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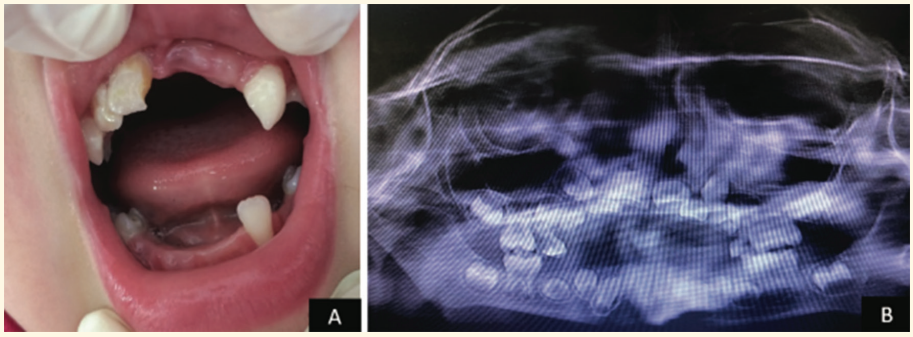
The Journal of Pediatric Research



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Aims and Scope

The Journal of Pediatric Research is the official publication of Ege University and Ege Children's Foundation. It is a peer-reviewed journal published quarterly in March, June, September and December in English.

The Journal of Pediatric Research is a peer-reviewed, open-access journal, which publishes original research articles, invited review articles, clinical reports and case reports in all areas of pediatric research.

The journal publishes original research and review articles, which summarize recent developments about a particular subject based on standards of excellence and expert review and case reports. The target audience includes specialists in general pediatrics and pediatric subspecialties (Emergency Medicine, Allergy and Immunology, Endocrinology, Gastroenterology, Hepatology and Nutrition, Genetics, Cardiology, Hematology-Oncology, Infectious Diseases, Metabolism, Nephrology, Neurology, Rheumatology, Pulmonology, Social Pediatrics, Newborn, Critical Care Medicine, Ethics and Health Service Research), as well as relevant specialities such as Pediatric Surgery, Child and Adolescent Psychiatry, Pedodontics, Pediatric Nursing and Family Physicians.

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Address for Correspondence & Permissions
Özgür Çoğulu
Ege University Faculty of Medicine, Department of Pediatrics, Bornova, 35100 İzmir, Turkey
Phone: +90 232 390 10 05 - 390 10 31
Fax: +90 232 390 13 57
E-mail: ozgur.cogulu@ege.edu.tr

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Case reports: the CARE case report guidelines

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Correspondence

Prof. Dr. Özgür Çoğulu

Ege University Faculty of Medicine, Department of Pediatrics, Bornova, 35100 İzmir/Turkey

Phone: +90 232 390 10 05 - 390 10 31

Fax: +90 232 390 13 57

E-mail: ozgur.cogulu@ege.edu.tr

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Editorial

Dear JPR readers,

We are pleased to share with you the 2th issue of The Journal of Pediatric Research of 2022. Firstly, I would like to thank all the editorial board, reviewers, authors and the publishing house for their efforts. This issue consists of valuable studies of which eleven are original articles and four are case reports. Currently, COVID-19 studies continue in many areas all over the world, and in this issue, we share two studies related to impact of the COVID-19 pandemic on "pediatric intensive care unit admissions" and "flexible bronchoscopy implementation". We hope that you will be interested in reading the other studies that comprise different pediatric subjects. Dental anxiety, burden of asthma, oral health in ADHD, fecal calprotectin and IBH, MEFV mutations and allergic diseases, maternal and neonatal B12 and folate deficiencies, reducing the pain and stress of newborns during the venipuncture, Kawasaki disease, increasing breastfeeding rates, nasal actinomyces, extrarenal Wilms' tumour, asfotase alfa treatment in hypophosphatasia, GVHD treatment with ruxolitinib are the main topics in the content of this issue.

As the JPR family, we are glad to bring together the new issues of the journal with you. We look forward to your valuable studies and scientific contributions.

Best wishes,
Dr. Yeliz Çağan Appak

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Original Article

Evaluation of the Risk Factors of Dental Anxiety in Children

İlhan Uzel[1], Bengi Aydınel[2], Aslı Topaloğlu Ak[3]

[1]Ege University Faculty of Dentistry, Department of Pediatric Dentistry, İzmir, Turkey
[2]Private Dentist, İzmir, Turkey
[3]İstanbul Aydın University Faculty of Dentistry, Department of Pediatric Dentistry, İstanbul, Turkey

ABSTRACT

Aim: Dental anxiety is described as state anxiety due to dental treatment procedures. The present study aimed to evaluate the etiological factors of dental anxiety in children aged 7-8 and 11-12 years old.

Materials and Methods: A total of 370 children of both genders were enrolled in this study. The parents' socio-economic status, education levels and family income, oral hygiene habits, and the caries status of the children were recorded on a structured questionnaire. The dental anxiety of both the children and their mothers was evaluated by the administration of a questionnaire based on Corah's dental anxiety scale (DAS). The children's fear survey schedule-dental subscale (CFSS-DS) was also used to assess the dental anxiety levels of the children. Data were analyzed using the SPSS (SPSS Inc., Chicago, IL, USA) 19.0 software program. Descriptive statistics were used for socio-demographic data. Parametric and non-parametric tests were used to compare means/medians, whereas the chi-square test was used to compare proportions. Student's t-test and one-way ANOVA with Bonferroni correction were employed to compare the anxiety scale results. All significance levels were set at 0.05.

Results: A negative correlation was found between the age groups and dental anxiety levels in the children (p=0.02). The difference between gender and dental anxiety was statistically significant (p=0.01). Boys were found to be more anxious than girls. The differences between the dental anxiety and the education levels of the mothers and the family income of the parents were not statistically significant (p>0.05). It was detected that the maternal dental anxiety level strongly affected the anxiety level of the children in the group of 7-8 years (p=0.01), while no significant difference was found in the group of 11-12 years (p>0.05). A positive correlation was found between the dental caries scores of the children and their dental anxiety level (p=0.01).

Conclusion: Dental anxiety is multifactorial and is far more complex than can be explained by a single contributing factor.

Keywords: Dental anxiety, CFSS-DS, DAS, children

Introduction

Dental anxiety is a common problem for a considerable number of patients and often results in inadequate oral health due to less frequent dental visits, avoidance of dental treatment, and poor cooperation (1,2). It is a widespread dental health problem affecting many people worldwide. The prevalence of dental anxiety has been reported to range from approximately 5-30%, depending on the population and measurement method (3-5).

The etiology of dental anxiety is multifactorial. It has been suggested that both exogenous and endogenous constituents play a significant role in its etiology (6,7).

Address for Correspondence
İlhan Uzel, Ege University Faculty of Dentistry, Department of Pediatric Dentistry, İzmir, Turkey
Phone: +90 505 772 12 25 E-mail: uzel_ilhan@hotmail.com ORCID: orcid.org/0000-0002-0540-2821
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Socio-economic factors, age and gender, previous traumatic experiences, environmental factors, and social interactions are the major contributing factors to dental anxiety (6-9).

The age of the subject is considered one of the factors which has a substantial impact on dental anxiety among children. There is almost total agreement in the literature that younger children tend to be more anxious in the dental office compared to older children (10,11).

Evidence regarding differences in dental anxiety between boys and girls is inconsistent (12,13). Most investigators reported higher levels of dental anxiety among girls (12-15). However, certain other studies reported that there were no differences between the genders regarding dental anxiety (16,17).

The education level and the social status of the parents have long been considered as factors which affect the dental anxiety level of children. Children from families with low socio-economic status and low educational levels tend to experience more dental anxiety. This could be due to decreased dental awareness (18,19). It was speculated that the parents' dental anxiety levels might exert an influence on their children's dental anxiety through modeling and information. It has been reported that over 40% of parents gave their children a negative attitude about their previous dental visit (20,21).

It has been stated that high dental anxiety affects the oral health of the patients and therefore causes negativity in their quality of life. Dental anxiety is associated with an increased level of dental caries and poor oral hygiene in children. Children with high dental anxiety also demonstrate poor cooperation during dental visits, which compromises the treatment outcomes, creates occupational stress on dental staff, and causes discord between dental professionals and the parents (22-24).

There are many methods for determining dental anxiety, such as physiological, psychometric, projective tests and observing and scoring behaviors (13,16,25,26). Corah's dental anxiety scale (CDAS), developed by Corah in 1969, is one of the most common methods used to assess the dental anxiety of patients. It consists of four questions, each question containing five answers. Items are scored on a scale of 1 (no anxiety) to 5 (extreme anxiety) and summed to give an overall anxiety score ranging from 4 to 20. The cut-off points for the CDAS were as follows: dental anxiety of the study participants was classified as low (4-12) or high (≥13) (25). The children's fear survey schedule-dental subscale (CFSS-DS), proposed by Cuthbert and Melamed (26) in 1982, is another widely used measure of dental anxiety in children. It consists of 15 items, including various aspects of dental and medical situations, and scored from 1 (not afraid) to 5 (very afraid) on a 5-point Likert scale, with total scores ranging from 15 to 75. The cut-off points for the CFSS-DS are as follows; dental anxiety of the children was classified as low (15-38) or high (>38) (26).

The present study aimed to evaluate the etiological factors of dental anxiety in children aged 7-8 and 11-12 years old.

The null hypotheses to be tested were as follows;

1) Gender has no influence on the presence of dental anxiety in children.

2) There is no statistically significant difference between socio-demographic factors and dental anxiety.

3) Maternal dental anxiety level does not correlate with the dental anxiety level of their children.

Materials and Methods

The present study was approved by the Ethical Committee of the Ege University Faculty of Medicine (approval no: 17-4/18, date: 20.04.2017). A total of 370 children aged 7-8 and 11-12 years old of both sexes were enrolled in this study. All children and their parents received written and verbal information about the procedure, and written informed consent was obtained before the study.

The study was carried out among patients who were referred for their first dental visit to Ege University Pediatric Dentistry Department. Patients with any systemic or genetic diseases were not included in this study.

The survey consisted of two sections. The first section requested socio-demographic and dental information of the patient, including age, gender of the children, socio-economic status, education level (low: ≤8 years, high: >8 years), and family income (low, moderate, high) of the parents, oral hygiene (brushing regularly twice a day, irregular, none) and dental caries scores [Caries free: decayed, missing, and filled teeth (DMFT + dmft)=0, low caries: DMFT + dmft=1-4, high caries: DMFT + dmft>4] of the children. The second section comprised the CDAS (low, high) for both the children and their mothers and the CFSS-DS (low, high) for only the children (Table I, II).

Data were analyzed using the SPSS (SPSS Inc., Chicago, IL, USA) 19.0 software program. Descriptive statistics were used for socio-demographic data. Parametric and non-parametric tests were used to compare means/medians, whereas the chi-square test was used to compare proportions. Student's t-test and one-way ANOVA with Bonferroni correction

were employed to compare the anxiety scale results. All significance levels were set at 0.05.

Results

The age and gender distribution of the children in the study population are shown in Table III. 51.4% (n=190) of the 370 patients were in the group of 7-8 years, while 48.6% (n=180) were in the group of 11-12 years. 183 (49.5%) of the patients were girls, while 187 (50.5%) were boys in the present study.

The mean ± standard deviation of the CDAS and CFSS-DS scores of the children according to their age and gender groups are shown in Table IV. A negative correlation was found between the age groups and dental anxiety levels in the children (p=0.02). The difference between gender and dental anxiety was statistically significant (p=0.01). Boys were more anxious than girls. No significant difference was found between the CDAS and CFSS-DS scores in children (p>0.05). 88 children in the 7-8 years group and 43 children in the 11-12 years group were recorded as having high anxiety according to the results of both anxiety scales.

The differences between the dental anxiety and the education levels of the mothers and the family income of the parents were not statistically significant (p>0.05).

Table I. Corah's dental anxiety scale (CDAS) (25)

1. If you had to go to the dentist tomorrow, how would you feel? (1) Look forward to it as a reasonably enjoyable experience (2) I wouldn't care one way or the other (3) I would be a little uneasy about it (4) I would be afraid that it would be unpleasant and painful (5) I would be very frightened of what the dentist might do
2. When you are waiting in the dentist's office for your turn in the chair, how do you feel? (1) Relaxed (2) A little uneasy (3) Tense (4) Anxious (5) So anxious that I sometimes break out in a sweat or almost feel physically sick
3. When you are in the dentist's chair waiting while he gets his drill ready to begin working on your teeth, how do you feel? (Same alternatives as Q.2)
4. You are in the dentist's chair to have your teeth cleaned. While you are waiting and the dentist is getting out the instruments which he will use to examine your teeth around the gums, how do you feel? (Same alternatives as Q.2)

Table II. The children's fear survey schedule-dental subscale (CFSS-DS) (26)

	Not afraid	A little afraid	Fairly afraid	Quite afraid	Very afraid
Dentists					
Doctors					
Injections					
Having somebody examine your mouth					
Having to open your mouth					
Having a stranger touch you					
Having somebody look at you					
The denstist drilling					
The sight of the dentist drilling					
The noise of the dentist drilling					
Having somebody put instuments in your mouth					
Choking					
Having to go to the hospital					
People in white uniform					
Having the dentist clean your teeth					

No significant association was found between the oral hygiene habits of the children and their dental anxiety level (p>0.05). It was detected that the maternal dental anxiety level strongly affected the anxiety level of the children in a group of 7-8 years (p=0.01), while no significant difference was found in a group of 11-12 years (p>0.05). The distribution of the dental anxiety levels of the patients according to their caries index scores is given in Table V. A positive association was found between the dental caries scores of the children and their dental anxiety levels (p=0.01).

Discussion

Dental anxiety regarding dentists and dental treatment is one of the most common dental treatment problems. Although dental anxiety can be observed at any age, it can usually be seen in childhood (1-3). Despite technological developments and a modern approach to the prevention and management of oral diseases, dental anxiety is still one of the main reasons for avoiding dental treatment (4,5).

The etiology of dental anxiety is complex and multifactorial. Many different factors have been discussed as influences on dental anxiety in children, including socio-demographic factors, oral hygiene habits, oral health, parental dental anxiety level, etc. (6-9). This study explored the possible associations between dental anxiety in children and its related factors.

Several valid psychometric questionnaires can be used to evaluate dental anxiety levels. Assessing the level of anxiety can be very useful in providing good quality dental services and to allow for the better management of the behavior of patients. The ideal measurement methods should be valid, allow for limited cognitive and linguistic skills, and be easy to administer and score in a clinical situation (13,16). According to these, the CDAS and the CFSS were used to assess dental anxiety levels in the present study. Both scales have demonstrated good reliability and acceptable validity and have been used to estimate the prevalence of dental anxiety and evaluate the behavior management procedures used for children in previous reports (13,16,25,26).

Several studies have suggested the relationship between age and dental anxiety to be a decrease in dental anxiety with increasing age (10,11). The contributing factors to dental anxiety in children were studied in two different age groups in the present study. According to the results of the present study, it was seen that dental anxiety seems to decrease with increasing age (p=0.01). This finding is in agreement with previous studies (10,11).

