbidity and mortality. Children and adolescents with PIDs are expected to have a higher prevalence of psychopathologies and a lower level of the health quality of life In this text, we aim to review and summarize the current literature.

Keywords: Primary immunodeficiency, psychiatry, quality of life, children, adolescent

Introduction

Although primary immunodeficiencies (PIDs), which may be fatal in the childhood period, can be recognized early and appropriate treatment modalities can be applied, they can lead to chronic diseases later in life. The improvements in intravenous immunoglobulin (IVIG) therapy have led to improved survival, so that psychosocial, school, academic difficulties and health quality of life (HR-QOL) have become a current issue nowadays.

In this text; the effects that the widespread use of IVIG to treat the physical health of PID children and adolescents has on daily life adaptations, comorbid psychiatric problems and quality of life have been reviewed and summarized.

Definition and characteristics of primer immunodeficiency: PID is a heterogeneous group of rare hereditary diseases of the immune system (1) PIDs have clinical importance due to having high mortality and morbidity rates (2). Among the classic clinical findings of immunodeficiencies are infections poorly responsive to treatment or having complications besides being susceptible to infections of low virulence microorganisms. PIDs can also occur with autoimmunity, autoinflammatory or hemophagocytosis syndromes (3).

Congenital diseases usually start in early childhood and lead to morbidity and mortality. For this reason, early diagnosis of these diseases may be life-saving and increase the quality of life in the long term. Considering PIDs more frequently in the differential diagnosis and evaluating patients immunologically makes it possible for these patients to be diagnosed at an early stage to reach early treatment opportunities and protective measures (2).

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It is estimated that over 300 genetic disorders have an effect on the immune system. These diseases, especially the autosomal recessive ones, are more commonly seen in Turkey due to higher the prevalence of consanguineous marriages (4).

PIDs are categorized on the basis of their disruptive mechanisms. The most common immunodeficiencies in these five groups are humoral immunodeficiencies (50-60%), predominantly selective IG A deficiencies. This is followed by cellular deficiencies (10-15%), combined deficiencies (15-30%), phagocyte defects (10-15%) and complex defects (1-3%) (5). Humoral deficiencies are also the most common in Turkey (2).

The treatment in primary immunodeficiency: As a result of disrupted immune system function, frequent and multiple infections, autoimmune disorders and malignancies are commonly seen in patients with PIDs. Untreated or under-treated PID can lead to life-threatening infections, chronic organ damage, or a marked reduction in life expectancy (6). However, early diagnosis and replacement therapy allow long and better life conditions for patients. The approaches used in the treatment of patients with PIDs include prophylactic treatments which significantly reduce the risk of infections. IVIG therapy is used to prevent recurrent infections and limits the progression of complications. Furthermore, if this therapy is given early and appropriately, it prevents tissue damage from infections and inflammations. It generally takes 4 to 6 hours and is administered at monthly intervals in hospital (7).

Individuals with PIDs may frequently experience infections secondary to their disease affecting their physical and psychological well-being (8). The prognosis of PIDs varies from benign conditions, such as respiratory tract infections, to complex conditions, such as malignancies with lethal outcome (9). Some patients need lifelong IVIG treatment and/or frequent courses of antibiotics as a treatment and/or prophylaxis. Especially patients with PIDs have a higher incidence of autoimmune diseases and experience long-term complications of infections and/or treatment (10). As a result of advances in PIDs treatment, mainly related to IVIG therapy, morbidity and mortality rates have improved remarkably in recent years, and the majority of children with PIDs can survive into adulthood (7).

Although long-term IVIG infusion is shown to be effective, there are some disadvantages. Firstly, the most common side effects of IVIG infusion in the first 30 minutes are lower back pain, nausea, chills, low body temperature, and vomiting. In the later hours of the infusion, headache, myalgia and syncope can be seen. Secondly, IVIG requires patients to be treated in inpatient clinics, regular visits to hospital resulting in a loss of school and family time, and a high cost of health care. That is to say, although IVIG treatment has enhanced the life-expectancy of such a chronical disease, it has caused secondary problems for children with PIDs and their families (11).

Conclusion

Psychological impact and quality of life in primer immunodeficiencies: Taking into account the studies on children and adolescents with PID, it is seen that psychosocial characteristics and HR-QOL are related and have been evaluated together in the literature (Table I). It is noteworthy that these studies are usually cross-sectional and descriptive (10,12-14). Except for a few studies, psychiatric examinations of children and adolescents have not been performed and they have been generally evaluated with scales (15-19). One of these studies that was conducted in Turkey evaluated PIDs with psychiatric interviews and a modified version of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version. The study was carried out on patients with JIA and healthy children selected as a control group. Although patients with PIDs were differentiated from healthy controls in terms of psychiatric diagnoses, there were no differences between JIA and PID (20). Similarly, the QOL scores were found to be similar in both JIA and PID, and both groups of chronic disease had lower scores compared to healthy controls (21).

Although healthy children frequently have been taken as a control group, in some studies, children with various chronic diseases, mostly children with juvenile rheumatoid arthritis (JRA) have been selected as a control group because healthy children as a control group would not be sufficient to determine the differences between PIDs and other chronic diseases (7,14,22).

Some studies have suggested that children with chronical disorders may be at risk of school absenteeism, participation in school and sporting activities, and the development of behavior and emotional disorders (23,24). Taking into consideration such challenges and the chronic nature of the disease, it is expected that the psychosocial development of children with PIDs, such as self-perception, self-esteem, interpersonal relationships and social activities are affected as with other pediatric situations and adults with PIDs (10,25,26). Consequently, there is evidence that patients with PIDs should be evaluated not only with simple clinical/disease parameters, but

Table I. The general characteristics of the studies related to the subject are given

Author, year	Methodology	Patients	Controls	Instrument	Findings
Cole, 2013 (18) but require lifelong treatment with immunoglobulin replacement. Some carry risks of inflammatory complications even with optimal treatment. Quality of life (QoL)	Cross sectional quantitative survey	47 Chronic Granulomatous Disease	0	PedsQL (HR-QOL), strength and difficulties questionnaire (emotional and behavioral difficulties)	Parent and self-reported QoL for non-transplanted children were significantly lower than HC. Parents reported increased emotional difficulties compared to published norms. PedsQL and SDQ scores for transplanted children were not significantly different from healthy norms
Kuburovic et al. (14)	Cross sectional quantitative survey	25 Mixed PID	139 50 JIA[1] 89 HC[2]	PedsQL[3] (HR-QOL), SCARED[4] (anxiety), Mood and Feeling Questionnaire (depression)	Children with PIDs had significantly lower HRQOL total score compared to children with JIA and healthy children on child rated and parent-rated assessments. Specifically, they had significantly lower emotional functioning compared to children with JIA, and social functioning compared to both children with JIA and healthy children. Only parent-rated school functioning scores were significantly lower among children with PIDs. For parent-rated assessments, six of 25 children with PIDs reported significant anxiety symptoms, and five had significant depressive symptoms
Zebracki et al. (7),	Cross sectional quantitative survey	36 Mixed PID	63 36 JIA 36 HC	CHQ-PF50[5] (HR-QOL)	Compared with children with JIA, children with PIDs were similar in many aspects of their HR-QOL. However, parents of children with PIDs reported greater limitations in their personal time, poorer general health of their children, greater limitations in their children's physical functioning and family activities, and less bodily pain than children with JIA. In contrast, children with PIDs scored lower on most HR-QOL domains compared with HC
Soresina et al. (22),	Cross-sectional quantitative case control survey	25 X-linked agammaglobulinemia	311 80 HC 231 rheumatic disease	PedsQL (HR-QOL)	The agammaglobulinemia subjects perceived a lower global quality of life than the healthy subjects, but significantly higher than the rheumatic diseases controls.
Abolhassani et al. (15),	Cross sectional quantitative survey	26 Mixed PID	0	Causes of anxiety (study specific)	Most significant causes of anxiety include long duration of disease, lack of cure, and side effects and complications from treatment
Mozaffari et al. (12),	Cross-sectional quantitative case control survey	50 Mixed PID	100 HC	PedsQL (HR-QOL)	Patients with PID had great limitations in physical functioning and psychological well-being compared with children without a chronic health condition. Patients had lower QoL scores in all age groups compared with normal sample. Long duration of disease significantly correlated with low psychological score

Table I. Continued

Author, year	Methodology	Patients	Controls	Instrument	Findings
Titman et al. (16),	Cross sectional quantitative survey	43 Primary antibody deficiency syndromes	0	PedsQL SDQ[6] Illness severity scale (study specific)	Higher rates of psychological difficulties, particularly emotional and peer-relationship difficulties were found in children with PAD when compared with healthy controls. QoL was poorer than in HC, and also worse than in children affected by diabetes mellitus. Variations in QoL and the degree of psychological difficulties were found between specific diagnostic groups, with children affected by transient hypogammaglobulinemia of infancy being amongst those with the lowest scores of QoL
Stephenson et al. (19)	Cross sectional quantitative survey	76 22q11 deletion syndrome/ DiGeorge syndrome	55 HC	MAS[7] CDI[8] BASC[9]	The 22q11.2DS youth were more likely to be in anxiety, depressed or comorbid clusters than the typically developing youth. Children with 22q11.2DS comorbid for anxiety and depression exhibited the worst functional outcomes
Kayan Ocakoğlu et al. (20,21)	Cross-sectional quantitative case control survey	48 Mixed PIDs on IVIG treatment	64 34 JIA 30 HC	K-SADS-PL[11] (psychiatric examination) PedsQL CDI SCARED	70.45% of the children with PIDs, 62.5% of those with JIA and 23.3% of HC have a psychiatric problem. There is no statistically difference between PIDs and JIA. The most frequent psychiatric diagnoses in PIDs and JIA groups were depressive disorders, anxiety disorders and disruptive behavior disorders. PIDs and JIA groups had lower quality of life scores than health controls. PIDs and JIA groups were not found statistically different from each other

[1]Juvenile idiopathic arthritis, [2]Healthy Control, [3]Pediatric Quality of Life Inventory, [4]Screen for Child Anxiety Related Emotional Disorders, [5]Child Health Questionnaire, parent form, [6]Strengths and Difficulties Questionnaire, [7]Multidimensional Anxiety Scale for Children, [8]Children's Depression Inventory, [9]Behavioral Assessment Scales for Children, [10]The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, *(Kayan Ocakoğlu B. et al., İntravenöz İmmunglobulin Tedavisi Alan Ağır İmmün Yetmezlikli Hastalarda Ruhsal Durum Özellikleri, Yaşam Kalitesi ve Aile Özellikleri Ocak, 2016'. Yayınlanmamış Uzmanlık Tezi. Ege Üniversitesi)
PID: Primary immunodeficiency, HR-QOL: Health quality of life, IVIG: Intravenous immunoglobulin,

also with patient-reported prognostic measures, how the child copes with his/her disease, and how the family perceives the effect of the disease across different life domains (14). Therefore, HR-QOL should be a part of the multidimensional evaluation and treatment in children with PIDs, because it provides direct information about disease and treatment. However, HR-QOL of children with PIDs have remained largely unstudied.

Whereas Kuburovic et al. (14) found that HR-QOL scores of children with JRA were higher than PIDs, the studies conducted by Zebracki et al. (7) stated that the quality of life scores in patients with PIDs were higher than those with rheumatologic disease (20).

Both the requirements of treatment and the complications of PID increase the burden on the family and the patient. However, since PIDs are seen rarely and contained a huge number of different disorders, the literature is not sufficient to determine emotional and behavioral problems in this group (14,15,17). The available research highlights those children with PIDs who have serious problems in different areas of life such as discontinuity in the educational system, limited participation in social and sport activities, and anxiety and depression symptoms (14). Other studies have also found that these children have emotional problems, difficulties in relationships with their friends and

hyperactivity (16,27). In addition, social and attention problems are found as the most common difficulties in children with 22q.11 deletion syndrome (17). As a result, these findings demonstrate that patients with PIDs tend to develop psychosocial problems.

With the advancements in treatment options in chronic diseases, the importance of psychosocial adjustment, quality of life and rehabilitation services has begun to rise. An appropriate care for these patients requires a team of psychiatrists, psychologists, social workers, and teachers working together to handle all the aspects that should be considered to improve these patients' life quality. Consequently, psychosocial disorders as well as psychiatric symptoms have increased in children with PIDs. For this reason, psychosocial problems should be taken into consideration and, if needed, a psychiatric assessment should be made.

As seen in this review, published literature in this area is very limited. Multi-centered, longitudinal research is needed to evaluate and monitor the child's emotional, social, family, and school functioning. The treatment of comorbid psychiatric illnesses and appropriate psychosocial interventions will help to improve the functioning and the quality of life in children with PIDs.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.E., N.E.K., G.A., Design: S.E., B.K.O., Data Collection or Processing: B.K.O., Analysis or Interpretation: S.E., N.E.K., G.A., B.K.O., Literature Search: B.K.O., Writing: B.K.O.

Conflict of Interest: I and my partners have had no potential conflict of interest.

Financial Disclosure: I and my partners have had no relevant financial interests.

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Original Article

Do We Have to Obtain Rebound Bilirubin Levels and What is the Optimal Time?

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ABSTRACT

Aim: We aimed to determine the frequency of rebound hyperbilirubinemia (RHB) needing treatment and therefrom, to clarify the clinical importance of routinely checking serum total bilirubin (STB) levels after the cessation of phototherapy and to define an optimal time to check STB levels for the detection of RHB.

Materials and Methods: Term and late preterm babies who received phototherapy were included in this study. The demographic and clinical features, time of onset of jaundice, phototherapy time and results to determine the etiology of jaundice were recorded for all babies. Serum "rebound" bilirubin measurements were performed two times at 12 and at 24 hours after the cessation of phototherapy. The re-initiation of phototherapy according to the 12th and 24th hour STB levels was accepted as "early rebound" and "late rebound", respectively. IBM SPSS 22 was used for statistical analyses.

Results: Data was available for 110 infants. The rebound rate requiring phototherapy was 9.1% (n=10) and all had a risk factor. Most of the babies (9/10) rebounded at the 12th hour after the termination of phototherapy. Hemolysis and prematurity were found to be statistically significant for RHB (p=0.008; p=0.048).

Conclusion: Post-phototherapy bilirubin follow-up may be incorporated using a combined approach of individualization, evaluation of risk factors, and application of common sense before discharge. Our study showed that STB levels could be measured after the cessation of phototherapy, especially in patients with a risk factor, at the 12th hour before discharge. Randomized controlled studies with larger sample sizes are still needed for definitive recommendations.

Keywords: Hyperbilirubinemia, newborn, phototherapy, rebound bilirubin

Introduction

Phototherapy is the most effective method to lower serum total bilirubin (STB) levels in newborns with hyperbilirubinemia. A sudden increase in bilirubin levels, depending on the cause of hyperbilirubinemia, may be observed after the cessation of phototherapy (1,2). This situation brings forth a discussion on whether to re-check bilirubin levels after phototherapy has been stopped. As the results of the studies about this dilemma are assessed, there is still no consensus about this subject (2-11). The American Academy of Pediatrics (AAP) recommends to measure bilirubin levels within 24 hours after cessation of phototherapy only in cases caused by hemolysis or in those who needed phototherapy in the first three to four days of life (2).

The incidence of hyperbilirubinemia varies with ethnicity and geography and our country is located in this high-risk area (12). Sarici et al. (13) reported the incidence of hyperbilirubinemia as 10.5% for term and 25.3% for near-

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term infants in Turkey, which is much higher than the incidence reported from European countries and the United States (14,15). Thus, guidelines developed from those countries may not apply to our country.

The primary aim of our study is to determine the frequency of rebound hyperbilirubinemia (RHB) needing treatment and therefrom, to clarify the clinical importance of routinely checking STB levels after the cessation phototherapy and to define an optimal time to check STB levels for the detection of RHB.

Materials and Methods

Term and late-preterm babies, who received phototherapy due to hyperbilirubinemia in the Neonatal Intensive Care Unit of Marmara University Medical Faculty between January 2015 and December 2015, were enrolled in this prospective study. Newborns with congenital anomalies or any disease accompanying hyperbilirubinemia (sepsis, pneumonia, perinatal asphyxia, etc.) were excluded. The study was approved by the Ethics Committee of Marmara University Medical Faculty (approval number: 09.2016.231). Informed consent was obtained from the parents of the infants. Infants with RHB and without RHB were compared in terms of their demographic and clinical characteristics, at the 12th and 24th hour STB levels after inpatient phototherapy, time of onset of jaundice, phototherapy period and in terms of the results of all tests performed to determine etiology (blood type of the mother and the baby, reticulocyte count, direct Coombs test, peripheral smear, glucose-6 phosphate dehydrogenase enzyme levels, thyroid function tests, reducing substances in urine). Late preterm infants are defined as those born at 34-0/7 to 36-6/7 weeks' gestational age (16). Phototherapy and exchange transfusion decisions were made according to the AAP's guidelines and high-density light-emitting diode phototherapy was initiated on all patients (2).

The "early treatment group" consisted of the infants who received phototherapy before the 72nd hour of life and the "late treatment group" consisted of the infants who received phototherapy after the 72nd hour of life. Phototherapy was discontinued when STB levels dropped below 13-14 mg/dL in the late treatment group and 3 mg/dL or more below the phototherapy threshold level according to postnatal age in the early treatment group (17,18). RHB was defined as the return of TSB to the phototherapy threshold within 72 hours of phototherapy. Serum rebound bilirubin levels were measured twice; at the 12th hour and 24th hour after the cessation of phototherapy. The need for the re-initiation of phototherapy at the 12th or 24th hour STB levels were accepted as "early rebound" and "late rebound", respectively.

Statistical Analysis

The distribution of the data was determined by Kolmogorov-Smirnov test. Independent samples t-test and Mann-Whitney U tests were used to compare normally-distributed (parametric) and not normally-distributed (non-parametric) data, respectively. Results were expressed as "mean ± standard deviation" for parametric data and "median (minimum-maximum)" for non-parametric data. Fisher's exact test and chi-square test was performed for non-parametric variables between groups where appropriate. IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, ABD) was used for statistical analyses. For all statistical analysis, p values <0.05 were considered significant.

Results

One hundred and ten infants who were treated due to hyperbilirubinemia were included in the present study. Ninety-one infants (83%) were term and 19 (17%) were late-preterm with mean birth weights of 3.273±462 g and 2.513±534 g; respectively. When the cases were assessed for jaundice etiologies, the number of cases with hemolysis due to ABO or Rh incompatibility, excessive weight loss (loss of ≥10% of birth weight) and prematurity were 17 (15%), 20 (18%) and 19 (17%), respectively. No etiological factor could be detected in 59 (53%) infants (Table I). Six babies received exchange transfusion (n=1, Rh incompatibilities and n=5, ABO incompatibilities).

The rebound rate was 9.1% (10/110). There were no significantly statistically differences between rebound and non-rebound groups in terms of birth weight, gestational weeks, gender, prematurity, delivery route, history of jaundice in sibling or excessive weight loss after birth (Table 2). The presence of hemolysis and early phototherapy were found to be statistically significant between the groups, (p=0.008, p=0.034) respectively (Table II). When the two groups were compared according to the rebound frequency, the number of RHB cases in the early phototherapy group was significantly higher (8/10; p<0.001). Nine of the 10 infants (90%) with RHB were detected at testing of STB levels at the 12th hour. Hemolysis and being late-preterm were found to be statistically significant higher for those babies with RHB (p=0.008, p=0.048) (Table II). The clinical features of those infants with RHB are shown in Table III.

Discussion

The necessity of rebound STB measurements in newborn jaundice cases is controversial (2,5,9,10). In our research, the RHB rate was calculated at 9.1%. In recent studies, the occurrence of RHB was reported to be between 0.7%-19.6% (7,19). The wide range of the reported frequencies could be

Table I. Demographic and clinical features of the study population

	Study group (n=110)
Male, n (%)	68 (61.81)
Late preterm, n (%)	19 (17.27)
Birth weight, (g)	
Term/late-preterm	3.273±462/2.513±534
Cesarean delivery, n (%)	53 (48.18)
Family history of jaundice, n (%)	11 (10.00)
Etiology, n (%)	
Hemolysis, n (%)	17 (15.45)
Late-preterm, n (%)	19 (17.27)
Excessive weight loss, n (%)	20 (18.18)
No etiological factor, n (%)	59 (53.63)
Exchange transfusion, n (%)	6 (5.45)
Early-treated hyperbilirubinemia, n (%)	56 (50.90)
Time of initiation of phototherapy (hours)	97 (3-192)
Serum total bilirubin level at the beginning of phototherapy (mg/dL)	17.63±5.52
STB level at the end of phototherapy (mg/dL)	11.33±1.87
Duration of phototherapy (hours)	24 (8-72)
12th hour rebound STB (mg/dL)	11.53±2.42
24th hour rebound STB (mg/dL)	11.38±2.30

Data of variables are expressed as mean ± standard deviation or median (minimum-maximum) or absolute number and its frequencies n (%); STB: Serum total bilirubin

Table II. The comparison of the demographic and clinical features of the ınfants with and without rebound hyperbilirubinemia

	Rebound group (n=10)	**No-rebound group (n=100)**	**p value**
Birth weight (g)	3045±536	3201±508	0.358
Gestational weeks	37.61±2.01	38.32±1.43	0.144
Male, n (%)	5 (50.00)	63 (63.00)	0.501
Term, n (%)	6 (60.00)	85 (85.00)	-
Late-preterm, n (%)	4 (40.00)	15 (15.00)	0.048
Vaginal delivery, n (%)	5 (50.00)	52 (52.00)	1.000
Hemolysis, n (%)	5 (50.00)	12 (12.00)	0.008
Weight loss of ≥10% of birth weight, n (%)	3 (30.00)	17 (17.00)	0.385
No etiological factor, n (%)	0 (0)	59 (59)	<0.001
Early phototherapy, n (%)	8 (80.00)	48 (48.00)	0.034
STB level at the beginning of phototherapy (mg/dL)	19.48±7.29	17.70±5.11	0.315
STB level at the end of phototherapy (mg/dL)	11.24±2.13	11.10±2.01	0.822
12th - hour rebound, n (%)	9 (90)	0	<0.001
24th - hour rebound, n (%)	1 (10)	0	0.001

Data of variables are expressed as mean ± standard deviation (range) or median (minimum-maximum) or absolute number and its frequencies n (%); STB: Serum total bilirubin

Table III. Clinical features of the patients with rebound hyperbilirubinemia

	Early treatment group	**Late treatment group**	**12th rebound**	**24th rebound**	**Hemolysis**	**Excessive weight loss**	**Late preterm infant**
Case 1	+	-	+	-	-	+	+
Case 2	+	-	+	-	-	+	+
Case 3	-	+	+	-	-	+	-
Case 4	+	-	+	-	+	-	-
Case 5	+	-	+	-	-	-	+
Case 6	+	-	+	-	+	-	-
Case 7	-	+	-	+	-	-	+
Case 8	+	-	+	-	+	-	-
Case 9	+	-	+	-	+	-	-
Case 10	+	-	+	-	+	-	-

explained by the differences in the definitions of RHB, sample sizes, jaundice etiologies and risk factors (3-5,8-10,19). Chang at al. (18) reported a 4.6% rate of RHB; whereas, Barak et al. (19) found a higher rate (19.6%) and this increased rate was attributed to the discontinuation of phototherapy in cases with higher STB levels (18). In another study, the rebound frequency was recorded as 0.7% and the exclusion of risk groups was held accountable for this low prevalence (7). The RHB rate has been reported as 5.1% in Turkey, which is lower than our study (5). The lower mean bilirubin levels at which to start and stop phototherapy and the lower number of cases with blood group incompatibility and hemolysis, compared to our study group, might explain the difference.

Waiting for a rebound STB level measurement prolongs hospital stay; therefore, AAP recommends rebound measurements only in babies with certain risk factors (2). In one study, gestational and postnatal age, and STB levels at the time of cessation of phototherapy were reported as the three most important risk factors for RHB (18). It has been reported in many studies that late-preterm infants, who have the highest risk for newborn jaundice, also have a high "rebound" rate (5,10). In our study, RHB developed in 4 of the 19 late-preterm babies (21%), which was higher compared to term babies (6%). Two of these "rebounding" late preterm babies were in the early treatment group, both having excessive weight loss. Kaplan et al. (9) reported that the majority of their rebound cases were late-preterm infants and all had hemolysis.

Those infants who received phototherapy before their initial discharge after birth (in the first three days of life) were reported to have a higher rate of being hospitalized for a second course of phototherapy (7,9). In our study, 9 of the infants with RHB (90%) were in the early treatment group. Kaplan et al. (9) stated that neonates with hemolysis, late prematurity and onset of phototherapy within 72 hours should be regarded as high risk. In our study, all of the cases with RHB had at least one of these risk factors (hemolysis, excessive weight loss or prematurity).

In the literature, the measurements of rebound STB levels were performed between 8 to 36 hours after the cessation of phototherapy (3,5,7,9,10). AAP recommends the measurement of rebound STB levels within 24 hours of phototherapy cessation, without specifying an exact period (2). "The National Institute for Health and Clinical Excellence", and the "Canadian Paediatric Society Fetus and Newborn Committee" do not refer to the evaluation of rebound STB levels in their guidelines (17,20). In our study, rebound STB levels were measured in two-time sections (12th hour and 24th hour) and it was detected that most of the "rebounds" had developed by the 12th hour measurement and all of them had risk factors. Nine of 10 infants with RHB were detected with the measurement of 12th hour rebound STB levels, thus enabling an early initiation of treatment. Even though AAP recommends outpatient evaluation for rebound STB level, in our country, as we are concerned about the lack of follow-up, we suggest checking rebound STB levels at the 12th hour, before discharge.

Conclusion

Post-phototherapy bilirubin follow-up may be incorporated using a combined approach of individualization, evaluation of risk factors, and application of common sense before discharge. Our study showed that STB levels can be measured after the cessation of phototherapy, especially in patients with a risk factor, at the 12th hour before discharge. Randomized controlled studies with larger sample sizes are still needed for definitive recommendations.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Marmara University Medical Faculty (approval number: 09.2016.231).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Ö., A.M., Z.A.Ü., Concept: H.Ö., H.S.B., Design: H.Ö., H.S.B., Data Collection or Processing: H.Ö., A.M., Z.A.Ü., Analysis or Interpretation: H.Ö., H.S.B., E.Ö., Literature Search: H.Ö., H.S.B., Writing: H.Ö., H.S.B.

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Original Article

Effect of Short-term Probiotic Yogurt Consumption on Caries Risk Factors in Infants

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ABSTRACT

Aim: We aimed to evaluate the effect of short-term probiotic yogurt consumption on pH, buffering capacity, and *Streptococcus Mutans*, Lactobacilli and secretory immunoglobulin A (sIgA) levels in saliva of 6-8 months old healthy infants.

Materials and Methods: Twenty healthy infants and their mothers were enrolled in the study. They were randomly allocated into two groups as study and control. In the study group, probiotic yogurt (*Bifidobacterium Longum* BB536, *Bifidobacterium Bifidum* Bb12, *Lactobacillus Rhamnosus* HN001) was given to infants for 3 weeks whereas, in the control group, home-made yogurt was consumed. A dental saliva pH-Indicator strip (GC, Japan) was used for salivary pH measurements. Buffering capacity was determined using CRT buffer (Ivoclar Vivadent, Liechtenstein). The counts of salivary mutans streptococci and lactobacilli were evaluated using CRT bacteria (Ivoclar Vivadent,Liechtenstein). ELISA was used for sIgA. Scores at baseline and three weeks after were statistically evaluated by Wilcoxon test using the IBM SPSS 20.0 program.

Results: Salivary pH, IgA, *S. mutans* and lactobacilli values showed no significant change after 3 weeks of probiotic yogurt consumption, however there was a statistically significant increase in the buffering capacity of saliva (p=0.04).

Conclusion: Short-term probiotic yogurt intervention in infants during the early stages of life might have benefits for oral health. Further studies with both short- and long-term use of probiotics must be implemented in infants to confirm the results and see the effects on other caries risk factors.

Keywords: Infants, short term probiotics, caries risk factors

Introduction

In today's market, the consumption of probiotic dairy products namely yogurt, cheese or ice cream is increasing with the media drawing attention to their promotive effects on general health (1,2). The beneficial effect of probiotic bacteria on intestinal microbial balance and immunity is still a matter of interest for researchers (3-5). These products come into contact with the oral cavity and so their immune overall effect may possibly cause an alteration of oral microbiota as well. Today, many studies have demonstrated not only their general health benefits but also their oral health benefits (2,6-15).

In recent decades, dental researchers have investigated probiotic-containing products as a preventive measure in dentistry. From an oral health perspective, these studies have been mainly conducted on children and young adults with various different probiotic containing foods, drinks and supplements (2,6-15). Today, probiotic is also integrated into infant nutrition such as infant formulas. They are on the

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shelves of markets aiming to compensate for human breast milk. In addition, natural probiotic containing baby foods like yogurts, gruel and cereals are gaining in popularity by promising good health from the first years of life (16).

Particularly, the first year of life plays a vital role in the formation of oral microbiota which may affect the probability of caries risk of the child. Lately, researchers have examined initial microbial colonization and salivary components of healthy full-term infants (17-19). Thus, researches have conducted studies on infants to see the effect of early intervention of probiotics on caries formation in the long-term. One such study by Stensson et al. (20) showed that daily supplementation of probiotics from birth and during the first year of life results in decreased caries prevalence and improved periodontal health even after 9 years. The other interesting finding was that children receiving probiotics tend to have a higher secretory immunoglobulin A (sIgA) level in the long term, however, there was no statistically significant difference noted.

Although the outcomes of studies present variations due to different strains of probiotics use, and methodologies, the idea that the consumption of probiotics can alter the oral microbiota and salivary components remains worthy of investigation.

To our knowledge, there is very limited data that demonstrates the effect of probiotic consumption on caries risk factors and salivary antibodies in infants. Hence, the present study aims to evaluate the effect of short-term probiotic yogurt consumption on pH, buffering capacity, and *Streptococcus mutans*, Lactobacilli and sIgA levels in the saliva of 6-8 month-old healthy infants.

Materials and Methods

This case control study was a part of short-term research that was supported by Ege University Scientific Research Committee, performed at the Department of Pediatric Dentistry and Healthy Baby Clinic of the Faculty of Medicine. Ethical approval was given by the Research Ethics Committee Faculty of Medicine at Ege University (approval number: 12-8/24). Healthy 6-8 month-old infants who were referred to the healthy baby clinic for their periodic examination and their mothers were recruited for the study. Prior to the commencement of the investigation, informed consent for infants was provided by their parents. The inclusion criteria were full term born and caesarean section infants with no systemic disease or immunological deficiency whereas exclusion criteria for both infants and mothers were; use of antibiotics within 1 month prior to the study.

At the start of the study, 27 babies and their mothers were recruited. The baby-mother pairs were randomly allocated into two groups as study and control. In the study group, probiotic yogurt (*Bifidobacterium Longum* BB536, *Bifidobacterium Bifidum* Bb12, *Lactobacillus Rhamnosus* HN001) was given to infants as a morning snack for 3 weeks, whereas, in the control group, home-made yogurt was consumed. A consumption chart with a 3-week duration was given in order to ensure that the babies were consuming the assigned yogurts at the proper amounts. Unfortunately, 7 of the baby-mother pairs were taken out of the study due to antibiotic use, irregular yogurt consumption or failure to follow up. As a result, findings for twenty 6-8 month-old infants and their mothers were reported.

Prior to dental examination, the head diameters, weight and height of the infants were measured and reported as 40.87±1.27 cm, 7.69±0.81 kg and 64.56±2.73 cm respectively. Two trained examiners conducted all the clinical oral examinations. At baseline, the infants were examined using a sterile dental mirror and artificial light in the knee-to-knee position.