Relating dental anxiety to gender is a controversial issue in the literature (12,13). Dental anxiety has been linked more to girls than boys, as reported by some studies (12-15). Only a few studies showed no significant relationship between dental anxiety and gender (16,17). The difference between gender and dental anxiety was found statistically

Table III. The distribution of the age and gender of the patients

Age groups		
Gender	**7-8 years group**	**11-12 years group**	**Total**
Girls	92	91	183
Boys	98	89	187
Total	190	180	370

Table IV. The mean ± standard deviation scores of the CDAS and CFSS-DS anxiety scales according to the age and the gender of the patients

Age groups				
Anxiety scales		**7-8 years group (Mean ± SD)**	**11-12 years group (Mean ± SD)**	**p-value**
CDAS	Girls	13.75±7.02	11.56±6.12	0.01
	Boys	15.91±8.22	13.45±7.18	
CFSS-DS	Girls	29.12±12.35	23.92±10.60	0.01
	Boys	32.52±14.23	28.86±11.07	

CDAS: Corah's dental anxiety scale, CFSS-DS: The children's fear survey schedule-dental subscale, SD: Standard deviation

Table V. The distribution of the dental anxiety levels of the patients according to the caries index scores

		Caries free DMFT + dmft=0 (n)	**Low caries DMFT + dmft=1-4 (n)**	**High caries DMFT + dmft>4 (n)**	**p-value**
CDAS	Low anxiety	93	102	44	0.001
	High anxiety	12	23	96	
CFSS-DS	Low anxiety	101	106	32	0.001
	High anxiety	9	20	102	

CDAS: Corah's dental anxiety scale, CFSS-DS: The children's fear survey schedule-dental subscale, DMFT: Decayed, missing, and filled teeth (caries index for permanent teeth), dmft: Decayed, missing, and filled teeth (caries index for primary teeth)

significant in the present study (p=0.01). Boys were more anxious than girls. Contradictory research findings may be explained by different study designs and methods of data collection. Moreover, gender influences should be regarded in combination with other factors such as local culture and the socio-economic status of the family. In our society, girls were thought to become more mature as they get older.

It has been reported that children from low socio-economic status families are more anxious than children of high socio-economic status families. This may be because parents from low socio-economic status transferred their fear and anxiety to their children due to a lack of awareness (18,19). The differences between dental anxiety and the education level of the mothers and the family income of the parents were not statistically significant in the present study (p>0.05).

There are numerous studies investigating the relationship between child and parental dental anxiety. The literature has also reported a positive association between children's dental anxiety and parental, particularly maternal, anxiety. Children, especially those under 8 years old, who see dental anxiety in their parents, or learn about it through stressful information provided by parents, are more likely to develop a similar attitude, thus resulting in the development of dental anxiety. Parents have an influence on their children's anxiety level. As the child grows, they are less affected by their family and environmental factors come into play (20,21). In the present study, it was seen that the maternal dental anxiety level strongly affected the anxiety level of the children in a group of 7-8 years (p=0.01), while no significant difference was found in a group of 11-12 years (p>0.05).

It has been shown that there is a significant correlation between anxiety and poor oral health, poor oral hygiene, and esthetics (22,23). In the present study, no significant correlation was found between the oral hygiene habits of the children and their dental anxiety level (p>0.05). The avoidance of dental treatment was higher in dentally anxious children, and they also had increased caries scores (22-24). A positive correlation was found between the dental caries scores of the children and their dental anxiety levels in the present study (p=0.01).

Dental anxiety is a serious problem that negatively affects oral health in people of all ages. Early detection of the etiology of the reasons for this anxiety is the key to solving this problem.

Study Limitations

The first limitation of this study is that the sample was from a single medical institution. The group of children represented were also more inclined to show the behavior of visiting a dentist, probably because of lower levels of dental anxiety.

The second limitation of this study was that the children included in the present study were selected among those who had their first dental visit. Therefore, only the effects of demographic and socio-economic factors and maternal dental anxiety on the dental anxiety levels of the children could be examined.

Conclusion

Dental anxiety is multifactorial and is far more complex than can be explained by a single contributing factor.

Acknowledgments

We would like to thank Assoc. Prof. Dr. Timur Köse for the statistical analysis.

Ethics

Ethics Committee Approval: The present study was approved by the Ethical Committee of the Ege University Faculty of Medicine (approval no: 17-4/18, date: 20.04.2017).

Informed Consent: Written informed consent was obtained before the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Design: İ.U., B.A., A.T.A., Data Collection and/or Processing: İ.U., B.A., Analysis or Interpretation: İ.U., A.T.A., Literature Research: İ.U., B.A., Writing: A.T.A.

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Original Article

The Burden of Asthma in Children Aged 0-14 Years in Asia: A Systematic Analysis for the Global Burden of Disease Study 2019

Elham Goodarzi[1], Kobra Rashidi[2], Zahra Zare[3], Vectoria Momenabadi[4], Zaher Khazaei[5]

[1]Social Determinants of Health Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[2]Social Determinants of Health Research Center, Department of Nursing, Boroujerd School of Nursing, Lorestan University of Medical Sciences, Khorramabad, Iran
[3]Tabriz University of Medical Sciences, Department of Statistics and Epidemiology, Faculty of Health, Tabriz, Iran
[4]Assistant Professor of Health Education and Health Promotion, School of public Health, Bam University of Medical Sciences, Bam, Iran
[5]Nahavand School of Allied Medical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran

ABSTRACT

Aim: Asthma is the most common chronic disease in children and it imposes a huge burden on the health systems of countries. The aim of the present study was to investigate the burden of asthma disease in children in Asia during the period of 1990-2019.

Materials and Methods: All data sources from the Global Burden of Disease study (2019) were used to estimate the breast cancer prevalence, mortality, disability-adjusted life years and asthma rates in children aged 0-14 years in Asia from 1990 to 2019. We estimated all-cause and cause-specific mortality, years of life lost (YLLs), years lived with disability (YLDs), disability-adjusted life years and attributable risks.

Results: There is a positive and significant correlation between the human development index (HDI) and disease incidence in both sexes ($r=0.417$, $p<0.05$), and this correlation is positive and significant in both females ($r=0.401$, $p<0.05$) and males ($r=0.420$, $p<0.05$). There is also a significant negative correlation between HDI and disease mortality in both sexes ($r=-0.475$, $p<0.05$) and this negative correlation is significant in both females ($r=-0.459$, $p<0.05$) and males ($r=-0.483$, $p<0.05$). The study of YLDs showed that there is a significant negative correlation between HDI and YLL in both sexes ($r=-0.474$, $p<0.05$), and in women ($r=-0.456$) and men ($r=-0.483$, $p<0.05$).

Conclusion: Given that YLL is higher in countries with low HDI, greater attention must be paid to reduce the incidence of and premature deaths attributable to asthma in these countries.

Keywords: Burden of disease, asthma, children, Asia

Introduction

Pediatric asthma is a serious public health problem worldwide, which can wield considerable influence on quality of life. Globally, asthma ranks 16th among the leading causes of years lived with disabilities and 28th among the primary causes of burden of disease, as assessed by disability-adjusted life years (DALYs) (1). The global prevalence, disease, and mortality of asthma in children have increased significantly over the past 40 years. The World Health Organization (WHO) estimates that approximately 300 million people worldwide suffer from asthma, and this figure is expected to reach 400 million by 2025 (2). Globally, the mortality rate of pediatric asthma ranges from 0 to 0.7 per 100,000 people (3,4). Among children, asthma is the

Address for Correspondence
Zaher Khazaei, Nahavand School of Allied Medical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran
Phone: +989183514874 E-mail: zaherkhazaei@yahoo.com ORCID: orcid.org/0000-0001-8619-3664
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most common chronic disease, ranking among the top 20 causes of DALY worldwide in children of all ages (5).

There is considerable geographical variation in the prevalence, severity, and mortality of asthma. While the prevalence of asthma is greater in high-income countries, more asthma-related deaths are reported in low-income countries (6). It has been shown that the prevalence of asthma in children and adults may have peaked in some areas, particularly in high-income countries, but it may have experienced an upturn in low- and middle-income countries (LMICs) (7).

The prevalence of asthma varies significantly in countries worldwide (7). In particular, the lifetime prevalence of asthma has remained constant or even declined in high-income countries, while in the majority of LMICs, especially in Eastern Europe, Latin America and North Africa, it has been on the rise (7,8). In Europe, the prevalence of this disease has escalated in children (9).

Children in LMICs cope with a disproportionately higher burden of disease, especially in terms of illness and mortality. One of the main reasons of hospitalization, especially in children under 5 years of age in LMICs, is asthma, the prevalence of which has risen over the past two decades. In recent years, the prevalence of asthma symptoms in children and adolescents, especially in LMICs, has taken an upturn worldwide. It seems that host (genetics, atopy) and environmental factors (microbial exposure, passive smoking exposure, and air pollution) are involved in this process. The rising prevalence observed in metropolitan areas compared to rural areas, and generally in industrialized countries, highlights the role of air pollution in the onset of asthma (10,11).

Some of the highest rates of asthma-related deaths are reported in the Asia-Pacific region (2). Despite the availability of effective drugs since the 1990s (12), most adult and pediatric patients worldwide, especially in the Asia-Pacific region (13,14), have been unsuccessful in the control of asthma. According to a 2013 study, 7.6% of asthma patients in the study population had properly controlled their asthma, with the highest rates reported in Singapore (14%) and the lowest rates in India (0%) and China (2%) (15). Therefore, the present study aimed to investigate the burden of asthma disease in children aged 0-14 in Asia in 2019.

Materials and Methods

This is a correlational analytical study designed to investigate the burden of asthma in children in Asia during the period 1990 to 2019. The incidence, prevalence, death toll, years of life lost (YLLs) (i.e. years that a person could have led a useful life, but were lost due to premature death), years lived with disability (YLDs) (i.e. the number of years the patient was incapacitated or disabled due to illness), the burden of disease and DALYs (i.e. the sum of YLLs and YLDs due to prevalent cases of the disease or health condition in a population) were analyzed to create an index. This index was defined and employed in the Global Burden of Disease (GBD) study to estimate disease burden based on age and sex from 1990 to 2019.

Data on pediatric asthma in Asia, which are available from the GBD 2019, were taken from online sources (https://vizhub.healthdata.org/gbd-compare/) and subjected to analysis. The burden of asthma was also taken into account in the Human Development Index (HDI), which is estimated annually for all developing and developed countries and is made publically available on the WHO website for researchers.

Countries are divided into several categories based on their degree of human development (very high human development, high human development, medium human development and low human development). The numerical value of HDI, which is between zero and one, exhibits how far each country has been successful in attaining the highest possible value (i.e. one), and therefore allows for comparisons between countries to be made. As an overview of human development, HDI measures the average success achieved in a country in the three main dimensions, namely a long and healthy life, access to education and living standards.

Statistical Analysis

In this study, the two-variable correlation method was used to analyze the extracted data to examine the correlation between the burden of asthma and HDI. The significance level was considered to be $p<0.05$. The analyses were made using Stata 12 software (Stata Corp, College Station, TX, USA).

Results

The results of the study suggest that the incidence of asthma in children aged 0-14 years in the world is 1,030.3 per 100,000, with this being higher in males than females (1,109.9 vs. 945.38 per 100,000). The mortality rate of this disease in children is 0.05 per 100,000, which is almost equal in both sexes. In the study of asthma burden of disease in children, the results showed that the asthma-related YLL is 42.1 per 100,000, which is greater in females

than in males. Also, the asthma-related YLD is 167.16 per 100,000, which is higher in males than in females (184.2 vs. 150.5 per 100,000). Finally, asthma-related DALY is 210 per 100,000, and this index is higher in males than in females (Table I).

Figure 1 displays the epidemiology of incidence, mortality and burden of disease indices in each continent and globally in 1990 and 2019. As depicted in the figure, the incidence of pediatric asthma in the world and all continents (except continental Europe) was lower in 2019 compared to 1990. Additionally, the highest incidence of asthma cancer in 1990 and 2019 was reported in the Americas and the lowest in Asia.

As for the mortality rate of asthma, the results showed that the mortality rate of this disease was lower in the world and all continents (except continental Europe) in 2019 compared to 1990. The highest mortality rates in 1990 and 2019 were reported in Africa.

With regard to the burden of disease indices, the results showed that asthma-related YLDs were lower in 2019 relative to 1990 in the world and all continents (except continental Europe). The highest asthma-related YLDs in children in 1990 and 2019 were reported in the Americas.

The results also demonstrated lower YLL in 2019 compared to 1990 in the world and all continents. The highest rates of YLL in 1990 and 2019 were seen in Africa.

The results of analysis showed that the DALY index had plummeted in the world and all continents by 2019 when compared to 1990. The highest rate of pediatric asthma-related DALYs in 1990 and 2019 were reported in the Americas (Figure 1).

Table I. Burden of asthma in children 0-14 years in the world in 2019 (Source: GBD Compare)

Index	Rate per 100,000		
	Male	**Female**	**Both**
Incidence	1109.9 (736.6, 1568.3)	945.38 (627.3, 1315.1)	1030.3 (683.6, 1449.5)
Death	0.5 (0.4, 0.62)	0.511 (0.39, 0.69)	0.0505 (0.4, 0.62)
YLL	41.5 (33.3, 52.02)	42.6 (32.5, 58.03)	42.1 (33.6, 52.4)
YLD	184.2 (107.7, 298.8)	150.5 (88.66, 242.2)	167.9 (98.96, 270.3)
DALY	225.8 (145.8, 342.3)	193.2 (128.9, 289.9)	210 (137.8, 314.83)

DALY: Disability-adjusted life years, YLL: Years of life lost, YLD: Years lived with disability

Table II shows the incidence and mortality of asthma in children aged 0-14 for Asian countries in 2019. The highest incidence of pediatric asthma was reported in the Philippines (1,686.9 per 100,000), the United Arab Emirates (1,500 per 100,000) and Kuwait (1,343,700 per 100,000) and the lowest rates in Nepal (176.5 per 100,000), Bangladesh (224.4 per 100,000) and Bhutan (237.9 per 100,000).

The results show that the highest mortality rates of pediatric asthma in Asia were reported in the Philippines (2.5 per 100,000), Myanmar (2.3 per 100,000) and Timor-Leste (2.04 per 100,000) and the lowest mortality rates were in Armenia (0.008 per 100,000) and Tajikistan (0.01 per 100,000).

Table III shows the burden of asthma disease (DALY, YLL, YLD) in children aged 0-14 by country and sex in Asia. As can be seen, the highest YLL in both sexes (218.8 per 100,000), females (202.3 per 100,000), and males (220.6 per 100,000) were reported in the Philippines. The highest rates of YLD in both sexes (594.8 per 100,000), in males (686.5 per 10,000) and in females (499.1 per 100,000) were reported in Georgia. Finally, the highest DALY index in both sexes (616.9 per 100,000), in males (713.3 per 100,000) and in females (516.4 per 100,000) were also reported in Georgia.

Figure 2 shows the trend of asthma-related indices in children aged 0-14 years from 1990 to 2019. As is depicted, the incidence rate of asthma is higher in countries with high income while the mortality rate of asthma is declining in all income level countries.

During the period 1990 to 2019, the mortality rate was lower in high-income countries than in low-income countries. According to the graph of the YLD index, the highest YLD between 1990 and 2019 was reported in high-income countries. The graph of the YLL index also suggests that the highest YLL was related to low-income countries. Finally, according to the graph of the DALY index, the highest DALYs from 1990 to 2019 were recorded in low-income countries.

Figure 3 shows the relationship of HDI with incidence, mortality, disease burden (DALY), YLD and YLL in children aged 0-14 years in Asia in 2019. As depicted in this figure, there is a positive and significant correlation between HDI and the incidence of disease in both sexes ($r=0.420$, $p<0.05$) and this correlation is positive and significant in both females ($r=0.401$, $p<0.05$) and males ($r=0.417$ $p<0.05$).

According to the results, there is a significant negative correlation between HDI and disease mortality in both sexes ($r=-0.483$, $p<0.05$), and this negative correlation is

significant in both females (r=-0.459, p<0.05) and males (r=-0.475, p<0.05).

The results reflect that there is a significant negative correlation between HDI and YLL in both sexes (r=-0.483, p <0.05), females (r=-0.456, p<0.05) and males (r=-0.474, p<0.05).

The findings also suggested a positive correlation between HDI and YLD in both sexes (r=0.219, p>0.05), females (r=0.230, p>0.05) and males (r=0.225, p>0.05), but these correlations were not statistically significant.

According to the findings, there was no significant relationship between HDI and burden of disease (DALY) in both sexes (r=0.08, p >0.05), females (r=0.05, p>0.05) and males (r=0.07, p>0.05).

Table IV shows the association between the components of HDI and each asthma index in children aged 0-14 years. As can be seen, the incidence of asthma is positively and significantly related to the gross national income per 1,000 capita (r=0.424, p<0.05) and the life expectancy at birth (r=0.409, p<0.05), whereas this correlation was not significant for mean years of schooling (r=0.281, p>0.05) or expected years of schooling (r=0.255, p>0.05).

The results revealed that the asthma mortality was negatively related to gross national income per 1,000 capita (r=-0.302, p<0.05), life expectancy at birth (r=-0.472, p<0.05), mean years of schooling (r=-0.484, p<0.05) and expected years of schooling (r=-0.397, p<0.05).

Moreover, asthma-related YLD was significantly negatively correlated with gross national income per 1,000

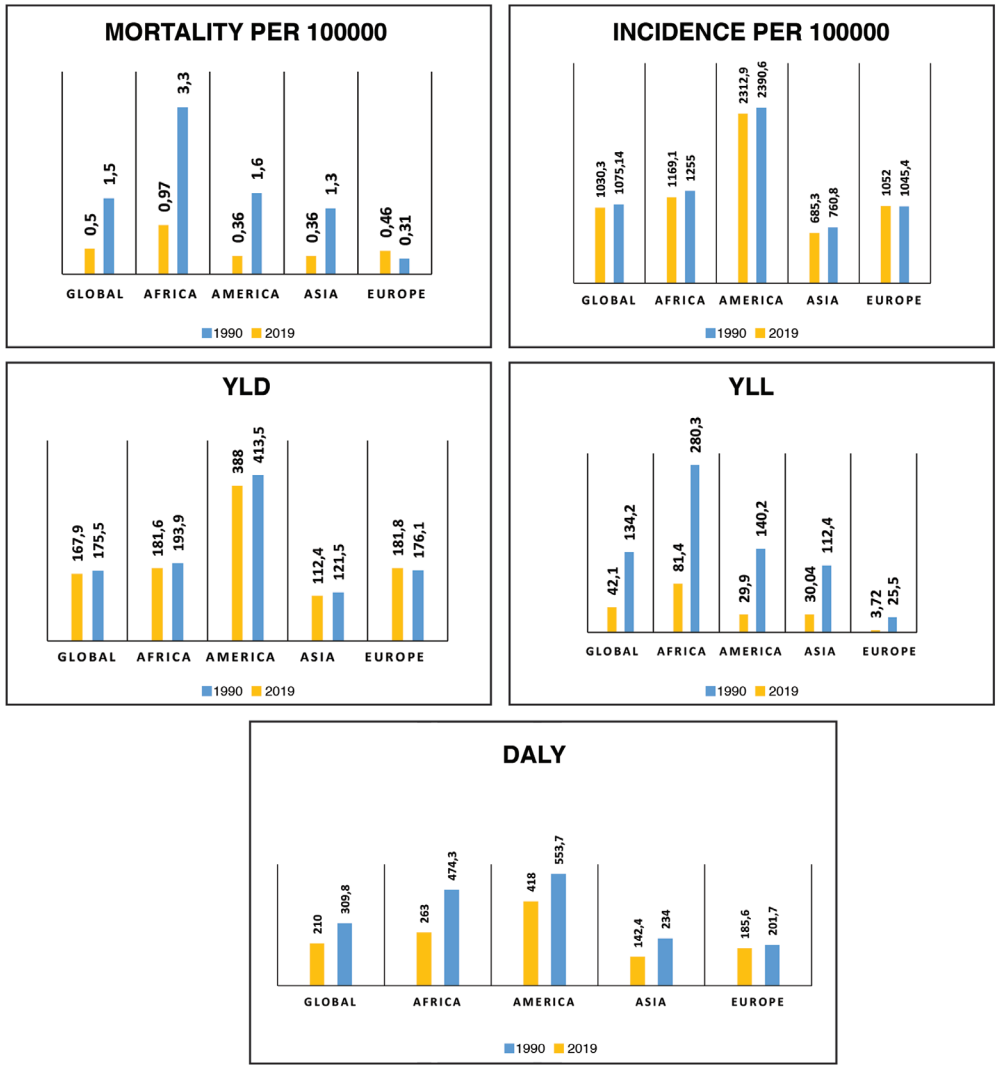

Figure 1. Distribution incidence, mortality and burden of asthma in children 0-14 years by continent (Source: GBD Compare)
YLL: Years of life lost, YLD: Years lived with disability, DALY: Disability-adjusted life years

Table II. Incidence and mortality of asthma in children aged 0-14 years in Asia in 2019 (Source: GBD Compare)

Country	Incidence			Mortality		
	M	F	Both	M	F	Both
Afghanistan	1095.57	1050.07	1138.54	0.87	0.86	0.88
Armenia	737.48	704.34	766.55	0.01	0.01	0.01
Azerbaijan	710.06	682.12	734.35	0.03	0.04	0.02
Bahrain	1180.14	1108.76	1248.46	0.23	0.32	0.14
Bangladesh	224.49	270.82	179.31	0.22	0.12	0.31
Bhutan	237.99	273.75	202.82	0.25	0.26	0.25
Brunei	1042.79	982.09	1098.53	0.10	0.07	0.13
Cambodia	853.74	778.31	925.43	0.67	0.64	0.70
China	869.18	736.66	981.91	0.02	0.02	0.03
Georgia	755.34	629.29	870.24	0.28	0.22	0.33
India	385.20	302.34	460.71	0.21	0.20	0.21
Indonesia	1024.11	839.07	1199.00	0.96	1.07	0.85
Iran	1106.55	984.06	1222.88	0.14	0.15	0.14
Iraq	1145.07	1072.42	1213.96	0.15	0.14	0.16
Israel	874.59	740.42	1001.93	0.04	0.05	0.04
Japan	1239.91	1304.99	1178.14	0.03	0.03	0.03
Jordan	1289.69	1171.23	1401.98	0.11	0.11	0.11
Kazakhstan	594.31	591.43	597.05	0.06	0.04	0.07
Kuwait	1343.76	1189.99	1488.56	0.13	0.14	0.11
Kyrgyzstan	812.35	790.94	832.70	0.02	0.02	0.01
Lao People's Democratic Republic	692.05	557.58	821.88	2.01	1.99	2.04
Lebanon	1312.68	1226.16	1392.43	0.08	0.07	0.08
Malaysia	925.17	909.90	939.54	0.13	0.13	0.14
Maldives	990.26	919.97	1056.31	0.36	0.45	0.27
Mongolia	723.72	692.47	753.82	0.06	0.05	0.07
Myanmar	604.46	473.67	731.07	2.35	2.14	2.54
Nepal	176.60	215.18	139.73	0.09	0.13	0.06
Oman	1290.30	1187.35	1389.27	0.02	0.03	0.02
Pakistan	317.93	358.60	280.05	0.39	0.15	0.60
Philippines	1686.94	1598.00	1770.21	2.53	2.41	2.65
Qatar	1163.38	1187.36	1140.40	0.10	0.08	0.12
Saudi Arabia	1002.70	815.25	1172.31	0.09	0.10	0.08
Singapore	1087.16	970.54	1202.16	0.04	0.04	0.04
Sri Lanka	852.69	805.80	898.10	0.29	0.28	0.30
Syrian Arab Republic	1036.33	1021.54	1050.44	1.40	1.00	1.79
Tajikistan	724.16	690.97	755.46	0.01	0.02	0.01
Thailand	1057.84	955.38	1154.75	0.33	0.26	0.38
Timor-Leste	988.45	948.68	1025.96	2.05	2.03	2.06
Turkey	1198.22	1173.47	1221.65	0.13	0.11	0.15
Turkmenistan	732.89	704.16	759.80	0.06	0.06	0.07
United Arab Emirates	1500.03	1386.60	1607.38	0.14	0.10	0.17
Uzbekistan	829.09	831.76	826.59	0.03	0.04	0.03
Vietnam	887.71	758.54	1007.34	0.28	0.22	0.33
Yemen	1104.65	1037.75	1168.20	0.68	0.78	0.59
Republic of Korea	1006.14	936.26	1071.96	0.02	0.01	0.02
Democratic People's Republic of Korea	952.92	822.80	1076.48	0.08	0.07	0.09

DALY: Disability-adjusted life years, YLL: Years of life lost, YLD: Years lived with disability, M: Male, F: Female

Table III. Burden of asthma in children aged 0-14 years in Asia in 2019 (Source: GBD Compare)

Country	YLL			YLD			DALY		
	M	F	Both	M	F	Both	M	F	Both
Afghanistan	71.21	70.65	71.74	167.81	157.84	177.23	239.02	228.49	248.97
Armenia	0.72	0.53	0.89	108.54	102.29	114.02	109.26	102.81	114.91
Azerbaijan	2.18	3.16	1.32	105.62	99.92	110.57	107.80	103.09	111.89
Bahrain	18.77	26.63	11.26	199.77	186.14	212.81	218.54	212.76	224.07
Bangladesh	17.50	9.63	25.18	35.09	42.90	27.47	52.59	52.53	52.65
Bhutan	20.94	21.24	20.64	36.95	43.17	30.84	57.89	64.41	51.48
Brunei	8.36	5.79	10.71	181.11	166.48	194.55	189.47	172.26	205.27
Cambodia	55.78	53.91	57.55	133.91	120.75	146.41	189.69	174.67	203.96
China	1.90	1.56	2.20	130.89	109.95	148.70	208.15	178.71	235.38
Georgia	27.34	22.06	32.15	594.84	499.20	686.54	616.95	516.45	713.32
India	22.41	18.11	26.51	542.03	453.92	626.14	87.91	67.22	106.76
Indonesia	79.43	89.05	70.33	163.75	133.43	192.42	243.18	222.48	262.75
Iran	11.60	11.93	11.29	166.53	145.02	186.96	178.13	156.95	198.26
Iraq	12.57	11.76	13.34	186.75	173.16	199.63	199.32	184.92	212.98
Israel	3.36	3.82	2.92	170.43	135.69	203.39	173.79	139.52	206.31
Japan	2.43	2.69	2.17	205.14	212.89	197.79	207.57	215.58	199.96
Jordan	8.92	8.79	9.06	219.42	195.81	241.80	228.34	204.59	250.85
Kazakhstan	4.48	3.18	5.73	83.06	81.88	84.18	87.55	85.06	89.91
Kuwait	10.40	11.56	9.31	224.70	194.79	252.86	235.10	206.36	262.17
Kyrgyzstan	1.50	1.91	1.10	117.71	113.36	121.85	119.21	115.27	122.95
Lao People's Democratic Republic	167.34	166.32	168.33	104.93	82.77	126.32	272.27	249.09	294.65
Lebanon	6.25	5.93	6.55	214.46	197.69	229.92	220.72	203.62	236.47
Malaysia	10.76	10.04	11.43	152.52	151.52	153.46	163.28	161.56	164.90
Maldives	29.90	37.40	22.86	156.73	145.04	167.72	186.63	182.44	190.58
Mongolia	4.93	3.86	5.96	100.60	95.27	105.73	105.53	99.13	111.69
Myanmar	197.21	180.44	213.44	92.07	70.48	112.97	289.28	250.92	326.41
Nepal	7.72	10.99	4.60	26.69	33.09	20.57	34.41	44.08	25.17
Oman	1.98	2.09	1.88	164.43	139.26	188.23	207.94	188.66	226.47
Pakistan	32.10	12.57	50.29	48.16	54.05	42.67	80.26	66.62	92.96
Philippines	211.81	202.37	220.66	282.30	270.34	293.51	494.12	472.71	514.16
Qatar	8.23	6.60	9.80	188.99	194.30	183.91	197.22	200.89	193.71
Saudi Arabia	7.38	8.11	6.72	160.72	126.88	191.33	168.09	134.98	198.05
Singapore	2.91	2.94	2.88	181.82	157.25	206.05	184.73	160.19	208.93
Sri Lanka	23.35	22.48	24.19	142.38	133.86	150.62	165.73	156.34	174.82
Syrian Arab Republic	114.52	81.61	145.90	172.33	170.09	174.47	286.85	251.70	320.36
Tajikistan	1.01	1.23	0.81	103.08	97.04	108.76	104.09	98.28	109.57
Thailand	26.64	21.59	31.41	185.40	165.77	203.97	335.94	302.03	368.86
Timor-Leste	171.68	170.92	172.41	162.13	156.47	167.47	333.82	327.39	339.88
Turkey	10.92	9.09	12.65	205.23	199.44	210.72	216.15	208.52	223.37
Turkmenistan	5.05	4.57	5.49	105.14	99.24	110.66	110.18	103.81	116.15
United Arab Emirates	10.94	7.89	13.82	267.34	244.85	288.62	278.28	252.74	302.44
Uzbekistan	2.52	2.96	2.10	121.58	121.10	122.03	124.10	124.07	124.13
Vietnam	23.17	18.45	27.53	145.51	122.62	166.71	168.68	141.07	194.24
Yemen	55.85	63.80	48.30	175.47	162.02	188.25	231.32	225.82	236.54
Republic of Korea	1.48	1.14	1.79	176.15	159.25	192.07	177.63	160.39	193.86
Democratic People's Republic of Korea	6.38	5.70	7.02	153.62	130.97	175.12	159.99	136.67	182.14

DALY: Disability-adjusted life years, YLL: Years of life lost, YLD: Years lived with disability, M: Male, F: Female

capita (r=-0.303, p<0.05), life expectancy at birth (r=-0.473, p<0.05), mean years of schooling (r=-0.481, p<0.05) and expected years of schooling (r=-0.393, p<0.05).

According to the results, there was no significant correlation between DALY index and components of HDI (p>0.05). Moreover, the correlation between YLD and components of HDI was not significant (p>0.05).

Discussion

Global asthma deaths are estimated to have dropped by about a third between 1990 and 2010. However, there are great discrepancies between countries. Data from the United States, Canada, New Zealand, Australia, Western Europe, Hong Kong, and Japan show that the peak of their mortality rate (0.62 per 100,000 people) in the mid-1980s in children and adults had declined progressively by the mid-2000s, dropping to less than 0.23 per 100,000 people. These findings, accompanied by the formation of national and international asthma management guidelines, demonstrate the potential positive impact of policy measures to reduce asthma mortality (16,17). Most asthma deaths are reported in low-income countries (6). In this regard, the results of the study showed that the mortality rate of this disease was lower in 2019 compared to 1990 in the world and all continents (except continental Europe), with the highest mortality rate in 1990 and 2019 being seen in Africa. On the other hand, while the general asthma mortality rate in the United States declined between 1999 and 2015, the mortality rate in children aged 1 to 14 years remained unchanged (18).