The mothers and infants were asked not to receive food or drink for 30 minutes prior to the first visit. An unstimulated saliva sample was collected from each child with a sterile cotton roll. The cotton roll was kept in the mouth of the infant until it was completely wet. Then these rolls were put in a small polypropylene tube with holes at the bottom. The small tube was then placed in a larger sterile polypropylene tube with a conical bottom. Both of the tubes, one within the other, were centrifuged to drain the saliva through the cotton roll. Subsequently, 1 mL of the saliva sample was sent to a laboratory within half an hour and stored at -92 °C until investigated for IgA.

The remaining saliva samples were used immediately to obtain the baseline buffering capacity, pH and bacterial counts using chair-side tests. The same procedures were conducted at third week recall.

In the mothers' group, un-stimulated saliva samples were collected for pH measurements. The mothers were asked to sit still for 5 minutes and then asked to lean their head downward and split the accumulated saliva into sterile tubes for 5 minutes. For stimulated saliva, mothers were asked to chew a paraffin block and after swallowing the first accumulated saliva, they were asked to spit as they chewed for 5 minutes. 1 mL of this stimulated saliva was sent to the laboratory for IgA evaluation.

A pH-Indicator strip (GC, Japan) was used for salivary pH measurements. Unstimulated saliva samples from the mothers and infants were examined and the color changes on the pH strips were recorded immediately.

Buffering capacity was assessed using CRT buffer (Ivoclar Vivadent AG, Schaan, Principality of Liechtenstein).

According to the manufacturer's instructions, stimulated samples were used. This was possible for the mothers but for the infants unstimulated saliva was used. The test strip was placed on a stable, absorbent paper with the yellow test field facing upwards. The entire yellow test field was wetted with saliva using a pipette to prevent the formation of bubbles. To determine the buffer capacity of saliva, the color of the test field was compared with the color samples after exactly 5 minutes of reaction time. A blue color indicated high, while green showed medium and finally yellow demonstrated low buffer capacity of saliva.

The CRT bacteria (Ivoclar Vivadent AG Schaan, Principality of Liechtenstein) was used in accordance with the manufacturer's instructions to determine the counts of salivary mutans streptococci and lactobacilli. Stimulated saliva was collected from the mothers but un-stimulated saliva of the babies was used for bacterial evaluation.

Briefly, saliva samples both from the infants and mothers were inoculated on a dip-slide with selective agar media and it was cultivated at 37 °C for 48 hours. The colony forming unit density for both salivary mutans streptococci and lactobacilli was compared against a chart provided by the manufacturer. Samples were categorized as group 1, 2, 3 and 4 (Figure 1 and 2).

1 mL saliva samples were kept in -92 °C. After all the sampling was done for infants and their mothers, both for baseline and third week recall, ELISA (IBL International GMBH, Hamburg, Germany) was used for the measurement of salivary sIgA.

Statistical Analysis

Baseline and three-weeks-after scores were statistically analyzed by the Wilcoxon test using IBM SPSS 20.0 program.

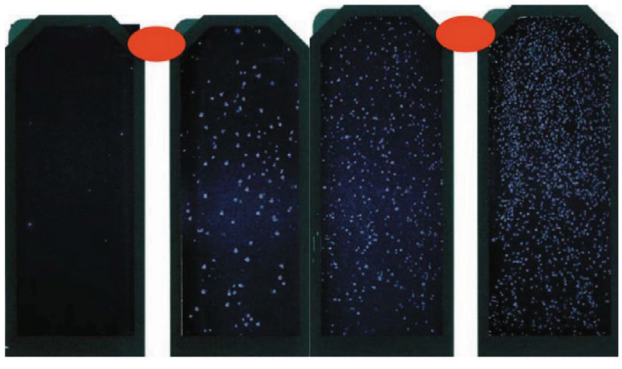

Figure 1. Pictures of colony-forming units show the differences in *Streptococcus mutans* between group 1, 2, 3, 4 as score 1, 2, 3, 4

Results

The mothers' sIgA, pH values, buffering capacity, *S. mutans* and Lactobacilli scores are given in Tables I, II and III. There was no statistically significant difference between the study and the control group of the mothers (p>0.05).

The infants' salivary IgA and pH values did not show any statistically significant difference after 3 weeks in both groups (Tables IV and V) (p>0.05). *S. mutans* and Lactobacilli score changes showed no statistically significant difference (Table VI). However, there was a statistically significant increase in the buffering capacity of saliva after 3 weeks of probiotic yogurt consumption (p=0.04) (Table VI).

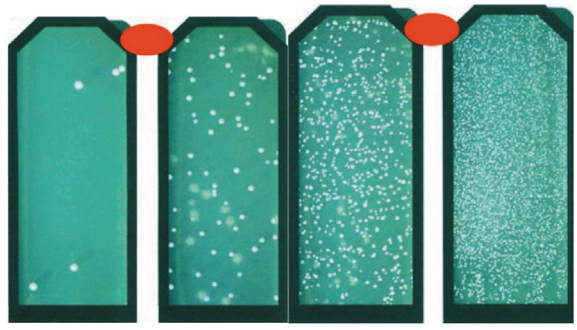

Figure 2. Pictures of colony-forming units show the differences in lactobacilli between group 1, 2, 3, 4 as score 1, 2, 3, 4

Table I. pH and sIgA values of mothers

	pH	sIgA
	pH ± Standard deviation	**µg/mL ± Standard deviation**
Mothers in control group (n)	7.27±0.52	32.24±19.77
Mothers in study group (n)	6.87±0.35	46.88±22.98

sIgA: Secretory immunoglobulin A

Table II. Buffering capacity of saliva of mothers

Saliva buffer capacity	Low	Medium	High
Mothers in control group (n)	0	0	10
Mothers in study group (n)	0	4	6

Table III. Scores of *Streptococcus mutans* and lactobacilli of mothers

	***Streptococcus mutans* scores**				**Lactobacilli scores**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Mothers in control group (n)	2	2	5	1	2	3	3	2
Mothers in study group (n)	0	5	3	2	2	0	3	5

Table IV. Infants' sIgA values at baseline and after 3 weeks

	Baseline IgA levels (µg/mL)	3 weeks after IgA levels (µg/mL)
Control group, (p>0.05)	13.11±14.98	14.05±19.20
Study group, (p>0.05)	15.49±17.95	18.87±35.24

sIgA: Secretory immunoglobulin A

Table V. Infants' pH values at baseline and after 3 weeks

	Baseline pH ± SD	3 weeks after pH ± SD
Infants in control group (n), (p>0.05)	7.11±0.53	7.23±0.77
Infants in study group (n), (p>0.05)	6.98±0.57	7.08±0.68

SD: Standard deviation

Table VI. Infants' *Streptococcus mutans*, Lactobacilli and buffering capacity changes after 3 weeks of probiotic yogurt consumption

	Study group (n=11)	Control group (n=9)
Streptococcus mutans, (p>0.05)	3[a] 2[b] 6[c]	1[a] 0[b] 8[c]
Lactobacilli, (p>0.05)	2[a] 3[b] 6[c]	0[a] 0[b] 9[c]
Buffering capacity of saliva, (p=0.04)	0[a] 4[b] 7[c]	1[a] 4[b] 4[c]

[a]: Values after 3 weeks < baseline values, [b]: Values after 3 weeks > baseline values, [c]: Values after 3 weeks=baseline values

Discussion

It is well known that the dynamics of immature microbiota colonization of infants can be changed with diet to the benefit of oral health (21). During the last decades, an intake of probiotics has been suggested to prevent dental caries (22). However, oral probiotic bacteria colonization in the oral cavity were defined as temporary. However, so far, studies have been carried out on subjects with mature microbiota. Currently, infants with immature oral microbiota have become the center of attention since early probiotic intervention may have a prolonged benefit on oral health (7,23).

The uniqueness of our study is that full term infants with immature oral microbiota were subjected to a short-term administration of probiotics. Our hypothesis was short-term early exposure to probiotics may alter oral microbiota and eliminate eliminate caries risk factors in infants. The duration of the intake of probiotics in studies varies however, both short- and long-term use have been reported to affect oral health and reduce caries risk factors (2,6-15). Hence, the present study was designed to see the short-term effect of probiotics.

Breast milk is the most important probiotic sources which promotes colonization with lactobacilli more effectively than formula feeding (24). In the present study, all included infants were breastfed. During weaning, a probiotic existence in the gut and oral cavity is maintained through products like yogurt which is effective, practical and acceptable among infants (25). Hence, probiotic containing yogurt was consumed in our study. In this product, there are triple probiotic cultures (*B. Longum* BB536, *B. Bifidum* Bb12, *L. Rhamnosus* HN001) and prebiotic fiber feeding these cultures, all of which help to regulate the digestive system and support the immune system of the baby (25). As for the oral health benefit, a study by Lin et al. (26) showed that short-term intake of yogurt in children resulted in a significant rise in pH value and cariostatic effects on biofilm acidogenicity. In general, the underlying mechanism of action is considered the same as those described for the intestine. Oral colonization competition with oral pathogens for adhesion sites, nutrients and enhancing the host immune responses can be counted as the mechanism of action. The bactericidal and bacteriostatic characteristics of probiotics derive from their production of antimicrobial agents like organic acids, hydrogen peroxide, antifungal compounds (23).

The lack of a difference between baseline and 3 weeks after probiotic intake can be due to the short duration of use. Our findings are not in line with several studies that suggest probiotic lactobacilli or Bifidobacterial decrease salivary mutans streptococci (7). Nevertheless, unchanged scores for salivary mutans streptococci have also been reported (10). Likewise, Hasslöf et al. (27) reported that early intervention with probiotic had no long-term effect on the caries experience.

Salivary oral immune response after probiotic use was also investigated by researchers. However, total sIgA levels were reported to be unaffected (28). In our study, salivary sIgA levels were measured by ELISA test after starting probiotic yogurt use in 6-8 month-old infants. Although there was a slight increase in sIgA levels after 3 weeks of probiotic yogurt consumption, the difference was not statistically significant. Likewise, in a study by Stensson et al. (20), sIgA was reported to show a tendency to increase, however, this was not found to be statistically significant. Our result is also in line with the dental literature (28). However, the long-term effect is worth investigating with a higher sample size.

Probiotic bacteria produce many substances that affect the mouth flora. These substances can alter the pH or oxidation/reduction potential of dental plaque and saliva, affecting cariogenic bacteria survival. Subsequently, saliva pH and buffering capacity may be affected (29). In the present study, buffering capacity rose after 3 weeks of probiotic yogurt consumption. Since buffering capacity is a measure to alleviate the caries condition, the use of probiotic can be recommended to increase the buffering capacity of children with high caries risk.

We had several study limitations. Firstly, bacteriological evaluation was assessed by chair side kits. Secondly, this study was limited to a three-week period. The effect of the long-term use of probiotic products is still challenging to research. Thirdly, *B. Longum* BB536, *B. Bifidum* Bb12, *L. Rhamnosus* HN001 was assessed in this study. However, different strains of probiotics may have different effects on oral health.

Conclusion

Consequently, even short-term probiotic yogurt intervention in infants during the early stages of life might have benefits for oral health. Further studies with both short- and long-term use of probiotics must be implemented in infants to confirm the results and see the effects on other caries risk factors.

Ethics

Ethics Committee Approval: Ethical approval was given by the Research Ethics Committee at Ege University Faculty of Medicine (approval number: 12-8/24).

Informed Consent: Informed consent for infants was provided by their parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.E., A.T.A., E.ER., G.A., F.Ö., Concept: E.E., A.T.A., E.ER., G.A., F.Ö., Design: E.E., F.Ö., G.A., Data Collection or Processing: E.E., A.T.A., E.ER., Analysis or Interpretation: E.E., A.T.A., E.ER., G.A., F.Ö., Literature Search: A.T.A., E.E., Writing: A.T.A., E.E.

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Original Article

A Game-based Nutrition Education: Teaching Healthy Eating to Primary School Students

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ABSTRACT

Aim: The purpose of this study is to determine eating habits of school age children and to evaluate the effect of nutrition education via games on promoting healthy eating habits.

Materials and Methods: The study, which is quasi-experimental with a pre-test/post-test experimental model without a control group, was conducted at a primary school. The population of the study includes 8-year-old 2nd-grade students. The population consists of 59 primary school students who participated in all nutrition education and completed one pre-test and two post-tests. The data were analyzed via descriptive statistics, Wilcoxon-Mann-Whitney-test, McNemar's test, Shapiro-Wilk tests.

Results: According to the findings, 59.3% of the students didn't have any nutrition education and 84.7% of them considered their eating habits as good. It was also found that 50.8% of the students before the training, 30.5% of them right after the training and 40.7% of them 3 months after the training stated they skipped one of their meals and that lunch which was the most frequently skipped meal. Although there was no change in consumption of fresh fruit and fruit juice, there was an increase in the consumption of fresh vegetable and vegetable meals, but this was not transformed into behavior. The consumption of dairy products increased and transformed into behavior. The average nutrition knowledge of the students increased both right after the training and 3 months later.

Conclusion: It is thought that the nutrition education given to the school age children had a positive effect on their nutrition knowledge, attitudes, behaviors.

Keywords: Eating habits, nutrition education, school age children, game

Introduction

Schools are an important source for giving nutrition education (1,2). Providing nutrition education by traditional methods only provides information, but actively participating in nutrition education is an interesting and effective method of teaching healthy nutrition behavior (3). With the help of game-based nutrition education instead of straight narration methods and recommendations, students can acquire healthy nutrition behavior (4,5). In nutrition education, the educator and the developer effect of the game are utilized.

Today, increasingly, gaming activities are becoming an integral part of educational programs, and educational and learning activities become effective, permanent, meaningful and enjoyable structures (6). Playing a game and education are complementary methods for healthy eating habits and active lifestyles because the game is motivational, enjoyable, recognizable, irregular, secure and active in the learning process. Making choices, making their own decisions, taking

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responsibilities and entertainment are the components of learning via playing games (3,7,8).

Children, communities, school-based individuals, teachers and health personnel must be conscious and educated about nutrition in order for the school child to be fed an adequate, balanced and healthily diet (9). For this reason, educators, sociologists, economists and planners, physicians and school health nurses should work in a multi-disciplinary team approach (1).

The most important person who will implement health education to raise and protect the health of the school-aged child is the nurse (10). Nurses are a key point in determining the nutritional behavior of children and the factors that affect them, determining the children who have inadequate and unbalanced feeding, teaching healthy eating habits to children and getting support in this field by working with other institutions and organizations in the society (11).

In this study, it is thought that the determination of eating habits of the school children and the evaluation of nutrition education via playing are important in terms of raising the health of the school-aged child. The aim of this research is to increase the knowledge of students by giving nutrition education via playing a game and to transform the education information into behavior.

Materials and Methods

Research Design

The study was carried out with semi-experimental, pre-test and post-test trial models without a control.

Sample

One hundred twenty 8-year-old 2nd grade students going to Murat Reis Primary School between March-June 2014 were invited to participate in the study and they received the nutrition education. Children with chronic illnesses that affect food allergy or food intake and children with any medical nutrition restriction were not included in the study. In the collection of the data, a survey, prepared by the researcher in accordance with the literature, containing the descriptive characteristics, nutritional habits and nutrition information of the school children was used. Expert opinions were received from four academician nurses for the information form and pre-test, post-test forms.

The research was planned to be done with 120 second grade students, the research data were collected before, immediately after and three months after the nutrition education, but the evaluations were based on the 59 students who participated in all three of the questionnaires.

Intervention

After the preliminary study was completed and the permits were received, the study was carried out for a total of 2 weeks as one lesson per week (45 minutes). The surveys of the study which were done as pre-test and post-test were repeated after three months and the efficiency of nutrition education via playing a game was examined.

In the first week of the education via the game, the course was given as a slide show and general nutrition information was given and then this information was reinforced with a card game. Healthy morning breakfast, lunch and dinner menus were prepared together with the students using food cards.

In the second week of nutrition education, baskets of food groups were prepared and taken to the school. By mixing food groups, it was required that students put the correct food in the correct basket. In addition, a food group game was played to find food not belonging to the basket. Then, the students were selected for a role play; the scenario was explained and the roles were distributed. The role play was improvised. One of the scenarios was portraying the mother and the child who go to the market and shop for healthy food, and the other one was discussions about healthy and adequate nutrition of family members eating dinner. After the role play, the game was discussed. Each class had a "Healthy Nutrition Post", and students were given the opportunity to examine it, and the main points of healthy eating were consolidated with posters.

After the education was completed, nutrition education was conducted with the voluntary participation of the school administration, class teachers and parents. The education was conducted in the form of questions and answers.

The canteen and environmental conditions were evaluated in terms of hygiene, food cleaning, preparation, and food variety.

Instruments

Two data collection tools were used in the study, namely the "Questionnaire for the Nutrition Habits of the School Age Child" which includes eighteen questions and the "Questionnaire for the Nutrition Knowledge of the School Age Child" which includes twenty questions. The questionnaires were developed by researchers and expert opinions were obtained.

Ethical Explanations

The required permission was obtained from the Scientific Ethics Committee of Ege University Nursing Faculty, the Ministry of Education and the Primary School attached to the Ministry of National Education (approval

number: 27344949-1267). Verbal and written approvals were obtained from parents and children prior to the children's education, by explaining the purpose of the study, benefits to be obtained from the study and time to be spent for the education.

Statistical Analysis

In data analysis with SPSS 16.0 statistical program, numbers and percentages, Wilcoxon-Mann-Whitney test and McNemar's test were used for the evaluation of information on the children and their nutrition habits before and after the intervention, Shapiro-Wilk test was used for the evaluation of nutrition information before and after the intervention.

Results

According to the obtained data, 54.2% of the students participating in the study were female, and 45.8% of them were male. The average weight of the females was 27 kg±4, and average height was 130±4 cm. The average weight of the males was 29 kg±5 average height was 130±6 cm. 16.9% of the students were thin, 54.2% were normal, 28.8% were overweight. It was determined that 84.7% of the students identified their pre-education own eating habits as "good". This rate increased to 93.2% after the education.

It was determined that 78% of the students stated that they consumed at least three meals a day before the education. This ratio was 64.4% after education and 61% three months after the education. Although there seems to be a decrease in the frequency of food consumption, the frequency of food consumption of three meals and above increased.

According to the obtained data, 50.8% of the students stated that they had skipped meals during the day before the education. This ratio was 30.5% after the education and 40.7% three months after the education. There is a significant difference between pre-education and post-education mean skipping behaviors of the students ($p=0.004$).

There was no significant change between those who consumed fruit and freshly squeezed juice before (55.9%) and after training (54.2%) and three months after the education (55.9%), and the individuals who consumed vegetables and vegetable foods did not translate into behavior despite the increase after the education from 49.2% to 54.2%. Students were consuming milk and dairy products predominated before the education (78%), after the education (79.7%) and three months after the education (86.4%) (Figure 1). The egg consumption rate showed an increase after education from 57.6% to 62.7 but decreased three months after the education (50.8%).

There was no significant change in the consumption of red meat and poultry which was at a level of 2-4 times a week. Seafood consumption increased after the education (27.1%) and decreased three months after the education (20.3%) (Table I). Dry legume consumption increased after the education (35.6%) and three months after the education (39%) (Figure 2).

The percentage of those who did not consume sugar and sweetened foods did not change after the education (18.6%), and decreased three months after the education (10.2%). The percentage of those who did not consume energy drinks increased significantly three months after the education (79.7%).

There was no significant difference between pre-education, post-education, and 3 months' post-education in the general sense among the food consumption frequency of the students ($p<0.05$).

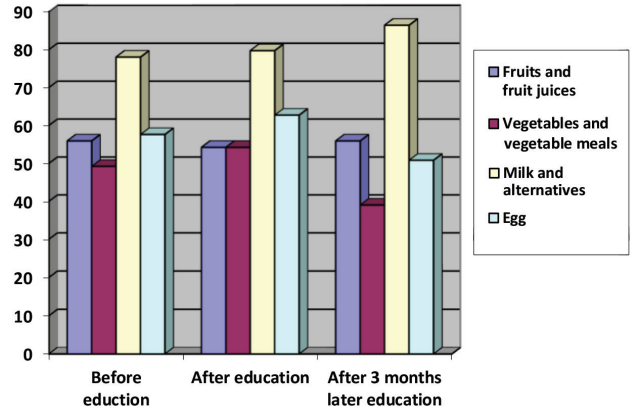

Figure 1. Consumption graphic of foods being consumed daily by school-age children

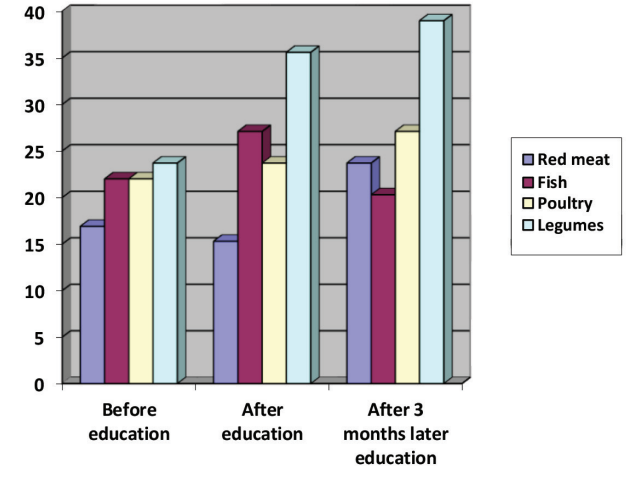

Figure 2. Consumption graphic of foods being consumed 2-4 times a week by school-age children

Before the education, 30.5% of the students had been doing something while eating. This rate did not change after the education (28.8%) but increased three months after the education (47.5%). This ratio did not show a significant change after the education and three months after the education.

According to the obtained data, 44.1% of the students take a lunch box to school. 54.2% of those who take a lunch box do not consume the food inside it. It was determined that 50.8% said they did not consume these foods because they did not like them. After the education and three months after the education, there was no change in the ratio of students who took a lunch box to school. The percentage of students who buy food from the canteen was 61%, this percentage dropped to 44.1% after the education but did not transform into behavior three months after the education. Fatty foods such as toast, sandwich and hamburger were the most commonly consumed ones from the canteen (37.3%), but this ratio decreased after the education (27.1%), but it did not change three months after the education.

Before the education, 86.4% of the students took part in daily physical activity. There was no significant change in physical activity after the education and three months after the education.

The average score of pre-education nutrition information for students is 79, 81 after education, 89 three months after the education. Nutrition information of 62.7% of the students was "very good" before the education, nutrition information of 71.2% after education and 91.5% three months after the education was "very good" (Table II).

Table I. School-age children's frequency of food consumption

	Before education										**After education**									
Food	**None**		**Once a month**		**Once a week**		**Two-four times a week**		**Every day**		**None**		**Once a month**		**Once a week**		**Two-four times a week**		**Every day**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Meat	25	42.4	11	18.6	13	10.2	9	16.9	9	11.9	13	22.0	15	25.4	13	22.0	9	15.3	9	15.3
Fish	8	13.6	14	23.7	15	25.4	13	22.0	9	15.3	4	6.8	15	25.4	12	20.3	16	27.1	12	20.3
Poultry	8	13.6	12	20.3	15	25.4	13	22.0	11	18.6	7	11.9	14	23.7	14	23.7	14	23.7	10	16.9

p<0.05

Table I. Continued

	Three months after education										**Wilcoxon t-test Asymp. Sig. (2-tailed**				
Food	**None**		**Once a month**		**Once a week**		**Two-four times a week**		**Every day**		**Before education – after education**	**Before education-after three months**	**After education-after three months**	**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**				**n**	**%**
Meat	19	32.2	8	13.6	13	22	14	23.7	5	8.5	0.055	0.252	0.613	59	100.0
Fish	1	1.7	17	28.8	28	47.5	12	20.3	1	1.7	0.113	0.507	**0.026**	59	100.0
Poultry	5	8.5	15	25.4	17	28.8	16	27.1	6	10.2	0.829	0.717	0.793	59	100.0

p<0.05

Table II. Nutritional information scores for school-age children

Questionnaire for the Nutrition Knowledge of the School Age Child	**n=59 x**	**n=59 minimum**	**n=59 maximum**	**Shapiro-Wilk test p score**
Before education	79	40	100	0,026
After education	81	30	100	**0,000**
After 3 months later	89	69	100	**0,002**

p<0.05

Discussion

Behaviors that can last for life are gained mostly in the school-aged child period (12). Nutrition education is important for improving the quality of life and health of future generations.

In our study, 40.7% of the students had nutrition education before the study. Sabbağ (13), with 5th and 6th grade students (n=549), stated that only 29.1% of the students received nutrition education. The majority of the students did not receive education in nutrition, indicating that nutrition education is still inadequate in our country.

In our study, the three-meal consumption rate decreased compared to the pre-education, but the ratio of those who consumed four meals a day or more increased. The school-aged child should be fed at least three meals or four to five meals a day. This result shows that the given education is not only in the level of knowledge but also transformed into positive behavior. The study of Kılıç and Uzunçakmak (14) on feeding behavior and nutrition education habits of students (n=305), 79.4% of the students eat three meals or more. In the study by Oğuz and Derin (15) on the investigation of nutrition habits, it was stated that 34.4% of the students had three meals or more. Our study results are higher when compared to the study done by Oğuz and Derin (15).

For an adequate, balanced, healthy and moral boosting nutrition, it should be consumed in the required quantities giving importance to various food types instead of eating one or two types of food (16).

In our study, consumption of vegetable and vegetable dishes increased after education but dropped three months after education. Although there was no change in the consumption of dairy products after education, there was an increase three months after education. Increasing the consumption of dairy products into behavior is a positive result in terms of bone and dental health since the school-aged child has a high calcium requirement. The egg is a very important protein, it is positive that most of the students consume eggs every day. Seafood consumption increased after education but decreased three months after education. There is a negative difference between post-education results and results 3 months after the education according to Wilcoxon-Mann-Whitney test (Table I). The fact that the consumption rate of seafood in İzmir, which is a coastal province, is lower than expected is thought to be because the region in which the research was conducted is influenced by migration.

No significant changes in food consumption in general after the education and 3 months after the education requires more serious consideration of nutrition education and coordination of the Ministry of National Education, the entire school team, the family, the school health nurse and other health workers.

Compared with the studies of Özilbey (17) and Derin et al. (18), the consumption of eggs each day was found to be higher. Consumption of milk and dairy products is higher than the study of Kutlu and Çivi (19).

As a result of the research named "bring a little fruit to the school" conducted in an elementary school in Italy by Panunzio et al. (7), it was determined that there is an increase in the consumption of fruit-vegetable-legume after nutrition education.

There is also a strong relationship between nutritional programs and school meals and the cognitive performance of students and educational achievement (20).

Nutrition information of 62.7% of the students was "very good" before the education, nutrition information of 71.2% after education and 91.5% three months after education was "very good". A statistically significant difference was found between pre-and post-education nutrition knowledge levels of the students. In the study of Choi et al. (21), the analysis for nutrition attitude and dietary behavior (n=493) showed that nutrition attitude was slightly higher in girls with 7.59 points than in boys with 7.31 points, which showed a statistically significant difference ($p<0.05$). In the study by Lin et al. (22), the nutrition knowledge of elementary school children was fair. On average, 1st to 3rd graders and 4th to 6th graders answered 67.3% and 71.4% of the nutrition knowledge questions correctly. In a study of 1.704 Indian American children by Davis et al. (23), nutrition education was found to increase nutrition knowledge and physical activity levels. In a study conducted by Nguyen (24) to investigate the effect of nutrition education, nutrition knowledge significantly increased from 15.58 to 19.73, baseline, and post intervention respectively. With Kandiah and Jones (25), their study showed the effectiveness of a nutrition education program on nutrition knowledge scores and the healthy food choices of fifth grade children. In another study conducted by Drummond (26), students increased their understanding of the principles of healthy eating and developed skills to make decisions about healthy eating behaviors. Our study and other studies show that nutrition education can improve the knowledge of students significantly (27,28).

Conclusion

According to these results, it can be said that the individual and therefore society should be conscious about nutrition. With nutrition education, the society should feed itself according to adequate and balanced nutrition rules ensuring that the community is healthy, and in this way the expenditures made for health should be minimized. This

can only be achieved through education. The earlier the start of nutrition education, the more successful it will be. The family is also very important in giving children a positive nutritional habit during the school years. For this reason, it is suggested that family education should be included in the pattern of studying similar studies.

Within the scope of community health protection, school administrations can work in cooperation with the relevant government institutions. Country policies can be established and applied in the fields of health, nutrition, and physical activity.

Ethics

Ethics Committee Approval: Required permissions were obtained from the Scientific Ethics Committee of Ege University Nursing Faculty, the Ministry of Education and the Primary School attached to the Ministry of National Education (approval number: 27344949-1267).

Informed Consent: Verbal and written approvals were obtained prior to the children's education, by explaining the purpose of the study, benefits to be obtained from the study and time to be spent for the education.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: Z.D.B., H.U., Design: Z.D.B., H.U., Data Collection or Processing: H.U., Analysis or Interpretation: H.U., Literature Search: H.U., Writing: H.U.

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

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Original Article

Evaluation of Anticoagulant Proteins and Fibrinolytic System Markers in Children with Pneumonia

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ABSTRACT

Aim: Coagulation and fibrinolytic systems play an important role in the pathogenesis of complicated pneumonia. This study aims to evaluate and compare coagulation and fibrinolytic system markers and natural anticoagulant proteins with acute phase reactants, such as C-reactive protein or procalcitonin in children with pneumonia.

Materials and Methods: One hundred and fifteen patients and 87 healthy children were included in the study. Patients were separated into two groups based on viral and bacterial pneumonia diagnosis. Coagulation and anticoagulation system markers were compared with acute phase reactants in children with pneumonia.

Results: White blood cell numbers and D-dimer (DD) levels were higher in the pneumonia patients compared to the control group (p=0.001 and 0.001 respectively). Protein C activity and antithrombin activity in the patient group were significantly lower compared to the control group (p=0.001 and p=0.011 respectively). Acute phase reactants and DD levels in the bacterial pneumonia patients were higher compared to the viral pneumonia patients (p<0.05). Protein S activity, fibrin monomers and fibrin degradation products were not significantly different between the bacterial and viral pneumonia patients. Protein S activity in the bacterial pneumonia patients was lower compared to the viral pneumonia patients (p=0.040). There was no difference in terms of antithrombin activity and protein C activity.

Conclusion: As a result, the relationships among acute phase proteins, anticoagulation proteins, and fibrinolytic system markers show that the coagulation and fibrinolytic system has an important role in pneumonia pathogenesis and associated inflammation. Evaluation of the coagulation system may help determine the severity of pneumonia in children and be used to monitor its clinical progress.

Keywords: Pneumonia, children, anticoagulant reactants, fibrinolytic system

Introduction

Pneumonia is an acute inflammation that develops in lung parenchyma as a result of changes in at least one of the lower respiratory defence mechanisms (1). More than 150 million children are diagnosed with pneumonia every year in developing countries. 23% of outpatient children and 29-38% of hospitalized children (33-50% if younger than one) are diagnosed with pneumonia. 37% of community-acquired pneumonia (CAP) occurs in children (2). In developed countries, the prevalence of CAP among children is 5 million cases per year, which creates a substantial demand for health services. The most common causative agents of CAP are *Streptococcus pneumoniae*, *Haemophilus influenza*, *Mycoplasma pneumoniae*, respiratory syncytial virus, rhinovirus, and parainfluenza viruses (1,3,4).

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Coagulation and fibrinolytic system plays an important role in the pathogenesis of serious lung diseases such as complicated pneumonia and respiratory distress syndrome (5,6). Activation of the coagulation system is triggered by endotoxin and other inflammatory mediators. As a reaction to these mediators, vascular congestion develops and alveoli are filled with fibrin during pneumonia. Fibrin residue products are secreted throughout the fibrinolytic enzyme degradation process by the fibrinolytic system. Heavy sepsis may cause disseminated intravascular coagulation (DIC) as a result of coagulation system activation through pulmonary inflammation in pneumonia. This may cause an increase in coagulation activation markers, D-dimer (DD), and fibrin degradation products (FDPs), and a decrease in natural anticoagulant proteins (5-8).