The prevalence and incidence of asthma varies considerably in different countries. The incidence of pediatric

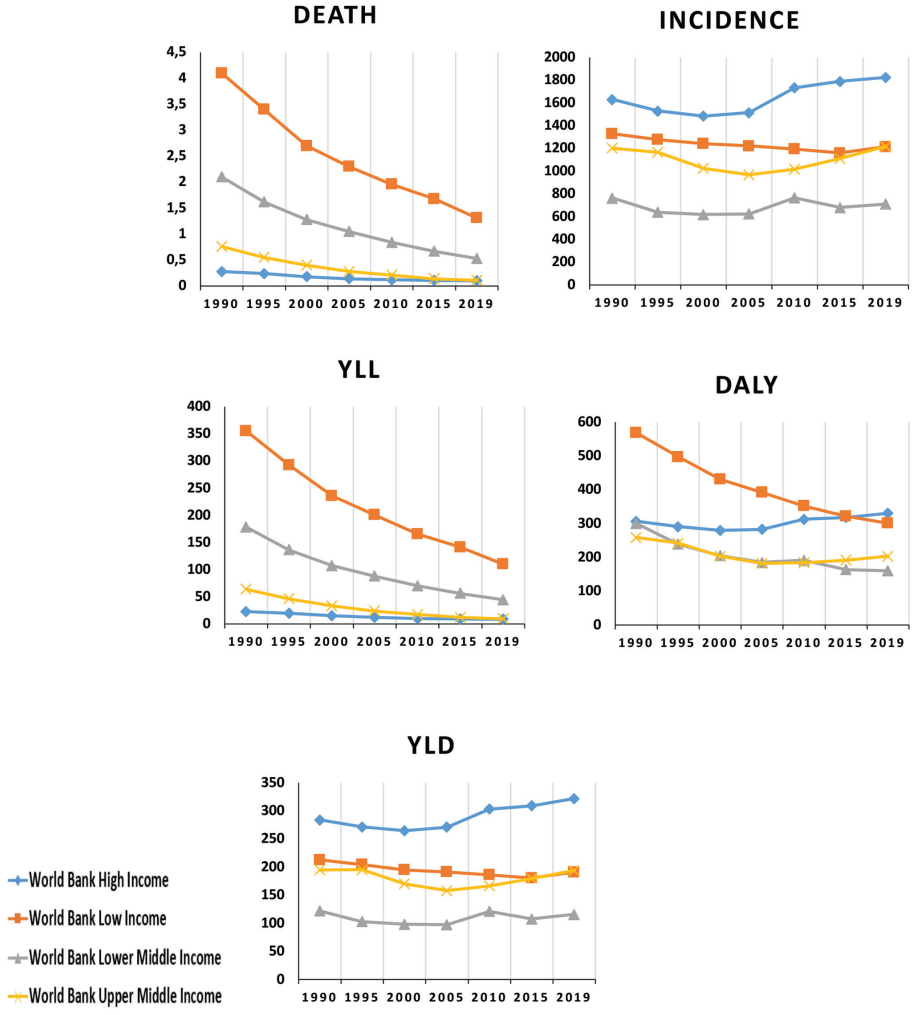

Figure 2. Trend incidence, mortality and burden (YLL, YLD, DALY) of asthma in children aged 0-14 years by world bank income level during 1990-2019
YLL: Years of life lost, YLD: Years lived with disability, DALY: Disability-adjusted life years (Source: GBD Compare)

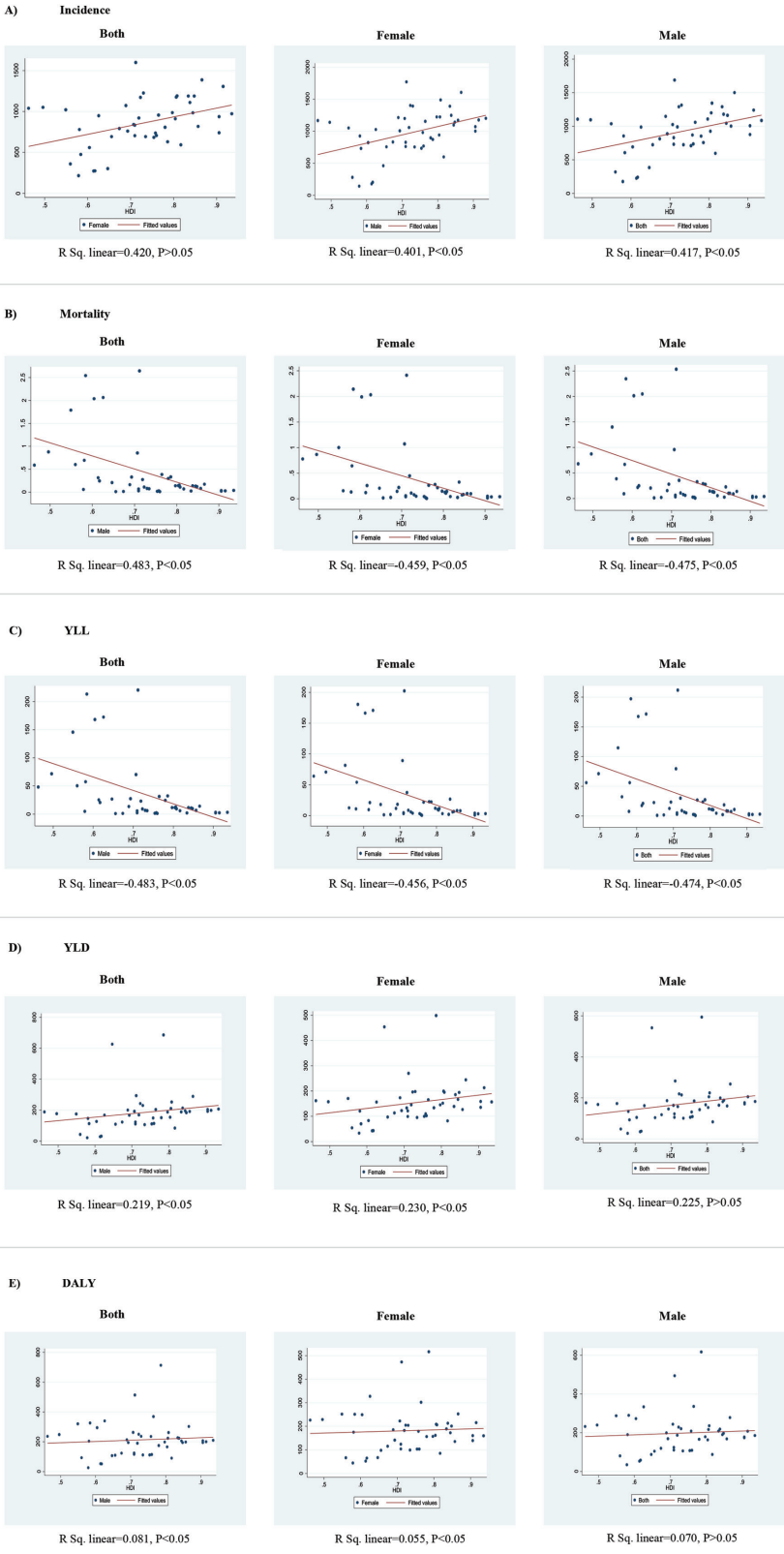

Figure 3. Relationship of HDI with: A) Incidence, B) Mortality, C) YLL, D) YLD and E) DALY in children aged 0-14 years in Asia in 2019
YLL: Years of life lost, YLD: Years lived with disability, DALY: Disability-adjusted life years

Table IV. Correlation of asthma in children aged 0-14 years white decomposites of human development index in Asia in 2019

HDI	Incidence		Mortality		DALY		YLL		YLD	
	r	P	r	P	r	P	r	P	r	P
Gross national income per 1000 capita	0.424	**	-0.302	**	0.038	*	-0.303	**	0.138	*
Mean years of schooling	0.281	*	-0.484	**	0.048	*	-0.481	**	0.232	*
Life expectancy at birth	0.409	**	-0.472	**	0.034	*	-0.473	**	0.150	*
Expected years of schooling	0.255	*	-0.397	**	0.102	*	-0.393	**	0.241	*

Significant level is less than 0.05.
*= Not significant, **= Significant
DALY: Disability-adjusted life years, HDI: Human development index, YLL: Years of life lost, YLD: Years lived with disability, M: Male, F: Female

asthma has risen sharply since the mid-1900s, especially in Western Europe. According to Phase 1 of the International Study of Asthma and Allergies in Children, asthma prevalence varied by more than 15 fold between English-speaking countries and other parts of the world, such as Eastern Europe and Asia in 1994 and 1995 (7). The incidence and prevalence of asthma also vary by gender during the lifetime. Pre-adolescent males have a higher incidence and prevalence of asthma as well as hospitalization rates than females of the same age, but this trend reverses during adolescence (19,20).

These results, aligned with the present study, show that the incidence of pediatric asthma is higher in males than females. This gender difference may be attributed to the more limited airway capacity of men in comparison to women in early life, which is caused by the effects of various hormonal factors (21). According to the results of the present study, the incidence of pediatric asthma had declined in the world and all continents (except Europe) by 2019 in comparison to 1990, and the highest incidence of asthma in 1990 and 2019 was reported in the Americas and the lowest in Asia.

Therefore, a decreased prevalence of asthma may reflect improved asthma control by augmented medication intake and a more rigorous follow-up or compliance. It is difficult to register the declining incidence of asthma because, in order to develop patterns that could be compared to an ideal group in the same geographical area, a parallel cohort study with specific age groups is required. These challenges may partly explain why studies in Australia and the United Kingdom have not consistently shown a fall in asthma prevalence, and why time trends in European and Asian countries between the 1970s and mid-2000s have been inconsistent (22).

According to the global ranking of asthma DALY in children in 1990 and 2010, asthma was among the top 20 causes of DALY at all ages, and the most common cause of DALY in the 10-19 age group (20). Asthma was ranked 23rd as a cause of disease burden in 2015 (23). In keeping with these results, the present study revealed that the burden of disease had dropped in 2019 when compared to 1990.

According to the results of the 1990-2019 GBD study, the age-standardized mortality rate of asthma in 2015 was higher in men than in women. Conversely, the age-standardized DALY rates for asthma were identical in men and women. In 2015, more women than men had asthma, which indicates a reversal of the higher male-to-female ratio in adolescence. Given the prevalence of asthma at all ages, YLDs account for more than 60% of the DALYs (23). However, the results of the present study showed equal mortality rates in both sexes while DALY and YLD rates were higher in males than females.

The present study displayed a significant negative correlation between mortality and HDI. In this regard, based on the results of the global burden of asthma study in 1990-2019, the highest reduction in the prevalence and aged-standardized mortality was recorded in countries included in the high-middle socio-demographic index (SDI) quintile and low-middle-SDI quintile between 1990 and 2015 (23).

Informed by the results of the global asthma burden of disease study in 1990-2015, the age-standardized DALYs dropped between 1990 and 2015, which are consistent with the results of the present study.

This reduction in DALY was to a larger degree attributed to decreased mortality and to a lesser degree to reduced YLDs. These results reflect a huge improvement in mortality reduction rather than a change in the prevalence and incidence of asthma (23). Indeed, the reduced mortality rate observed in high-income countries reflects better access to health care following the application of the International Asthma Guidelines (24), which indicates a strong link between SDI and mortality rather than the prevalence of asthma (23).

Decreased age-standardized DALY rates of asthma were observed in the moderate-low SDI quintile. The analysis of expected association between SDI and DALY rates of all ages reflected a decrease in asthma rates with elevated SDI in both sexes. The present study, however, demonstrated a significant relationship between HDI and DALY. The age-standardized asthma rates are estimated at more than 1,200 per 100,000 in Afghanistan, Central African Republic, Fiji, Kiribati, Lesotho, Papua New Guinea and Swaziland. In Eastern and Central European countries, China, Italy and Japan, asthma DALY rates are between 100 and 200 per 100,000 people (23).

Also, based on the results of the global burden of asthma study in 1990-2019, asthma-related DALYs in both sexes fell uniformly with an increase in SDI. The relationship between asthma-induced DALY levels and SDI largely reflects changes in YLLs. In 1990 (when SDI was at its lowest), asthma-related DALYs in South Asia exceeded expectations, but later matched those projected in 2019. Asthma-related DALY rates were lower than expected in Central Europe, Eastern Asia, and sub-Saharan Africa (23).

Conclusion

Given that there is a positive correlation between the incidence of asthma and HDI, it is worth considering factors that can exacerbate pediatric asthma in some countries. Moreover, since YLL is higher in countries with low human development, greater attention should be allocated to prioritizing and planning health services in these countries to decrease premature deaths.

Ethics

Ethics Committee Approval: This study was approved by the ethics committee of Lorestan University of Medical Sciences, Ethics Committee (number: IR.LUMS.REC.1399.219, date: 2020.11.16).

Informed Consent: This is a correlational analytical study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Design: E.G., K.R., Z.Z., V.M., Z.K., Data Collection and/or Processing: E.G., Z.K., Analysis or Interpretation: E.G., K.R., Z.K., Literature Review: E.G., K.R., Z.Z., V.M., Z.K., Writing: E.G., K.R., Z.Z., V.M., Z.K.

Conflict of Interest: There are no conflicts of interest.

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Original Article

Oral Health and Oral Health-related Quality of Life in Children with Attention Deficit Hyperactivity Disorder

Eda Arat Maden[1], İpek Süzer Gamlı[2]

[1]Sarıyer Oral and Dental Health Center, Clinic of Pediatric Dentistry, İstanbul, Turkey
[2]İstanbul Erenköy Training and Research Hospital, Clinic of Child and Adolescent Psychiatry, İstanbul, Turkey

ABSTRACT

Aim: Oral diseases can affect various aspects of life in children with attention deficit hyperactivity disorder (ADHD). The aim of this study was to evaluate the oral health conditions, oral health behaviors, and the oral health-related quality of life (OHRQoL) of children with ADHD.

Materials and Methods: A sample of 76 children with ADHD who were treatment naive was compared to 71 healthy children, with ages ranging from 6 to 13 years. Through an intraoral clinical examination, the numbers of decayed, missing, and filled teeth (DMFT, dmft index), the plaque index, the gingival index, occlusion status, overjet, overbite and parafunctional oral habits were determined. The children's parents completed the Turkish version of early childhood oral health impact scale (T-ECOHIS) and questionnaires regarding oral health behaviors and dental care.

Results: The child impact score (CIS) of the T-ECOHIS were significantly higher among those children with ADHD compared to the control group patients (16 versus 12), consistent with poorer OHRQoL. The children with ADHD also had more dental trauma in both dentitions and more frequent nail-biting habits compared to the participants in the control group.

Conclusion: In our study, T ECOHIS-CIS scores showed that those children with ADHD were affected more when compared to those children without ADHD in terms of oral health problems.

Keywords: Attention-deficit/hyperactivity disorder, DMFT, oral health-related quality of life, plaque index

Introduction

Attention deficit hyperactivity disorder (ADHD) is among the most common neurodevelopmental disorders in childhood with a worldwide prevalence of 7.2% (1,2). ADHD is clinically characterized by persistent patterns of inattention and/or hyperactivity-impulsivity symptoms, which are not consistent with the age and developmental stage of the child (1). Children with ADHD often make careless mistakes, have difficulty in sustaining attention and organizing daily activities, are easily distracted in tasks and are forgetful in everyday duties. Additionally, children with hyperactivity and impulsivity symptoms show signs of excessive motor activities, such as being fidgety and restless, which result in an inability to perform activities quietly and properly. These children usually have lower quality of life as well as functionality problems at school, at home and in social settings (1,3,4).

In the literature, it is hypothesized that these children are at risk of having more dental problems compared to typically developed children due to these clinical

Address for Correspondence
Eda Arat Maden, Sarıyer Oral and Dental Health Center, Clinic of Pediatric Dentistry, İstanbul, Turkey
Phone: +90 532 736 04 70 E-mail: edamaden1980@gmail.com ORCID: orcid.org/0000-0003-2562-3928
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presentations. There are several explanations put forward regarding this hypothesis. First of all, children with ADHD are shown to have worse dental hygiene related to their lack of attention and hyperactivity. Secondly, they are stated to have a tendency to forget to brush their teeth regularly or may not brush with a proper attitude. Also, when compared to typically developed children, they may have poorer eating practices, such as consuming sweet foods more, and/or bad habits, such as tobacco or alcohol use (5,6). In addition, due to hyperactivity, they are more prone to traumatic dental injuries, which may result in worse dental states (7). Therefore, it is recommended that children with ADHD should visit dental clinics more often in order to prevent oral health problems (8).

In the literature, the relationship between ADHD and dental hygiene has been investigated in detail. However, in most of these studies, there is limited data as to whether those children with ADHD were treatment naive, had treatment in the past or were under medication during the investigation. Medications used to treat ADHD may have oral side effects, such as dry mouth, bruxism, dental erosion or periodontitis, which may have additional effects on the dental hygiene in these patients (9). Therefore, in this study, treatment naive patients were selected and included to minimize the possibility of medication-related adverse effects. The aim of the study was to investigate oral health parameters, oral health behaviors and OHRQoL of children with or without ADHD, with ages ranging from 6 to 13 years, in Turkey.

Materials and Methods

Ethical Aspects

This study was approved by the Non-Invasive Research Ethics Committee of the Gaziosmanpaşa Training and Research Hospital (process no: 147/2020). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from the parents or caregivers of the children taking part in this research.

Study Design

Patients of Gaziosmanpaşa Training and Research Hospital Child and Adolescent Psychiatry Unit were included in this study. The main objectives were to characterize the oral health conditions and the oral health-related quality of life (OHRQoL) of children with ADHD. Patients were included in this study if they: 1) were aged between 6-13 years, 2) were diagnosed as having ADHD based on the Turkish version of the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Aged Children-Present and Lifetime (K-SADS-PL) (10), 3) were treatment naive, 4) did not have any comorbid psychiatric disorders which needed psychotropic medication, 5) had no organic pathology and 6) had given consent for participation in this study. Mental retardation and autism spectrum disorders were determined to be the exclusion criteria. Healthy children between the ages of 6-13 years with no physical or psychological disorder and who referred to the department of pediatric dentistry were included in this study as the control group. Those children who were eligible for this study were referred to the child and adolescent psychiatry unit for further examination.

The participants were interviewed by a child and adolescent psychiatrist, using the K-SADS-PL, and psychiatric diagnoses were made in accordance with the text of the DSM-IV-TR. Patients with comorbid psychiatric disorders were not included in this study. A socio-demographic form, which included the participant's age, gender, birth history, developmental stages, family features, socioeconomic status, medical and academic histories and peer relationships, was filled out by the child and adolescent psychiatrist in accordance with the parents' responses. All participants were examined, diagnosed and referred to the dental clinic for further examination.

Clinical Assessment

All participants were examined to assess their oral health conditions as well as oral hygiene and dietary habits. The oral characteristics of the children were recorded as a global value for the whole mouth during the dental examination using the following scoring systems: plaque index (PI) (0= no plaque, 1= isolated plaque deposits, 2= generalized plaque deposits, 3= heavy plaque deposits); gingival index (GI) (0= no inflammation, 1= inflammation with no bleeding on probing, 2= inflammation with bleeding on probing, 3= spontaneous bleeding). For the statistical examination, the plaque and gingival inflammation scores were recorded as either 0 (not present) or ≥1 (present). The diagnosis of dental caries was based on the detection of carious lesions at the cavitation stage, as recommended by the World Health Organization (11). The most common indices of caries experience were used: the decayed, missing, and filled teeth (DMFT) for permanent teeth and dmft for primary teeth. Additionally, occlusion status, overjet,

overbite and parafunctional oral habits were assessed. The Angle's molar classification method for the classification of malocclusion was used. The recorded variables for dental occlusion included: (1) molar occlusion [normal (class I), distal (class II) or mesial (class III)]. The amount of overjet (how much the upper front teeth protrude forward with regards to the lower front teeth; normally ~3 mm) and overbite (the overlap of the top teeth and bottom teeth, normally ~30%) were measured horizontally and vertically, respectively, using a probe.

Questionnaires

A comprehensive questionnaire was developed and used in this study to assess oral hygiene, oral health behaviors and dietary habits. The questions included diet and bottle use during infancy, duration of breastfeeding, frequency of intake of acidic/sugary beverages, intake of sweet snacks, and sugary medications between meals per day, tooth-brushing habits, frequency of dental visits, whether or not any treatment was performed during these visits and any history of dental trauma.

The ECOHIS was first developed and validated in the USA to assess oral health-related negative impacts in children between 3-5 years of age and their families (12). In this study, the Turkish version of the ECOHIS (T-ECOHIS) was used to assess the OHRQoL of the children by interviewing the families (13). Since the original version of the ECOHIS was designed for children under 8 years of age, some modifications were made to ensure applicability, content and face validity for an older age group by Buldur and Güvendi (14). Both the original ECOHIS used for children under 8 years and the modified ECOHIS for children over 8 years were found to be equal. The revised version for children over 8 years of age, which was modified by Buldur and Güvendi (14), was used to assess the OHRQoL of the children between 8-13 years of age.

T-ECOHIS consists of 13 questions divided into two sections: the child impact section and the family impact section. The child impact section is composed of four sub-domains: child symptoms, child function, child psychology and self-image/social interaction. In the family impact section, there are two sub-domains: parental distress and family function. ECOHIS is scored using a simple 5-point Likert scale: 0= never; 1= hardly ever; 2= occasionally; 3= often; 4= very often; 5= don't know. The total score ranges from 0 to 52: from 0 to 36 in the child section, and from 0 to 16 in the family section. ECOHIS scores are calculated as a simple sum of the response codes for the child and family sections separately, after recoding all "don't know" responses to missing. Higher ECOHIS data scores indicate greater impact and/or more problems for OHRQoL (12).

All the parents interviewed in this study completed the questionnaire about oral hygiene, oral health behaviors and dietary habits as well as the T-ECOHIS questionnaire.

K-SADS-PL

As a semi-structured interview technique, K-SADS-PL detects former and existing psychopathologies with respect to DSM-III and DSM-IV-TR. It was developed in 1997 by Kaufman et al. (15). Initially, questions are asked regarding main headings, and answers are scored between 0 and 3 based on severity and/or frequency. Symptoms with higher scores are reappraised in more detail, and it aims to get more information on dysfunctional areas related to the pathology of the patient. The validity and reliability of the Turkish version were established by Gökler et al. (10) in 2004.

Statistical Analysis

The data was analyzed with IBM SPSS Statistics 22 (SPSS IBM, Turkey) for this study. The suitability of quantitative variables for normal distribution was examined using the Kolmogorov-Smirnov test. The independent groups were compared using the Mann-Whitney U test. The relationship between qualitative variables was investigated by chi-square test. The relationship between quantitative variables was analyzed using Spearman's correlation analysis. The descriptive statistics of quantitative variables were shown as mean ± standard deviation, median (25^{th}-75^{th} percentile) and minimum-maximum. The descriptive statistics for qualitative variables were expressed as frequency (%). Values of $p<0.05$ were considered to be statistically significant.

Results

Study Population Characteristics

The participants, who were 6-13 years of age, consisted of 76 children in the ADHD group and 71 children without ADHD in the control group. Table I shows the characteristics of all these participants. The children in both groups were of similar economic status: the majority of family income levels were low in both groups. In the ADHD group, the majority (48 participants; 63.2%) were male and 28 participants (36.8%) were female. The control group consisted of 37 males (52.1%) and 34 females (47.9%). The mean ages were 9.57±2.47 years and 8.82±2.21 years in the ADHD group and the control group, respectively.

Oral Health

Table II shows the comparison results of dmft and DMFT between the control and ADHD groups. The mean dmft of the participants with ADHD was 3.74±3.56 (median=0, min=0/max=16) and the mean dmft of the participants without ADHD had a value of 3.37±3.21 (median=0, min=0/max=14). In the ADHD group, the mean DMFT was 2.07±2.75 (median=1, min=0/max=16) and the control group had a mean DMFT of 1.13±1.29 (median=1, min=0/max=5). According to this, there were no statistically significant differences between the two groups in terms of both dmft (p=0.655) and DMFT (p=0.115). Additionally, the comparison results of PI and GI variables, Angle's molar classification, overjet and overbite status and parafunctional oral habits are presented in Table II. There was no statistically significant difference between control and ADHD groups in terms of GI (p>0.05). However, the PI values of the ADHD group were significantly higher than the control group (p=0.025). While there was no difference between the ADHD group and control groups in terms of overjet (p=0.212), significant differences were found concerning occlusion, overbite and parafunctional oral habits (p=0.040, p<0.001 and p<0.001, respectively). The nail-biting habit was statistically significantly higher in the ADHD group (p<0.001), while the absence of parafunctional oral habits was statistically significantly higher in the control group (p<0.001).

Results of the Questionnaires Relating to Oral Health Behavior and Dental Traumatic Injures

Table III shows the comparison results of the control and ADHD groups regarding oral hygiene habits, dietary habits, breastfeeding time, frequency of dental visit, whether or not any treatment was performed during the visit and any history of dental trauma. According to this, a statistically significant difference was found between the control and ADHD groups in terms of diet during infancy, sweetening of food during infancy, duration of breastfeeding, bottle feeding during infancy, frequency of dental visit, status of visiting the dentist before examination, whether or not the family helps tooth brushing and any history of dental trauma (p<0.05).

The diet during infancy was mostly breast milk in the control group (91.5%) as opposed to bottle feeding in the ADHD group (35.5%). The control and ADHD groups differ in sweetening of foods during infancy: while 32.9% of the ADHD group's parents sweetened foods, the majority of the control group (87.3%) did not, which resulted in a significant difference between the two groups. In the control group, a breastfeeding duration rate of more than 12 months was found to be 80.3%, which was more than the rate for the ADHD group at 40.8%.

In the control group, the frequency of dental visit was more compared to the ADHD group (75%). The control and ADHD groups differ from each other in terms of whether the family helps with brushing of the teeth or not. In the ADHD group, the families help with brushing of the teeth more than the families in the control group (35.5%). Additionally, the ADHD group had more traumatic dental injuries compared to the control group.

Table I. Characteristics of the study population

	Study groups	
	Control (n=71)	**ADHD (n=76)**
Family income	**n (%)**	**n (%)**
Less than two minimum wages	43 (60.6)	49 (66.2)
More than two minimum wages	28 (39.4)	25 (33.8)
Gender		
Female	34 (47.9)	28 (36.8)
Male	37 (52.1)	48 (63.2)
	$\overline{X}$ ± SD (min.-max.)	**$\overline{X}$ ± SD (min.-max.)**
Age in years	8.82±2.21 (6-13)	9.57±2.47 (6-15)

$\overline{X}$: Average, SD: Standard deviation, min.-max.: Minimum-maximum, ADHD: Attention deficit hyperactivity disorder

Assessment of OHRQoL

Table IV displays the distribution of responses to T-ECOHIS according to each question. Trouble in sleeping, being irritable or frustrated, avoiding smiling or laughing and avoiding talking, which are the items in child psychology and child self-image/social interaction domains, were the most frequently reported items of the child impact section in the ADHD group. In Table IV, the control and ADHD groups were compared in terms of T-ECOHIS. The comparison of the child impact scores (CIS), a sub-dimension of T-ECOHIS which includes child symptoms, child functions, child psychology and child self-image/social interaction, showed that those children with ADHD had significantly higher CIS scores compared to ones in the control group (p=0.008, p=0.015, p=0.014, respectively).

The CIS, child psychology and child self-image scores of those children with ADHD were significantly higher compared to the children in the control group (p=0.008, p=0.015, p=0.014, respectively).

The control and ADHD groups were also compared in terms of total family impact factor (FIS) and its sub-dimensions: parental distress and family function. Accordingly, the groups were similar to each other in terms of FIS and parental distress (p>0.05). However, the family function score of the control group was found to be significantly higher than the score of the ADHD group (p=0.016). Total ECOHIS scores were similar in both groups (p=0.416).

Discussion

In this study, we examined the oral health, oral health behaviors, and OHRQoL of children with ADHD in comparison to healthy controls. No statistically significant difference in the oral health parameters of those children with or without ADHD was found regarding dmft, DMFT, GI, and oral hygiene habits. In some studies (16,17), significantly higher decay surface (DS) scores were found in those children with ADHD compared with the

Table II. Descriptive statistics and comparison results of variables related to oral health status

Oral health parameters	**Study groups**		χ^2	**p**
	Control (n=71)	**ADHD (n=76)**		
Occlusion	**n (%)**	**n (%)**		
Class 1	53 (74.6)[a]	61 (80.3)[a]	5,764	**0.040**
Class 2	18 (25.4)[a]	11 (14.5)[a]		
Class 3	0 (0)[a]	4 (14.5)[a]		
Overbite				
Normal	65 (91.5)[a]	51 (67.1)[b]	23,657	**<0.001**
Increased overbite	2 (2.8)[a]	14 (18.4)[b]		
Tetatet	0 (0)[a]	10 (13.2)[b]		
Anterior open bite	4 (5.6)[a]	1 (1.3)[a]		
Overjet				
Normal	51 (71.8)[a]	55 (72.4)[a]	4,398	0.217
Increased overjet	16 (22.5)[a]	15 (19.7)[a]		
Anterior cross-bite	4 (5.6)[a]	2 (2.6)[a]		
Posterior cross-bite	0 (0)[a]	4 (5.3)[a]		
Parafunctional oral habits				
None	56 (78.9)[a]	33 (43.4)[b]	33,661	**<0.001**
Nail biting	2 (2.8)[a]	27 (35.5)[b]		
Finger sucking	1 (1.4)[a]	2 (2.6)[a]		
Bruxism	4 (5.6)[a]	5 (6.6)[a]		
Nail biting + Finger sucking	4 (5.6)[a]	1 (1.3)[a]		
Nail biting + Bruxism	4 (5.6)[a]	8 (10.5)[a]		
	$\overline{X}$ ± SD (min.-max.)	**$\overline{X}$ ± SD (min.-max.)**	**Z**	**p**
DMFT	1.13±1.29 (0-5)	2.07±2.75 (0-16)	-1,578	0.115
dmft	3.37±3.21 (0-14)	3.74±3.56 (0-16)	-0,448	0.655
Plaque index	1.24±0.27 (0.96-1.95)	1.31±0.31 (0.29-2)	-2,239	**0.025**
Gingival index	1.12±0.17 (0.85-1.56)	1.09±0.31 (0-1.81)	-0,150	0.881

χ^2: Chi-square test statistics, $\overline{X}$: Average, SD: Standard deviation, min.-max.: Minimum-maximum, ADHD: Attention deficit hyperactivity disorder
Similar letters in the same lines indicate similarity between groups, different letters indicate difference between groups

Table III. Descriptive statistics and comparison results related to oral health behaviors and dietary habits				
	Study groups		χ^2	**p**
	Control (n=71)	**ADHD (n=76)**		
Diet during infancy				
Breast milk	65 (91.5)[a]	49 (64.5)[b]	13,941	**<0.001**
Formula	6 (8.5)[a]	27 (35.5)[b]		
Daily sugar consumption				
None	8 (11.3)	8 (10.5)	6,265	0.099
Only in main meals	4 (5.6)	12 (15.8)		
1-2 times a day	40 (56.3)	45 (59.2)		
More than 3 times a day	19 (26.8)	11 (14.5)		
Sweetened food during infancy				
Yes	9 (12.7)[a]	25 (32.9)[b]	7,341	**0.007**
No	62 (87.3)[a]	51 (67.1)[b]		
Bottle feeding				
Yes	20 (28.2)[a]	58 (76.3)[b]	34,165	**<0.001**
No	51 (71.8)[a]	18 (23.7)[b]		
Any previous dental visits				
Yes	55 (77.5)[a]	46 (60.5)[b]	4,142	**0.042**
No	16 (22.5)[a]	30 (39.5)[b]		
Receiving treatment during dental visit				
Yes	39 (70.9)	37 (75.5)	0.094	0.759
No	16 (29.1)	12 (24.5)		
Frequency of brushing teeth				
Less than once a day	33 (46.5)	35 (46.1)	0.916	0.632
Once a day	24 (33.8)	30 (39.5)		
2-3 times a day	14 (19.7)	11 (14.5)		
Brushing teeth without help				
Yes	63 (88.7)	69 (90.8)	0.019	0.889
No	8 (11.3)	7 (9.2)		
The family helps tooth brushing				
Yes	14 (19.7)[a]	30 (39.5)[b]	5,921	**0.015**
No	57 (80.3)[a]	46 (60.5)[b]		
Previous dental trauma				
Yes	4 (5.6)[a]	16 (21.1)[b]	6,171	**0.013**
No	67 (94.4)[a]	60 (78.9)[b]		
Frequency of dental visit				
None	4 (5.6)[a]	19 (25)[b]	13,810	**0.003**
When toothache	39 (54.9)[a]	35 (46.1)[a]		
Once in a year	24 (33.8)[a]	14 (18.4)[b]		
Once in every 6 months	4 (5.6)[a]	8 (10.5)[a]		
Duration of breastfeeding				
Less than 6 months	6 (8.5)[a]	27 (35.5)[b]	24,750	**<0.001**
6-12 months	8 (11.3)[a]	18 (23.7)[b]		
More than 12 months	57 (80.3)[a]	31 (40.8)[b]		

χ^2: Chi-square test statistics, ADHD: Attention deficit hyperactivity disorder
Similar letters in the same lines indicate similarity between groups, different letters indicate difference between groups

subjects in the control groups, whereas other studies (8,18), did not find significant differences in DS/DMFS scores between children with or without ADHD. The results regarding dmft and DMFT in this study are in agreement with the study by Blomqvist et al. (8), Chau et al. (18), Hidas et al. (19) and Lorber et al. (20). Some studies (21-23) have reported that children with ADHD have a higher risk of caries than healthy controls, with statistically significantly higher DMFT scores due to their medication. In these previous studies, the lack of data as to whether or not children with ADHD were under medication may have affected the results. Thus, in our study, treatment naive children with ADHD were included to minimize the possibility of any adverse effects of medication on oral health. Those patients with ADHD in our study had a significantly higher PI and had more orthodontic class III occlusions. Chandra et al. (24) found statistically significant differences in plaque indices between ADHD and control group participants, which is in agreement with our study. In spite of these findings, the participants with ADHD tended to have higher dmft/DMFT values than those participants without ADHD. In addition to higher plaque indices, the tendency of having higher incidences of dental caries in individuals with ADHD may also be attributed to an inability to brush teeth effectively. In children, the high values of PI, which is the real determinant of oral hygiene, point out the need for significant improvement, including better instructions for caregivers and parents (6). Health professionals must be aware that medications to treat ADHD in these children may increase the risk for future caries development (21,22).