This study aims to evaluate and compare coagulation and fibrinolytic system markers and natural anticoagulant proteins with acute phase reactants, such as C-reactive protein (CRP), high-sensitive (hs)-CRP, and procalcitonin in children with CAP.

Materials and Methods

One hundred and fifteen patients and 87 healthy children (as the control group), between two months and 18 years of age, were included in the study. The study groups were selected from individuals who were admitted to the Ankara Training and Research Hospital Clinic of Paediatric and Paediatric Emergency. This study was obtained from the Ethics Committee of Ankara Training and Research Hospital with file (approval number: 0533 date: 10.01.2014). The parents of the study groups were given informed consent forms and written consent was obtained from the participants prior to the study.

Patients were divided into two groups based on viral and bacterial pneumonia diagnoses. Bacterial type pneumonia was defined based on at least two of the following three criteria: 1) plasma CRP level > 0.8 mg/dL, 2) leukocyte number > $15x10^9$/L, and 3) alveolar infiltration in posterior-anterior chest x-ray. Patients who did not conform to these criteria were defined as viral pneumonia patients (1). Patients who started antibiotic treatment prior to the study, hematologic patients, and patients with a chronic illness were not included in the study.

Blood samples were taken from the patients before treatment. White blood cell (WBC) count, CRP, hs-CRP, procalcitonin, fibrinolytic system markers including DD, FDPs, fibrin monomers, and natural anticoagulant proteins such as protein C, protein S, and antithrombin III were investigated by means of the blood samples.

Statistical Analysis

The results were analysed using (SPSS Inc. Chicago, IL, USA) 16.0 statistical analysis package program. Chi-square test was used for qualitative variables in statistical comparisons. Normal distribution of quantitative variables (protein C, protein S, antithrombin III, DD, FDPs, CRP, hs-CRP, WBC count, procalcitonin) was evaluated using Kolmogorov-Smirnow Z (K - S) test. Student's t-test was used to investigate differences with variables that showed normal distribution. Mann-Whitney U test was used for comparing two independent groups if the distributions were not a normal distribution. A "p" value of less than 0.05 was considered statistically significant.

Results

One hundred and fifteen patients diagnosed with pneumonia (55.7% male and 44.3% female) were compared with the healthy control group of 87 children (57.5% male and 42.5% female). The median age of study group was 36 months (2-216 months). The age distribution of the viral and bacterial pneumonia patient groups was similar [median age of the viral pneumonia group was 16.5 months (2-120 months) and median age of the bacterial pneumonia group was 17 months (2-168 months), p=0.396]. 26.9% of the patients (n=31) were diagnosed with bacterial pneumonia while 73.1% (n=84) of them had viral pneumonia.

WBC numbers and DD levels in the pneumonia patients ($10.3\pm4.082x10^9$/L and 0.47 µg/mL) were significantly higher compared to the control group ($8.116\pm2.876x10^9$/L and 0.18 µg/mL; p=0.001 and 0.001 respectively as shown

Table I. Comparison of white blood cell numbers, anticoagulant proteins, and fibrinolytic system values between the patient and the control groups

	Patient group, (n=115)	Healthy control, (n=87)	p value
White blood cell count (/L)	10.3±4.082	8.116±2.876	**0.001***
Antithrombin III activity (%)	59.7±10.2	84.6±10.3	0.001*
Protein C activity (%)	67.6±17.1	87.0±22.1	**0.001***
Protein S activity (%)	62.7±18.8	61.0±24	0.570*
D-dimer (µg/mL)	0.47 (0.06-2.80)	0.18 (0.01-3.78)	**0.001†**
Fibrin degradation products (µg/mL)	3.8±2.8	3.9± 1.9	0.482*
Fibrin monomers (µg/mL)	4.5 (0.33-184)	3.9 (0.48-6)	0.152†

*mean ± standard deviation; †median (minimum-maximum)

in Table I). At the same time, protein C activity in the pneumonia patients was lower compared to the control group (67.6±17.1% and 87.0±22.1% respectively, p=0.001). Antithrombin III activity was found to be lower in the patient group compared to that of the control group (p=0.001). When protein S activity, FDPs, and fibrin monomers were evaluated, there was no significant difference between the patient group and the control group (Table I).

WBC count (13±5.255x10^9/L and 9.286±3.019x10^9/L, p=0.001), CRP levels (3.9 mg/dL and 0.4 mg/dL, p=0.000), hs-CRP levels (10.6 mg/dL and 3.8 mg/dL, p=0.000), and procalcitonin levels (0.13 mg/dL and 0.10 mg/dL, p=0.014) were significantly higher in the bacterial pneumonia patients compared to the viral pneumonia patients (Table II). DD levels (0.56 µg/mL and 0.044 µg/mL) were also higher in the bacterial pneumonia patients (p=0.046); however, other fibrinolytic marker FDPs and fibrin monomers were not significantly different (Table II). One of the natural anticoagulant proteins, protein S activity in the children with bacterial pneumonia (68.6±20.8%) was lower compared to the viral pneumonia patients (60.5±17.7%, p=0.040), while there was no difference in terms of antithrombin III activity and protein C activity (Table II).

Table II. Comparison of acute phase reactants, anticoagulant proteins, anticoagulant proteins, and fibrinolytic system markers between the bacterial and viral pneumonia patient groups

	Bacterial pneumonia, (n=31)	**Viral pneumonia, (n=84)**	**p value**
White blood cell count (/L)	13±5.255	9.286±3.019	**0.001***
CRP (mg/dL)	3.9 (0.3-24)	0.4 (0.09-6)	**0.000†**
hs-CRP (mg/dL)	10.6 (4.6-21)	3.8 (0.2-10.6)	**0.000†**
Procalcitonin (mg/dL)	0.13 (0.06-7.02)	0.10 (0.02-2.23)	**0.014†**
Antithrombin III activity (%)	97.3±18.8	103±13.4	0.074*
Protein C activity (%)	66.9±18.1	67.9±16.8	0.782*
Protein S activity (%)	68.6±20.8	60.5±17.7	**0.040***
D-dimer (µg/mL)	0.56 (0.12-2.80)	0.044 (0.06-1.95)	**0.046†**
Fibrin degradation products (µg/mL)	4.9±2.7	3.6±28	0.150*
Fibrin monomers (µg/mL)	4.6 (0.6-184)	4.46(0.33-170)	0.847†

*mean ± standard deviation; †median (minimum-maximum)

A positive correlation was determined between DD and CRP (p=0.001; r=0.497); DD and hs-CRP (p=0.030; r=0.217), and DD and procalcitonin (p=0.026; r=0.208) in the pneumonia patients. At the same time, there was negative correlation between procalcitonin and protein C (p=0.044; r=-0.189); antithrombin III and CRP (p=0.018; r=-0.220), while antithrombin III and protein C (p=0.001; r=0.417) and protein C and protein S (p=0.010; r=0.240) had positive correlation.

Discussion

Vascular congestion that develops in pneumonia cases may cause fibrin accumulation in alveoli. Recent studies support that intravascular and extravascular coagulation show a relationship with acute and chronic lung injury (9,10). Enzymatic degradation of fibrin by the fibrinolytic system may cause FDPs, such as DD, to be released into the circulation (8,9,11). Also, plasma DD levels may increase as a result of the activation of the blood coagulation process; which is caused by endotoxins in the gram-negative pathogens that trigger CAP (12). During pulmonary infections, the formation of fibrins could enhance host protection by containing infectious agents. Such protection may also be enhanced by protecting the endothelial-epithelial barrier. On the other hand, coagulation as well as thrombin and fibrin products exhibit proinflammatory features that could negatively influence the reliability and function of the pulmonary system in severe acute respiratory distress syndrome (13).

In CAP patients, the relationship between coagulation activators and inhibitors and biomarkers is still not well known. In a study conducted with adults, DD was found to be high in heavy pneumonia cases while protein C and antithrombin III were found to be low in CAP patients (8). Studies conducted with paediatric pneumonia cases are rare (14,15). Långström et al. (14) found that in 28 CAP children with bacterial pneumonia, protein C and antithrombin III were low while DD was significantly high.

Guo et al. (15) studied 52 children and found that lobar pneumonia and interstitial pneumonia patients had higher levels of thrombomodulin and DD levels compared to a control group. This suggests damage to the vascular endothelial cells and blood hypercoagulability may be involved in the pathogenesis of pneumonia.

In our study, protein C and antithrombin III levels were found to be significantly low in CAP patients compared to the control group while the DD level was significantly high. At the same time, in bacterial pneumonia cases, the DD level was significantly high while the protein S level was low. Fibrin monomers and FDPs levels were higher in bacterial

pneumonia cases, but the difference was not statistically significant.

Antithrombin III inhibits activated coagulation proteins by binding to them. Decreased antithrombin III levels are related to an elevated risk of thrombosis. It is reported that pulmonary vascular endothelium has a significant role in the catabolism of antithrombin III (8,16). In heavy pneumonia cases, antithrombin III and protein C levels are found to be especially low (8). In our study, antithrombin III levels were found to be low in the pneumonia patients. Unlike other studies, this study found that protein S levels, a natural anticoagulant, were lower in the bacterial pneumonia cases. These findings suggest that natural anticoagulants have an important role in pneumonia pathogenesis.

The best laboratory procedure that shows coagulation activity is DD level analysis, which may elevate in acute coronary illness, deep vein thrombosis, peripheral vein diseases, pulmonary emboli, coronary failure, and DIC, which are situations in which fibrin formation and degradation increase. Studies show that DD levels increase in sepsis and pneumonia as well. Levi et al. (13) reported that DD levels in heavy pneumonia cases are not related to CRP and leucocyte numbers; however, they correlate with the existence of pleural liquid, the severity of pneumonia, and radiologic appearance. They also determined that DD levels are higher in alveolar or interstitial pneumonia patients compared to bronchopneumonia patients.

In studies conducted with adults, DD levels are reported to increase in bronchoalveolar lavage fluid of patients with pneumonia compared with controls, and DD levels increase progressively with severity, being higher in patients who require mechanical ventilation than in those with uncomplicated pneumonia (11). Other studies reported that DD levels in CAP patients are related to the severity of pneumonia, mortality, and morbidity (8,11,17-19).

In our study, DD levels were found to be high in pneumonia cases and they were higher in bacterial pneumonia cases than in viral pneumonia ones with a statistically significant difference. At the same time, the FDPs and fibrin monomer levels, which are important parameters of the fibrinolysis system, were higher in pneumonia patients. More research in this area needs to be done.

Pereira et al. (20) conducted their study with adults and reported that in severe CAP sepsis situations, high DD levels are important for an early diagnosis; DD, procalcitonin and CRP levels are correlated with each other. In the present study, DD levels in bacterial pneumonia patients were significantly higher and correlations among DD, FDPs and acute-phase proteins CRP, hs-CRP, and procalcitonin were observed.

Långström et al. (14) conducted their study on children with pneumonia and reported that, in bacterial pneumonia, DD and CRP are related. Other studies show that serum CRP concentration at the time of diagnosis correlates with the severity of CAP. Inflammatory response increases in pneumonia patients. Activated macrophages discharge inflammatory mediators such as interleukin-6 which induces acute-phase proteins, like CRP (8). In this study, a positive correlation among DD and CRP, hs-CRP and procalcitonin levels in pneumonia patients was found; while a negative correlation between procalcitonin and protein C, and antithrombin III and CRP was observed.

Study Limitations

This study is limited to 115 patients presenting at the paediatric clinic of a major training and research hospital in Ankara, Turkey.

Conclusion

As a result, the relationships among acute phase proteins, anticoagulation proteins, and fibrinolytic system markers show that the coagulation and the fibrinolytic system has an important role in pneumonia pathogenesis and associated inflammation. Evaluation of the coagulation system may help determine the severity of pneumonia in children and be used to monitor its clinical progress. Further studies are needed on this subject.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Ankara Training and Research Hospital with file (approval number: 0533, date: 10.01.2014).

Informed Consent: All participants' parents provided written informed consent.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.Y., N.Ç., Concept: Z.Y., B.A., F.İ.A., Design: Z.Y., B.A., F.İ.A., Data Collection or Processing: N.Ç., Analysis or Interpretation: F.I.A, B.A., Literature Search: Z.Y., Writing: Z.Y., N.Ç.

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Original Article

The Effect of Gonadotropin-releasing Hormone Analog Treatment on Body Mass Index and Height in Female Patients with Central Precocious Puberty

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ABSTRACT

Aim: Gonadotropin-releasing hormone agonists (GnRHa) are widely used in the treatment of central precocious puberty (CPP). There is concern that GnRHa treatment, whose positive effects on the adult height are known, may cause weight gain and body mass index (BMI) increase. The aim of this study was to assess the effect of the GnRHa treatment on BMI and height in female patients with CPP.

Materials and Methods: Ninety-two patients diagnosed with idiopathic CPP and 22 patients diagnosed with organic CPP, who received GnRHa treatment were included in the study. Data taken on the treatment start date, 6th month, 1st and 2nd year for height, weight, BMI and bone age were obtained retrospectively from the file records.

Results: BMI standard deviation score (SDS) increased during the treatment period in all the patients. In the second year of GnRHa treatment, BMI SDS was higher in the organic CPP, compared to the idiopathic CPP (0.66±0.84 and 1.35±0.72, p=0.007). In both groups, at the beginning of GnRHa treatment, the BMI SDS increase was higher in those patients with normal weight compared to those who were overweight/obese. In both groups, the prevalence of obesity was higher than the reference population at the beginning of treatment. An increase was determined in the height SDS and predicted adult height in both groups according to bone age.

Conclusion: In patients with CPP, the prevalence of obesity was higher in the first application compared to the reference population. In CPP, BMI SDS increased with GnRHa treatment. The weight of the patients at the beginning of the treatment affected the weight and BMI change with GnRHa treatment. Those patients with organic CPP were more prone to weight gain and BMI increase.

Keywords: Central precocious puberty, gonadotropin-releasing hormone analogues, obesity

Introduction

Precocious puberty (PP) is defined as the initiation of secondary sex characters before the age of eight in girls and nine in boys. PP may be true (central-gonadotropin dependent) or pseudo (peripheral-gonadotropin independent). Central PP (CPP) occurs with sex steroids released by the gonads as a result of an early activation of the hypothalamic-pituitary-gonadal (HPG) axis. Increased sex steroids cause an acceleration in pubertal progression, height increase and bone maturation and may lead to a reduced final adult height, early menarche, and psychological disorders (1). The purpose of treatment in CPP is to stop the progression of secondary sex characters by suppressing the HPG axis, to slow the skeletal maturation, to slow the bone epiphyseal closure, to increase adult height, and to benefit

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psychosocial well-being (2). For this purpose, gonadotropin-releasing hormone agonists (GnRHa) have been widely used for the treatment of CPP for more than 30 years. GnRHa treatment that effectively inhibits gonadotropin secretion is generally a reliable treatment in children (2). In long-term follow-up studies, it was shown to increase final adult height in children with CPP but it did not show significant changes in reproductive activity (3). Studies on the efficacy and the auxological effects of GnRHa treatment in CPP are mainly related to revealing adult height gain (4). The results in the studies conducted about the effect of GnRHa treatment on body weight are contradictory. While it is reported in some studies that GnRHa treatment is linked with body fat mass, body weight and body mass index (BMI) increases (4-10), it is reported not to be linked in other studies (3,5,9,11,12). Even some studies reported that it was associated with a decrease in BMI (12,13).

Nutrition and body fat mass during childhood are closely related to PP. Considering the increasing prevalence of obesity all over the world and the higher prevalence of PP in obese people compared to the normal population (14-17), the importance of the effects of GnRHa treatment used for PP on body weight is increasing day by day.

The aim of this study was to evaluate the effects of the GnRHa treatment on BMI and height in female patients with central PP.

Materials and Methods

In this study, the records of patients who were diagnosed with PP in the Konya Training and Research Hospital, Clinic of Paediatric Endocrinology Outpatient between 2010 and 2018 were retrospectively reviewed. The study was approved by the Necmettin Erbakan University Faculty of Medicine Local Ethics Committee (approval number: 2017/1100). Informed consent was obtained. A total of 114 female patients, who received GnRHa treatment and were in the age group of 1.65-8.9 years, including 22 patients (19.3%) followed up with the diagnosis of PP (organic CPP) associated with an organic disorder of central neural system and 92 patients (80.7%) followed up with the diagnosis of idiopathic CPP were included in the study.

Idiopathic CPP criteria were taken as follows: 1) the onset of budding in the breasts before the age of 8 years in girls, 2) determination of at least 1 year advanced bone age compared to chronological age, 3) the peak luteinizing hormone (LH) ≥5 mIU/mL examined with chemiluminescence immunoassay method after the exogenous GnRH (gonadorelin 100 µg) intravenous injection, 4) a lack of a history suggesting a central nervous system disease and the presence of normal cranial magnetic resonance imaging (MRI) symptoms (18). Patients with a history of central nervous system disease and/or pathological cranial MRI symptoms were accepted as central PP (organic CPP) developing secondarily to central nervous system pathologies. Patients who used drugs that might affect anthropometric measures and had a systemic disease such as hypothyroidism, congenital adrenal hyperplasia or Cushing's disease were excluded from the study.

From the outpatient clinic file records of patients, their weight, height, puberty phase according to Marshall and Tanner (19), basal serum LH, follicle stimulating hormone (FSH), and estradiol studied from venous blood samples taken between 08.00-12.00 after 12-hour fasting, peak serum LH level after exogenous GnRH (gonadorelin 100 µg) intravenous injection, bone age assessment according to the Greulich and Pyle (20) method, and cranial imaging results were recorded. BMI of the patients was calculated with weight (kg) - height $(cm)^2$ by using their weight and height measurements; height standard deviation score (SDS), BMI SDS, BMI percentile values were obtained using standardized data prepared for Turkish children based on age and gender (21). BMI ≥95 percentile was considered as obese, 85-95 percentile as overweight, and <85 percentile as normal body weight. Predicted adult height (PAH) according to bone age was calculated according to the Bayley and Pinneau (22) method. Height SDS, BMI SDS, BMI percentile, and PAH value were calculated from the height, weight and bone age data for the 6^{th} month, 1^{st} year and 2^{nd} year of the treatment in the follow-up of patients.

In the routine protocol of the clinic of paediatric endocrinology outpatient; for the treatment of PP, GnRHa leuprolide (Lucrin 3.75 mg depot) or triptorelin (Decapeptyl 3.75 mg depot) is intramuscularly administered every 28 days with a dose of 3.75 mg if the patient has a weight >20 kg and with a dose of 1.875 mg if the patient has a weight <20 kg (2). Patients undergoing treatment are followed up with anthropometric measurements, pubertal symptoms, and serum LH levels every 3 months. During the follow-up, serum LH level <3 mIU/mL at 60^{th} minute after GnRHa injection is accepted as suppressed HPG axis; serum LH level ≥3 mIU/mL is accepted as non-suppressed axis (23) and the axis is checked again in terms of suppression with a standard GnRH test in the 3^{rd} week of GnRHa injection. A peak LH level <2 mIU/mL in the standard GnRH test is considered as suppressed HPG axis, while a peak LH ≥2 mIU/mL is considered as non-suppressed axis (24) and GnRHa treatment dose is increased to 7.5 mg/28 days. The data of the patients, who were followed up in this context and were diagnosed with central PP, and received GnRHa treatment, were included in the study.

Serum LH, FSH, and estradiol levels were studied in the Biochemistry Laboratory of Konya Training and Research Hospital using an ADVİA Centaur XP (Siemens Healthcare Diagnostics, Camberley, UK) device with a chemiluminescence immunoassay method.

Statistical Analysis

For statistical analyses, the IBM SPSS Statistics version 22 (IBM Corporation, Armonk, NY, United States of America) program was used. The data were determined as mean ± standard deviation and the significance limit for all statistics was accepted as $p<0.05$. In order to decide the appropriate test statistics in the evaluation of data, first the suitability of data to normal distribution was tested by applying Kolmogorov-Smirnov test statistics. In the numeric data, Student's t-test in two-group comparisons for the data meeting normal distribution; Mann-Whitney U test for comparison of data that did not show normal distribution; ANOVA in the comparison of repeated measurements in the same group; chi-square and Fisher's exact tests in the comparison of qualitative data were used.

Results

The admission average age was 7.84±1.01 years in the idiopathic CPP group and 7.57±1.03 years in the organic CPP group. The bone age at the beginning of the treatment was 9.99±1.13 years in the idiopathic CPP group and 9.44±1.5 years in the organic CPP group. Admission age, age at beginning of treatment, bone age, bone age-age at beginning of treatment difference, basal LH, FSH, estradiol and peak LH responses to classical GnRH test were similar in the idiopathic and organic CPP groups (Table I).

The mean BMI SDS increased during treatment in all patients receiving GnRHa treatment. BMI SDS statistically significantly increased in the 1st year (0.67±0.12 and 0.88±0.12, $p<0.001$) and 2nd year (0.67±0.12 and 0.89±0.11, $p=0.005$) compared to the beginning of treatment, in the 1st year (0.73±0.12 and 0.88±0.12, $p=0.014$) and 2nd year (0.73±0.12 and 0.89±0.11, $p=0.023$) compared to the 6th month of treatment (Table II).

When examining the mean BMI SDS values of the patients in the groups, it was determined that there was a significant increase in BMI SDS in the 1st year compared to the beginning of treatment in patients with idiopathic CPP (0.63±0.88 and 0.76±0.84, $p=0.006$). A significant increase was found in BMI SDS in the 2nd year compared to the beginning of treatment in the organic CPP group (0.80±0.97 and 1.35±0.72, $p=0.029$). Although BMI SDS was not different between the two groups in the 6th month and 1st year of GnRHa treatment, BMI SDS was significantly higher in the organic CPP group compared to the idiopathic CPP group in the 2nd year of the treatment (0.66±0.84 and 1.35±0.72, $p=0.007$) (Table III).

In the patients who had normal weight at the beginning of GnRHa treatment in both groups, the BMI SDS increase

Table I. Characteristics of the patients before gonadotropin-releasing hormone agonists treatment

	Idiopathic CPP, (n=92)	Organic CPP, (n=22)	p value
Admission age (year)	7.84±1.01	7.57±1.03	0.201
Tb age (year)	8.14±0.98	7.79±1.15	0.161
Bone age (year)	9.99±1.30	9.44±1.50	0.203
Δ KY-Tb age (year)	1.78±0.97	1.72±0.92	0.802
Basal FSH (IU/L)	3.50±2.2	3.69±2.2	0.686
Basal LH (IU/L)	0.70±0.9	0.71±1.0	0.909
Basal estradiol (pg/mL)	29.5±23.1	27.7±18.8	0.956
Peak LH (IU/L)	12.9±10.3	13.8±6.7	0.126

Values are given as mean ± standard deviation, CPP: Central precocious puberty, Tb: Gonadotropin-releasing hormone agonists treatment beginning, FSH: Follicle stimulating hormone, LH: Luteinizing hormone

Table II. Average body mass index standard deviation score values of all patients in the beginning, 6th month, 1st year and 2nd year of the treatment

	Idiopathic CPP+Organic CPP (n=114)
BMI SDS tb	0.67±0.12
BMI SDS 6th month	0.73±0.12
BMI SDS 1st year	**0.88±0.12[a,c]**
BMI SDS 2nd year	**0.89±0.11[b,d]**

CPP: Central precocious puberty, BMI: Body mass index, tb: GnRHa treatment beginning, [a]$p<0.001$: First year BMI SDS change compared to the beginning of treatment, [b]$p=0.005$: Second year BMI SDS change compared to the beginning of treatment, [c]$p=0.014$: First year BMI SDS change compared to 6th month of treatment, [d]$p=0.023$: Second year BMI SDS change compared to 6th month of treatment

Table III. Body mass index standard deviation score changes in patients in the follow-up before and after gonadotropin-releasing hormone agonists treatment

	Idiopathic CPP, (n=92)	Organic CPP, (n=22)	p value‡
BMI SDS tb	0.63±0.88	0.80±0.97	0.077
BMI SDS 6th month	0.64±0.84	1.01±0.91	0.058
BMI SDS 1st year	**0.76±0.84***	1.19±0.75	0.057
BMI SDS 2nd year	0.66±0.84	**1.35±0.72†**	**0.007**

CPP: Central precocious puberty, BMI: Body mass index, SDS: Standard deviation score, tb: Gonadotropin-releasing hormone agonists treatment beginning, *$p=0.006$: First year BMI SDS change compared to the beginning of treatment; †$p=0.029$: Second year BMI SDS change compared to the beginning of treatment, ‡statistical difference between groups

was higher in these patients compared to overweight-obese ones. BMI SDS at the beginning of treatment in patients who had a normal weight at the beginning of treatment in the idiopathic CPP group significantly increased in the 1st year (0.02±0.57 and 0.26±0.66, p=0.008) and 2nd year (0.02±0.57 and 0.28±0.65, p=0.011) of the treatment. Likewise, BMI SDS at the beginning of treatment in patients who had a normal weight at the beginning of treatment in the organic CPP group significantly increased in the 1st year (0.02±0.56 and 0.36±0.36, p=0.048) and 2nd year (0.02±0.56 and 0.63±0.43, p=0.03) of GnRHa treatment. No significant change was observed in the BMI SDS during treatment of those patients who were overweight-obese at the beginning of GnRHa treatment in both groups (Table IV).

While 34% of the patients in the idiopathic CPP group were overweight- obese (21%, 13%) at the beginning of the treatment, this rate was determined as 45% (25%, 20%) in the organic CPP group. An increase was observed in the frequency of overweight-obese patients undergoing GnRHa treatment in the organic CPP group (50%, 59%, and 64%, respectively in the 6th month, 1st year, and 2nd year of treatment). The frequency of obese patients with BMI >95 percentile was higher in the organic CPP group compared to the idiopathic CPP group both in the 1st year (47% and 18%, p=0.039) and 2nd year (57% and 19%, p=0.02) of GnRHa treatment (Figure 1).

In patients with idiopathic CPP, the 2nd year height SDS was significantly lower compared to the beginning of treatment (1.05±1.03 and 0.74±1.06, p=0.024); whereas, height SDS according to bone age was significantly higher in the 2nd year compared to the beginning of treatment (-1.01±0.95 and -0.68±0.72, p=0.03). Similarly, the height SDS of patients with organic CPP was significantly higher based on bone age compared to the beginning of treatment (-1.19±0.82 and -0.69±0.68, p=0.025). No difference was found in the height SDS in the organic CPP group in the 1st and 2nd years compared to the beginning of treatment. PAH was determined as 158.7±6.5, 162.3±6.1, and 164.4±6.6 cm, respectively for the beginning, 1st year and 2nd year of treatment (p>0.05) in patients with idiopathic CPP; and as 159.9±6.1, 161±5.3, and 164.7±4.6 cm, respectively (p>0.05) in the organic CPP group. There was no significant difference between the two groups in terms of 1st and 2nd year PAH. ΔPAH was determined to be on average 5.7 cm in the idiopathic CPP group and 4.8 cm in the organic CPP group following 2 years of GnRHa treatment. No difference was determined in both groups in terms of ΔPAH (Table V).

The average treatment durations were 2.8±0.84 and 2.62±0.51 years, respectively for the patients with idiopathic CPP and organic CPP (p>0.05). In both groups, 65% of the patients were using leuprolide and 35% were using triptorelin and there was no difference between the groups in terms of the frequency of medication usage. Cranial imaging was performed in 104 patients from 114 patients included in the study and pathological findings were determined in 22 of them (organic CPP). Pathologies detected in cranial imaging were: septum pellucidum anomaly (n=1), hypothalamic

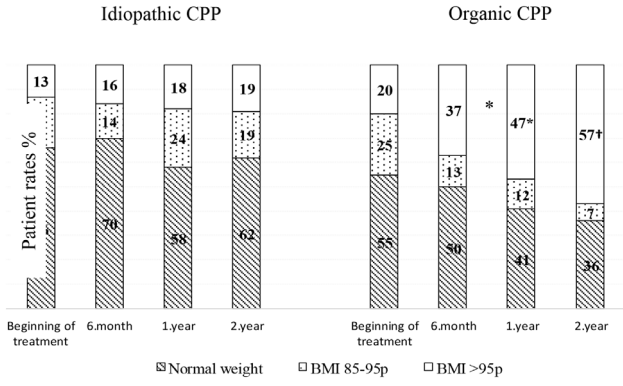

Figure 1. Overweight, obese patient rates and changes in the beginning, 6th month, 1st year and 2nd year of GnRHa treatment in patient groups with idiopathic and organic CPP. *p=0.039: Difference between the 1st year BMI >95p patient rates between two groups, †p: 0.02: Difference between the 2nd year BMI >95p patient rates between two groups
CPP: Central precocious puberty, BMI: Body mass index

Table IV. Body mass index standard deviation score changes with treatment in patients who were normal weight and overweight-obese before the gonadotropin-releasing hormone agonists treatment

	Idiopathic CPP		Organic CPP	
	BMI ≥85p, (n=61)	**Normal BMI, (n=31)**	**BMI ≥85p, (n=12)**	**Normal BMI, (n=10)**
BMI SDS tb	1.49±0.37	0.02±0.57	1.70±0.45	0.02±0.56
BMI SDS 6th month	1.40±0.59	0.14±0.62	1.76±0.45	0.13±0.49
BMI SDS 1st year	1.58±0.54	**0.26±0.66***	1.82±0.22	**0.36±0.36†**
BMI SDS 2nd year	1.47±0.56	**0.28±0.65+**	1.89±0.24	**0.63±0.43‡**

CPP: Central precocious puberty, BMI: Body mass index, SDS: Standard deviation score, tb: Gonadotropin-releasing hormone agonists treatment onset, *p=0.008: First year BMI SDS change compared to the beginning of treatment; †p=0.011: Second year BMI SDS change compared to the beginning of treatment; †p=0.048: First year BMI SDS change compared to the beginning of treatment; ‡p=0.03: Second year BMI SDS change compared to the beginning of treatment

Table V. Height standard deviation score and predicted adult height values of patients in the follow-up before and after gonadotropin-releasing hormone agonists treatment

	Idiopathic CPP, (n=92)	Organic CPP (n=22)	p value‡
Height SDS tb	1.05±1.03	0.92±1.04	0.572
Height SDS 1st year	1.01±0.98	1.02±1.05	0.427
Height SDS 2nd year	**0.74±1.06***	1.06±0.87	0.314
Height SDS tb compared to KY	-1.01±0.95	-1.19±0.82	0.420
Height SDS 1st year compared to KY	-0.83±0.85	-0.87±0.85	0.250
Height SDS 2nd year compared to KY	**-0.68±0.72†**	**-0.69±0.68+**	0.400
PAH tb (cm)	158.7±6.5	159.9±6.1	0.434
PAH 1st year (cm)	162.3±6.1	161±5.3	0.505
PAH 2nd year (cm)	164.4±6.6	164.7±4.6	0.066
ΔPAH 2nd year-tb (cm)	5.7	4.8	0.150

CPP: Central precocious puberty SDS: Standard deviation score, tb: Gonadotropin-releasing hormone agonists treatment beginning, PAH: Predicted adult height, ΔPAH: PAH difference; *p=0.024: Second year height SDS difference compared to the beginning of treatment, †p=0.03: Height SDS according to 2nd year KY compared to the beginning of treatment, +p=0.025: Height SDS according to 2nd year KY compared to the beginning of treatment, ‡difference between two groups

hamartoma (HH) (n=3), arachnoid cyst (n=5), porencephalic cyst (n=3), hydrocephalus/ventriculo-peritoneal shunt (n=5), periventricular leukomalacia-hypoxic ischemic encephalopathy sequelae (n=3), neuroepithelial cyst (n=1), and cerebral cortical atrophy (n=1).

Discussion

This study investigated the effect of GnRHa treatment on body weight and height in female patients suffering from central PP for both idiopathic and organic reasons.

The present study revealed that GnRHa treatment in patients with CPP caused an increase in BMI SDS, that patients with organic CPP were more prone to the increase in weight and BMI, and the patients had height gain after undergoing the GnRHa treatment.

In the literature, the results of studies evaluating the effects of GnRHa treatment on body weight and BMI are controversial and incompatible with each other. There are studies reporting that GnRHa treatment is linked with body weight, BMI and BMI SDS increases in patients with CPP (4,6,9,10,25-29), whereas with other studies, GnRHa treatment is not linked with them (3,5,9,11,12,30-33), and even GnRHa treatment decreases BMI (12,13). The reason for the inconsistency between studies is not clear. Possible causes may include different designs of studies, heterogeneous etiology including idiopathic and organic etiology, different gender and age ranges, different body weights at the beginning of GnRHa treatment, different treatment strategies, and different follow-up intervals. In a recent study conducted in Spain to evaluate BMI SDS of 333 patients with CPP who received GnRHa treatment, a significant increase was determined in BMI SDS during treatment and this increase was reported to continue after the interruption of the GnRHa treatment and reaching adult height (10). Similarly, in the present study, the average BMI SDS of all our patients who received GnRHa treatment increased both in the 1st and 2nd years compared to the beginning of treatment and in the 1st and 2nd years compared to the 6th month (Table II). These results suggest that GnRHa treatment is associated with an increase in BMI and weight gain. The mechanism of GnRHa treatment causing an increase in body weight and BMI is not exactly known. There is a need for further studies on this subject that will explain the mechanism and evaluate adipokine levels involved in the energy-gonad axis such as leptin, neuropeptide Y, insulin and ghrelin.

In the present study, 1st year BMI SDS in the idiopathic CPP group and 2nd year BMI SDS in the organic CPP group showed a significant increase compared to the beginning of treatment. In the organic CPP group, the 2nd year average BMI SDS values were higher than the idiopathic CPP group (0.66±0.84 and 1.35±0.72, p=0.007) (Table III). These results revealed that GnRHa treatment caused an increase in weight and BMI in patients with both idiopathic CPP and organic CPP and the weight gain and prevalence of overweight-obesity were higher in the organic CPP group compared to the idiopathic CPP group. In the literature, there are a limited number of studies comparing the effects of GnRHa treatment on body weight and BMI in idiopathic and organic CPP groups. Feuillan et al. (26) reported in their study conducted with 18 patients with CPP caused by hypothalamic hamartoma HH-CPP and 32 patients with idiopathic CPP who received GnRHa treatment that BMI SDS was higher in patients with HH-CPP compared to the idiopathic CPP group at the beginning of the treatment, termination of the treatment and during the follow-up period after the treatment. In another study conducted to evaluate the patients with HH-CPP who received GnRHa treatment, the prevalence of overweight and obesity was reported to be high in female patients with HH-CPP (34). The structural central disorder causing organic pathology in patients with organic CPP is likely to cause more weight gain by causing changes in the neuronal network of the central nervous system

associated with obesity and in neurotransmitters. There is a need for more related studies which include more organic CPP and control groups.

In addition to the studies reporting that the increase in BMI SDS and obesity prevalence for patients receiving GnRHa treatment is observed in those children who were overweight before the treatment (5,35), there are also other studies reporting that the patients who had normal weight at the beginning of treatment had more weight gain with GnRHa treatment compared to the overweight-obese patients (11,36,37). In the present study, a significant increase was observed in BMI SDS in both the 1st year and 2nd year of the treatment in patients who had normal weight at the beginning of treatment in both groups (Table IV). No significant change was observed in BMI SDS during the treatment period in those patients who were overweight-obese at the beginning of treatment in both groups. The present study showed that patients who had a normal weight at the beginning of treatment had the tendency to have more weight gain during the treatment and the weight at the beginning of the treatment affected the weight gain associated with GnRHa treatment. The fact that the patients who were overweight and obese at the beginning of the treatment, and their parents, were more susceptible to the possible weight gain that could develop with the treatment and so had the tendency to take measures such as diet, physical activity, and sleep regulation to prevent obesity is believed to contribute to the lower weight gain in this group.

According to the Cosi-Tur 2016 study conducted by the Ministry of Health (38), it was found that the prevalence of overweight and obesity was 24.2% in girls who were aged between 6-9 years in Turkey, the prevalence of overweight and obesity was higher with the rate of 34% in the idiopathic CPP group and 45% in the organic CPP group compared to the reference age group in our patients who were in a similar age group at the beginning of their treatment (Figure 1). Similar to the results of the present study, Anık et al. (35) reported that the overweight prevalence and obesity prevalence in patients with PP before GnRHa treatment were higher than the average of the population with rates of 37.5% and 21.9%, respectively. The high obesity prevalence determined at the beginning of GnRHa treatment in the present study shows a correlation between the obesity and PP.

In the literature, the results reporting on the prevalence of overweight-obesity in the follow-ups of GnRHa treatment in patients with CPP are contradictory similar to BMI and BMI SDS results (11,12,35). In the present study, the rate of overweight-obese patients in the idiopathic CPP group which was 34% before the treatment, increased by 42.2% in the 1st year and by 38% in the 2nd year but it was not found to be statistically significant. In the organic CPP group, the prevalence of overweight-obese patients showed a significant increase at the beginning, the 1st year, and the 2nd year of the treatment with 45%, 59% and 64%, respectively. The rate of overweight-obese patients was found to be higher in the 1st and 2nd year of the treatment in the organic CPP group compared to the idiopathic CPP group (Figure 1). Especially cases with organic CPP receiving GnRHa treatment should be monitored more carefully in terms of weight gain and risk factors that may contribute to weight gain should be eliminated during the follow-up.

In the present study, while height SDS did not change in the idiopathic and organic CPP groups in the 1st year of GnRHa treatment, it decreased in the 2nd year of treatment in the idiopathic group compared to the basal. In both groups, an increase was observed in height SDS according to bone age (Table V). Similarly, Weise et al. (39) reported that height SDS decreased according to age and height SDS increased according to bone age in 100 female patients with CPP receiving GnRHa treatment. Slowness in the rapid height increase, decrease in height SDS according to age and increase in height SDS according to bone age as a result of the rapid bone maturation stopping with GnRHa treatment are expected results and reflect the positive effect of this treatment on the height prognosis.

There are no randomized controlled studies evaluating the efficacy of GnRHa treatment in terms of height gain and most studies are conducted by comparing final height and the height before PAH treatment (4). Klein et al. (40) showed that a gain in adult height was achieved with basal PAH 149.3±9.6 cm, final height 159.8±7.6 cm compared to pre-treatment PAH in patients with PP receiving a 2-year GnRHa treatment and the height prognosis was affected positively by treatment. In the present study, an average of 5.7 cm height gain in the idiopathic CPP group and 4.8 cm height gain in the organic CPP group were obtained with a 2-year GnRHa treatment, which is compatible with the literature (Table V). It was thought that GnRHa treatment was effective in terms of height gain in patients with CPP with the height SDS and PAH increase according to bone age.

Study Limitations

The limitations of the present study are that the study was retrospective, the number of patients was low especially in the organic CPP group, there was no control group, it included a relatively short duration of treatment, and there was a lack of follow-up after the completion of the treatment. There is a need for prospective studies which also include serum adipokine levels for the interpretation of

weight changes associated with GnRHa treatment including more patients and control groups.

Conclusion

The prevalence of obesity was higher in patients with CPP during the admission, and obesity is a risk factor for PP. BMI SDS increases with GnRHa treatment in CPP. Patients with organic CPP are more prone to weight and BMI increase. Patients with CPP receiving GnRHa treatment should be followed up for weight gain and obesity development and necessary precautions should be taken.

Ethics

Ethics Committee Approval: The study was approved by the Necmettin Erbakan University Faculty of Medicine Local Ethics Committee (approval number: 2017/1100).

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Original Article

An Evaluation of Platelet Parameters and Neutrophil/Lymphocyte Ratios in Children with Acute Rheumatic Fever

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ABSTRACT

Aim: Acute rheumatic fever (ARF) is an inflammatory disease developing as a response to group A streptococcal infection. Platelet parameters and neutrophil/lymphocyte ratios (NLRs) have been used as markers of inflammation severity in various inflammatory diseases in recent years. The purpose of this study was to evaluate platelet parameters and NLRs of patients with a diagnosis of ARF under monitoring by our clinic, and to compare these with a healthy control group.

Materials and Methods: Fifty patients diagnosed with ARF (37 with carditis and 13 without carditis) and 50 age- and sex-matched healthy children were included in the study. The subjects' demographic characteristics, complete blood count values, acute phase reactants, and transthoracic echocardiography findings were recorded.

Results: NLR, leukocyte, neutrophil, and platelet numbers were statistically significantly higher in the ARF group than in the control group ($p<0.001$), while hemoglobin, lymphocyte, and mean platelet volume (MPV) values were significantly lower ($p<0.001$). No statistically significant difference in MPV, plateletcrit (PCT) or NLR values was observed between the ARF subgroups with or without carditis. Platelet distribution width (PDW) was significantly higher in those ARF patients with carditis ($p=0.003$). Correlation analysis revealed that platelet count was positively correlated with leukocyte and neutrophil numbers, MPV was negatively correlated with leukocyte numbers, and PCT was significantly positively correlated with leukocyte and neutrophil numbers. PDW exhibited negative correlation with lymphocyte count and positive correlation with NLR.

Conclusion: MPV values were significantly lower and NLR values significantly higher in patients with ARF. Thus, it is thought that these parameters can be used as markers in patients diagnosed with ARF.

Keywords: Acute rheumatic fever, neutrophil/lymphocyte ratio, mean platelet volume, platelet distribution width, platelet

Introduction

Acute rheumatic fever (ARF) is an inflammatory disease resulting from an autoimmune response to group A beta haemolytic streptococcal infection in sensitive individuals. It is particularly important due to being capable of leading to rheumatic heart disease associated with high morbidity and mortality (1-4). Cytokines produced by lymphocytes and macrophages that are differentiated following an antigenic stimulus play an important role in the triggering of immunological and inflammatory reactions and therefore in the pathogenesis of ARF (5-7).

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New findings have shown that platelets are not only involved in haemostasis, but also constitute an important component of the inflammatory response (8,9). Chemokines, cytokines and other inflammatory mediators are released by activated platelets (10). Mean platelet volume (MPV) indicates platelet size and the rate of platelet production in bone marrow, and can be used as a marker of severity of inflammation and platelet activation (6,8,11-13). Platelet distribution width (PDW), another marker of platelet activation, indicates variation in platelet dimensions (14,15). Plateletcrit (PCT) provides more comprehensive information about the total thrombocyte mass (16). It is similar to the red cell haematocrit and indicates the percentage of the blood produced by the platelets (17,18). The neutrophil/lymphocyte ratio (NLR), which is easily calculated from blood count parameters, has also become increasingly important as an inflammation marker in recent years (19-24). Although C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), widespread and classic markers of inflammation for many years, continue to be valuable, modern studies suggest that MPV and NLR are also promising as novel acute phase reactants (21,23).

Some studies have examined MPV and NLR together in various disease groups with an inflammatory pathogenesis, including Familial Mediterranean Fever (19), Behçet's disease (20), Henoch-Schönlein purpura (24), rheumatoid arthritis (RA) (25), and systemic lupus erythematosus (26), but there have been no studies on the subject involving ARF, another inflammatory disease. The purpose of this study was to evaluate platelet indices and NLR in children diagnosed with ARF and to reveal the relation between these parameters and the disease.

Materials and Methods

Patients

Fifty patients, 26 boys and 24 girls, with a mean age of 10.7±2.4 years with patient files opened following inpatient treatment for ARF between 2010 and 2014 were retrospectively included in the study. Fifty healthy children, 27 boys and 23 girls, with a mean age of 10.8±2.8 years presenting to our outpatient clinic for routine monitoring were enrolled as the control group. This study was approved by the University of Health Sciences, Haseki Training and Research Hospital Ethics Committee (approval number: 137, date: 20.08.2014). All of the parents gave their informed consent prior to their inclusion in the study.

ARF was diagnosed on the basis of modified Jones criteria (27). Patients with chronic kidney disease, abnormal liver function, acute or chronic infection, haematological disease, chronic systemic inflammatory or autoimmune disease, malignity, or with a history of anti-inflammatory drug use within the previous one month were excluded from the study. These exclusion criteria were also applied to the control group.

Patient group files were scanned and acute phase reactant, and complete blood count (leukocyte, neutrophil, lymphocyte, NLR, hemoglobin, haematocrit, platelet, MPV, PDW, and PCT) values were recorded. Complete blood count (neutrophil, lymphocyte, NLR, hemoglobin, haematocrit, platelet, MPV, PDW, and PCT) values were also recorded for the control group.

The ARF group was also divided into two subgroups with or without carditis. Diagnosis of carditis was based on physical examination, auscultation and echocardiography (ECHO) findings.

Laboratory Analysis

Blood samples were collected (without stasis after morning fasting) from all participants and placed into tubes with gel, and tubes containing K2 EDTA (Becton Dickinson, UK) on the first day of admission. Complete blood counts were performed using an ABX Pentra DX 120 (Horiba Medical, Montpellier, France) haematology analyser. The NLR was calculated as a simple ratio between absolute neutrophil and absolute lymphocyte counts. Serum CRP levels were measured using an immune turbidimetric method with an AU-2700 autoanalyzer [(Beckman Coulter, United States of America (USA)]. An ESR automated analyser 120 device was used for ESR.

Transthoracic Echocardiography

Cardiac dimensions were measured by M-mode and two-dimensional ECHO. They were acquired on a General Electric (GE) Vivid S5 (GE Medical Systems, USA) ultrasound platform using an S3 curved-array transducer. Echo images were acquired from the third or fourth intercostal space according to the patient's age, and with the subject in the supine or left lateral decubitus position.

Statistical Analysis

Statistical analysis was performed on SPSS 15.0 for Windows software. Descriptive statistics were expressed as number and percentage for categorical variables, and as mean and standard deviation for numerical variables. Student's t-test was used to compare two independent groups if normal distribution was established, and the Mann-Whitney U test was used if normal distribution was not established. Comparisons between more than two independent groups were performed using ANOVA when normal distribution was established and with the Kruskal-Wallis test in the absence of normal distribution. Subgroup analyses were performed with the parametric Tukey test

and the non-parametric Mann-Whitney U test, and were interpreted with Bonferroni correction. Proportions of categorical variables between groups were tested using chi-square analysis. Relations between numerical variables were examined using Spearman correlation analysis since parametric test conditions were not established. Statistical alpha significance was set at $p<0.05$.

Results

There was no statistically significant difference between the groups in terms of sex or age (p=0.757, p=0.841, respectively). Leukocyte and neutrophil counts, NLR, and platelet count were significantly higher in the ARF group, while lymphocyte count and hemoglobin and MPV values were significantly lower (p=0.005 for lymphocytes, p<0.001 for the others). No significant difference was determined between the groups' mean PCT and PDW values (p=0.063, and p=0.133, respectively) (Table I).

Carditis was present in 37 (74%) of the children with ARF. PDW was significantly lower in the ARF patients with carditis than in the subjects without carditis (p=0.003). No statistically significant difference was determined in

Table I. A comparison of the demographic features and laboratory results of the acute rheumatic fever and control groups

Parameters	**ARF (n=50), n (%)**			**Controls (n=50), n (%)**			**p value**
Gender							
Male	26 (52.0)			27 (54.0)			0.841
Female	24 (48.0)			23 (46.0)			-
	Mean ± SD	**Minimum**	**Maximum**	**Mean ± SD**	**Minimum**	**Maximum**	**p value**
Age (years)	10.7±2.4	5	15	10.8±2.8	5	15	0.757
Leu (10^3/mm^3)	11.8±4.2	5.3	25	7.7±1.7	5.0	12.0	**<0.001**
Neu (10^3/mm^3)	8.2±3.7	2.5	20.3	4.2±1.5	1.9	9.0	**<0.001**
Lym (10^3/mm^3)	2.4±0.9	0.76	5.2	2.7±0.7	1.12	4.48	**0.005**
NLR	3.7±2.0	1.1	10.3	1.7±1.0	0.58	5.3	**<0.001**
Hgb (g/dL)	10.5±1.4	6.3	13.8	12.9±1.1	11.1	16.6	**<0.001**
Plt (10^3/mm^3)	419.2±123.0	218	775	333.9±86.5	132	523	**<0.001**
MPV (fL)	8.1±1.1	6.3	11.7	9.1±1.1	6.9	11.8	**<0.001**
PCT	0.33±0.08	0.16	0.59	0.30±0.07	0.14	0.49	0.063
PDW (fL)	14.9±4.4	0.1	38.6	15.7±0.3	15	16.6	0.133

ARF: Acute rheumatic fever, Leu: Leucocyte, Neu: Neutrophil, Lym: Lymphocyte, NLR: Neutrophil to lymphocyte ratio, Hgb: Hemoglobin, Plt: Platelet, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width; fL: Femtoliters, SD% Standard deviation

Table II. A comparison of cases of carditic and non-carditic acute rheumatic fever

	ARF without carditis, (n=37)	**ARF with carditis, (n=13)**	**p value**
	Mean ± SD	**Mean ± SD**	
Leucocyte (10^3/mm^3)	13.0±4.4	11.3±4.1	0.138
Neutr (10^3/mm^3)	8.8±3.5	8.0±3.8	0.419
Lymph (10^3/mm^3)	2.9±1.0	2.2±0.8	0.020
NLR	3.2±1.2	3.9±2.2	0.347
Hemoglobin (gr/dL)	10.9±1.3	10.4±1.4	0.335
Platelet (10^3/mm^3)	460.9±98.5	404.5±128.6	0.052
MPV (fL)	8.0±1.2	8.2±1.1	0.329
PCT	0.36±0.07	0.32±0.09	0.224
PDW (fL)	13.3±1.9	15.5±4.9	**0.003**

ARF: Acute rheumatic fever, SD: Standard deviation, Neutr: Neutrophil, Lymph: Lymphocyte, NLR: Neutrophil-lymphocyte ratio, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, fL: Femtoliters

terms of the other parameters, with the exception of PDW, between the subgroups with and without carditis (Table II).

A significant inverse correlation was determined in the ARF patients between MPV and platelet and leukocyte counts (rho=-0.469, p=0.001; rho=-0.306, p=0.031, respectively). PCT was significantly positively correlated with platelet, leukocyte and neutrophil counts (rho=0.793, p<0.001; rho=0.383, p=0.006; and rho=0.352, p=0.012, respectively) (Table III). PDW was significantly inversely correlated with lymphocyte count (rho=-0.344, p=0.014) (Table III) and significantly positively correlated with NLR (rho=0.389, p=0.005) (Table IV).

Discussion

Neutrophils, lymphocytes, and platelets occupy an important place in the control of inflammation. Ours is the first study to examine platelet indices and NLR together in children with ARF. Significantly lower MPV and higher NLR values in patients with ARF compared to the control group were determined.

Platelet indices are simple and practical parameters that can be easily investigated at routine complete blood count (8). When platelet numbers fall, platelet production in bone marrow increases, and the resulting new platelets are larger and more reactive, and MPV values are therefore higher (6).

MPV has been used as a simple marker of severity of inflammation in various studies, but the results have been inconsistent. Yazici et al. (28) reported an increase in MPV values in an active RA group compared to a control group. In contrast, Kisacik et al. (29) determined significantly lower MPV values in patients with active RA compared to patients with osteoarthritis and healthy controls. Kim and Kim (30)

Table III. Correlation between platelet indices and neutrophil-lymphocyte ratio, and with other parameters, in the acute rheumatic fever and control groups

	Platelet		MPV		PCT		PDW	
ARF	**rho**	**p value**	**rho**	**p value**	**rho**	**p value**	**rho**	**p value**
Leukocyte	0.508	**<0.001****	-0.306	0.031*	0.060	**0.006****	0.060	0.679
Neutrophil	0.454	**0.001***	-0.247	0.084	0.214	**0.012***	0.214	0.136
Lymphocyte	0.266	0.062	-0.292	0.039*	0.344	0.251	-0.344	**0.014***
Hemoglobin	-0.118	0.416	-0.020	0.131	0.893	0.192	0.131	0.364
ESR	0.164	0.255	-0.079	-0.007	0.115	0.426	-0.007	0.959
CRP	0.052	0.721	0.189	-0.129	0.155	0.283	-0.129	0.372

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, ARF: Acute rheumatic fever, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein

Table IV. Correlation between neutrophil to lymphocyte rate and platelet indices, and with other parameters, in the acute rheumatic fever group

Neutrophil to lymphocyte rate n=50		
ARF group Parameters	**rho**	**p value**
Leucocyte	0.517	**<0.001****
Neutrophil	0.739	**0.001****
Lymphocyte	-0.583	**0.001****
Hemoglobin	0.019	0.895
Platelet	0.147	0.309
MPV	0.020	0.890
PCT	0.174	0.228
PDW	0.389	**0.005****
ESR	0.149	0.302
CRP	0.117	0.417

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). ARF: Acute rheumatic fever, MPV: Mean platelet volume, PDW: Platelet distribution width, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein

also reported lower MPV in their active RA group compared to the control group, and showed that MPV increased after treatment compared to pre-treatment values. Studies have also determined that MPV increases in low-grade inflammatory conditions such as myocardial infarction, atherothrombotic diseases and arteriovenous thrombosis (10,31), and that it decreases in high-grade inflammatory conditions (6,29).

Very few studies have investigated platelet parameters in patients with ARF, an inflammatory disease. Sert et al. (13) investigated 40 children with ARF (32 with carditis) and 40 healthy children. MPV values were lower in the acute period in those children with ARF compared to the healthy control group, and also MPV values were inversely correlated with WBC, ESR, and platelet count before ARF treatment. In contrast, another study of 53 patients diagnosed with acute rheumatic carditis and 53 control subjects determined no significant variation in MPV and PDW values between the patient and control groups (6). In addition, no significant difference was observed with the comparison of MPV and PDW values before and after treatment in the patient group with acute carditis (6). Another study determined high MPV values in children with rheumatic heart disease (1). However, that study was not performed in the acute phase.

In agreement with Sert et al. (13), MPV values in our study were significantly lower in the ARF group than in the control group ($p<0.001$). However, although PDW values were lower than in the control group, the difference was not statistically significant. Comparison of the ARF groups with and without carditis revealed no significant difference in terms of MPV values, while PDW was significantly lower in the ARF patients without carditis than in the ARF group with carditis.

Various mechanisms may be responsible for the low MPV values in our ARF patients. Blood mononuclear cell cultures from rheumatic children have been reported to produce more tumor necrosis factor-α (TNF-α) than those from a control group (32). Together with Interleukin (IL)-6 and IL-8, TNF-α is thought to play a pathogenic role in rheumatic fever (33). In addition, significant elevation of IL-1 levels has been determined in patients with ARF and rheumatic heart (34). Another study described IL-1α as a minor criterion for the diagnosis of carditis and IL-6 for arthritis (35). Decreased MPV values in conditions in which inflammatory markers increase, such as the active stage of ARF, can indicate the severity of the inflammatory process. Overproduction of cytokines such as IL-6 and acute phase reactants can suppress the size of platelets released from bone marrow by affecting megakaryopoiesis (6,9). Previous studies have shown that IL-6 causes a decrease in MPV values in addition to an increase in platelet numbers (36,37). Serum IL-6 levels have been shown to increase significantly during ARF attacks (5). Low MPV values during ARF attacks can be attributed to IL-6 due to its effect on platelets. This is important in terms of low MPV stimulating ARF activation.

Another explanation of low MPV in ARF may be associated with intensive consumption of large platelets in the area of inflammation. Large platelets are more active than small platelets in the release of various pro-inflammatory and thrombotic agents, and consumption of these increases during the acute phase of inflammation (13).

MPV was inversely correlated with leukocyte and platelet counts in our ARF patient group. These results suggest that MPV exhibits negative acute phase reactant characteristics.

PCT, which is another platelet parameter, did not differ between the groups. In the correlation analysis, PCT and platelet had a positive correlation with leucocyte and neutrophil. There is a limited number of studies about PCT. Ozturk et al. (17) showed that PCT values and platelet increased in infectious conditions accompanied by leukocytosis. As far as we are aware, there is no study examining PCT in children with ARF.

NLR is a novel inflammatory marker frequently used in clinical practice and it can easily be calculated from blood count parameters. Its associations with various diseases, particularly cancer and cardiovascular diseases, have begun to be investigated in recent years (18,19,20,38). The number of studies investigating the relation between rheumatic diseases and NLR is limited. One meta-analysis published in 2017 examined a total of 17 studies on the subject (six involving patients with ankylosing spondylitis, three involving RA, four involving Behçet's disease, and four involving SLE), and NLR increases were determined in patients with ankylosing spondylitis, RA and Behçet's disease. However, no association was observed between SLE and NLR (39). Several studies of patients with rheumatic mitral valve stenosis have reported NLR elevation (22,23). These studies have involved adult patients and the chronic phase of the disease. No previous studies have investigated NLR in children with ARF. NLR elevation in inflammatory diseases has been linked to neutrophilia and relative lymphopenia caused by increased cortisol (23). We also determined significantly high neutrophil and low lymphocyte counts in our ARF cases compared to the control group.

The principal limitations of our study are its retrospective nature and the fact that measurements were not repeated after treatment, meaning that the effectiveness of treatment could not be assessed. However, our study is nevertheless

important in being one of the rare investigations of the subject in children with ARF and in eliciting significant findings.

Conclusion

MPV values were significantly low and NLR were significantly high in patients with ARF. Therefore, it is thought that these parameters can be used as markers in the early evaluation of patients with suspected ARF. Further, wide-scale, prospective studies are now needed for a definitive conclusion to be drawn.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences, Haseki Training and Research Hospital Ethics Committee (approval number: 137-20/14).

Informed Consent: All of the parents gave their informed consent prior to their inclusion in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.S.D., Design: N.S.D., Data Collection or Processing: A.A., M.E., Analysis or Interpretation: N.S.D., Literature Search: A.A., N.S.D., Writing: N.S.D.

Conflict of Interest: None of the authors had conflict of interest.

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Original Article

Oral Bacteria of Children with Turner Syndrome

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ABSTRACT

Aim: Turner syndrome (TS) is a genetic disorder caused by a numerical or structural aberration of the X chromosome, which is associated with a female phenotype. Concerning oral status, several studies have revealed that girls with TS have dental anomalies and periodontal problems. The aim of this study was to evaluate the effects of oral bacteria on caries prevalence and periodontal status in pediatric patients with TS.

Materials and Methods: Twenty TS patients and 17 healthy girls were examined for cariological and periodontal status. The levels of mutans streptococci (MS), lactobacilli (LB), yeast and 10 different periodontal bacteria were determined by using culture and microarray techniques in children's stimulated saliva samples.

Results: There was no difference in salivary flow rate and buffering capacity, decayed-missing-filled teeth, MS, LB, or yeast levels between the groups. Plaque index and gingival index levels were significantly higher in the Turner group and dft was significantly higher in the control group ($p<0.05$). As a result, microarray analysis, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens*, *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus* were detected at high levels in the Turner group ($p<0.05$).

Conclusion: Besides dental and craniofacial anomalies, clinicians should be alert to the early diagnosis and treatment of periodontal problems in patients with TS.

Keywords: Turner syndrome, microarray analysis, oral bacteria

Introduction

Turner syndrome (TS) is one of the most common chromosomal disorders characterized by typical findings, such as pubertal problems and internal organ anomalies, accompanied by a total or partial loss of an X chromosome (1).

Dental anomalies and orthodontic disorders affecting the maxilla and mandible have been frequently mentioned in TS patients (1-3). Researchers have reported an increased incidence of gingivitis in patients with TS, as well as higher plaque index (PI) and gingival index (GI). Disorders of growth hormone and sex hormones in these patients are thought to increase the susceptibility to gingivitis and periodontitis (3,4). In several other studies, the relationship between changes in sex hormones and gingival diseases has been extensively reported, and the variable effects of oral microbiota due to hormone changes have been reported (5,6).

Besides traditional microbiological studies such as medium, planting, culture and microscopy; molecular genetic studies have also determined the levels of specific species to understand the oral microbiota. Molecular studies in line with new technological possibilities have determined that complex bacterial communities are

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significant in tooth caries and gingival diseases associated with biofilm (7,8).

The aim of this study was to evaluate the oral and dental health of children with TS by determining the bacterial levels related to tooth decay and periodontal diseases.

Materials and Methods

Study Population

The study included 20 children with TS aged between 6-18 years and 17 girls aged between 4-18 who were not mentally or physically suffering, followed by the Department of the Pediatric Endocrinology, Department of Child Health and Diseases of the İstanbul Medical Faculty. Mothers and their children, who agreed to participate in the study, were referred to the clinics of the Pediatric Dentistry Department of İstanbul University Faculty of Dentistry. The aim of the study was explained to these children and parents verbally, and the parents signed an informed volunteer form. In the prepared patient information form, the child's age, general health status and medications used were questioned. For this study, ethics committee approval was obtained from the Ethics Committee of İstanbul University Medical Faculty (approval number: 2013/690).

Clinical Examination

In-mouth examinations of children were performed by the same physician in the reflector light of the dental unit in a sitting position using mirror and sond. The oral and dental health status was assessed according to the recommendations of the World Health Organization by using the decayed-missing-filled teeth (DMFT)-decayed-missing-filled surface (DMFS) / milk teeth: dft, dfs) index (9), PI (10) and GI (10) scores. The periodontal index calculation was removed from the study because alveolar bone loss and periodontal pocket formation were not observed in any of the children participating in the study.

Microbiological Sampling

Patients who underwent an oral examination were directed to the Oral Microbiology Laboratory of İstanbul University Faculty of Dentistry, Department of Basic Medical Sciences for microbiological examination. Saliva samples stimulated by means of sugar-free chewing were taken from all children in the patient and control groups. The patient's saliva-buffering capacities (S-BC) and salivary-flow rates (S-FR) were determined.

Culture

The saliva samples were 10-fold diluted and then samples of 0.1 mL were plated on Mitis Salivarius Bacitracin agar (MSB) (Acumedia Man Inc., Baltimore, Maryland) for mutans streptococci (MS); on Rogosa Agar (Merck, KgaE, Damstadt, Germany) for lactobacilli (LB) and on Sabouraud Dextrose Agar (Merck) for yeast counts. MSB Agar and Rogosa Agar plates were incubated in air supplemented with 5-7% CO_2, while Sabouroud Dextrose Agar plates were incubated aerobically. The samples were incubated for 48 hours at 37 °C. The typical colonies were counted and calculated (cfu/mL). High detection level was; $\geq 10^5$ cfu/mL for MS, $\geq 10^4$ cfu/mL for LB and $\geq 10^2$ cfu/mL for yeasts; whose detection limits were 10^3, 10^2 and 10 cfu/mL respectively (11).

Microarray

The remaining 0.5 mL saliva sample was separated for DNA extraction and stored at -20 °C. DNA extraction from the stored samples was performed according to the protocol of a ready kit (High Pure polymerase chain reaction (PCR) Template Preparation Kit, Roche, Mannheim, Germany). The obtained DNA samples were stored at -20 °C. For PCR amplification and hybridization, 10 periodontal bacteria were analyzed in accordance with the instructions of the manufacturer using a specially engineered 16S rRNA microarray system (ParoCheck10®, Greiner Bio-One GmbH, Frickenhausen, Germany). The results were directly created using a scanner (CheckScanner™, Greiner Bio-One GmbH) and operated by the ParoReport software (ParoCheck® Kit, Gene Pix®, Axon Instruments Inc.). It produces a semi-quantitative labelling scheme. The results were classified according to signal levels: no detection, low, moderate, high and very high. The 10 species identified by the ParoCheck10® microarray detection system were as follows: The red complex: *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*; the orange complex: *Campylobacter rectus*, *Fusobacterium nucleatum*, *Parvimonas micra* and *Prevotella intermedia*; the green complex: *Aggregatibacter actinomycetemcomitans* and *Eikenella corrodens*; the blue complex: *Actinomyces viscosus* (12).