Furthermore, those children with ADHD had more dental trauma in both dentitions and more parafunctional oral habits compared to others in the control group. The presence of bruxism was similar in both groups, which is in agreement with Hidas et al. (19), whereas Chau et al. (18) found a statistically significant higher percentage of bruxism in children with ADHD. In this study, the most common parafunctional oral habit was nail biting in these children. The prevalence of dental trauma in both dentitions was higher among those children between 11 and 13 years of age with ADHD compared to the healthy controls in a previous Swedish study (25) on dental trauma, which is in line with our study. Therefore, behavioral problems can be an additional risk factor for traumatic dental injuries (TDI) in children with ADHD.

Earlier studies have shown that ADHD is associated with dysfunctional eating patterns, such as eating more snacks and junk food in comparison to peers (26,27). Blomqvist et al. (8) found a higher percentage of eating sweet snacks between meals in children with ADHD compared to children in a control group. However, this reported effect is controversial, since Kim and Chang (28) conducted a study on 107 school-aged Korean children, with only 8.5% categorized as having ADHD, and found no significant association between an increased risk of ADHD and the consumption of simple sugars, including those in sweets and sugar-sweetened beverages. Similarly, in this study, no significant differences in the frequency of sugar consumption were found between ADHD patients and control group patients when dietary habits were considered. This similarity regarding acidic/sugary beverages and sweet

Table IV. Descriptive statistics and comparison results for T-ECOHIS scores

Item	Domain	Study group				Z	p
		Control (n=71)		ADHD (n=76)			
		$\bar{X}$ ± SD	min.-max.	$\bar{X}$ ± SD	min.-max.		
1-9	**Total child impact**	14.76±6.68	9-32	16.39±5.54	9-33	-2,649	**0.008**
1	Child symptom	2.10±1.21	1-5	2.25±0.99	0-5	-1,300	0.194
2-5	Child function	7.03±3.31	4-17	7.55±3.06	3-16	-1,434	0.152
6-7	Child psychology	3.07±2.13	2-10	3.50±1.79	0-8	-2,435	**0.015**
8-9	Child selfimage/social interaction	2.56±1.20	2-6	3.09±1.58	0-9	-2,452	**0.014**
10-13	**Total family impact**	8.30±3.02	4-14	7.63±3.12	4-16	-1,404	0.160
10-11	Parental distress	3.94±1.61	2-6	3.00±2.00	2-9	-0.481	0.630
12-13	Family function	4.35±1.82	2-8	3.72±1.87	2-10	-2,419	**0.016**
1-13	**Total ECOHIS score**	22.06±8.26	13-41	24.03±7.50	13-44	-0.814	0.416

Z: Mann-Whitney U test statistics, $\bar{X}$: Average, SD: Standard deviation, min.-max.: Minimum-maximum, ADHD: Attention deficit hyperactivity disorder

snacks intake in the present study might be explained by the low-income level of families, where cheap and unhealthy snacks are consumed more frequently. Regarding nutrition during infancy, while breastfeeding rates were significantly less in children with ADHD compared to healthy controls, bottle-feeding was markedly more in these children. The levels of sugar-added food intake during infancy were also significantly more in those children with ADHD compared with the children in the control group.

Tooth brushing frequencies did not differ between the two groups in the present study, as in other studies (19,22). The majority of the participants reported that they brushed their teeth less frequently than once a day. These individual risk factors might lead to a future increased risk of being in the elevated caries risk group. In contrast, in a previous study (24), it was observed that the ADHD children brushed their teeth statistically significantly less often than the children in the healthy group. We also found that the families were more likely to help their children with ADHD brush their teeth because they could not brush their teeth effectively.

The dental visit frequency in the ADHD group was statistically lower compared to the control group. Although both groups often had dental visits in the presence of toothache, the ADHD group had a higher tendency of not going to dental visits at all compared to the control group. The reason as to why families with a child diagnosed with ADHD do not tend to visit dentists may be explained by the therapeutic difficulties encountered with children affected by ADHD, due to a short attention span and lack of cooperation during dental treatment compared to control participants (6).

To the best of our knowledge, the present study is the first attempt to evaluate OHRQoL in children with ADHD. In this study, the negative effects on the quality of life of children due to oral health problems was high when examined in terms of the items in the child sub-dimension of the scale. The T-ECOHIS CIS, child psychology and child self-image and the family function score of the children differ between the two groups in the present study. In the study of Naidu et al. (29), it was reported that among those children who had negative impact on their quality of life and those with early childhood caries, higher percentages were observed in the items of being angry and anxious, and hesitating to smile, laugh and talk, which are the sub-dimensions of the CIS. Similarly, the child psychology and child self-image scores were higher in the ADHD group compared to the control group. Thus, when the aesthetics, sense of self-confidence and social relations, and the desire to talk and laugh are considered, it is of great importance to treat any problems that occur in the oral health of the child with dental treatments (30). The results of this study showed that this is more important in those children with ADHD. Obtaining permission for time-off from work and financial problems in the family function domain, which is a sub-dimension of the FIS, were observed more in the control group in comparison to the ADHD group. These results may be due to the higher frequency of dental visits of the control group when compared to the ADHD group.

In our study, T-ECOHIS-CIS scores showed that children with ADHD were affected more than those children without ADHD in terms of oral health problems. This shows that ADHD has an additional negative impact on the OHRQoL of children. Improving oral health by increasing parental awareness through public health programs may help lower T- ECOHIS scores, which will be a sign of a better quality of life for the parents and the children.

Study Limitations

One of the limitations of the present study was that the sample group consisted of families with low income. It is important to mention that if the study had been conducted on mostly middle and high family income populations, the results might have been different due to different health-related behaviors, social interactions and an increased awareness of the children as well as their families regarding oral hygiene. The second limitation was that no dental radiographs could be taken; thus, it was not possible to identify non-cavitated lesions reliably. Further population-based research is required to assess the oral health status and OHRQoL of children aged 6-13 years old with ADHD to confirm the present study results.

Conclusion

The findings of the present study showed that the ADHD participants had higher PI and T-ECOHIS CIS scores. They also had more dental trauma and parafunctional oral habits. There was statistically no significant difference in the oral health behaviors of children with or without ADHD. This study shows the need for considerable improvement of oral hygiene and dietary habits in all of the study participants. Parents and caregivers need better guidance for a more adequate control of oral hygiene and dietary habits both in children with and without ADHD. Appropriate preventive dental care seems to be of great importance for the prevention of future dental caries due to medication, especially for those children with ADHD.

Increased awareness among clinicians is also important to facilitate better caries and trauma management. Moreover, those professionals conducting psychiatric follow-ups of children should educate parents and caregivers about the increased need of preventive dental care in children with ADHD.

Ethics

Ethics Committee Approval: This study was approved by the Non-Invasive Research Ethics Committee of the Gaziosmanpaşa Training and Research Hospital (process no. 147/2020).

Informed Consent: Informed consent forms were obtained from the parents or caregivers of the children taking part in the research.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: E.A.M., İ.S.G., Design: E.A.M., İ.S.G., Data Collection and/or Processing: E.A.M., İ.S.G., Analysis or Interpretation: E.A.M., İ.S.G., Writing: E.A.M., İ.S.G.

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Original Article

Impact of Fecal Calprotectin Measurement for Inflammatory Bowel Disease in Children with Alarm Symptoms

Burcu Güven[1], Fatma İssi[1], Elif Sağ[1], Kurtuluş Buruk[2], Murat Çakır[1]

[1]Karadeniz Technical University Faculty of Medicine, Department of Pediatric Gastroenterology, Trabzon, Turkey
[2]Karadeniz Technical University Faculty of Medicine, Department of Microbiology, Trabzon, Turkey

ABSTRACT

Aim: The differentiation of inflammatory bowel diseases (IBD) from other gastrointestinal diseases in pediatric patients is highly important and the definitive diagnosis of IBD is established by endoscopic examination. The use of non-invasive methods (clinical symptoms and laboratory tests) allows for the early and accurate referral of patients from first step health centers to advanced health centers. We aimed to investigate the effectiveness of fecal calprotectin (FC) in the differentiation of IBD from other gastrointestinal diseases in children.

Materials and Methods: This retrospective study included patients who had undergone FC testing and colonoscopy. The demographic characteristics, alarm symptoms (AS), and abnormal laboratory findings (ALF) were recorded for each patient. A negative calprotectin result was considered to be less than 50 µg/g, and a second cut-off value for FC was accepted as 150 µg/g. Definitive diagnosis was established by colonoscopy in each patient.

Results: The study included 88 consecutive patients (mean age, 10.2±6.1 years; 51.1% female). Of these, 20 (22.7%) patients were diagnosed with IBD. No significant difference was found between IBD and non-IBD patients with regard to the presence of AS except for involuntary weight loss ($p<0.001$). The prevalence of increased C-reactive protein and hypoalbuminemia was significantly higher in the IBD patients ($p=0.002$ and $p=0.026$, respectively). FC>50 µg/g [80.0 vs 39.7%, $p=0.044$, odds ratio (OR): 6.07, 95% confidence interval (CI) 1.83 to 23.42] and >150 µg/g (60.0 vs 16.2%, $p=0.002$, OR: 7.78, 95% CI 1.83 to 20.14) was significantly higher in the IBD patients compared to the non-IBD patients. AS combined with ALF and FC>150 µg/g had the highest specificity (95.12%).

Conclusion: Although primary care clinicians often use AS and laboratory parameters in the differentiation of IBD from non-IBD diseases, FC was found to have a relatively higher diagnostic value.

Keywords: Alarm symptoms, fecal calprotectin, pediatric

Introduction

Differentiation of inflammatory bowel diseases (IBD) from other gastrointestinal diseases in pediatric patients is highly important and the definitive diagnosis of IBD is made by endoscopic and histopathological examinations. Although the definite diagnosis of IBD is established by colonoscopy and histopathologic examinations, the use of non-invasive methods (clinical symptoms and laboratory tests) allows for the early and accurate referral of patients from family physicians or general pediatricians to pediatric gastroenterology centers. Additionally, the use of these methods will be helpful in the differentiation of IBD from

Address for Correspondence
Burcu Güven, Karadeniz Technical University Faculty of Medicine, Department of Pediatric Gastroenterology, Trabzon, Turkey
Phone: +90 532 475 71 69 E-mail: burcuguven55@gmail.com ORCID: orcid.org/0000-0002-5142-8168
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other organic diseases such as polyps, solitary rectal ulcer, and allergic colitis (1).

Fecal calprotectin (FC), which is a neutrophil-derived protein released in the stool in response to mucosal inflammation, has recently emerged as a practical, simple, and non-invasive test in the diagnosis of IBD. FC plays an immunoregulatory role in the interaction with the zinc-dependent metalloproteinases responsible for the activation of proinflammatory cytokines. Moreover, its fecal excretion is highly correlated with the severity of intestinal inflammation (2). FC is resistant to colonic bacterial degradation and can be stored at -20 °C without decomposition and is stable for up to seven days at room temperature, which increases its use in clinical practice (3). Its sensitivity is remarkably high (usually 100% at <50 µg/g, ranging from 83% to 100% at 50 µg/g) since it has a broad range (0-3,000 µg/g) and increases as a result of numerous factors including celiac disease, infectious gastroenteritis, and the use of proton pump inhibitors and non-steroidal anti-inflammatory drugs (4-6). Moreover, its specificity ranges between 51% and 100% (6), with this being relatively lower in children (7) and thus, additional findings are needed to enhance its specificity in children.

In the present study, we aimed to investigate the effectiveness of FC measurement in the differentiation of IBD and other colonic diseases in children with clinical and laboratory findings.

Materials and Methods

This retrospective study included 88 patients who presented to Karadeniz Technical University Medical School Pediatric Gastroenterology outpatient clinic with gastrointestinal symptoms [chronic diarrhea (>14 days), rectal bleeding, weight loss of unknown origin, perianal lesions] and underwent colonoscopy with a pre-diagnosis or exclusion of organic gastrointestinal disease and who also had FC measurement. The demographic characteristics, alarm symptoms (AS), and abnormal laboratory findings (ALF) were recorded for each patient. AS included rectal bleeding, involuntary weight loss, chronic diarrhea, perianal lesions, extraintestinal findings, or a family history of IBD (8,9). ALF included anemia, hypoalbuminemia (<3.5 g/dL), increased erythrocyte sedimentation rate (ESR) (>20 mm/sec), or increased C-reactive protein (CRP) level (>1 mg/dL), thrombocytosis (>450,000/µL) (10). Anemia was defined as a hemoglobin (Hb) level of <-2 standard deviations (SD) from the mean for age and gender for the entire population (8).

The concentration of FC in the stool samples was measured semi-quantitatively using a CalFast XT immunochromatographic assay with a mixture of anticalprotectin monoclonal and polyclonal antibodies (Eurospital, Trieste, Italy). A negative calprotectin result was considered to be less than 50 µg/g (4), and a second cut-off value for FC was accepted as 150 µg/g (6). Its range varied from 0 to 300 µg/g. Within one-week, a definitive diagnosis of colonic disease was established by colonoscopy and histopathological examination in each patient.

Non-specific colitis was defined as an inflammatory condition of the colon which microscopically lacks the characteristic features of any specific form of colitis (11).

Statistical Analysis

Data were analyzed using SPSS version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Quantitative variables were expressed as mean, SD, and minimum-maximum values. Categorical variables were expressed as frequencies (n) and percentages (%). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated for the diagnosis of IBD in patients with AS and FC using binary logistic regression. The Area under the curve (AUC) was used to assess the value of AS and FC in the prediction of IBD in children with gastrointestinal symptoms.

This study was approved by Karadeniz Technical University Medical School Ethical Committee (approval no: 2020-127).

Results

This study included 88 consecutive patients (mean age, 10.2±6.1 years; range, 2 months - 18 years; 51.1% female). Of these, 20 (22.7%) patients were diagnosed with IBD, including 12 (13.6%) patients with ulcerative colitis and 8 (9.1%) patients with Crohn's disease. The remaining 68 (77.3%) patients had no IBD, including 16 (18.2%) patients with lymphonodular hyperplasia, 11 (12.5%) patients with non-specific colitis and 10 (11.4%) patients with allergic colitis. Thirty-one (35.2%) patients had normal histopathological findings (Figure 1). The patients' endoscopic and histopathological findings are summarized in Table I. Five patients with non-specific colitis did not come for follow-up. The other 6 patients recovered with probiotics and diet.

AS was present in 55 (62.5%) patients and the most common AS was rectal bleeding (n=37; 42.0%), followed by

involuntary weight loss (n=31; 35.2%), family history of IBD (n=7; 7.79%) and perianal lesions (n=3; 3.4%, anal fissure in 2, skin tag in 1) (Table II). No extraintestinal symptoms were detected in any patient. No significant difference was found between IBD and non-IBD patients with regard to AS except for involuntary weight loss, which was significantly greater in IBD patients compared to non-IBD patients [75.0 vs 23.5%, p<0.001, odds ratio (OR): 9.75, 95%, confidence interval (CI) 3.0 to 31.00] (Table II).