Statistical Analysis

The data were analyzed using the IBM SPSS statistics 22 (IBM SPSS, TURKEY) software. Shapiro-Wilk test was used for normality of data analysis. Student's t-test was performed for the analysis of groups according to age, S-FR and buffering capacity, DMFT and dft levels ($p<0.05$). Mann-Whitney U test was performed to compare results for both groups for the PI and GI value ($p<0.05$). Mann-Whitney U test was used to assess the presence and levels of bacterial species in samples according to periodontal bacterial associations ($p<0.05$). The bacterial relationship among the groups based on the detection of the target bacteria in each subject was

determined by Spearman's (rho) Correlation Coefficient, irrespective of their proportional recovery in samples.

Results

There was no significant difference in age distribution among the children in the groups. Considering the parameters related to salivary, the groups showed no significant difference for the S-FR (p=0.45) and S-BC (p=0.37) (p>0.05). PI (p=0.01) and GI (p=0.03) were significantly higher in the Turner group and dft was significantly higher in the control group (p<0.05) (Table I).

All the children in the Turner group were taking growth hormone. Some patients (n=9) were taking estrogen in addition to growth hormone. However, there was no significant relationship between the drugs used and PI/GI levels (p>0.05).

As a result of the culture examination, there was no significant difference between groups in terms of MS (p=0.14), LB (p=1.00) and yeast (p=0.19) levels (p>0.05) (Table II).

Figure 1 shows the prevalence and target levels in different complexes determined by the ParoCheck10® study:

- Red complex bacteria were seen at low rates in all groups.
- Orange complex bacteria *P. intermedia* was found to be significantly higher in the Turner group. *F. nucleatum* was frequently detected in both groups and was significantly higher in the Turner group.
- Green complex bacterium *E. corrodens* was found to be significantly higher in the Turner group, although it was frequently detected in both groups. *A. actinomycetemcomitans* was significantly higher in the Turner group.
- Blue complex bacterium *A. viscosus* was found to be significantly higher in the Turner group.
- Orange complex bacteria *C. rectus* was not detected in the Turner group. (p<0.05) (Figure 1)

Table III shows the results of the correlation analysis between bacteria:

Table I. Clinical examination findings

	Control (n=17)	Turner (n=20)	
	Mean ± SEM	Mean ± SEM	p value
Age	12.47±3.64	13.65±3.30	0.291
S-FR	0.66±0.49	0.77±0.49	0.459
S-BC	5.00±0.57	5.16±0.50	0.370
PI[b]	0.46±0.60	0.96±0.70	0.019*
GI[b]	0.17±0.39	0.47±0.59	0.033*
DMFT	2.41±1.77	2.10±2.27	0.411
dft	2.24±2.02	0.30±0.73	0.000*

Student's t-test, b: Mann-Whitney U test *p<0.05. SEM: Standard error of the mean, S-FR: Salivary flow rates, S-BC: Saliva buffering capacities, PI: Plaque index, GI: Gingival index, DMFT: Decayed-missing-filled teeth

Table II. Distribution of caries-inducing microorganism levels in TS and control group

		Control	Turner	p value
		n (%)	n (%)	
MS	Low	1 (5.9%)	4 (20.0%)	0.149
	Medium	5 (29.4%)	8 (40.0%)	
	High	11 (64.7%)	8 (40.0%)	
LB	Low	5 (29.4%)	6 (30.0%)	1.000
	Medium	7 (41.2%)	8 (40.0%)	
	High	5 (29.4%)	6 (30.0%)	
Yeasts	Low	10 (58.8%)	17 (85.0%)	0.98
	Medium	6 (35.3%)	2 (10.0%)	
	High	1 (5.9%)	1 (5.0%)	

MS: Mutans streptococci, LB: Lactobacilli, Mann-Whitney U test *p<0.05

- In the Turner group, a positive correlation between *P. gingivalis* and *T. denticola* (p=0.768) was observed in red complex bacteria.

- In both groups, a positive correlation was found between *T. denticola* from red complex bacteria with *P. intermedia* (control p=0.483, Turner p=0.584), and *P. micra* (control p=0.654, Turner p=0.684) from orange complex bacteria.

- A positive correlation between *F. nucleatum* with *P. intermedia* (p=0.547) and *E. corrodens* (p=0.638) was observed in the control group.

- There was also a positive correlation between *P. intermedia* and *E. corrodens* (p=0.643) in the control group.

- *A. actinomycetemcomita*n was positively associated with *A. viscosus* (p=0.606) in the control group and *E. corrodens* (p=0.652) in the Turner group.

- A negative relationship between bacteria was observed but it was not statistically significant. (p<0.05) (Table III).

Table IV shows the relationship between the PI and GI values and bacteria:

- In the Turner group, all of the red complex bacteria and *P. intermedia* from orange complex bacteria were significantly correlated with the GI.

- *P. gingivalis* (p=0.490), *T. denticola* (p=0.593) and *P. intermedia* (p=0.701) were significantly correlated with PI in the Turner group (p<0.05) (Table IV).

Discussion

The first detailed study evaluating the mouth symptoms of patients with TS was performed by Flipsson et al. (13) in 1965. There are many unknowns in the craniofacial and dental findings as well as the diagnosis and treatment of this syndrome which has attracted many researchers to date.

Although the DMFT index in patients with TS was significantly lower than in control groups (3,14,15), López et al. (16) found a higher index of caries in the deciduous teeth. Kusiak et al. (14) has reported that these patients may have a higher concentration of S-BC and antibacterial factors such as immunoglobulin A, lactoferrin, lysozyme and a lower DMFT index may be due to salivary properties (3),

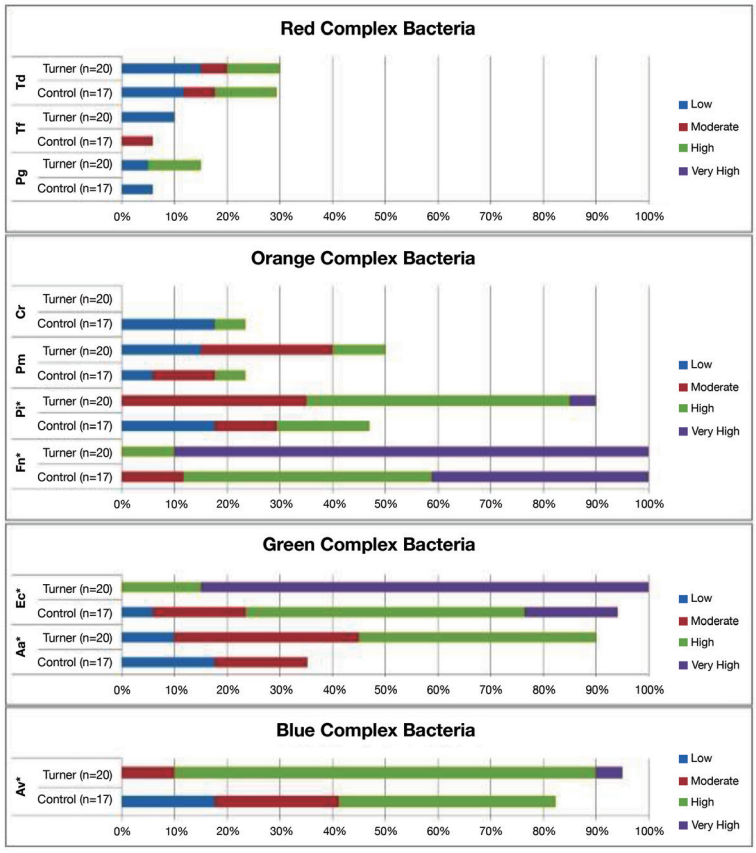

Figure 1. Presence and levels of bacterial species in samples according to periodontal bacterial associations
Mann-Whitney U test *p<0.05
Td: Treponema denticola, Tf: Tannerella forsythia, Pg: Porphyromonas gingivalis, Cr: Campylobacter rectus, Pm: Parvimonas micra, Pi: Prevotella intermedia, Fn: Fusobacterium nucleatum, Ec: Eikenella corrodens, Aa: Aggregatibacter actinomycetemcomitans Av: Actinomyces viscosus

Table III. Relations between the bacteria in the sample

		MS	LB	Yeats	Pg	Tf	Td	Fn	Pi	Pm	Cr	Aa	Ec	Av
MS	Control	1.000*	0.093	0.384	-0.304	-0.304	-0.277	-0.241	0.000	-0.453	0.150	-0.491*	-0.162	-0.425
	Turner	1.000	0.722*	0.468*	-0.460*	-0.124	-0.475*	-0.373	-0.120	-0.541*		0.193	0.104	0.520*
LB	Control		1.000	0.288	0.000	-0.326	-0.331	0.430	0.102	0.000	-0.211	0.000	0.563*	0.386
	Turner		1.000	0.541*	-0.351	-0.215	-0.194	0.000	0.178	-0.260		0.271	0.000	0.425
Yeats	Control			1.000	0.264	-0.206	-0.003	-0.151	-0.043	0.065	-0.209	-0.141	-0.078	-0.408
	Turner			1.000	-0.175	-0.140	-0.269	0.140	-0.144	-0.131		0.232	0.176	0.104
Pg	Control				1.000	-0.063	0.381	0.281	0.389	0.343	-0.138	0.421	0.056	0.268
	Turner				1.000	-0.349	0.768*	0.140	0.318	0.610*		0.242	0.176	-0.177
Tf	Control					1.000	0.476	-0.140	0.250	0.446	0.413	0.421	0.056	-0.027
	Turner					1.000	0.214	0.111	0.253	0.016		0.093	0.140	0.083
Td	Control						1.000	0.034	0.483*	0.654*	-0.021	0.382	0.141	-0.003
	Turner						1.000	0.214	0.584*	0.684*		0.220	0.270	-0.092
Fn	Control							1.000	0.547*	0.113	-0.091	0.238	0.638*	0.477
	Turner							1.000	0.016	0.312		-0.093	-0.140	-0.083
Pi	Control								1.000	0.263	-0.072	0.293	0.643*	0.081
	Turner								1.000	0.215		-0.046	-0.093	-0.008
Pm	Control									1.000	0.000	0.206	0.122	0.011
	Turner									1.000		0.125	0.223	-0.219
Cr	Control										1.000	0.351	-0.202	0.246
	Turner													
Aa	Control											1.000	0.349	0.606*
	Turner											1.000	0.652*	0.320
Ec	Control												1.000	0.421
	Turner												1.000	0.278
Av	Control													1.000
	Turner													1.000

Spearman's (rho) Correlation Coefficient *p<0.05 *Correlation is significant at the 0.05 level. MS: Mutans streptococci, LB: Lactobacilli, Pg: *Porphyromonas gingivalis* Tf: *Tannerella forsythia* Td: *Treponema denticola*, Fn: *Fusobacterium nucleatum*, Pi: *Prevotella intermedia*, Pm: *Parvimonas micra*, Cr: *Campylobacter rectus*, Aa: A*ggregatibacter actinomycetemcomitans*, Ec: *Eikenella corrodens*, Av: *Actinomyces viscosus*

although the S-FR is slower. In this study, the dft value was significantly higher in the control group ($p<0.05$), although there was no significant difference between the TS and the DMFT values determined in the control group. When the parameters related to saliva were examined, there was no significant difference between groups in terms of S-FR and S-BC ($p<0.05$).

In patients with TS, Ogiuchi et al. (4) found that the rate of incidence of gingivitis increased, and Szilágyi et al. (3) found that PI and GI were significantly high. Väisänen et al. (17) reported that the GI and calcular index values of these patients were lower and their periodontal health was better. In this study, PI and GI Turner groups were found to be significantly higher, but no relationship was found between growth hormone and/or estrogen use and PI and GI indexes ($p<0.05$). It is thought that gingival problems in TS patients are not due to imbalances in growth hormone and estrogen levels but are a consequence of oral ecosystem damage due to a lack of oral hygiene (4), even if changes in sex hormones increase gingivitis and periodontitis susceptibility (6).

In this study, the intra-oral findings of children with TS who were seldom examined in terms of child dentistry were examined and the effect of oral microbiology and hormones on dental and periodontal tissues were evaluated. In this study, no significant difference was found between groups

in terms of caries-causing microorganisms MS, LB and yeast levels ($p<0.05$) (Table II).

In this study, the presence and levels of periodontal bacteria in the groups were determined using the semi-quantitative microarray system ParoCheck10®, which allows for the rapid and reliable detection of 10 different periodontal pathogens (9).

Periodontal diseases are bacterial infections related to the complex microbiosis of tooth biofilm, mainly composed of anaerobic gram-negative species. Although *A. actinomycetemcomitans* and *P. gingivalis* are the most common periodontal pathogens (18), Socransky et al. (19) have identified five consecutive microbial complexes in the subgingival biofilm of individuals with or without periodontal disease, demonstrating that periodontal diseases are due to the co-operation of periodontal microorganisms rather than individual pathogens. In particular, red complex bacteria formed by *P. gingivalis*, *T. denticola* and *T. forsythia* have been reported to show a strong association with periodontal disease and each other.

Table IV. Relation to plaque index and gingival index of bacterial entities

		PI	GI
MS	Control	-0.113	0.086
	Turner	-0.380	-0.509*
LB	Control	-0.392	-0.400
	Turner	0.022	-0.151
Yeats	Control	-0.169	-0.224
	Turner	0.060	-0.272
Pg	Control	0.160	-0.137
	Turner	0.490*	0.551*
Tf	Control	0.426	0.549*
	Turner	0.377	0.464*
Td	Control	0.299	0.061
	Turner	0.593*	0.657*
Fn	Control	-0.429	-0.444
	Turner	0.363	0.359
Pi	Control	-0.075	-0.022
	Turner	0.701*	0.659*
Pm	Control	-0.012	0.075
	Turner	0.341	0.338
Cr	Control	0.587	0.721
	Turner	-	-
Aa	Control	0.381	0.196
	Turner	-0.107	-0.160
Ec	Control	-0.278	-0.384
	Turner	-0.207	-0.277
Av	Control	0.218	-0.085
	Turner	-0.384	-0.230

PI: Plaque index GI: Gingival index, MS: Mutans streptococci, LB: Lactobacilli, Pg: *Porphyromonas gingivalis*, Tf: *Tannerella forsythia*, Td: *Treponema denticola*, Fn: *Fusobacterium nucleatum*, Pi: *Prevotella intermedia*, Pm: *Parvimonas micra*, Cr: *Campylobacter rectus*, Aa: *Aggregatibacter actinomycetemcomitans*, Ec: *Eikenella corrodens*, Av: *Actinomyces viscosus*, Spearman's (rho) Correlation Coefficient *p<0.05

Griffen et al. (20) found that *P. gingivalis* and *T. denticola* were related to the disease, and that different species such as Spirochetes and Filifactor alocis should be investigated in relation to periodontitis. da Silva-Boghossian et al. (21) reported that red complex bacteria and *A. actinomycetemcomitans* were highly associated with periodontal disease. Topcuoglu and Kulekci (12) examined different types of periodontitis patients with ParoCheck10® and found that red complex bacteria were common, and that these pathogens were associated with periodontitis.

In this study, red complex bacteria associated with periodontal disease were seen at low rates in all groups. We think that this result is related to the presence of only gingivitis in the children in this study. In this study, *P. intermedia* and *F. nucleatum* from orange complex bacteria, *A. corrodens* and *A. actinomycetemcomitans* from green complex bacteria and *A. viscosus*, which is more related to periodontal health, were significantly higher in the Turner group ($p<0.05$). Previous studies have shown that some of the disease-related species are also present in samples from healthy individuals and these bacteria, which normally become part of the oral microflora, increase pathogenicity with the degradation of the oral ecosystem (20,22). These results support complex bacterial associations with high gingivitis levels in the Turner group.

In this study, while the red complex bacteria was positively correlated between the each other in the Turner group, a positive correlation was found between the red and orange complex bacteria in both groups. There was also a positive correlation between green complex bacteria in the Turner group. These results support the relationships of bacterial complexes as described by Socransky et al. (19).

In the presented study, all of the red complex bacteria and *P. intermedia* from the orange complex bacteria were significantly associated with the GI ($p<0.05$). There was a significant correlation between the PI and periodontal pathogens such as *P. gingivalis*, *T. denticola*, *P. intermedia* in the Turner group ($p<0.05$). As a result, it should be kept in mind that anaerobic and gram (-) populations increase in

matured dental plaque biofilm and this ecology poses a risk for gingivitis and periodontitis (23).

Study Limitations

The research findings of this study were limited by the small number of subjects due to the fact that the children with TS were gathered from just one institution. Therefore, larger studies including more subjects from other institutions may be planned.

Conclusion

A higher incidence of *P. intermedia* and *F. nucleatum*, *A. corrodens*, *A. actinomycetemcomitans* and *A. viscosus* as well as higher PI and GI scores suggest an increased susceptibility to periodontal diseases in patients with TS.

Acknowledgment

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Ethics

Ethics Committee Approval: For this study, ethics committee approval was obtained from the Ethics Committee of İstanbul University Medical Faculty (approval number: 2013/690).

Informed Consent: Informed consent was obtained from the parents or guardians of all eligible children.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: O.A., G.K., G.Ü., N.T., Design: G.Ü., N.T., Data Collection or Processing: Ş.P., G.Ü., Y.G., Analysis or Interpretation: N.T., G.K., Literature Search: G.Ü., O.A., Y.G., Writing: G.Ü., N.T.

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

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Original Article

Diagnostic Value of the Mean Platelet Volume in the Prediction of Respiratory Syncytial Virus in Acute Bronchiolitis

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ABSTRACT

Aim: Respiratory syncytial virus (RSV) is a viral pathogen that causes lower respiratory system infections in childhood. The purpose of this study was to examine whether mean platelet volume (MPV) changes are significant in the prediction of RSV bronchiolitis.

Materials and Methods: One hundred and eighty-four infants who were diagnosed with acute bronchiolitis were divided into groups based on being RSV positive and other respiratory viruses positive. Using the receiver operating characteristic (ROC), diagnostic accuracy was evaluated according to the areas under the curves (AUC) for the diagnosis of bronchiolitis. A p value of <0.05 was considered statistically significant.

Results: MPV was significantly lower in patients with single RSV (6.6±1.1 vs 7±1.2, p<0.05). The MPVs were similar in patients diagnosed with positive and negative RSV bronchiolitis (6.8±1.5 vs 7±1.3, p>0.05) and other viruses. ROC curve analysis indicates that the MPV level cut-off point for making the diagnosis of single RSV bronchiolitis was 6.63 fL with a sensitivity and specificity of 55% and 63% respectively. The median AUC was 0.384 for the MPV (95% CI 0.270-0.499, p=0.04).

Conclusion: Volume of MP may be a useful marker to provide a prediction on single RSV bronchiolitis. However, the measurement of MPV might not be correct and sufficient to provide a prediction on the types of respiratory viruses in bronchiolitis.

Keywords: Mean platelet volume, acute bronchiolitis, respiratory syncytial virus, prediction

Introduction

Respiratory syncytial virus (RSV) is a pathogen that causes lower respiratory system infection in children and infants. RSV associated respiratory infection is a major burden for children and is related with new acute lower respiratory infection episodes in children especially at 5 years of age (1,2). It is the most common etiologic pathogen; however, other viral pathogens such as adenovirus, coronavirus, parainfluenza, influenza, rhinovirus, human metapneumovirus, human bocavirus, and human coronaviruses cause acute bronchiolitis (3,4). Many different medical studies based on different diagnostic and predictive strategies are currently being developed to predict RSV related acute bronchiolitis that is likely to have high mortality and morbidity.

Thrombocytes play an important role in inflammation, allergic reactions, angiogenesis, repair and renewal of tissues through several mediators such as chemokines,

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cytokines and coagulation factors. These mediators provide a strong inflammatory response and tissue regeneration. Thrombocytes produced from megakaryocytes in the bone marrow increase their production and also change their volume and distribution range in the bone marrow during any inflammation (5-8). This thrombocyte volume is called mean platelet volume (MPV) in laboratory records. Several studies in recent years have reported that the MPV could be a useful biochemical marker in chronic and/or acute inflammatory diseases (9-12). In a study conducted by Renshaw et al. (12), it was noticed that some patients infected with RSV had relatively low MPVs. However, the clinical importance of MPV in RSV or other virus-related bronchiolitis has not been clearly defined yet.

The aim of this study was to evaluate the changes in MPV on patients diagnosed with RSV related bronchiolitis, to define whether the MPV could be a predictive marker in RSV bronchiolitis, and also to identify whether the type of viral infection affects MPVs in bronchiolitis.

Materials and Methods

Study Patients

One hundred eighty-four patients younger than 24 months (excluding newborns) who were hospitalized with acute bronchiolitis between August 2015 and August 2017 at the General Pediatrics Ward of the Children's Hospital were included in this study. The diagnosis of acute bronchiolitis was based on at least 2 of the following signs: chest retractions, tachypnea, and wheezing or rales on auscultation following viral upper respiratory tract infection in children less than 24 months of age for the first time (13). We excluded those infants who had been hospitalized within a 2-week period prior to the current admission, who developed nosocomial acute bronchiolitis, or who had a known history of any chronic disease. The Local Ethics Committee of Ege University approved this study (approval number: E.155324). Infants who were hospitalized with acute bronchiolitis were recruited with informed, written, parental consent.

Data Acquisition, Blood Samples, and Detection of Respiratory Viruses

The age, gender, clinical findings, values of MP, white blood cell count (WBC), C-reactive protein (CRP), and lymphocyte percentage were recorded from each patient's chart. Complete blood counts were performed on presentation for all patients using a commercially available analyzer [CELL-DYN Ruby, Abbott Park, Illinois, United States of America (USA)].

A nasal smear was obtained from each infant and tested for the presence of RSV, influenza virus types A and B, adenovirus, parainfluenza viruses, human rhinovirus, human coronavirus, human metapneumovirus, and human bocavirus with multiplex reverse-transcription polymerase chain reaction (PCR) methods (RealAccurate, Respiratory RT PCR, PathoFinder, Netherlands, and Seeplex RV15 ACE Detection, Seegene, South Korea). Nasal samples were obtained more commonly by a nurse or sometimes a research assistant on all subjects within 48 hours of admission using a standardized protocol (14). Samples were frozen at -20 °C and transported in ice to the department of clinical microbiology and virology laboratory of our university for viral nucleic acid amplification.

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 21.0 for personal computers (Chicago, IL, USA). The categorical variables were analyzed using the Fisher's exact test group if there were different groups. The Mann-Whitney U test was used to analyze non-normally distributed data. As for the receiver operating characteristic (ROC) curves for the biomarkers, calculation of their sensitivity and specificity in acute bronchiolitis was evaluated by drawing the discriminative ability of MPV in respiratory viruses. A value of $p<0.05$ was considered as statistically significant.

Results

In this study, the population included 184 children with acute bronchiolitis. One hundred and fourteen (62%) of them were male, and 70 (38%) were female. The median age was 12±14.5 months. One hundred and twenty-six (68.5%) children had at least one viral respiratory agent and the most common two viruses were RSV (18%; n=51) and rhinovirus (16.2%; n=46). The distribution of respiratory viruses is shown in Figure 1. RSV was the most common agent. The MPVs were similar in patients with positive

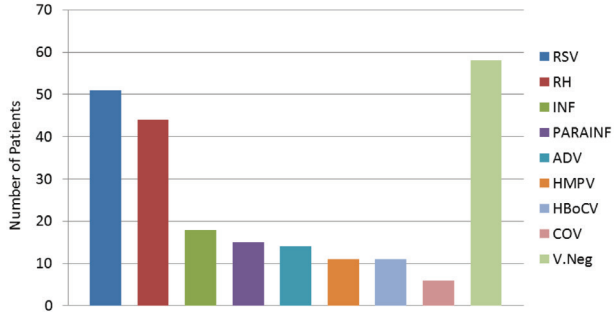

Figure 1. Distribution of respiratory viruses
RSV: Respiratory syncytial virus, RH: Rhinovirus, INF: Influenza virus, PARAINF: Parainfluenza virus, ADV: Adenovirus, HMPV: Human metapneumonia, HBoV: Human bocavirus, COV: Coronovirus, V. Neg: Virus negative

and negative RSV bronchiolitis (6.8±1.5 vs 7±1.3, p>0.05). The comparison of the lymphocyte percentage showed a significantly higher lymphocyte percentage in infants with RSV bronchiolitis (p<0.05).

The differences that were found regarding the laboratory findings between patient groups with respiratory viruses are given in Table I. The MPV was significantly lower in patients with single RSV bronchiolitis versus their negative counterparts (6.6±1.1 vs 7±1.2, p=0.04). The groups who had non-single RSV bronchiolitis tended to possess a statistically significant higher WBC (p=0.02). Being positive or negative in terms of respiratory viruses did not make any statistical difference in MPV and other laboratory findings.

ROC curve analysis indicated that the cut-off of MPV level point for making the diagnosis of single RSV bronchiolitis was 6.63 fL with sensitivity and specificity of 55% and 63% respectively. The AUC value of MPV was lower than WBC, CRP and lymphocyte (Table II and Figure 2).

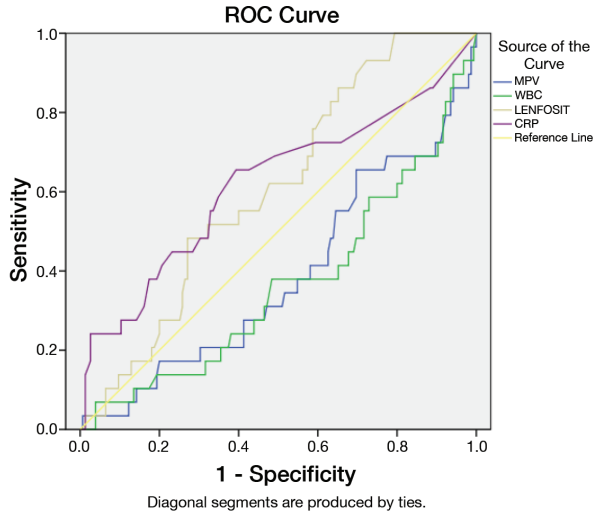

Figure 2. Receiver operating characteristic curve of mean platelet volume, white blood cell, and C-reactive protein count for single respiratory syncytial virus bronchiolitis
MPV: Mean platelet volume, WBC: White blood cell, CRP: C-reactive protein, ROC: Receiver operating characteristic, RSV: Respiratory syncytial virus

Table I. Comparison of laboratory data of patients in terms of respiratory virus groups

Viruses	MPV (med ± IR) (f/L)	WBC (med ± IR)/mm³	Lymphocyte (med ± IR) (%)	CRP (med ± IR) (mg/dL)	p value
RSV					
Single, (n=29) Non-single, (n=97)	6.6±1.1 7±1.2	8960±6420 11760±6540	51±30 37.9±37	0.9±4.3 0.3±1.2	[a]0.04, [b]0.02, [c]NS, [d]0.04
Rhinovirus					
Positive, (n=46) Negative, (n=28)	7±1.2 6.8±1.3	12690±6602 1100±5110	29±31.2 44±30	0.3±1.6 0.6±1.9	[a]NS, [b]NS [c]0.01, [d]NS
Adenovirus					
Positive, (n=18) Negative, (n=60)	7±0.9 6.9±1.4	11900±6375 11220±6092	41±50.5 40.4±34.2	0.5±1.4 0.4±1.7	[a]NS, [b]0.03 [c]NS, [d]NS
Virus					
Positive, (n=136) Negative, (n=58)	6.9±1.3 6.9±1	11300±5300 12300±7500	40±37 35±34	0.4±1.7 0.3±1	[a]NS, [b]NS [c]NS, [d]NS

MPV: Mean Platelet Volume, WBC: White Blood Cell, CRP: C-reactive protein, RSV: Respiratory syncytial Virus, NS: Not significant, [a]: shows the statistical difference of MPV in virus groups, [b]: shows the statistical difference of WBC in virus groups, [c]: shows the statistical difference of lymphocyte percentage in virus groups, [d]: shows the statistical difference of CRP in virus groups

Table II. Results of the receiver operating characteristic curve of mean platelet volume, C-reactive protein, white blood cell and lymphocyte for single respiratory syncytial virus bronchiolitis

Variable	Area under the curve	Standard error	p value	95% CI
MPV	0.384	0.059	0.04	0.270-0.499
CRP	0.364	0.059	0.02	0.249-0.479
WBC	0.608	0.051	0.06	0.509-0.778
Lymphocyte	0.620	0.064	0.04	0.494-0.745

MPV: mean platelet volume, CRP: C-reactive protein, WBC: White blood cell, CI: Confidence Interval, p value of <0.05 was considered statistically significant

Discussion

Our current study of the infants hospitalized with bronchiolitis has shown that the respiratory pathogens which cause acute bronchiolitis cannot be diagnosed or predicted by MPV. Our results indicated that bronchiolitis with RSV is associated with a reduced MPV which was not statistically significant. However, we demonstrated that a single RSV had lower MPV compared to a non-single RSV bronchiolitis with an MPV under 6.63 fL that was relatively sensitive and specific for the single RSV infection. Other studies that investigated the differences of the laboratory markers of acute bronchiolitis have demonstrated a negative correlation between MPV and acute bronchiolitis (11,12). Similar to our study, Renshaw et al. (12) reported that MPV was lower in patients hospitalized with RSV compared to a control group by rapid RSV assays and viral cultures in 158 patients, 112 of which were aged <18 years. In this study, it is also reported that the MPV under 8.9 fL with a sensitivity of 71% and specificity of 49% is a useful marker for RSV bronchiolitis in children undergoing bronchoscopy.

A systematic review of the current literature for studies concerning MPV and pediatrics, published up to 2017 in databases such as Pubmed using search terms including "MPV", "pediatrics", to identify reports that presented data on these topics was carried out. In Pubmed, more than 100 articles were identified. However, the topics of MPV, pediatrics and acute bronchiolitis combined were found in only one article (11). Although the MPV measurements in autoimmune, cardiac conditions and most infections were reported in adult studies, there is a lack of information on MPV for pediatric patients with acute bronchiolitis diagnosed with RSV or other respiratory viruses.

Actually, the current study into changes of MPV has proved that there is no relationship between respiratory viruses and MPV. However, infants with single RSV bronchiolitis have significantly decreased MPVs compared to those with RSV accompanied by other respiratory viruses in our study. Thus, we suggest that the impact of single RSV on MPV might be specific to this virus condition and MPV may be a useful predictor for diagnosing single RSV bronchiolitis.

Study Limitations

There are several limitations to this study. We only investigated hospitalized patients with acute bronchiolitis, and not those infants who applied to the emergency services or out-patients policlinics, so our study group was limited. Prospective studies with a larger number of patients are needed to assess the role of MPV values in acute bronchiolitis.

Conclusion

MPV may be utilized for the diagnosis of single RSV bronchiolitis. However, we think that MPV is not a reliable marker in specifying the cause of acute bronchiolitis; and that its diagnosis could simply be made by conventional microbiological methods. Rather than guessing the virus type according to laboratory findings, we should use more accurate diagnosis methods that will allow for treatment.

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Ethics

Ethics Committee Approval: The Local Ethics Committee of Ege University approved this study (approval number: E.155324).

Informed Consent: Infants who were hospitalized with acute bronchiolitis were recruited with informed, written, parental consent.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: Ş.G., S.P., Design: Ş.G. S.P., Data Collection: Ş.G., S.P., Analysis or Interpretation: C.Ç. Literature Search: Ş.G., Z.K., G.K., A.A.

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Original Article

A Reliability and Validity Study of the Turkish Version of the Parenting Scale

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ABSTRACT

Aim: This study was conducted for the purpose of determining the validity and reliability of the Parenting scale in a Turkish sample.

Materials and Methods: The study was conducted as a methodological-descriptive-cross sectional study. The study sample consisted of 355 parents who had applied to Child and Adolescent Psychiatry Polyclinic of Uludağ University. However; as 85 parents did not precisely fill the scales, they were excluded from the sample and the analyses were conducted on the basis of 270 parents. The study data were collected using the Demographic Data Collection Form and the Parenting scale. Validity analyses of the scale were examined via explanatory and confirmatory factor analysis. The internal consistency of the scale was evaluated via Cronbach alpha, Spearman-Brown and Guttman split-half coefficients. The relationship between item-total score and item-subscale total score was examined via Pearson correlation analysis.

Results: The Cronbach alpha values of the Parenting scale were determined as; 0.935 in the lower dimension of Laxness, 0.916 in the lower dimension of Over-reactivity, 0.770 in the lower dimension of Hostility (use of verbal or physical force) and 0.829 in the total scale. The factor loads varied between; 0.52 and 0.98 in the lower dimension of Laxness, 0.75 and 0.92 in the lower dimension of Over-reactivity and 0.46 and 0.95 in the lower dimension of Hostility. It was determined that the total scale scores and correlations of items in the scale varied between 0.20-0.66. It was also determined that item-subscale total score correlations varied between; 0.61-0.96 in the lower dimension of Laxness, 0.70-0.86 in the lower dimension of Over-reactivity and 0.68-0.91 in the lower dimension of Hostility.

Conclusion: The Parenting scale is a valid and reliable tool that can be used in Turkish culture.

Keywords: Parent, parenting, scale, validity, reliability

Introduction

The family is the environment where the child's physical, social and psychological needs are met and personality development is experienced. The attitudes of parents towards their children in the family are very important in supporting children's socialization and autonomy (1,2).

Parental attitudes can be defined as the sum of parents' attitudes, beliefs, behaviours and expectations about raising children and they are formed by the interaction between mother, father and child (3). There are many factors affecting the child rearing attitudes (CRA) of parents and these factors vary from family to family, from culture to culture and from society to society (4).

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The studies of Diana Baumrind provided a basis for parental attitudes (5,6). Baumrind defined parenting style as a combination of parental values, attitudes, beliefs, and behaviours reflected towards the child. In 1971, she explained this concept in three different models as authoritarian, democratic and permissive parenting styles (5,6). According to Baumrind, emotional support, high expectations, toleration to autonomy and a bilateral net communication style are all together in democratic attitude. This type of attitude has been found to provide the skills necessary for a better balance of personal and social needs and responsibilities of children and adolescents (7). It is also stated that parents who are warmer and supportive towards their children are more aware of their life and are less oppressive (8). The authoritarian parents expect their children to follow and obey their rules. In such families, children are punished if they do not comply with the rules and parents do not exchange many views with their children (5,6). Authoritarian parents give great importance to establishing authority and immediately suppress the efforts of children to change it (9). Permissive parents give their children a lot of freedom, do not have expectations of their children, do not control their children in any way and behave with a negligent tolerance towards permission (5,6,9).

It is accepted that there are two factors on the basis of parental attitudes: how much expectation and how many different types of expectations the parents have; and how much they supported their children or how much they show awareness to them. Parents' expectations of their children show how much they are willing to provide socializing to their children. Sensitive education represents the level of acceptance of parents about the individuality of their children in a sense (7). Democratic parents have both high expectations and a high level of sensitivity. In authoritarian parents, again, expectations are high, but sensitivity to their children is at a low level (7). Maccoby and Martin (9) divided Baumrind's definition of permissive parental attitudes into negligent parents and permissive parents in terms of the dimensions of demandingness and sensitivity. Negligent parents are emotionally distant from their children except to meet their basic needs, do not control or care for what is happening in the lives of their children. However, permissive parents are always concerned about and thoughtful towards their children. They do not restrain their children in any case and never punish them.

Research on parental attitudes around the world and especially in western countries is one of the most studied subjects. Also, in Turkey, many studies have been done to evaluate parental attitudes and their possible side effects. When a search is performed in the Higher Education Board (HEB) Thesis Centre's search engine, if "mother and father (anne-baba) attitude" is researched, 40 studies are found, and similarly "mum and dad (ana-baba) attitude", 8 studies; and finally "parental (ebeveyn) attitude" 15 studies are present.

In our country, three scales have been developed to measure parental attitudes. The first one of them was the Parental Attitude Inventory developed by Kuzgun (10) in 1972, the second one is the Parental Attitude scale developed by Polat (11) (1986) and the third one is the CRA scale developed by Sümer and Güngör (12) in 1999. Data of these scales are collected from children and parental attitudes are classified.

In addition to these developed scales, there are six scales (obtainable) adapted to our language by conducting Turkish validity and reliability studies. These are the following studies: Parental Attitude Research Instrument (PARI), adapted by Le Compte et al. (13), Le Compte et al. (13) (1978); Parenting Style scale, adapted by Yılmaz (14) (2000); Parental Acceptance-Rejection Questionnaire-Child version (PARQ-Child version), its validity and reliability tested by Erdem (15) (1990); the same scale's Adult PARQ (Adult PARQ), adapted by Varan (16) (2005); the same scale adapted by Varan et al. (17) Yağmurlu (2008); and finally PARQ-Mother Form (PARQ-Mother version), adapted by Erkman and Rohner (18) in 2002. However, McMaster Family Assessment Device adapted by Bulut (19) (1990); Child-Rearing scale adapted by Yağmurlu et al. (20) (2005); Behavioral Control scale adapted by Kındap et al. (21). (2008) are among other scales in the literature. Data of the all other scales except for PARI were collected from children and parental attitudes were evaluated. At the same time, the fact that the scale items of PARI are too long makes it difficult for parents to fill in the scale and makes it difficult for researchers to use.

As is seen, although there are many scales that evaluate the attitudes of parents, it is thought that a new and more useful scale is needed to evaluate the attitudes of parents of children aged 0-12, in which data are collected from parents. Considering that the mum-dad-child interaction has differentiated qualitatively in different developmental periods, the development of individual scales that are sensitive to different age periods related to parental attitudes will be one of the most important contributions to this field (22,23).

The aim of the study was to adapt the Parenting scale, developed by Rhoades and O'Leary (24) in 2007, to Turkish and to perform validity and reliability studies.

Materials and Methods

This study was performed in a methodological-descriptive-cross sectional manner in order to evaluate the validity and reliability of the Parental scale in Turkey.

The research was conducted at the child psychiatry clinic between December 2014 and January 2016, in cooperation with the Faculty of Health Sciences of Uludağ University, located in the western part of Turkey and Uludağ University Research and Application Hospital.

Sample

The sample of the study was selected from parents who applied to Uludağ University Hospital Child and Adolescent Psychiatry Clinic and Polyclinic. The insertion criteria of the study was taken into consideration when selecting parents. Accordingly, the sampling characteristics are;

- Parents of children between 0-12 years of age who applied to child and adolescent psychiatry polyclinic,
- Parents of children without mental retardation, autism, psychosis, schizophrenia, bipolar disorder, obsessive compulsive disorder or pervasive developmental disorder,
- Request to participate in the study after reading informed consent form to be included in the study.

While determining the number of samples in validity and reliability studies, the literature refers to three rules: 5s, 10s and 100s rule. It is emphasized that the researcher should take at least five people per item for factor analysis. If there is no problem about reaching the sampling, it is recommended that the number of persons per item should be 10 (25). However, if there are serious limitations in reaching the sampling, it is recommended that the number of samples should be at least 100 persons (25). For the validity and reliability study of the Parenting scale consisting of 30 items, the sample size was calculated as 300 children by taking 10 children per item. Three hundred fifty-five children parents who met the research criteria were included in the sample. However, 85 parents were excluded from the sample because they did not fill out the scales fully, and the analyses were performed on 270 parents. The sampling rate is 76.1%.

Data Collection Tools

Data of the study were collected by using the Socio-Demographic Data Collection Form and Parenting scale. The researchers gave the two forms in the clinic and the completed forms were collected by the researchers. It took an average of 25-30 minutes to complete the forms.

Socio-Demographic Data Collection Form: In this form, there are 19 questions about the child's name and age and parents' marital status, profession and education.

Parenting Scale: The original name of the scale is the "Parenting scale". It was developed in 1993 by Arnold et al. (26). In 2007, it was reorganized by Rhoades and O'Leary (24) and validity and reliability studies were performed. In our study, the revised version of 2007 was used. This 30-item scale measures non-functional disciplinary methods by asking the probabilities of certain disciplinary methods that parents use. It gives a total score and three revised factors: laxness (permissive, inconsistent discipline); over-reactivity (strict emotional authoritarian discipline and anger); and hostility (use of verbal or physical force). The scale has sufficient internal consistency. The Cronbach's Alpha values of the scale were respectively 0.85 for the laxness sub-dimension; 0.80 for the over-reactivity sub-dimension and 0.83 for the hostility sub-dimension. It was found that the scale had a good test-retest reliability in order to find the difference between the parents of children with clinical diagnosis [total score M=3.1, [standard deviation (SD)=0.07] and parents of children not receiving clinical diagnosis (total score M=2.6, SD=0.06) and to establish a relationship between child behaviour, marital incompatibility and depression symptoms, and between child behaviour and non-functional observational measures. All 30 items are scored on a 7-point scale. Low scores indicate good parenting, and high scores indicate non-functional parenting. There are three factors in the Parenting scale: laxness, over-reaction and hostility. The items are sorted by factors and the side of the indicator of "ideal" is shown as the right or left side. If the indicator of "ideal" is on the left, 1 point is given to the left indicator. If the indicator of "ideal" is on the right, reverse scoring is performed and 1 point is given instead of 7 to the right indicator. The total score is calculated by dividing the sum of points of all items by 30. In order to calculate the factor score, the scores in that factor are added and this total is divided by the number of items in the factor.

Data Collection

1. Language Validity: For language validity, the scale was translated from English to Turkish independently by two English linguists whose native language is Turkish. Later, the researchers have developed a joint Turkish text by evaluating the most appropriate translation for each item. After being translated back to English by two linguists who are fluent in Turkish and English, the scale translated into Turkish was compared with its original form with the re-translation method. Inappropriate terms were reviewed and language validity was ensured.

2. Content Validity: For content validity, the draft scale was presented to 10 experts in their fields working

in psychiatric nursing, child and adolescent psychiatry nursing and paediatric nursing. The experts were asked to evaluate the items in terms of both language and content. In order to determine content validity, the scale items were evaluated by four points as being either (a) appropriate, (b) the item should be reviewed; (c) the item must be critically reviewed; or (d) inappropriate. According to these expert answers, content validity indices for item and scale were calculated by dividing the number of experts who marked (a) and (b) by the total number of experts.

3. Implementation Phase: For the pilot scheme of the developed scale, the comprehensibility and implementation process of the developed scale were evaluated by testing the scale on parents not included in the sample.

4. Construct Validity: Explanatory and confirmatory factor analysis was used for construct validity.

5. Determination of Reliability: Cronbach-Alpha reliability coefficient, bisection and item-total score analyses were performed.

Statistical Analysis

Evaluation of Data

Parental demographic data were analysed with percentage and average. The validity analyses of the scale were analysed with explanatory and confirmatory factor analysis. The internal consistency of the scale was evaluated with Cronbach's Alpha, Spearman-Brown and Guttman split-half coefficients. The relationship between item-total score and item-subscale total scores was analysed by Pearson correlation analysis. For the validity of the scale content validity index, descriptive and confirmatory factor analysis were used. The significance level was accepted as 0.05.

Ethical Side of Research

In order to adapt the Parenting scale to Turkish, permission was obtained via e-mail from Rhoades and O'Leary (24) (2007) who developed the scale.

In order to carry out the research, written permission was received from the Ethics Committee of Uludağ University Hospital (approval number: 2013-2/18). Written consent was obtained from the parents.

Results

Content and Language Validity

For language validity, opinions of 10 experts were received. For each item, the concordance between the experts' opinions (I-CVI) was found to be between 0.90-1.00 and 0.99 for the whole scale (S-CVI).

Explanatory Factor Analysis

In this study, Kaiser-Meyer-Olkin (KMO) value was found to be 0.660 and Bartlett's test was determined as x^2=5888.904 and p=0.000. As a result of explanatory factor analysis, the data were collected under three sub-dimensions. The first sub-dimension accounted for 36.7% of the total variance, the second sub-dimension accounted for 27.4% and the third sub-dimension 13.5%. The three sub-dimensional scales accounted for 77.6% of the total variance. The factor loads of the first sub-dimension ranged from 0.52 to 0.98. The factor loads of the second sub-dimension ranged between 0.75 and 0.92. The factor loads of the third sub-dimension ranged from 0.46 to 0.95 (Table I).

As a result of confirmatory factor analysis, factor loads in the first sub-dimension ranged from 0.43 to 0.99 in three sub-dimensional models. The factor loads of the second sub-dimension are between 0.49-0.99. The factor loads of the third sub-dimension ranged between 0.36 and 0.97 (Table II). Model fit indicators were determined as GFI=0.93, CFI=0.98, IFI=0.98, NFI=0.96, NNFI=0.97 and x^2=127.55, df=58, p=0.000 and RMSEA=0.067 (Figure 1).

Reliability Analysis

The total sub-dimensions of the scale were 0.829, 0.935, 0.916 and 0.770, respectively. In this study,

Table I. Explanatory factor analysis results

Items	Factor loads		
	Laxness	Overreaction	Hostility
3rd Item	-	0.93	-
6th Item	-	0.75	-
10th Item	-	0.75	-
12th Item	0.98	-	-
14th Item	-	0.92	-
16th Item	0.97	-	-
17th Item	-	0.91	-
18th Item	-	-	0.46
19th Item	0.52	-	-
21st Item	0.98	-	-
25th Item	-	-	0.95
28th Item	-	-	0.95
30th Item	0.98	-	-
Explained Variance (%)	36.7	27.4	13.5
Total Explained Variance (%)	77.6	-	-
Eigenvalue	4.771	3.564	1.751

Cronbach's Alpha, Spearman-Brown and Guttman split-half are above 0.70. In this study, the maximum and minimum values of the total and subscale scores of the scale are below 20%.

The correlations of the items in the scale with the total score of the scale were identified as ranging from 0.20 to 0.66. Item-subscale total score correlations ranged from 0.61-0.96 for the first sub-dimension; and 0.70-0.86 for the second sub-dimension and 0.68-0.91 for the third sub-dimension (Table III).

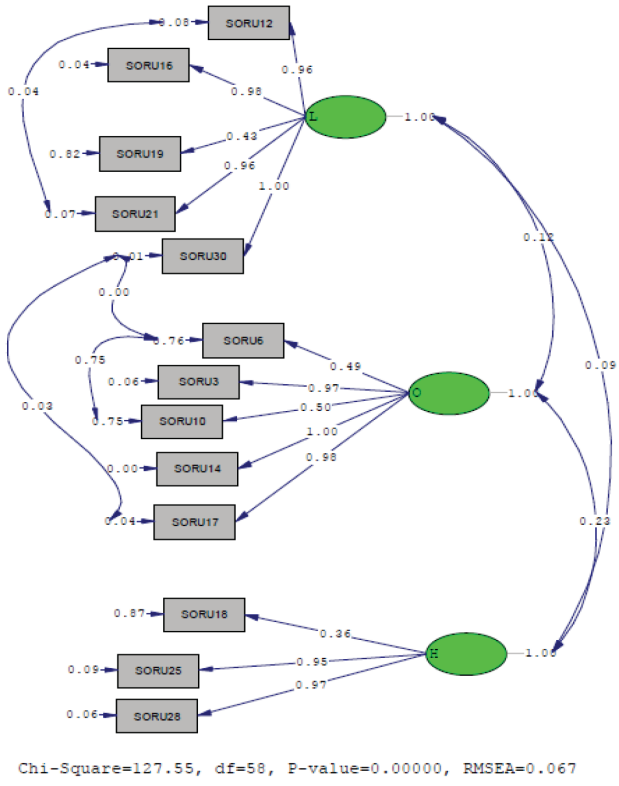

Figure 1. Confirmatory factor analysis

Discussion

The opinions of ten experts were obtained for the validity of language and content. For both language and content validity, the fit indices were found to be above 0.90 both on item and scale basis. The results of I-CVI and S-CVI in this study showed that there was concord between the experts, that the language validity of the scale was achieved, that the scale measures the subject adequately and that the content validity was achieved (27-29).

Validity Analysis

The appropriateness of the obtained data and the factor analysis of the sampling size were evaluated by use of the KMO coefficient and Bartlett's test. Being greater than 0.60 for the KMO value and the meaningfulness of the Bartlett's test indicates that the database is appropriate for factor analysis and the number of samples is sufficient for factor analysis. In this study, the KMO value was 0.60 and Bartlett's test was $p<0.05$. These results indicate that the data are appropriate for factor analysis. In this study, it was determined that the three sub-dimensions revealed 77.6% of the total variance. According to the explanatory factor analysis, the factor loads were found to be above 0.40 in all sub-dimensions. While the total variance explained in the literature is considered to be between 40-60%, to be above 50% for this value is accepted as evidence for a strong construct validity. In this study, both the greatness of the explained variance and being above 0.40 for all factor loads in all the sub-dimensions showed that the scale had a strong structure validity in the Turkish sample (25,27-33). In addition, the factor loads on the original scale were found to be over 0.30 for both mothers and fathers (24) and the original scale factor loads were compatible with the results of this study. Since the total variance was not given in the original study, the total variance rates of the two studies could not be compared.

Table II. Model fit indices of confirmatory factor analysis

Models	χ^2	Df	p	χ^2/df	RMSEA	GFI	CFI	IFI	NFI	NNFI	RFI
Model 3 (three sub-dimensional model)	127.55	58	0.000	2.19	0.067	0.93	0.98	0.98	0.96	0.97	0.95

Table III. Reliability analysis of scale and sub-dimensions (n=270)

Scale	Cronbach α	Spearman-Brown	Guttman split-half	mean ± SD	Min-Max	Floor effect %	Ceiling effect %
Laxness	0.935	0.919	0.939	3.45±1.71	1-7	5.9	3.3
Overreaction	0.916	0.842	0.846	3.62±1.48	1-7	5.6	2.2
Hostility	0.770	0.781	0.807	2.81±1.29	1-6.67	15.6	0.0
Total Scale	0.829	0.733	0.721	3.69±0.82	1.20-6.67	0.0	0.0

α: alpha, SD: Standard deviation, Min: Minimum, Max: Maximum

As a result of the confirmatory factor analysis in this study, factor loads in the first sub-dimension ranged from 0.43 to 0.99 in the three sub-dimensional models. The factor loadings of the second sub-dimension are between 0.49-0.99. It was determined that the factor loads of the third sub-dimension ranged between 0.36-0.97. Model fit indicators were found to be greater than 0.90 (GFI=0.93, CFI=0.98, IFI=0.98, NFI=0.96, NNFI=0.97), x^2/df ratio was less than five (5) and RMSEA was found to be less than 0.08 (Figure 1). As a result of DFA, it was determined that the factor loads of all sub-dimensions were over 0.30, the fit indices were above 0.90 and the RMSEA was below 0.08. If the Model fit indicators are >0.85, x^2/df is less than five and RMSEA <0.08, than it is considered as a good fit indicator in the literature. The CFA results in this study showed that the data were compatible with the model, that the data confirmed the three-factor structure, that the sub-dimensions were related to the scale and that the items in each sub-dimension defined their own factor as sufficient. On the original scale, the fit indices for both mothers and fathers were found to be above 0.90, RMSEAs were below 0.08 and x^2/df was below 5. The results of this study were similar to the results of the DFA for both mothers and fathers in the original study (24). This similarity showed that the Turkish version of the scale had a similar structure with the original scale and the structure validity of the Turkish version was obtained.

The results of the explanatory and confirmatory factor analysis in this study reveal that the scale is a valid tool by supporting the construct validity of the scale.

Reliability Analysis

The Cronbach Alpha coefficient indicates whether the items measure the same characteristics and whether the items are relevant to the subject to be measured. In the scales, this value is to be as close to 1 as possible. When this value is between 0.60 and 0.80, it indicates that the scale is fairly reliable; and between 0.80 and 1.00 means that it is highly reliable (25,27-34). In this study, the Cronbach Alpha coefficients of the total and sub-dimensions of the scale were found to be greater than 0.70. This result showed that both the full scale and the sub-dimensions of the scale are highly reliable. The values obtained from the study showed that the items were able to measure the desired level adequately, that the items were related to the subject and that the scale had a very good reliability (25,27-33). Also, in the original study, it was determined that the corrected Cronbach Alphas for both mothers and fathers were over 0.70 (24). This result shows that the scale is similar to the original structure and that the scale has a strong internal consistency.

In the split half method used in this study, it was found that the Cronbach's Alpha values of both sections were above 0.70; a strong and significant relationship was found between the two halves and both Spearman-Brown and Guttman Split-Half coefficients were found to be more than 0.80. These results showed that the scale has a high level of reliability (25,27-33). While these results showed that the internal validity of the scale was high; since these analyses were not given in the original study, the results of this study were not compared with the original scale results. In this study, maximum and minimum values of the total score and subscale scores of the scale were found to be less than 20%. In the literature, it is emphasized that the maximum and minimum values are indicators of the homogeneity of the scale, that this value should be below 20% and that provides evidence for both validity and reliability (25,27-32,34). Also, in this study, the fact that this value is below the limit indicates that the scale is a reliable tool by supporting the construct validity of the scale (25,27-33).

The item-total score analysis shows the relationship between the scores of the scale items and the total score of the scale. It is evidence that the items in the scale measure the desired quality (25,27-33). This value should be greater than 0.20 and in a positive direction (25). In this study, the correlations of the items with the scale total score ranged between 0.20-0.66; and the correlations of the items with the subscale total score ranged between 0.61-0.96. It was found that the correlation coefficients of both the item-total score and the item-subscale were in a positive direction and greater than 0.20. According to these results, it was found that all items of the scale had a high correlation with the total score and total score of their sub-dimensions, that the scale was able to measure the desired quality and that the reliability of the scale and the sub-dimensions were high. Since the item-total score and item-subscale total score correlations were not given in the original study, the scale results could not be compared with the original scale (24).

Study Limitations

Despite all its strengths, the scale has a few limitations. These limitations are that the study was conducted only in the western part of the country and with the use of a random sampling method. These limitations may affect the generalization of the results of the study.

Conclusion

The results of this study show that the scale is a valid and reliable measurement tool for the Turkish sample. The scale is a valid and reliable tool that can be used to

examine the disciplinary methods used by Turkish parents. Using this scale is recommended to conduct studies in both healthy and clinical samples and to plan studies in which intercultural comparisons can be made.

Ethics

Ethics Committee Approval: Written permission was received from the Ethics Committee of Uludağ University Hospital (approval number: 2013-2/18).

Informed Consent: Written consent was obtained from the parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.A., M.B., C.Ö., N.Ö., P.V., Concept: B.A., M.B., C.Ö., Design: B.A., M.B., Data Collection or Processing: B.A., N.Ö., P.V., Analysis or Interpretation: B.A., M.B., C.Ö., Literature Search: B.A., M.B., C.Ö., N.Ö., Writing: B.A., M.B., C.Ö., N.Ö., P.V.

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Original Article

Preseptal and Orbital Cellulitis in Childhood: The Experience of Ankara Training and Research Hospital

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ABSTRACT

Aim: Evaluation of the etiology, clinical and laboratory findings, treatment and complications of preseptal and orbital cellulitis in patients and to show that these complications can be prevented with early diagnosis and effective treatment.

Materials and Methods: Thirty-eight patients with orbital and preseptal cellulitis who had been admitted to Ankara Training and Research Hospital, Clinic of Paediatric between September 2015 and February 2017 were retrospectively studied.

Results: Thirty-five patients (92.1%) were diagnosed with preseptal cellulitis and 3 patients (7.9%) were diagnosed with orbital cellulitis. The mean age at diagnosis of the patients [24 girls (63.2%) and 14 boys (36.8%)] was 4.01±3.72 years. The most frequent etiologic factor was conjunctivitis (28.9%). Twenty-five patients (65.7%) were treated with intravenous ampicillin-sulbactam alone as the first treatment. No patient underwent surgery. All patients recovered completely without any eye illnesses and no complications were observed.

Conclusion: Orbital infections can be healed through early diagnosis and effective antibiotic therapy in childhood and ampicillin-sulbactam therapy alone should be preferred over combination therapy due to its high effectiveness and relatively low side effects.

Keywords: Preseptal cellulitis, orbital cellulitis, child

Introduction

Orbital site cellulites are common eye infections which can be treated by the effective use of antibiotics. However, a delay in diagnosis and treatment can cause serious complications such as loss of vision, cavernous sinus thrombosis (CST), meningitis and/or sepsis (1). The major infections of the ocular adnexal and orbital tissues are defined as a preseptal cellulitis and orbital cellulitis depending on the site of the infection.

Preseptal cellulitis is characterized by swelling and redness of the orbital septum that can also spread to the upper cheek and forehead (2). On the other hand, orbital cellulitis is associated with proptosis and a limitation of eye movements. Other accompanying findings are inflammation in the conjunctiva, orbital pain, decreased visual acuity (VA), and afferent pupillary defect. Although the differences between preseptal and orbital cellulitis are understood, it is sometimes difficult to differentiate (3).

In this study, the demographic and clinical features, underlying etiology, laboratory and radiological findings, treatment and complications in patients who had been diagnosed with preseptal or orbital cellulitis during childhood were evaluated retrospectively.

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Materials and Methods

Thirty-eight patients with orbital or preseptal cellulitis who had been admitted to the Ankara Training and Research Hospital, Department of Paediatrics between September 2015 and February 2017 were retrospectively studied.

The patients with periorbital edema and/or redness, and normal eye movements were diagnosed with preseptal cellulitis, whereas at least one of following findings of limited eye movement, inflammation signs in the conjunctiva, orbital pain, decreased VA (for at least 2 lines on the Snellen chart), afferent pupillary defect signs or radiological imaging detecting inflammation in the orbital region, were accepted for a diagnosis of orbital cellulitis (1-3).

The patients' demographic, clinical features and underlying diseases such as age, gender, season of diagnosis, upper respiratory tract infection, sinusitis, dental infection, conjunctivitis, skin infection, and trauma history were obtained from the database.

The serum white blood cell count (WBC), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were recorded.

All results were evaluated using the SPSS. The collected data were analysed with respect to frequency (%), mean ± standard deviation, median, minimum and maximum values.

The authors obtained approval from the Ethics Committee of Ankara Training and Research Hospital with file for this study (approval number: 0002-21 on 15.03.2017). The study was conducted in accordance with the principles of the Declaration of Helsinki. The inform consent was taken from the patients' parents.

Results

Thirty-five patients (92.1%) were diagnosed with preseptal cellulitis and 3 other patients (7.9%) were recognized as suffering from orbital cellulitis. These diagnoses were collected from 24 female (63.2%) and 14 male (36.8%) individuals with a mean age of 4.01±3.72 years old. The patients' demographic and clinical features such as age, diagnoses results, seasonal distribution, cellulitis location, hospitalization and total treatment duration are presented in Table I. By evaluation of the underlying diseases, the most common causes were listed as conjunctivitis, tooth infections, sinusitis, skin infections, upper respiratory tract infection and trauma (Table II). The patients with orbital cellulitis had decreased VA (for at least 2 lines of Snellen charts). None of the patients had preauricular lymphadenopathy.

The WBC counts were similar in preseptal (13.890±3.485/mm^3, minimum: 6.900-maximum: 21.300/mm^3) and orbital cellulitis (13.633±1.137/mm^3, minimum: 12.700-maximum: 14.900/mm^3) patients, while the serum CRP and ESR levels were found to be increased in orbital cellulitis patients. The serum CRP levels were 3.91±6.18 mg/dL (minimum: 0.12-maximum: 31) in preseptal cellulitis patients and 9.64±0.31 mg/dL (minimum: 9.4-maximum: 10) in orbital cellulitis patients. The serum ESR levels were 20.2±19.2 mm/hour (minimum: 2-maximum: 78) in preseptal cellulitis patients and 36.3±26.4 mm/hour (minimum: 13-maximum: 65) in orbital cellulitis patients (Table III). However, statistical comparison was not applicable due to the insufficient number of patients.

Through magnetic resonance imaging (MRI) and/or computed tomography in the orbital region, 13 patients (34.2% of all patients) were diagnosed as follows: sinusitis

Table I. Demographic and clinical characteristics of patients with orbital and preseptal cellulitis

	Orbital cellulitis	**Preseptal cellulitis**
Average age (years) (mean ± SD)	10.85±2.49 (min: 8.19-max: 13.14)	3.43±3.2 (min: 0.11-max: 10.10)
Girl gender (%)	66.6	62.8
Cellulitis locations (%)		
Right	66.6	40
Left	33.4	54.3
Bilateral	-	5.7
Eye findings (%)		
Edema	100	100
Propitosis	66.4	22.8
Discharge	-	25.7
Ecchymosis	-	14.2
Haemorrhage	-	2.8
Redness	33.3	8.7
Ophtalmoplegia	66.4	-
Season (%)		
Autumn	33.4	42.8
Winter	66.4	20
Spring	-	11.4
Summer	-	25.7
Duration of hospitalization (days) (mean ± SD)	23.33±8.62 (min: 14-max: 31)	7.71±6.93 (min: 3-max: 38)
Days of antibiotic use (mean ± SD)	28±12.76 (min: 14-max: 39)	12.17±7.15 (min: 4-max: 45)

SD: Standard deviation, min: Minimum, max: Maximum

in 6 patients (46.1%), subperiosteal abscess in 3 patients (23%), preseptal cellulitis in 8 patients (61.5%), and orbital cellulitis in 3 patients (23%). There was evidence of sinusitis in the maxillary sinus for all the patients while 2 patients had isolated sphenoid sinusitis and another 2 children had frontal and ethmoidal sinusitis at the same time. There was one patient diagnosed with maxillary sinusitis by direct X-ray.

For detecting the microorganisms, blood cultures were performed on 21 patients (55.2%) and conjunctival culture on 2 patients (5.2%).

Of the treatment methods, 25 patients (65.7%) were given ampicillin-sulbactam treatment (100 mg/kg/day, q6hr) alone as the first option. In addition, ampicillin-sulbactam treatment was given in combination with metronidazole (30 mg/kg/day, q8hr), clindamycin (30 mg/kg/day, q8hr) or amikacin (15 mg/kg/day, q12hr) in 9 patients (23.6%). Four patients (10.5%) used ceftriaxone (75 mg/kg/day, q12hr) as first-line treatment. Two of these were given ceftriaxone alone and the other two were given metronidazole (30 mg/kg/day, q8hr) and vancomycin (60 mg/kg/day, q8hr).

Discussion

If preseptal and orbital cellulitis is not properly treated, it can lead to serious complications ranging from optic neuritis, optic atrophy, blindness, CST, superior orbital fissure syndrome, orbital apex syndrome, meningitis, brain abscess, subdural empyema and even death. Preseptal and orbital cellulitis are more common orbital infections during childhood rather than adulthood (4-6).

Studies have shown that preseptal and orbital cellulitis have some common risk factors such as trauma and respiratory infections, cellulitis, sinusitis, tooth loss, trauma, asthma or diabetes mellitus. These findings support that low standards of hand hygiene and health play a role in the development of orbital cellulitis in developing countries (7). The most common causes of preseptal and orbital cellulitis are sinusitis, conjunctivitis, skin infections and upper respiratory tract infections (8-11).