ALF was present in 38 (43.2%) patients and the most common ALF was increased CRP (n=15; 17.0%), followed by increased ESR (n=12; 13.6%), hypoalbuminemia (n=9; 10.2%), and anemia (n=8; 9.1%) (Table II). The prevalence of increased CRP (40.0 vs 10.3%, p=0.002, OR: 5.81, 95% CI 1.77 to 19.06), hypoalbuminemia (25.0 vs 5.9%, p=0.026, OR: 5.33, 95% CI 1.28 to 22.29), FC>50 µg/g (80.0 vs 39.7%, p=0.044, OR: 6.07, 95% CI 1.83 to 23.42) and >150 µg/g (60.0 vs 16.2%, p=0.002, OR: 7.78, 95%, CI 1.83 to 20.14) was significantly higher in the IBD patients compared to the non-IBD patients. The sensitivity, specificity, PPV, NPV, accuracy and AUC of the presence of AS, ALF, and FC>50 µg/g, FC>150 µg/g and their combinations are shown in Table III. AUC for FC>150 µg/g and AS+ FC>150 µg/g were significant in predicting IBD (AUC=0.715, p=0.011, 95% CI: 0.566-0.865 and AUC=0.702, p=0.016, 95% CI: 0.541-0.863, respectively).

FC was revealed to be a significant predictor in the differentiation of IBD and non-IBD diseases at a cut-off value of 207 µg/g (AUC=0.794, p<0.05, 95% CI: 0.658-0.930), with a sensitivity and specificity of 70.6% and 82.5%, respectively (Figure 2).

Discussion

The present study aimed to investigate the effectiveness of clinical and laboratory findings in the differentiation of IBD from other gastrointestinal diseases in pediatric patients. As proposed by Waugh et al. (6), two distinct cut-off values were determined for FC (50 µg/g and 150 µg/g) (6). FC<50 µg/g is known to rule out IBD (12). Holtman et al. (10) reported that FC had a high sensitivity (0.99; 95% CI, 0.81-1.00) and suggested that negative FC (50 µg/g) safely rules out IBD. Another study indicated that clinical follow-up is recommended in those patients with an FC level of 50-150 µg/g and those patients with these levels may develop IBD as well as diseases which do not require endoscopy such as irritable bowel syndrome (6). In such patients, additional findings are needed to make a decision for invasive procedures such as endoscopy.

The present study investigated the predictive role of AS and ALF in the diagnosis of IBD, among which involuntary weight loss was found to be the most significant predictor of IBD among AS cases. Heida et al. (8) reported that rectal bleeding and perianal lesions were accepted as high-risk factors for the diagnosis of IBD, while a family history of IBD, extraintestinal findings, and weight loss were accepted as low-risk factors. However, these low-risk factors were accepted as high-risk factors when combined with FC>50 µg/g. In our study, AS had a specificity of 39.71% and this level increased to 87.5% for AS combined with FC>150 µg/g.

Among the ALFs analyzed, CRP and hypoalbuminemia were found to be more effective than other ALFs in the diagnosis of IBD. Caviglia et al. (13) reported that CRP was significantly higher in IBD patients compared to controls, and FC was revealed to be the only significant factor on multivariate regression analysis. Holtman et al. (4) indicated

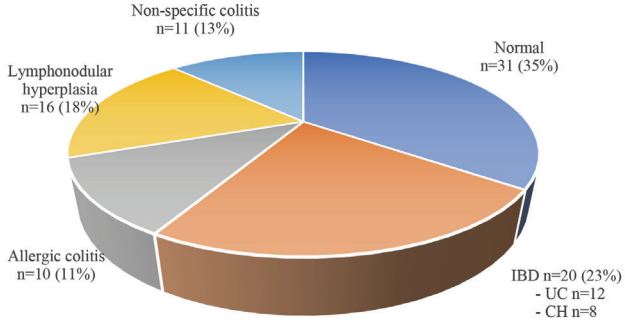

Figure 1. Final diagnosis of patients after colonoscopic examination

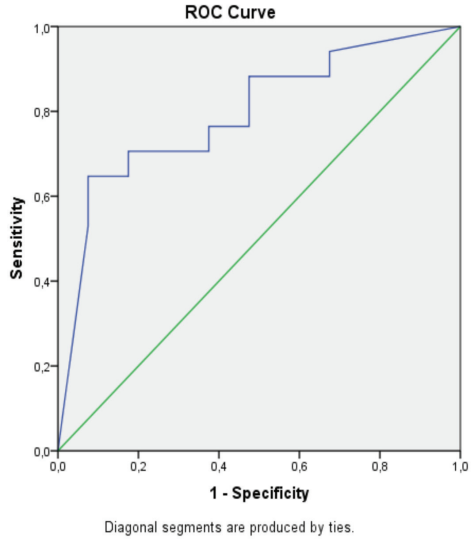

Figure 2. Fecal calprotectin for predicting IBD (AUC=0.794, p<0.05, 95% CI: 0.658-0.930) at a cut-off value of 207 µg/g

ROC: Receiver operating characteristic, IBD: Inflammatory bowel diseases, AUC: Area under the curve, CI: Confidence interval

that AS in combination with CRP had no remarkable benefit in the diagnosis of IBD, while AS in combination with FC had the highest benefit. In our study, AS had a specificity of 72.06% when combined with ALF and a specificity of 87.5% when combined with FC>50 µg/g. Moreover, the accuracy rates and AUC indicated that ALF had no significant effect in the differentiation of IBD and non-IBD diseases.

In a previous meta-analysis, Degraeuwe et al. (14) found the AUC value for FC 212 µg/g to be 0.94 (95% CI, 0.92-0.95) in the differentiation of IBD and non-IBD diseases. Another meta-analysis conducted in 2017 evaluated a large cohort of 1,120 pediatric patients and revealed that FC improved the AUC more than other laboratory markers (ESR, CRP, platelet count, Hb, albumine). Additionally, the pooled AUC of FC (6 studies) was 0.95 (95% 0.93-0.98) in this meta-analysis (9). In our study, the AUC for FC 207 µg/g was 0.794, which was lower than those of other studies. As proposed by Holtman et al. (9), there are numerous factors playing a role in the differentiation of IBD and non-IBD diseases. FC has a remarkably high AUC value, which could be associated with an overestimation of FC levels. Moreover, the contribution

Table I. Patients' endoscopic and histopathological findings

		IBD (n=20) n (%)	Non-IBD (n=68) n (%)	Total (n=88) n (%)
Endoscopic findings	Normal	0 (0)	22 (32.3)	22 (25)
	Nodularity (colon)	2 (10)	9 (13.2)	11 (12.5)
	Erosions	4 (20)	6 (8.8)	10 (11.3)
	Ulcers	10 (50)	2 (2.9)	12 (13.6)
	Nodularity (terminal ileum)	2 (10)	18 (26.5)	20 (22.7)
	Edema	6 (30)	4 (5.9)	10 (11.3)
	Hyperemia	10 (50)	7 (10.3)	17 (19.3)
Histopathological findings	Normal	0	31 (45.6)	31 (35.2)
	Chronic active colitis	4 (20)	0 (0)	4 (4.5)
	Acute active colitis	11 (55)	10 (14.7)	21 (23.9)
	Lymphoid aggregates and plasma cells	3 (15)	10 (14.7)	13 (14.8)
	Eosinophilia	0 (0)	10 (14.7)	10 (11.3)
	Noduler lymphoid hyperplasia	0 (0)	4 (5.9)	4 (4.5)
	Non-specific colitis	0 (0)	11 (16.2)	11 (12.5)
	Terminal ileitis	4 (20)	0 (0)	3 (3.4)

IBD: Inflammatory bowel diseases

Table II. Demographic and clinical characteristics

Variables	Total 88 (100)	IBD (+) 20 (22.7)	IBD (-) 68 (77.3)	p-value
Demographic characteristics				
Age, (years) mean ± SD (range)	10.26±6.15	10.66±4.99	10.14±6.47	0.740
Gender, female	45 (51.1)	10 (50.0)	35(51.2)	0.908
Alarm symptoms	55 (62.5)	14(70.0)	41(46.6)	0.431
Rectal bleeding	37 (42.0)	11 (55.0)	26 (38.2)	0.182
Involuntary weight loss	31 (35.2)	15 (75.0)	16 (23.5)	**<0.001**
Family history for IBD	7 (7.9)	4 (20.0)	3 (4.4)	0.073
Perianal lesions	3 (3.4)	2 (10.0)	1 (1.5)	0.251
Abnormal laboratory findings	38 (43.2)	11 (55.0)	27 (39.7)	0.225
CRP (>1 mg/dL)	15 (17.0)	8 (40.0)	7 (10.3)	**0.002**
ESR (>20 mm/hour)	12 (13.6)	5 (25.0)	7 (10.3)	0.216
Hypoalbuminemia (<3.5 g/dL)	9 (10.2)	5 (25.0)	4 (5.9)	**0.026**
Anemia (hemoglobin <-2 SD for age and gender)	8 (9.1)	4 (20.0)	4 (5.9)	0.075
Thrombocytosis (>450,000/µL)	14 (15.9)	3 (15.0)	11 (16.2)	1.000
FC positivity				
>50 µg/g	43 (48.9)	16 (80.0)	27 (39.7)	**0.044**
>150 µg/g	23 (26.1)	12 (60.0)	11 (16.2)	**0.002**

IBD: Inflammatory bowel diseases, SD: Standard deviation, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, FC: Fecal calprotectin

Table III. Impact of fecal calprotectin, alarm symptoms and abnormal laboratory findings on the diagnosis of IBD

Parameters	Sensitivity (%) (Lower - Upper CI)	Specificity (%) (Lower - Upper CI)	PPV (%)	NPV (%)	Accuracy (%)	AUC (%) (Lower - Upper CI)	p-value
AS (n=55)	70 (45.72-88.11)	39.71 (28.03-52.3)	25.75	81.59	46.67	0.439 (0.276-0.602)	0.469
ALF (+) (n=38)	55 (31.53-76.94)	60.29 (47.7-71.97)	29.27	81.77	59.08	0.418 (0.256-0.581)	0.333
AS+ALF (+) (n=25)	30 (11.89-54.28)	72.06 (59.85-82.27)	24.28	77.15	62.39	0.522 (0.356-0.688)	0.794
FC>50 µg/g (n=43)	94.12 (71.31-99.85)	32.5 (18.57-49.13)	37.41	92.80	50.99	0.367 (0.219-0.515)	0.114
AS+FC>50 µg/g (n=24)	58.82 (32.92-81.56)	65 (48.32-79.37)	41.87	78.65	63.15	0.619 (0.458-0.781)	0.158
FC>150 µg/g (n=23)	70.59 (44.04-89.69)	72.50 (56.11-85.4)	52.38	85.19	71.93	0.715 (0.566-0.865)	**0.011**
AS+FC>150 µg/g (n=14)	52.94 (27.81-77.02)	87.50 (73.2-95.81)	64.48	81.27	77.13	0.702 (0.541-0.863)	**0.016**
AS+ALF+FC>50 µg/g (n=12)	23.53 (6.81-49.9)	80.49 (65.13-91.18)	33.0	72.04	63.97	0.518 (0.351-0.684)	0.834
AS+ALF+FC>150 µg/g (n=6)	23.53 (6.81-49.9)	95.12 (83.47-99.4)	66.33	75.28	74.36	0.593 (0.422-0.764)	0.272

CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, AS: Alarm symptoms, ALF: Abnormal laboratory findings, FC: Fecal calprotectin

of ALF to the AUC value is relatively low due to the greater AUC values of AS and FC. On the other hand, the lower number of pediatric patients in the meta-analyses further complicates this evaluation (9), which was a limitation of our study as well.

Given that FC testing is not available in most primary healthcare centers despite being a useful marker of IBD, the present study also aimed to provide a basis which could aid primary care clinicians in the referral of patients with suspicious IBD to pediatric gastroenterology clinics. Accordingly, AS and ALF are more important than FC, although they were found to be inadequate for the prediagnosis of IBD in the present study. In a previous study, 17 (19%) out of 90 patients who had AS and were referred from a primary healthcare center were diagnosed with IBD (10). Similarly, in our patients, IBD was diagnosed in 25.4% of the patients with AS. Unfortunately, there are limited studies on this topic and thus further studies are needed to substantiate our findings.

Study Limitations

Our study was limited in several ways. Firstly, the number of IBD patients was significantly less than the number of non-IBD patients. Another limitation was that the age of the patients ranged from 2 months to 18 years. FC cut-off levels have been well established in children older than 4 years of age but FC values vary widely in infants and high FC levels levels may be normal in infancy (15). These limitations may have affected our results. Therefore, future larger scale randomized trials with different age ranges are needed.

Conclusion

In conclusion, although primary care clinicians often use AS and laboratory parameters in the differentiation of IBD and non-IBD diseases, FC was found to have a relatively higher diagnostic value. Moreover, although there are varying cut-off values of FC reported in the literature, a cut-off value of >150 µg/g was found to be highly effective in the diagnosis of IBD.

Ethics

Ethics Committee Approval: The study was approved by Karadeniz Technical University Medical School Ethical Committee (approval no: 2020-127).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.G., M.Ç., Design: K.B., E.S., Supervision: F.İ., Data Collection and/or Processing: B.G., E.S., Analysis or Interpretation: F.İ., Literature Review: B.G., E.S., Writing: B.G., M.Ç.

Conflict of Interest: The author(s) indicated no potential conflicts of interest.

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Original Article

A Negative Correlation Between *MEFV* Mutations and Allergic Diseases

Malik Ejder Yıldırım[1], Hande Küçük Kurtulgan[1], Hasan Kılıçgün[2], Fatma Duksal[3]

[1]Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Genetics, Sivas, Turkey
[2]Erzincan Binali Yıldırım University Faculty of Health Sciences, Department of Nutrition and Dietetics, Erzincan, Turkey
[3]Konya Training and Research Hospital, Clinic of Pediatric Immunology and Allergy, Konya, Turkey

ABSTRACT

Aim: Atopy is associated with a genetic predisposition to develop allergic diseases such as allergic rhinitis, asthma, and atopic dermatitis. In this study, we aimed to compare the prevalence of Familial Mediterranean Fever (FMF) mutations in asthma and allergic rhinitis patients with controls in the pediatric population and to analyse the positive or negative effect of *MEFV* mutations in the development of atopy.

Materials and Methods: For the detection of FMF mutations, 88 pediatric patients (51 allergic asthma, 17 allergic rhinitis and 20 both asthma and allergic rhinitis cases) and 92 controls were included in our study. Total genomic DNA was extracted from peripheral blood samples using DNA isolation kit. Then, the patient and control groups were screened for *MEFV* gene mutations by Reverse Hybridization procedure (Strip Assay).

Results: There were 9 carriers (heterozygous mutation) in the patient group. The control group had 21 carriers and 1 individual with a compound heterozygous mutation. It was not detected any homozygous mutation in both two groups. The number of individuals with mutation was statistically higher in the control group than in the patients of asthma and allergic rhinitis (p=0.015) and the mutation number (allelic frequency) in the control group was also higher than in the patients (p=0.014).

Conclusion: We suggest that FMF mutations are less frequent in allergic rhinitis and asthma cases than in the normal population. Asthma and allergic rhinitis may be more common in individuals without FMF mutation. It can be thought that *MEFV* gene mutations are effective to prevent allergic reactions on the basis of T helper 2 (Th2) suppression.