Sinusitis is an important underlying disease for orbital infections. Sinusitis can cause orbital complications in 74-85% of cases. This condition is most commonly seen as ethmoidal sinusitis (12). In various studies, it has been shown that 85-95% of orbital infections due to sinusitis are preseptal cellulitis and 5-15% are postseptal infections (13). In a study with 26 paediatric patients with sinusitis presenting with orbital complications, subperiosteal abscess was found in 11 patients (42.3%), preseptal cellulitis in 13 patients (50%) and orbital cellulitis was determined in 2 patients (7.7%). The authors suggested that the relatively low incidence of cellulitis may be due to the fact that some of the patients are followed in children's clinics and eye clinics (14).

Similarly, the most common etiologic factor in our study was conjunctivitis. Sinusitis was detected as the second most common etiological factor. Unlike other studies in the literature, maxillary sinusitis is the most common in our patients.

The WBC count, serum CRP and ESR levels, proptosis on examination, ophthalmoplegia, decreased VA, or abnormal pupillary reflex, conjunctival discharge and/or collection or inflammation in the orbital region on radiological imaging are indicated as signs of preseptal and orbital cellulitis (8-10). In our study, the most frequent examination finding was edema. Those patients with orbital cellulitis were found to have proptosis and ophthalmoplegia, and preseptal cellulitis was the most common presentation with redness

Table II. Risk factors and associated diseases in preseptal and orbital cellulitis

Risk factors/associated disease	Count, (n)	Percent, (%)
Conjunctivitis	11	28.9
Tooth infections	7	18.4
Sinusitis	7	18.4
Skin infections	5	13.2
History of upper respiratory tract infection	5	13.2
Trauma	4	10.5

Table III. Laboratory findings of patients

	(WBC/mm³)	CRP (mg/dL)	ESR (mm/hour)
Preseptal cellulitis (mean ± SD)	13890±3485 (min: 6900-max: 21300)	3.91±6.18 (min: 0.12-max: 31)	20.2±19.2 (min: 2-max: 78)
Orbital cellulitis (mean ± SD)	13633±1137 (min: 12700-max: 14900)	9.64±0.31 (min: 9.4-max: 10)	36.3±26.4 (min: 13-max: 65)
Total (mean ± SD)	13869±3352 (min: 6900-max: 21300)	4.3±6.12 (min: 0.12-max: 31)	21.5±19.92 (min: 2-max78)

WBC: White blood cell count, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, SD: Standard deviation, min: Minimum, max: Maximum

and discharge. The WBC count from laboratory findings was found to be similar in the preseptal and orbital cellulitis groups. CRP and ESR levels were higher in those patients with orbital cellulitis. However, there was no statistical comparison due to the insufficient number of patients. Those patients diagnosed with orbital cellulitis were screened and found to be compatible with orbital cellulitis.

It has been reported that the most frequently isolated microorganisms in blood culture and abscess cultures in patients with preseptal and orbital cellulitis are *Staphylococcus Aureus* and other *staphylococci* (7-10). In our study, no microorganisms were isolated in a total of 21 blood cultures and 2 conjunctival cultures. Furthermore, abscess drainage was not necessary in our patients; therefore, penicillinase resistant penicillin (oxacillin), ampicillin-sulbactam or amoxicillin-clavunate were given as the first choice of treatment in our paediatric patients (8-11).

Streptococcus anginosus was isolated as the most frequent cause in a study with 94 paediatric patients; 34% of these patients were treated with only ampicillin-sulbactam, while the majority of patients were treated with combination therapy (cephalosporin and clindamycin or vancomycin and ampicillin-sulbactam). When increased orbital signs and/or symptoms were observed after 48 hours of treatment, the patients underwent surgery and they received vancomycin therapy. Empirical vancomycin and combination therapy are not routinely used in childhood, emphasizing the usefulness of ampicillin-sulbactam therapy in the treatment of oral cirrhosis, and also in preventing resistant organisms, drug reactions and central venous catheter infections (15).

In our study, 25 patients (65.7%) were given ampicillin-sulbactam treatment alone as the first option. In addition, ampicillin-sulbactam treatment was given in combination with metronidazole, clindamycin or amikacin in 9 patients (23.6%). Four patients (10.5%) used ceftriaxone as the first-line treatment. Two of these were given ceftriaxone alone and the other two were given metronidazole and vancomycin.

In our study, subperiosteal abscess developed as a complication in 3 patients. Clinical improvement was achieved with appropriate systemic antibiotic therapy without any surgical intervention. Other complications such as decreased vision and intracranial complications did not develop in any patient. Under ophthalmological examination of one patient, orbital MR imaging revealed lacrimal gland hyperplasia. The patient was evaluated for a possible tuberculosis infection and a diagnosis of tuberculosis was excluded. Ampicillin-sulbactam therapy, which had previously been used after a significant decline in eye findings after 48 hours of systemic antibiotic therapy, was replaced with intravenous piperacillin-tazobactam (300 mg/kg/day, 3 doses); intravenous vancomycin (40 mg/kg/day, 4 doses) was added to the treatment when there was no adequate clinical response. Bone marrow aspiration and biopsy were performed on the patient for possible malignancy and no evidence of malignancy was found. In the third week of treatment, the patient, suffering from a high fever, underwent lumbar puncture and no pathology was detected. On the 25th day of treatment, drug eruption was considered on the basis of widespread maculopapular rash development starting from the face and proximal to the extremity and vancomycin therapy was discontinued and intravenous teicoplanin treatment (12 mg/kg dose 3 doses with 12-hour intervals followed by 6 mg/kg/day single dose) was begun. After teicoplanin treatment, the fever did not reoccur and the lesion became markedly regressed. During the 4th week of treatment, the patient whose eye symptoms had improved, was discharged from the hospital.

After discharge, 9 (23.6%) patients were treated with antibiotic therapy. Two of them (22.2%) were discharged with oral ampicillin-sulbactam, 5 patients (55.5%) with oral amoxicillin-clavunate, and 2 patients (22.2%) with oral ciprofloxacin and oral metronidazole combination. Acyclovir was also added to the treatment due to preseptal cellulitis, which was caused by zona zoster (Figure 1).

All patients underwent ophthalmology consultations at the ophthalmology department, and no surgical intervention was performed.

When the complications were evaluated, subperiosteal abscess was determined in 3 patients (7.89%). In these 3 patients, the abscess was seen incidentally during orbital imaging, and no clinical deterioration was observed in the patients. Figure 2 shows an orbital MR image of a

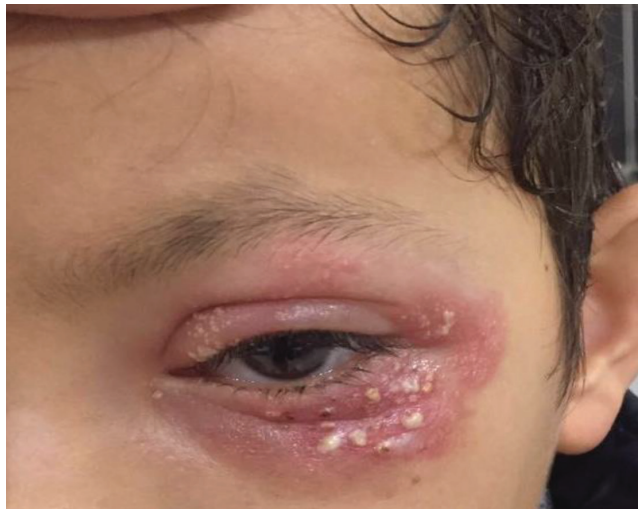

Figure 1. Ten-year-old boy with primary Herpes infection. Left periorbital erythema, edema and infected vesicular lesions

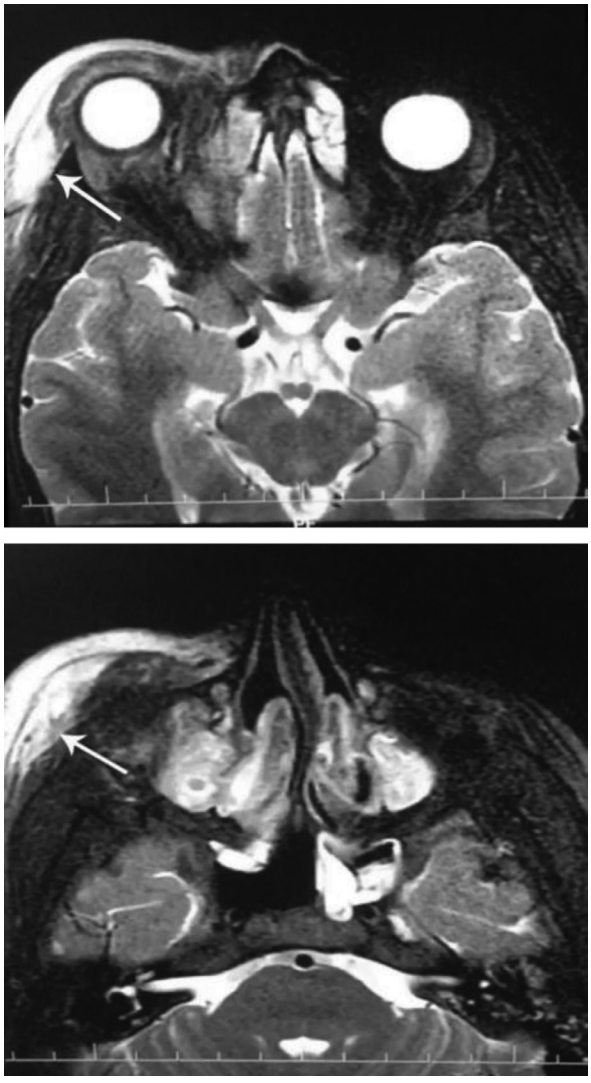

Figure 2. 11-year-old girl. Orbital magnetic resonance image of a patient with orbital cellulitis. A subperiosteal abscess of 28x9 mm dimensions is noted at the lateral extra-coronal area, which indented the lateral rectus muscle

patient who had been admitted to our clinic with orbital cellulitis at the age of 11 and who had a subperiosteal abscess on the orbital MR. This patient, who had been given clindamycin and ceftriaxone therapy, was determined not to have to undergo surgical abscess drainage due to regression in the clinical and laboratory findings. In the other 2 patients who developed subperiosteal abscess, drug eruption occurred due to ampicillin-sulbactam treatment. Consequently, one of these patients was given intravenous clindamycin (30 mg/kg/day, 3 doses) while discontinuing ampicillin-sulbactam therapy and the other patient was given intravenous ciprofloxacin (30 mg/kg/day, 2 doses) and intravenous metronidazole (30 mg/kg/day, 3 doses) was initiated. These two patients were discharged with the resolution of the rash. In a vancomycin-treated patient, extensive erythematous lesions developed and these lesions improved when the treatment was replaced with teicoplanin. The diagnosis of subperiosteal abscess was not made with the diagnosis of deterioration in the clinical findings of the other patient, and the orbital MRI made the diagnosis based on the appearance of an abscess. After the patient was diagnosed with subperiosteal abscess, he underwent consultations at the ophthalmology and neurosurgery departments; however, no surgical drainage was performed.

Conclusion

Orbital infections in childhood can be treated with early diagnosis and effective antibiotic treatment. However, the possibility of serious complications should be kept in mind in every patient. It should be taken into account that ampicillin-sulbactam therapy alone can be used as the preferred and initial treatment option rather than combination therapy due to its high efficacy and relatively low side effects.

Ethics

Ethics Committee Approval: The authors obtained approval from the Ethics Committee of Ankara Training and Research Hospital with file for this study (approval number: 0002-21 on 15.03.2017).

Informed Consent: The inform consent was taken from the patients' parents.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: Z.S.Ş., Design: Z.S.Ş., Data Collection or Processing: Z.S.Ş., T.T.K., S.K., G.Ö., F.Ö., B.A., Analysis or Interpretation: Z.S.Ş. T.T.K., B.A., F.Ö., Literature Search: Z.S.Ş., Writing: Z.S.Ş.

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

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Case Report

A Rare Cause of Diplopia: Idiopathic Orbital Myositis

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ABSTRACT

Orbital myositis is an entity affecting the ocular muscles, especially the medial rectus. These cases are usually referred to clinics with complaints such as diplopia, orbital/periorbital pain, limitation in ocular movements, increased pain with eye movements, proptosis, swelling of the eyelid and/or hyperemia in the conjunctiva. Orbital myositis is usually idiopathic and autoimmunity is often suspected in etiology. In this article, we present a 15-year-old girl who presented with diplopia, pain in both eyes, anomalous head posture, periorbital edema and was diagnosed with idiopathic orbital myositis through history, clinical findings and imaging methods.

Keywords: Orbital myositis, diplopia, anomalous head posture

Introduction

Idiopathic orbital myositis is a syndrome of the acute onset of single or multiple inflammation of the extraocular muscles. This type of myositis is represented in a broad clinical classification of idiopathic orbital inflammatory pseudotumor (1). However, the idiopathic orbital inflammation and non-specific orbital inflammation overlap considerably, and occasionally the two entities can be considered interchangeably. Idiopathic orbital myositis can be described as a clinical syndrome of inflammation (2). In addition to muscle tissue, the disease can also affect other structures of the orbit including fat, lacrimal glands or connective tissues (3). Its etiology is not clear yet. However, it is reported to be associated with polymyositis, thyroid diseases, juvenile idiopathic arthritis, and other rheumatological diseases. It has also been shown that there is an association with several diseases (4,5). Idiopathic orbital myositis accounts for 6-17% of cases in childhood (6,7). It is seen in females from two to four times more often than in males and causes inflammation in extraocular muscles. Due to the inflammation, orbital signs and symptoms, such as pain, proptosis, ptosis, periorbital pain, diplopia, ophthalmoplegia, conjunctival hyperemia or ocular injection can be seen (8). Diplopia is due to the inadequate contraction of the affected eye muscles that can bring about an anomalous head posture characterized by head tilting to the side opposite the inflamed muscles to prevent diplopia (9). Diagnosis of idiopathic orbital myositis can be done after ruling out many diseases that might present with orbital inflammation features. In the magnetic resonance imaging (MRI) of idiopathic orbital myositis cases, there are various patterns of extraocular muscle involvement (3). In this article, a 15-year-old girl with idiopathic orbital myositis, who had no form of chronic systemic illness, presenting with swelling-redness in the left eye lid, diplopia for fifteen days, and anomalous head posture for one week is discussed and a brief review of the literature is also given.

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Case Report

A previously healthy 15-year-old girl presented to our pediatric hospital with a 2-week history of swelling-redness, gradually progressing ptosis in the left eye lid, diplopia, and anomalous head posture. Her ophthalmologic examination revealed painful extraocular movement in all directions with normal visual acuity. The left eye was hyperemic and had alternating exotropia with proptosis. There was a motility restriction of the left eye towards the right side. On her examination of primary view position, she complained of diplopia and, in order to cope with the diplopia, she adopted a mild anomalous head position to the right and downwards with the jaw to the left. (Figures 1 and 2). Direct and indirect light reflexes of the patient were normal in both eyes. Frontal and posterior segment examinations of the eyes were performed. Hemoglobin, biochemical investigations, C-reactive protein and sedimentation rate were normal. Autoimmune tests such as antinuclear antibody, anti-deoxyribonucleic acid, rheumatoid factor, c-anti-neutrophil cytoplasmic antibody were negative. There was no positivity on serological investigations of lyme, *Brucella*, toxoplasma, toxocara and other viral agents. Gadolinium-enhanced T_1 and T_2-weighted MRI of the orbit revealed inflammation in bilateral extraocular muscles which was more pronounced in the left medial rectus suggesting orbital myositis (Figures 3 and 4). Cranial MRI was reported as normal. Thyroid ophthalmopathy, intracranial pathologies, rheumatologic diseases and infections were ruled out and the case was diagnosed with idiopathic orbital myositis. Oral 60 mg/day methylprednisolone therapy was started. On the third day of the treatment, eye edema and proptosis were decreased. Methylprednisolone therapy was stopped by gradually reducing dosages over a four-week period and policlinic follow-up was performed due to the possibility of recurrence. Informed consent was obtained from our patients' parents.

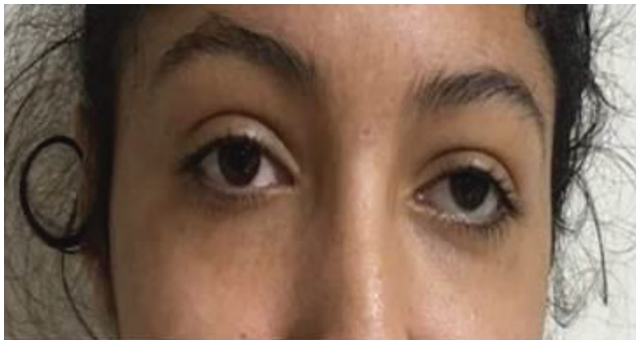

Figure 1. Primary view position of the left eye which was hyperemic and had alternating exotropia with proptosis

Figure 2. The patient's mild anomalous head position to the right and downwards, with the jaw to the left

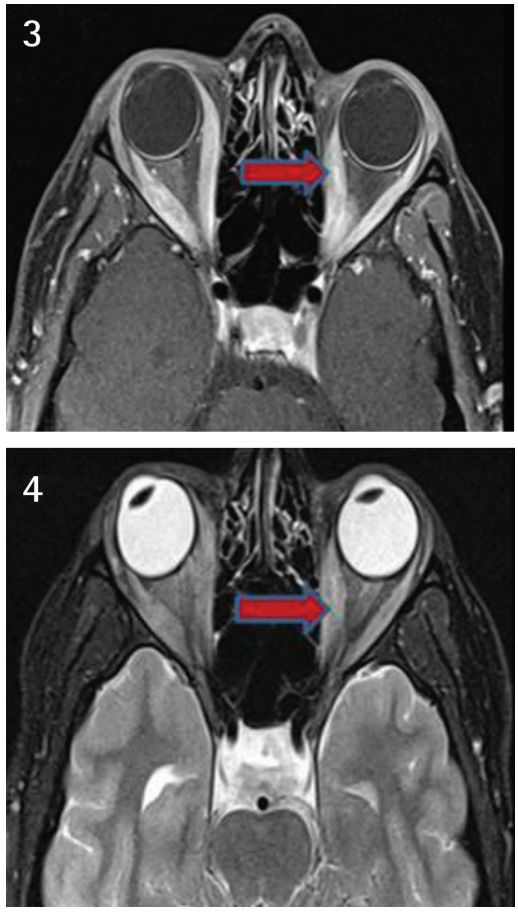

Figure 3, 4. Magnetic resonance imaging of the orbit showing inflammation in the bilateral extraocular muscles which was more pronounced in the left medial rectus

Discussion

This case report describes a young girl who developed idiopathic orbital myositis related to no systemic illness. Idiopathic orbital myositis, also called myositis subtype of idiopathic orbital inflammatory pseudotumor, first described by Gleason (10) in 1903, is a myositis resulting in the inflammation of the extraocular muscles without any local or systemic reason. The disease is represented in a broad clinical classification of idiopathic orbital inflammation termed by Birch-Hirchfield in 1905 as an orbital pseudotumor condition. The most frequent clinical features of idiopathic orbital myositis are acute or subacute exacerbated orbital pain. In childhood, it is also reported to be associated with non-specific signs (3). It is widely seen in the female gender between 18-40 years of age. In studies, it has been reported to be in coexistence with diseases (1,3,11).

Acute, chronic or recurrent forms of idiopathic orbital myositis may be seen. The patients might present with symptoms such as pain in the eyes, strabismus, diplopia, proptosis, conjunctival hyperemia, and swelling-pain in the eyelids. Additionally, patients may suffer from abnormal head position as a result of diplopia as seen in our case. In a study (3), 86.4% of the cases with idiopathic orbital myositis were most frequently referred to as ophthalmologists with oculomotor disorder and strabismus. They reported that proptosis (68.2%), congestion-edema (65.9%) in the conjunctiva, swelling in the eyelids, pain in the eyes, and an increased sensation of pressure may also be seen in idiopathic orbital myositis. In the same study, diplopia was found in 43.2% of the patients.

Diagnosis of idiopathic orbital myositis is based on the history and clinical findings with the detection of one or more extraocular muscular involvement in orbital computerized tomography or orbital MRI. Diagnosis should be confirmed after excluding thyroid disorders, other orbital inflammatory conditions, vasculitis, sarcoidosis, orbital cellulitis and orbital tumors. Some studies indicated that the medial rectus muscular involvement is the most common involvement (9).

In conclusion, although orbital myositis is a rare entity in childhood, it should be considered in patients presenting with complaints of diplopia, proptosis, periorbital edema and anomalous head posture in differential diagnosis.

Ethics

Informed Consent: Informed consent was obtained from our patients' parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ş.G., Data Collection or Processing: E.Ş., S.K.Y., Analysis or Interpretation: S.A., Literature Search: S.A., Writing: Ş.G., S.A.

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

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

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Case Report

A Myasthenia Gravis Case Diagnosed Simultaneously with Diabetic Ketoacidosis

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ABSTRACT

Juvenile myasthenia gravis (JMG) is an autoimmune disease caused by antibodies affecting the postsynaptic membrane at the neuromuscular junction. The association of JMG with Type I diabetes mellitus (DM), another autoimmune disease, is very rare and the pathogenesis has not been fully explained. Our case is the youngest patient where this association has been reported in the literature and presented at the age of 4 years when diabetic ketoacidosis developed together with the emergence of ocular myasthenia findings. She is the only case diagnosed with JMG among the 510 Type I DM patients followed-up at our clinic. Although an autoimmune process may have triggered both autoimmune diseases at the same time in this case, we believe the diabetic ketoacidosis was a triggering factor for the JMG and discuss this association.

Keywords: Type I diabetes mellitus, diabetic ketoacidosis, juvenile myasthenia gravis

Introduction

Type I diabetes is an organ-specific autoimmune disease which is characterized by the selective destruction of pancreatic beta cells. Genetic predisposition, autoimmunity and viral infections are the main etiopathological factors in the pathogenesis. Although, other autoimmune diseases such as autoimmune thyroid disorders, adrenal disorders, celiac disease and connective tissue disorders may be associated with Type I diabetes mellitus (DM), the association of myasthenia gravis and Type I diabetes is very rare (1,2).

Juvenile myasthenia gravis (JMG) is an autoimmune disease in which antibodies are directed against the postsynaptic membrane of the neuromuscular junction, resulting in muscle weakness and fatigability. The incidence of JMG is between 1.0 and 5.0 per 1.000.000 per year (3). In many patients, the autoimmune response is regulated with antibodies developing against acetylcholine receptors (AChR). Antibodies to muscle-specific kinase (MuSK) and to Leucine rich protein 4 have been reported in some seronegative patients (4,5). The diagnosis of JMG requires a detailed history, repeated physical examinations, neurophysiological investigations, and antibody evaluations (4,6).

JMG association was reported only in 1 (0.38%) of 260 children with Type I diabetes during 14 years of follow-up in a series (7). The coexistence of JMG, primary adrenal deficiency and Type I DM has previously been reported

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in a 12-year-old child (8). Our case has been reported as the coexistence of ocular myasthenia gravis and diabetic ketoacidosis at the age of 4 years with the aim of discussing the Type I DM and JMG association.

Case Report

A 4-year-old female patient presented to the emergency service due to rapid breathing and sleepiness for 2 days. We found no unusual feature about her personal and the family history. It was reported that patient had drank a lot of water and experienced increased urination for the last month resulting with weight loss that had become more evident in the prior week. Her physical examination revealed moderate general condition; body temperature: 36.7 °C, respiratory rate: 40/min, pulse rate: 120/min, oxygen saturation: 95%, and arterial blood pressure: 90/60 mm/Hg. Moreover, the patient had dry mouth, sunken eyeballs, acidotic breathing and she was weak. The laboratory test results of the patient showed that, hemoglobin: 13.2g/dL, white blood cells: 9.810/mm^3, platelets: 229.000 mm^3, blood glucose: 697 mg/dL, sodium (Na): 128 meq/L, corrected Na: 137.5 meq/L, potassium: 3.6 meq/L, chlor: 104 mEq/L, calcium: 8.4 mg/dL, phosphate: 2.7 mg/dL, blood urea nitrogen: 7 mg/dL, creatinine: 0.58 mg/dL, uric acid: 4 mg/dL, SGOT: 15 U/L, SGPT: 16 U/L, total bilirubin: 0.3 mg/dL, total protein: 6.8 g/dL, albumin: 4.2 g/dL, hemoglobin A1c: 14.8% (4.6-6.2), c peptide: 0.12 ng/mL (0.9-4), blood gas pH: 7.11, pCO_2: 15.6 mmHg, HCO_3: 8.7 mmol/L, base deficit: -22.4, and positive blood and urine ketones. On the basis of these findings, the patient was diagnosed as diabetic ketoacidosis. Moreover, with the evaluation of her dehydration; we decided that she had 2nd degree dehydration. Accordingly, we applied 0.9% sodium chloride which was loaded for 1 hour at 20 mL/kg. Then, she was admitted to the intensive care unit where we started infusion of intravenous fluids and insulin. The ketosis and acidosis improved at the 21st hour and then it was possible to feed her orally. Then, enteral nutrition and subcutaneous insulin were started. The bilateral ptosis that was found at presentation continued after the diabetic ketoacidosis had improved and became more evident at follow-up. Her history revealed that the ptosis had first started mildly 2 days before presentation and had increased in a short time. Also, her symptom became more severe after physical activity and it had minimal diurnal variation. We observed that the ptosis became worse in the evening and it improved after resting. We found no signs of weakness of the facial, neck, bulbar, limb and respiratory muscles. The ptosis was not present in pictures of the patient that were taken 1 month before the diabetic ketoacidosis developed, indicating that it had emerged during the diabetic ketoacidosis development period (Figures 1, 2). The fatigue test was performed with a preliminary diagnosis of ocular MG. The test revealed that the ptosis increased after prolonged upgaze and improved after the ice test which supported the preliminary diagnosis (Figure 3). Repetitive nerve stimulation of the median and ulnar nerves showed no significant amplitude decrements. We did not perform IV or IM edrophonium tests on our patient due to the possible side effects and lack of edrophonium in our country. We started oral pyridostigmine 0.5 mg/kg/day every 6 hours and gradually increased the dose to 1 mg/kg/day every 6 hours over several days. The third dose of pyridostigmine improved the bilateral ptosis. Serum AChR antibodies and muscle-specific tyrosine kinase antibodies were both negative. The diagnosis

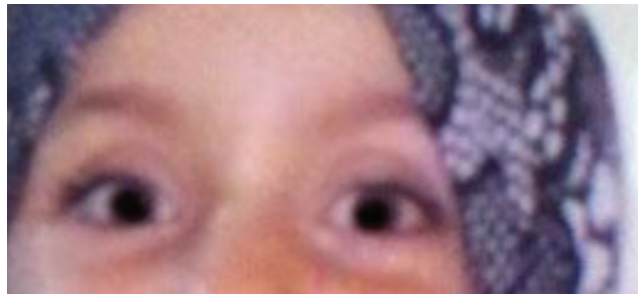

Figure 1. The patient before ptosis development

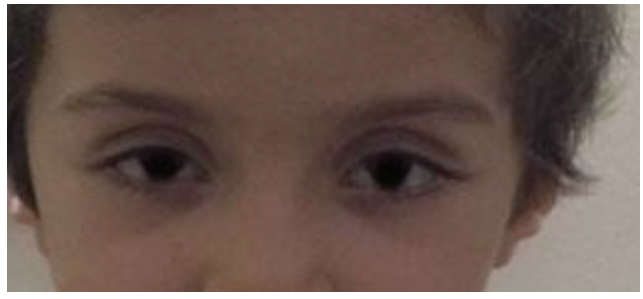

Figure 2. The ptosis that developed in the diabetic ketoacidosis period

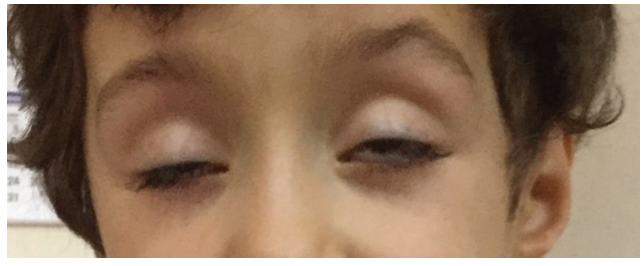

Figure 3. The fatigue test performed with diagnosis of ocular myasthenia gravis revealed that the ptosis increased after prolonged upgaze

was double-seronegative ocular myasthenia gravis. Magnetic resonance imaging of the brain revealed no abnormalities. Chest X-ray obtained focusing on thymoma was normal. Evaluation regarding possible concurrent autoimmune disease revealed the following: islet cell antibody: negative, anti-insulin antibody: 2.71% (0-7), anti-GAD: 37.06 U/mL (0-1), TSH: 1.93 µIU/mL (0.6-6.3), sT4: 1.18 ng/dL (0.9-2.1), sT3: 2.71 pg/mL (2-6.5), anti TPO: 41.3 IU/mL (0-60), anti-thyroglobulin: 17.1 (0-60), tissue transglutaminase immunoglobulin A (IgA): positive, tissue transglutaminase IgG: negative, anti-endomysium IgA: positive, anti-gliadin IgA: positive, anti-gliadin IgG: negative, cortisol: 18.11 µg/dL (5-22), and adrenocorticotropic hormone: 14.9 pg/mL (0-46).

The patient's blood glucose was regulated with intensive insulin treatment, the ptosis improved with pyridostigmine and diabetes education was completed. She was discharged to follow-up with a diagnosis of seronegative ocular MG. Informed consent from patient's family was received.

Discussion

Type I DM can be accompanied by other autoimmune diseases such as thyroid diseases, adrenal diseases, and celiac disease (1). JMG patients are also under increased risk of comorbid autoimmune disease such as thyroid disease, systemic lupus erythematosus and rheumatoid arthritis. The mechanism regarding the development of multiple autoimmune disorders is unclear. The human leukocyte antigen locus is still the risk factor showing the strongest association with autoimmune disorders including JMG (9).

The Type I DM and JMG association is very rare and only 2 cases have been reported so far (7,8). The Type I DM and JMG association was only found in 1 patient in a study where 260 Type I DM patients were monitored. This male case, whose age was not reported, presented with an acute myasthenia gravis attack and was treated with intravenous immunoglobulin and plasmapheresis (7). Primary adrenal deficiency and Type I DM association with JMG was reported in another 12-year-old patient (8). Our case is different from existing studies since our case is probably the youngest patient to be diagnosed and the symptoms appeared during the clinical diabetic ketoacidosis development period. Moreover, she is the only patient diagnosed with JMG among the 510 Type I DM patients followed-up at our clinic, resulting in an 0.19% rate for JMG among our Type I DM patients.

JMG is rare in Europe and makes up 10-15% of all myasthenia gravis cases in Caucasians (6). Peri- or postpubertal children presenting with JMG share more similarities with adult-onset MG and also show increased association with other autoimmune disorders (4). The coexistence of these two autoimmune disorders during the prepubertal period is interesting in our patient. The diagnosis in younger children constitutes a particular challenge as non-specific symptoms may be present or the antibodies are only minimally elevated or even normal. While anti-AChR and anti-MUSK were negative in our case; the JMG diagnosis was made with the presence of ocular symptoms that started during diabetic ketoacidosis development. Besides, the positive fatigue test and the improvement of ocular symptoms with pyridostigmine supported our diagnosis in the previously healthy patient. Some young children, as in our patient, who are negative for AChR antibodies will have "low affinity" antibodies to AChR that are not detectable using standard assays (10). Ocular symptoms only are seen in 15% of the patients with myasthenia gravis and this group makes up the pure ocular form. Patients who present with an ocular form are recommended to be followed-up for a minimum of 2 years before being classified as the pure ocular form (6). Presentation with ocular symptoms is more common in patients with prepubertal JMG, as in our patient. We will be following-up our patient in terms of generalized MG development.

It is interesting that the ocular MG symptoms of our previously healthy patient emerged with the diabetic ketoacidosis clinical picture. However, both autoimmune disorders can be seen together and the autoimmune process could have triggered both autoimmune disorders at the same time. Hence, it is possible that diabetic ketoacidosis may also be a triggering factor.

In conclusion, the Type I DM and JMG combination in the prepubertal period has not been reported until now as far as we know and it is important for clinicians to be aware of this combination and the possibility of an association between various autoimmune disorders in order to improve disease outcomes.

Ethics

Informed Consent: Informed consent from patient's family was received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: There are no financial conflicts of interest to disclose.

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Case Report

A Migrated-mesenteric Lymphangioma: An Unusual Case of Intrabdominal Cystic Lesion in a New-born

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ABSTRACT

Abdominal lymphangioma is a rare presentation of abdominal cystic lesions. They generally arise from small bowel mesentery. Migration of abdominal masses is an uncommon entity and usually occurs due to gossypiboma. There have been many reports of the radiologic appearance and unusual complications of mesenteric lymphangioma. However, to our knowledge, migration of mesenteric lymphangioma has not been reported in the literature. We present imaging findings of migrated-mesenteric lymphangioma from the intrauterine to postnatal period with histopathological correlation.

Keywords: Mesenteric lymphangioma, intraabdominal cyst, migration, CT, MRI

Introduction

Lymphangiomas are congenital benign malformations. They commonly occur in the head and neck region in children. Abdominal lymphangiomas are rare and defined as mesenteric lymphangioma, which is one of the subgroups of mesenteric cysts, in the case of origin from the mesentery (1). Although abdominal lymphangiomas are described most commonly in the small-bowel mesentery, they have been reported as less than 1% of all lymphangiomas (2).

There have been many reports of the radiologic appearance and unusual complications of mesenteric lymphangioma, however, to our knowledge, the migration of mesenteric lymphangioma has not been reported in the literature. We present imaging findings of migrated-mesenteric lymphangioma from the intrauterine to postnatal period with histopathological correlation.

Case Report

A 35-year-old primigravid pregnant woman at the 32nd week of gestation presented to the perinatology department for a routine check-up. On ultrasound, the foetus had a 42x35 mm cystic mass on the left side. The patient was referred to our clinic for determination of the origin of the cystic lesion by using foetal magnetic resonance imaging (MRI). The mass was located in mesentery and adjacent to the small bowel wall (Figure 1). There were no apparent

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fluid-fluid levels and fatty component, also the lesion did not originate from solid organs. The cystic lesion was determined as a mesenteric cyst. The patient was followed-up and a baby girl weighing 2.950 grams was born at the 38th week of gestation. Physical examination revealed a palpable, mobile mass on the left side of the abdomen. There was no physical abnormality, and routine laboratory tests were normal.

A contrast-enhanced computed tomography scan was performed on the postpartum second day and showed contrast enhancement of thin wall and septa. The left-sided intestinal bowel segment was surrounded by the cystic lesion (Figure 2). There were no air-fluid levels in the bowel segments. An operation was planned for one month later. Physical examination before surgery was confusing because the cystic lesion was not palpable on the left side. MRI was performed and it showed that the cystic lesion migrated to the right side while maintaining the same features and dimensions. The lesion was removed completely including the resection of the adjacent bowel involved by the cyst. Histopathological examination confirmed mesenteric lymphangioma. Informed consent was obtained from the parents.

Discussion

Foetal abdominal cystic lesions often arise from the genitourinary and gastrointestinal systems. Others are mesenteric cysts, meconium pseudocysts, and choledochal cysts. Location, relationships with abdominal organs and the internal structure of cysts are crucial for differential diagnosis. Mesenteric cysts are described as cysts which are located in the mesentery, omentum, and retroperitoneum and do not originate from solid organs (1,3). They are divided into six groups which were previously described by de Perrot et al. (4) Mesenteric lymphangiomas are reported in children and young adults, however a small number of cases with mesenteric lymphangioma in new-borns have been reported in the literature.

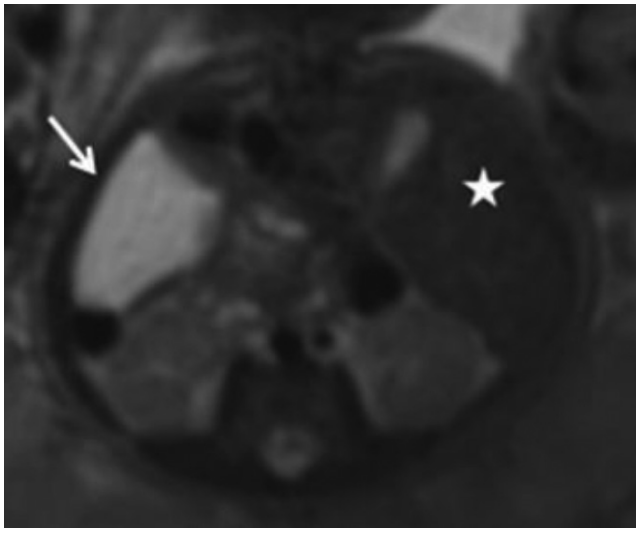

Figure 1. On axial T2-weighted foetal imaging shows hyperintense cystic lesion on the left side (arrow), note that liver is on the right side (star)

The clinical presentation of a mesenteric lymphangioma is usually asymptomatic, but abdominal distension, abdominal pain, infection, haemorrhage, and ileus may occur (5). The most common finding during physical examination is a palpable abdominal mass. Also, mesenteric cysts can move in transverse directions but have limited movement vertically (3).

Imaging techniques play a major role in the determination of cysts and their relationships with abdominal organs. Lymphangiomas are usually seen as well defined, thin-walled, multiloculated cysts. Fluid-fluid levels might be seen depending on cyst contents (5).

Bhullar and Orfanou (6) reported on a mesenteric pseudocyst that would disappear and then reappear in different locations within the abdomen. They hypothesized

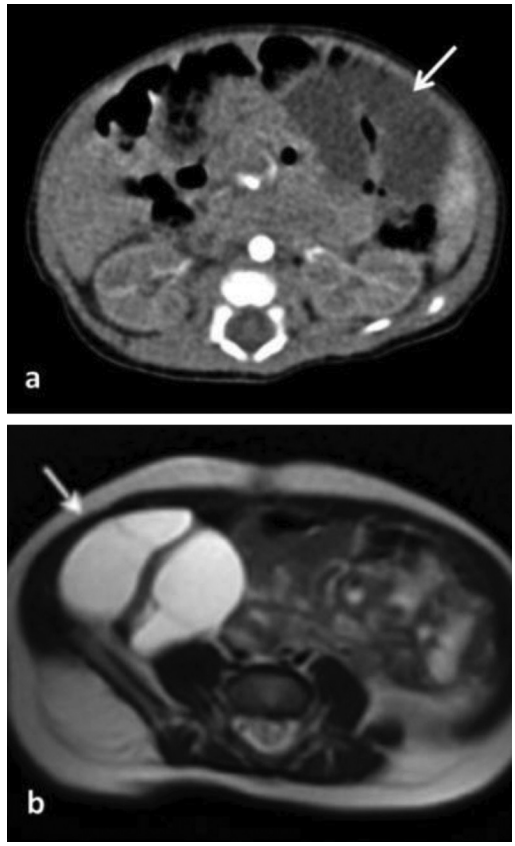

Figure 2. a) Axial computed tomography scan shows a well-defined, hypodense lesion on the left side (arrow) which surrounds the intestinal segment. b) Axial T2-weighted imaging shows migrated-cystic lesion from the left side to the right side (arrow). Note that internal septations and surrounded-intestinal segment are also seen

that this situation is associated with the mobile jejunum. In our opinion, intestinal peristalsis might cause migration of the cystic lesion contralaterally. As a known fact, mesentery allows for movement of the organs during digestion.

Foetal abdominal cystic lesions generally have their origin in the genitourinary system, such as hydronephrosis, renal dysplasia, ovarian cyst, and urachal cyst. Additionally, atresia or duplication of gastrointestinal system may be the cause of abdominal cysts. Differential diagnosis might be a problem due to the small abdominal capacity of foetuses. However, there are some significant signs which help with the distinction between them. Furthermore, cystic lesions rarely arise from the adrenal glands, pancreas or spleen.

The definitive treatment for abdominal mesenteric lymphangioma is complete surgical excision. Because of the risk of recurrence and malignant transformation, a bowel resection is the recommended surgical technique based on the intimate relationship between the cyst and the intestine (7).

In conclusion, determination and accurate anatomic location of lymphangiomas are important in surgical planning. Migrated mesenteric lymphangioma is distinct from most of the previous cases. Migrating of the lesion contralaterally and emphasizing with imaging findings are the values of this case report.

Ethics

Informed Consent: It was taken from family.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: F.C.S., Ö.Ö., Design: F.C.S., O.S., Data Collection or Processing: A.I.B., E.T., D.Ö., Analysis or Interpretation: F.C.S., Ö.Ö., Writing: F.C.S., O.S.

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

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

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Case Report

An Adolescent Boy with Steroid-responsive Ophthalmoplegic Migraine: A New Case and Systematic Review

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ABSTRACT

Ophthalmoplegic migraine (OM) is defined as recurrent attacks of headaches with migrainous characteristics, associated with the paresis of one or more ocular cranial nerves and with the absence of any demonstrable intracranial lesion, other than magnetic resonance imaging changes within the affected nerve. In OMs, the most common involvement is seen in the oculomotor nerves. The clinical features of OM including onset in the childhood period, headaches preceding and ipsilateral to the third nerve paresis, dilated pupil and/or ophthalmoplegia. Here, we report on an adolescent boy with OM, treated with steroid and showing a dramatic improvement. It is believed that this case report can add to the understanding and treatment options of OMs.

Keywords: Cranial nerve palsy, headache, migraine, ophthalmoplegy

Introduction

The International Headache Society (IHS) defines ophthalmoplegic migraine (OM) as recurrent attacks of headaches with migrainous characteristics, associated with the paresis of one or more ocular cranial nerves and with the absence of any demonstrable intracranial lesion, other than magnetic resonance imaging (MRI) changes within the affected nerve (1).

In OM, the most common involvement is seen in the oculomotor nerves. The clinical features including onset in the childhood period, headaches preceding and ipsilateral to the third nerve paresis, dilated pupil, ophthalmoplegia that may be permanent and rarely accompanied by aberrant oculomotor regeneration, a minimum of two episodes and a lack of evidence for a structural lesion are known as the criteria for oculomotor OM (2).

In this report, the aim is to present an adolescent boy with OM involving the third nerve, treated with steroid and showing a dramatic improvement. It is believed that this case report can contribute to the understanding and treatment options of OM.

Case Report

A 13-year-old boy presented with unilateral (left) ptosis and diplopia with sudden onset and a duration of about 12 hours (Figure 1). The patient had been suffering from such complaints as headache, pain in the left eye, nausea and recurrent vomiting during the previous three days. The patient's history revealed that he had experienced recurrent headache attacks five to six times in the previous six years, and that all episodes had been spontaneously resolved with in two or three days.

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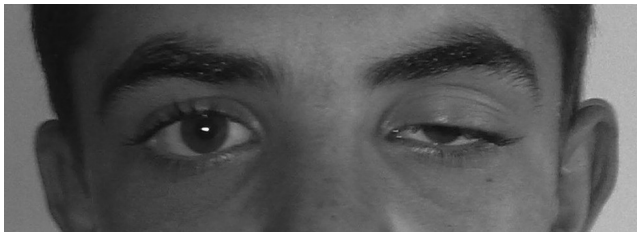

Figure 1. The incomplete ptosis of the left eyelid

However, it was noted that the patient developed eye findings for the first time.

Ophthalmologic examination revealed incomplete ptosis of the left eyelid and an inability to rotate the eye upward, downward, or inward. While normal in the right eye, pupil size was slightly dilated in the left eye. Pupillary constriction was normal in the right eye and sluggish in the left eye. Visual acuity and intraocular pressure in both eyes were within normal limits. Fundus examination showed no abnormal signs such as pallor or edema of the discs. An examination of anterior and posterior segments, as well as macula, vessels and periphery, showed no abnormalities.

Laboratory investigations revealed the following findings: hemoglobin 14.2 g/dL: leukocyte count: 6.400/mm^3; and platelet count: 345.000/mm^3. C-reactive protein and erythrocyte sedimentation rates were 2.97 mg/dL and 21 mm/h, respectively. Serum electrolytes, renal and liver function tests were normal. While brain MRI without contrast was normal, MRI with contrast revealed the diffuse contrast enhancement in the cisternal segment of the left oculomotor nerve. Diffusion MRI also showed a change of blood stream in the left cavernous sinus, suggestive of thrombus. Cerebrospinal fluid examination was normal. No viral agent was identified in the cerebrospinal fluid by polymerase chain reaction.

The patient was diagnosed with OM, based on clinical and laboratory findings. Prednisolone treatment (2 mg/kg/day) was started. The recovery in OM was observed on day three of treatment. The case was completely improved one week after the diagnosis. However, steroid treatment was continued for 10 days and then discontinued by tapering off. In order to prevent future attacks, antimigraine prophylaxis (flunarizine) was started, and no recurrence sign was observed during a 12-month follow-up period. The informed consent was taken from the patient's parents for publication.

Discussion

According to the IHS, OM is diagnosed when at least two or more attacks of migraine-like headaches lasting for 4 days from the onset are accompanied or followed by the paresis of one or more of the third, 4th or 6th cranial nerves. The pathophysiology of OM has yet to be clearly determined. However, different mechanisms, including compressive, ischemic and inflammatory processes, have recently been put forward (1). It is suggested that one or more ocular motor nerves may be compressed by edematous or dilated carotid artery. This is the most accepted theory.

Adult patients with OM often report that OM attacks are experienced at the rate of 1 in 1 or 1 in 2 of all headache attacks they have had. However, in children, OM may be the first manifestation of migraine (3). Therefore, it is usually attributed to aneurysm, trauma, central nervous system infection or immunization. The correct diagnosis is made generally when the clinical condition improves and relapses occur. The majority of the patients experience their initial attack in their first decade, mostly before 5 years of age. Rarely, patients with OM experience their first attacks in adulthood, but they mostly have either a history of typical migraine headaches with or without aura since childhood or a family history of migraine (3). While the onset of migraine attacks in the presented patient was about 7 years of age, the first attack of OM developed in his adolescent period.

Although cranial MRI without contrast is generally normal, approximately 86% of patients with OM have transient contrast enhancement of the affected cranial nerve in MRI with contrast (1). In this case, MRI with contrast revealed the diffuse contrast enhancement in the cisternal segment of the left oculomotor nerve. Contrast-enhanced studies also show that focal thickening takes place at the exit of the nerve in the interpeduncular cistern without enhancement of the cavernous sinus or adjacent dura. Diffusion MRI showed the change of blood stream in the left cavernous sinus which is suggestive of thrombus.

The differential diagnosis of OM includes intracranial aneurysms or tumours, orbital myositis, oculomotor nerve schwannoma, sphenoidalsinus mucoseles, raised intracranial tension with brain herniation and diabetic neuropathy (3,4). The diagnosis of these disorders was excluded through MRI. The fact that the previous history of recurrent headaches resolving spontaneously and other neurological examinations being normal also was supportive of an OM diagnosis.

Optimal treatment for OM still remains unclear. However, prophylactic medications, such as beta blockers and calcium channel blockers, have been proposed as a treatment. Most cases recover completely within days to

weeks (median time three weeks), but a minority are left with a persistent neurologic deficit (5,6). Some authors believe that immediate administration of steroids at the onset of an attack might reduce permanent sequelae of OM, including residual weakness of the third cranial nerve and pupillary dysfunction (7).

In conclusion, further studies are needed to determine the underlying mechanism and management of this unusual condition. This case met the IHS criteria for OM, and systemic steroid therapy showed promising results. Thus, it is believed that this report can add to the understanding and treatment options of OM.

Ethics

Informed Consent: The informed consent was taken from the patient's parents for publication.

Peer-review: External and internal peer-reviewed.

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

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Case Report

Diamond-Gardner Syndrome: Autoerythrocyte Sensitization Syndrome

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ABSTRACT

Diamond-Gardner syndrome (DGS) is an autoimmune disease characterized by painful ecchymoses that develop following emotional stress or trauma. The lesions are observed mostly in the extremities and these lesions are the result of autosensitization to extravasated erythrocytes after trauma. The majority of the patients diagnosed with this disease are composed of young women. In this case report, a teenager who had complaints of recurring painful ecchymoses with no related personal or familial background and later-diagnosed with DGS is presented. Although it is seen less commonly, DGS should be considered in the differential diagnosis of cutaneous lesions and haemorrhages of the childhood period, especially in adolescence.

Keywords: Diamond-Gardner syndrome, autoerythrocyte sensitization syndrome, Psychogenic purpura

Introduction

Diamond-Gardner syndrome (DGS) which is also known as autoerythrocyte sensitization syndrome, painful bruising syndrome or psychogenic purpura was first described by Diamond-Gardner in 1955 (1). They discovered recurrent, painful ecchymosis following emotional or physical stress in four women and named the syndrome. In the following years, more cases consisting of ecchymosis which are related with trauma but have no relationship with haematological disorders were reported. The syndrome is mostly seen among adult females but there are also cases in children and adult men (2,3). Although some theories have been put forward, the underlying mechanism of the syndrome remains unclear (4).

In this report, a case of a 16-year-old female patient with DGS is presented.

Case Report

A 16-year-old female patient was admitted to the paediatric clinics of TOBB ETU Hospital with a complaint of recurrent ecchymosis on both upper and lower extremities.

In her personal history, no other complaints other than ecchymosis which had continued for over 4 months was mentioned. There was no history of epistaxis or menorrhagia. No physical trauma or drug usage was described. Although she denied any emotional trauma, when the matter was more deeply investigated, it was learned that she had been suffering from a drop in success in school. No similar complaints were present among the other family members.

On physical examination, ecchymosis with different diameters and colours were observed, one on the right wrist and two on the shoulders. The one on the right wrist had a 3 cm diameter and was light pink in colour (Figure 1).

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Received: *02.01.2018* **Accepted:** *25.03.2018*

The one on the left shoulder had a 4 cm diameter and was purple, the one on the right shoulder was smaller and less prominent than the one on the left shoulder (Figure 2). The ecchymosis was smooth on the surface and was not painful with or without palpation. No other findings other than the ecchymosis were present.

Complete blood count, international normalized ratio, prothrombin time (PT), thrombin time, activated partial thromboplastin time (aPTT), bleeding time and fibrinogen were found to be normal. Antinuclear antibody was found to be negative. On peripheral blood smear, thrombocytes were found sufficient and clustering with no abnormalities.

Since autoerythrocyte sensitization syndrome was considered, the patient was intradermally given 1 mL of both whole blood and plasma obtained from the patient herself, and also saline was administered to the other arm as a control (Figures 3, 4). The skin was examined on the 1st and 24th hour after the injection (Figures 3-5). Although the skin reacted to both whole blood and plasma, the reaction to whole blood was more prominent.

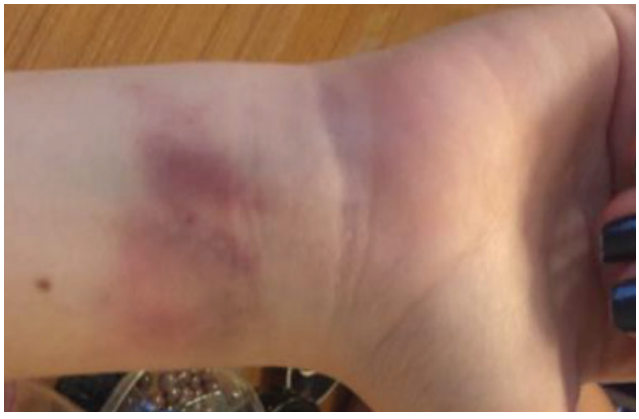

Figure 1. Ecchymosis on the right wrist

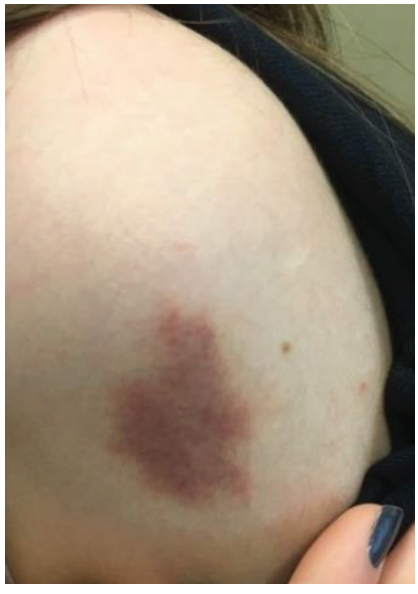

Figure 2. Ecchymosis on the left shoulder

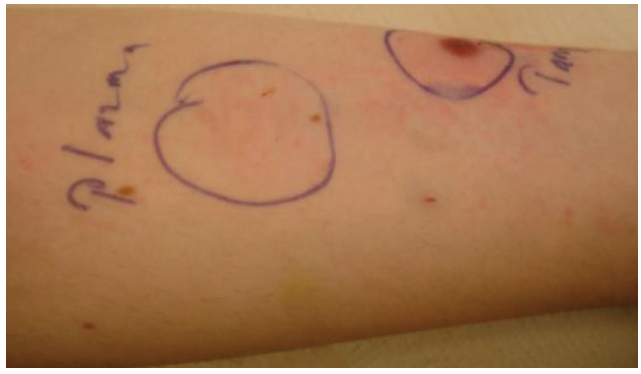

Figure 3. Skin one hour after the injection of plasma and whole blood

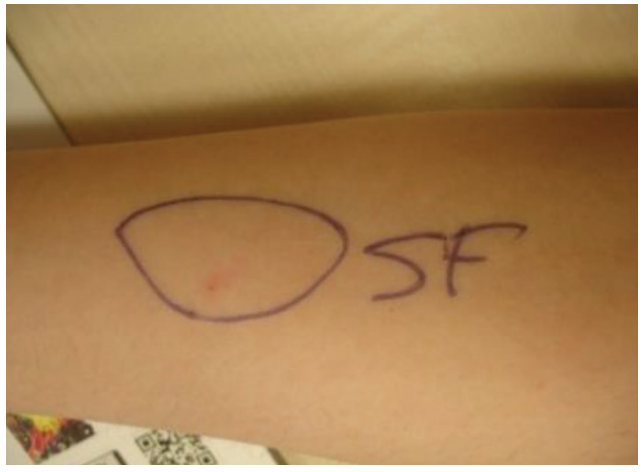

Figure 4. Control injection of saline

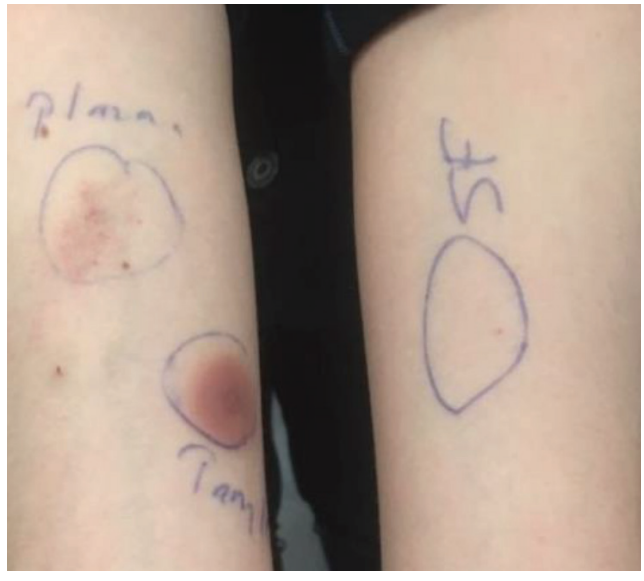

Figure 5. Skin observed 24 hours after the injection

The pre-diagnosis of DGS was confirmed according to the test result. The patient and her parents consented to the publication of this study.

Discussion

DGS is a hard-to-diagnose disease which is characterized by recurrent, unexplained bruising that mostly occurs on the extremities and/or face (1). The lesions are found to be well correlated with episodes of increased physical or mental stress (4).

Patients with DGS are vulnerable to trauma and are easily bruised. These bruises result in episodes of ecchymotic lesions which usually begins with burning, pain, and/or tenderness in the affected area. Patients may experience malaise or fatigue and, in some cases, fever, headache, or gastrointestinal symptoms (3). Bruises typically go away approximately in a week and the pain generally lessens when the bruises disappear. However, relapses and remissions of bruising episodes can last during the lifetime of the patient.

The underlying mechanism of this condition is thought to be increased sensitivity to phosphatidylserine in the stroma of the erythrocytes, however it has not been proven yet (4).

There is no specific laboratory test for the diagnosis of this syndrome, diagnosis is made mostly by detailed anamnesis and ruling out the other possible disorders for the lesions. A special skin test in which the patient's own plasma is injected to see the reaction may help in diagnosis, however a negative result of the test does not rule out the syndrome (5).

For the differential diagnosis, factitial purpura, coagulation and platelet disorders, von Willebrand disease, vasculitis and systemic lupus erythematosus should be considered.

Many drugs including antihistaminics, corticosteroids, immunosuppresives, hormones, anticoagulants are used for the treatment of DGS but none of them have been found to be efficient enough. Physiological treatment is seen as the most effective treatment. Although there is no specific treatment, the prognosis of DGS is good, most cases experience recurrent lesions that appear in times of increased stress, but mortality due to the syndrome is not reported.

Ethics

Informed Consent: The patient and her parents consented to the publication of this study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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

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Letter to the Editor

A Superficially Located Soft Tissue Mass in Upper Leg of a 7-Year-Old Boy

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Dear Editor;

A heterogeneous group of soft tissue masses including pseudotumours, benign and malignant neoplasms may occur in children. Most of them are benign and located in the arm, leg, or trunk. Leiomyomas constitute a small part of these neoplasms and can occur anywhere in the body where smooth muscle is found such as the skin, the eyes, the uterus, the bladder, and the gastrointestinal and respiratory tracts. As they are formed of spindle cells, they can resemble some pseudotumours or even malignant neoplasms (1). In the evaluation, the age of the child, location and duration of the tumour, rate of growth, consistency, and associated symptoms with radiological findings are helpful for determining differential diagnosis and treatment. Surgical excision provides both diagnosis and treatment and recurrence is rare (2).

A 7-year-old boy was referred to the paediatric outpatient department with a nodular lesion on the lateral of his right upper leg. It was noticed 2 months prior and followed by a change in size. During his first visit, the nodule was about 10x10 mm and was tender with palpation. The surface temperature was normal, the mass was smooth, firm with well-defined edges. The ultrasonographic examination showed an oval, well-shaped, 11x5.5x11.5 mm sized hypoechoic mass in the subcutaneous fat tissue with a suspicion of schwannoma and it was agreed to have a second examination three months later. The second ultrasonographic data showed that the size of the nodule had become 14x5.6x14 mm and an operation was planned. The preoperative laboratory tests were normal.

The nodule, which was described as located subcutaneously and attached to fascia by the surgeon, was resected for pathological evaluation. Microscopic examination revealed a well-circumscribed, unencapsulated tumour formed of bundles and fascicles of spindle cells with eosinophilic cytoplasm with variable cytoplasmic vacuole in one end, blunt ended elongated nuclei and indistinct nucleoli. Three mitotic figures per 10 high power views were observed. There was no coagulative necrosis.

The tumour cells showed diffuse and strong cytoplasmic staining with Smooth Muscle Actin antibody immunohistochemically. CD68 antibody stained macrophages, CD34 antibody stained small vessels throughout the tumour and S100 stained dendritic cells but not tumoral spindle cells. No staining was observed

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with S100, epithelial membrane antige, HMB45 and Desmin antibodies. The MIB-1 proliferative index was 10-15%.

The diagnosis was a spindle cell mesenchymal neoplasm consistent with a leiomyoma. Although it was totally excised, clinical and radiological follow-up were advised due to the hypercellularity, mitosis and MIB-1 proliferation index. In the follow-up of six months duration, the patient showed no signs of recurrence via ultrasonographic examination.

Soft tissue leiomyomas are smooth muscle-derived benign tumours with minimal atypia and few mitoses but with no coagulative necrosis. They are seldom reported as occurring in the lower extremities (2). They can be located in dermis, subcutis, and also in deep soft tissue. The skin is the second most common location for leiomyoma after the uterus, hosting ~5% of all leiomyomas. When they are located in the dermis, they are characteristically small and superficial, and they arise from arrector pili muscles (3). It is important to differentiate from leiomyosarcoma especially when mitotic figures and MIB-1 proliferation is higher than the expected. Leiomyosarcoma of soft tissue is relatively rare, and typically a tumour of adults and the elderly. They account for only 7 to 15 percent of all childhood soft tissue sarcomas (4). Soft tissue sarcomas can occur anywhere in the body, but most originate in an extremity (59%), the trunk (19%), the retroperitoneum (15%), or the head and neck (9%) (5).

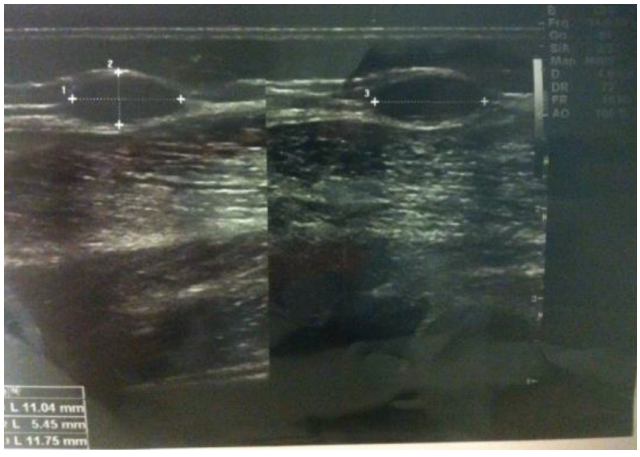

Figure 1. First ultrasonografic image

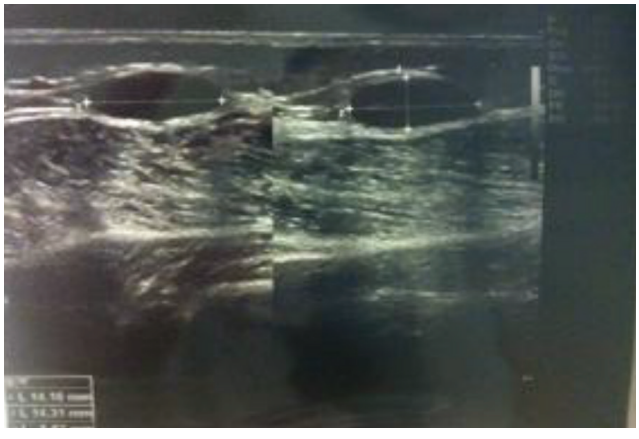

Figure 2. Ultrasonografic image before the operation

The resected nodule was diagnosed as a leiomyoma. The specimen didn't include adjacent normal skin or deep muscle tissue, but it is thought to have arisen from the arrector pili muscles considering its location (below the dermis and above the muscle). Although it had an MIB-1 proliferation index higher than 10% and some mitotic figures, it was considered as benign because of the young of age of the patient and its superficial location. Clinical and radiological follow-up is advised.

As in our case, not only the pathologic features but also the age of the patient, location and duration of the tumour, rate of growth and associated symptoms are important in the diagnosis of soft tissue tumours. The patient's family consented to the publication of this study.

Ethics

Informed Consent: The patient's family consented to the publication of this study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.M., Ş.A.B., Concept: N.A., Y.N.A.A., Ş.A.B., Design: Y.N.A.A., Data Collection or Processing: Ş.A.B., Y.N.A.A., Analysis or Interpretation: Ş.A.B., Literature Search: Y.M., Ş.A.B., Writing: Ş.A.B., Y.N.A.A., N.A.

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