Keywords: Allergic rhinitis, asthma, FMF, *MEFV* gene, mutation

Introduction

Allergy is among the most common chronic disorders of childhood and it is manifested primarily by symptoms associated with the nose, lungs, sinuses and skin. Allergic diseases such as asthma, eczema and allergic rhinitis affect approximately 20% of the population all over the world (1). These are complex conditions that are affected by different genetic and environmental factors (2). Asthma is a chronic, inflammatory disorder of the lower airways characterized by airflow obstruction, airway hyperresponsiveness and remodeling (3). Allergic rhinitis is a disease of the upper airways resulting from IgE-mediated inflammation caused by intolerance to allergens in the external environment (4). Allergic rhinitis and asthma often coexist in the same patients and constitute a significant global health problem (5). Immunotherapy is an important form of treatment in such allergic diseases (6). The main drugs used for the treatment of allergic rhinitis are oral antihistamines

Address for Correspondence
Malik Ejder Yıldırım, Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Genetics, Sivas, Turkey
E-mail: mey2002@gmail.com ORCID: orcid.org/0000-0003-4386-1583
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and nasal corticosteroids (7). These allergic disorders are associated with the activity of T helper 2 (Th2) cells involved in the production of interleukin 4 (8). Asthma is a Th2-cell driven inflammatory disease accompanied by eosinophilic inflammation, cytokine production and airway hypersensitivity (9).

Familial Mediterranean Fever (FMF) is an autosomal recessive genetic disorder that has the highest prevalence in people of Mediterranean origin (10). It is characterised by recurrent episodes of pain and fever, serosal inflammation including peritonitis, pleuritis, synovitis and erysipelas like erythema (11). Amyloidosis is an important problem in FMF and it can cause renal failure. Mutations in the *MEFV* gene cause FMF. This gene encodes a protein called pyrin (12). FMF mutations cause T helper 1 (Th1) polarization. The defective pyrin cannot suppress Th1 mediated inflammation. Th1 cells produce interferon-γ (IFN-γ) and they are associated with immune responses against intracellular viral and bacterial infections (13). IFN-γ inhibits IgE dependent reactions.

Atopy may be rare in people with MEFV gene mutations due to Th1 dominance. In a study by Yildiz et al. (14), the frequency of asthma, atopic dermatitis and allergic rhinitis in patients with FMF were found to be lower than their general prevalence in Turkey. However, Aydoğmuş et al. (15) found no difference in terms of the prevalence of asthma and allergic rhinitis between the FMF patients and control group. Therefore, they claimed that there was no proven antagonistic relationship between atopic diseases and FMF. Yazici et al. (16) stated that atopy was at a low frequency in FMF patients.

In this study, we aimed to evaluate the frequency of FMF mutations in allergic rhinitis and asthma cases in comparison to the normal population in Central Anatolia, Turkey where FMF is very common. Unlike the literature evaluating the atopy status in FMF patients, we screened *MEFV* mutations in allergic patients on the basis of Th1 and Th2 connections.

Materials and Methods

Study Design

This study was carried out between 2014 and 2019 in Cumhuriyet University Research Hospital, Department of Medical Genetics in Sivas, Turkey. Eighty-eight children diagnosed with allergic asthma and/or allergic rhinitis and ninety two healthy controls were included in the study in consultation with the biostatistics department. Respectively, 51 patients (58%) had allergic asthma, 17 patients (19.3%) had allergic rhinitis and 20 of them (22.7%) were suffering from both asthma and allergic rhinitis. This study was approved by the Cumhuriyet University Ethics Committee (approval number: 2014-06/09, date of the approval: 18.06.2014). Informed consent was obtained from the parents of the patients and control group.

Atopy Examination

The levels of total serum IgE were measured for the patients and a skin prick test panel including *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria*, cat dander, grass mix, tree mix, *Blattella germanica*, (histamine phosphate as positive control and 0.9% serum physiologic as negative control) was used to confirm the presence of atopy.

Mutation Screening

In our molecular laboratory, total genomic DNA was extracted from peripheral blood samples with a DNA isolation kit (Invitek Invisorb Spin Blood Kit, Germany). Multiplex polymerase chain reaction (PCR) amplification was performed with biotinylated primers. Those patients with asthma and allergic rhinitis and the controls were screened for *MEFV* gene mutations (E148Q, P369S, F479L, M680I(G/A), M680I(G/C), I692del, M694V, M694I, K695R, V726A, A744S, R761H) using a Reverse Hybridization procedure (Vienna Lab, FMF StripAssay, GMBH, Austria). PCR products were incubated on nitrocellulose strips and the process was completed with colour development and the detection of signals in Auto-LIPA (Auto-LIPA Innogenetics).

Statistical Analysis

Statistical analysis was performed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) to evaluate the differences between the patients and controls. Mean age was analysed via t-test and chi-squared test was used to compare the frequencies of *MEFV* mutations in the two groups. The odds ratios were calculated at a 95% confidence interval.

Results

The patient group consisted of 56 males (63.6%) and 32 females (36.4%). The mean age of the patients was 8.46±2.4 years (range: 5-14 years). The control group consisted of 56 males (60.9%) and 36 females (39.1%). The mean age of the controls was 9.02±1.6 years (range: 6-15 years). There was no difference between the patients and controls in terms of age and gender (Table I).

The distribution of FMF mutations in the patient and control groups is shown in Table II. There were 9 individuals with a single mutation (10.2%) in the patient group. There was no homozygous or compound heterozygous mutation in this group. The number of controls with mutation was 22 (23.9%). While 21 of them had a heterozygous mutation, only one had a compound heterozygous mutation. The controls also had no homozygous mutation. The most frequent mutation in both the patient and control groups was E148Q (3.4% in patients, 6.5% in controls). The compound heterozygous mutation in the control group was M694V+M680I (G/C).

There was no significant difference between asthma, allergic rhinitis and asthma+allergic rhinitis cases in terms of mutation frequency (Table III). The difference between the patients and the controls was significant in terms of the number of individuals with mutation (p=0.015). The mutation ratio of the controls was also significantly higher in comparison to the patients (p=0.014). Those individuals with mutation and the number of total mutations were presented in Table IV. The proportion of individuals with FMF mutations was 10.2% in the patient group and 23.9% in the control group. Allelic frequency was 5.1% in the patients and 12.5% in the controls. The mutation ratio of the control group was more than two-fold of each patient group (Figure 1).

Discussion

Allergy is one of the most common chronic problems in the world with the symptoms of nose, lungs, sinuses and skin. It is a medical condition caused by the hypersensitivity of the immune system and is associated with genes and the environment. Allergic asthma is a chronic inflammatory disease with airway hyper-responsiveness and air-flow obstruction. The inflammatory process that enhances eosinophil accumulation and IgE production is driven by Th2 cells (17). In this context, asthma is associated with an excessive Th2 response to allergic stimuli that cause airway inflammation. Allergic rhinitis is also an inflammatory disease characterized by an IgE-mediated hypersensitivity reaction and mucosal infiltration with inflammatory cells (18). Antihistamines are frequently used in the symptomatic treatment of allergic rhinitis.

Th1 cells are associated with the host immunity and directed by IL-12 and IL-2. One of the main cytokines in this process is IFN-γ. Th2 cells are usually triggered by IL-4 and their potent cytokines are IL-4, IL-5, IL-9, IL-10 and IL-13. It was demonstrated that some myeloid dendritic cells are effective in the differentiation of Th1 and Th2 cells during the immune response (19). While IL-12 directs Th1 differentiation, IL-4 induces the development of Th2 (20). Th1-type cytokines can contribute the pro-inflammatory responses to kill intracellular microorganisms. Th2 cells have a responsibility in the development of allergic diseases (21). Th2-type cytokines are associated with eosinophilic activity in atopy. Th2 cells play an important role in the activation of IgE antibody producing B cells, mast cells and eosinophils (22). Th2 cytokines, especially IL-13 have a critical role in the pathogenesis of asthma (23).

FMF is an auto-inflammatory genetic disease caused by *MEFV* gene mutations leading to interleukin-1β activation

Table I. The characteristics of the patients and controls

Characteristics	Patients	Controls	p-value
Age	8.46±2.4	9.02±1.6	NS*
Gender			
Male	56 (63.6%)	56 (60.9%)	NS*
Female	32 (36.4%)	36 (39.1%)	
*Non-significant			

Table II. *MEFV* mutations in the patients and controls

Mutation	Patients (%)	Controls (%)
M694V	2 (2.3)	5 (5.4)
E148Q	3 (3.4)	6 (6.5)
P369S	1 (1.1)	2 (2.2)
M680I (G/C)	2 (2.3)	4 (4.3)
V726A	1 (1.1)	2 (2.2)
A744S	-	2 (2.2)
M694V+M680I (G/C)	-	1 (1.1)
Individuals with mutation	9 (10.2)	22 (23.9)
Individuals with no mutation	79 (89.8)	70 (76.1)
Total mutation number	9	23

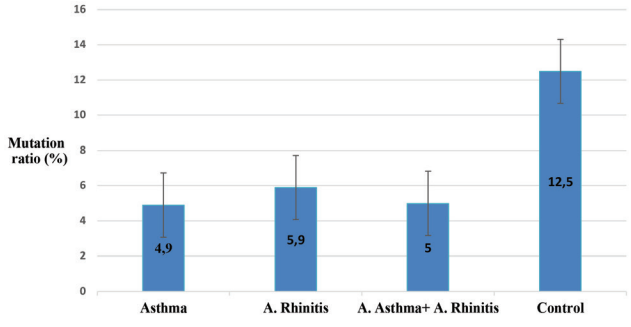

Figure 1. *MEFV* mutation ratio of the patients and control group

(24). This gene encodes pyrin, a protein that is effective in the regulation of apoptosis and inflammation (25). Pyrin is produced in some blood cells such as neutrophils, eosinophils, and monocytes. It may direct white blood cells to the site of the inflammation and reduce the inflammatory response if it is no longer needed.

FMF is an important cause of morbidity especially in the Jewish, Arab, Armenian, and Turkish populations (26). The etiology of FMF is not fully understood. Mutations in a single gene play a role in this disease, but some other factors may also be effective in its pathogenesis. Although FMF is regarded as an autosomal recessive disorder, some patients carry only one *MEFV* mutation and therefore, it can be thought that dominant inheritance is possible. On the other hand, some mutations may have not been identified yet and some rare mutations may be overlooked in the laboratory.

Pyrin coordinates caspase-1 activation and thus IL-1b production (27). Pyrin normally diminishes neutrophil-mediated inflammation by the downregulation of interleukin-1 (IL-1), but it is defective in FMF (28). The defective pyrin has been shown to enhance inflammation through IL-1β production but the precise physiopathology of FMF appears to be more complex (29). FMF inflammation is accompanied by Th1 polarization and the IFN-γ levels are usually higher in FMF patients. IFN-γ inhibits Th2 cells. On the basis of these facts, suppression of Th2 is expected in FMF.

The negative association between asthma and FMF mutations may originate from the suppression of Th2 activity with defective pyrin (30). Lidar et al. (31) suggest that normal pyrin is essential for eosinophil function and the mutated pyrin in patients with FMF attenuates eosinophil-mediated bronchial inflammation resulting in a reduction in the frequency of asthma. Similarly, on the basis of Th2 suppression, the prevalence of atopy in FMF patients was found to be significantly lower than in the normal population (8). However, Amet et al. (32) suggested that atopy frequencies were similar in children with or without FMF. On the other hand, Celiksoy et al. (33) asserted that FMF is a multi-systemic problem and atopic disorders are characteristic features of this disease. This claim does not appear to be compatible with the literature. According to Aypar et al. (34), Th1 polarization in patients with FMF and carriers may be protecting them from diseases of pronounced Th2 response but the decreased allergic responses in those patients with FMF are a result and not the cause of the underlying pathophysiology. The frequency of MEFV mutations in our study was significantly lower in the patient group compared to the control group. The relationship of *MEFV* mutations with atopy is a topic of great interest. The subject of allergy has been studied in FMF patients in the past. However, the screening of *MEFV* mutations in allergic patients is likely to be the first with this study.

Conclusion

Our study demonstrated that the allelic frequency of *MEFV* mutations in asthma and allergic rhinitis patients is statistically lower in comparison to the healthy population in Sivas, Central Anatolia. The mutation ratio in the healthy

Table III. The frequency of *MEFV* mutations in the patients (A. Asthma, A. Rhinitis and A. Asthma + A. Rhinitis)

Disease	A. asthma (n=51)	%	A. rhinitis (n=17)	%	A. asthma+A. rhinitis (n=20)	%	p-value
Mutation	5	9.8	2	11.8	2	10	>0.05*
Wild	46	90.2	15	88.2	18	90	
*Binary comparisons							

Table IV. The comparison of individuals with mutations and the allelic frequency in the patients and controls

Individuals	Patients	%	Controls	%	p-value
With mutation	9	10.2	22	23.9	0.015 p<0.05
Without mutation	79	89.8	70	76.1	
Odds ratio: 0.362 (0.157-0.839)					
Allelic frequency	**Patients**	**%**	**Controls**	**%**	**p-value**
Mutation	9	5.1	23	12.5	0.014 p<0.05
Wild	167	94.9	161	87.5	
Odds ratio: 0.377 (0.169-0.840)					

group was more than twice that of the patient groups. These data suggest that individuals with FMF mutations may be more resistant to allergic diseases. Although the current results were obtained with a different study plan (*MEFV* mutation screening in allergic diseases), they are compatible with most of the previous studies in this area. *MEFV* mutations may be an advantage for the protection against allergic diseases in the context of their relationship with Th2 activity.

Ethics

Ethics Committee Approval: This study was approved by the Sivas Cumhuriyet University Ethics Committee (approval number: 2014-06/09, date of the approval: 18.06.2014).

Informed Consent: Informed consent was obtained from the parents of all participants included in the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Project: M.E.Y., Molecular Study: M.E.Y., H.K.K., Analysis: M.E.Y., H.K.K., H.K., Writing the Manuscript: M.E.Y., Literature Search: H.K.K, F.D., Statistics: H.K., Patient Supply and Diagnosis: F.D.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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Original Article

Maternal and Cord Blood Vitamin B12, Folate and Homocysteine Levels

Selime Özen Bölük[1], Ebru Şahin[2], Oya Baltalı Halıcıoğlu[3], Feyza Koç[4], Sadık Aksit[4]

[1]Ege University Faculty of Medicine, Department of Pediatrics, İzmir, Turkey
[2]University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Clinic of Obstetrics and Gynecology, İzmir, Turkey
[3]University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Clinic of Pediatrics, İzmir, Turkey
[4]Ege University Faculty of Medicine, Department of Social Pediatrics, İzmir, Turkey

ABSTRACT

Aim: Nowadays, insufficiency and deficiency of vitamin B12 and folate are seen as an important health problem. The purpose of the present study was to determine the frequency of vitamin B12 and folate deficiencies in pregnant women and their babies at birth.

Materials and Methods: The study group consisted of 117 pregnant women and their single, term babies in İzmir Ege Maternity Hospital. Analysis of vitamin B12, folate and homocysteine levels were performed from venous blood samples which were obtained from the mother and cord blood at birth. Additionally, a questionnaire using a face-to-face interview method was performed with the pregnant women included in this study. The mean duration of pregnancy was 39.1±0.89 weeks and the mean age of the mothers was 28.2±6.2 years.

Results: Vitamin B12 deficiency (<130 pg/mL) was present in 88.9% of mothers and 56% of babies. Folate deficiency (<4 ng/mL) was found in 6.8% of mothers, but not found in any babies. The homocysteine levels were high (>8 µmol/L) in 58.1% of mothers and 63.2% of babies. There was a significant correlation between maternal and cord blood vitamin B12, folate and homocysteine levels (p<0.01). However, there was no correlation between maternal vitamin B12 and homocysteine levels (p=0.016, p=0.354).

Conclusion: Low maternal vitamin B12 levels are strongly associated with low cord blood vitamin B12 levels. This data reveals that vitamin B12 deficiency which can occur from the neonatal period is a preventable public health problem. Pregnant women and physicians should be made aware of the importance of vitamin B12 intake during pregnancy.

Keywords: Folate, homocysteine, vitamin B12

Introduction

Pregnancy is a period of rapid development for both the mother and the fetus and the requirement for some nutrients increases during pregnancy (1). Almost 20% percent of females have vitamin B12 and folate deficiency (2). Vitamin B12 is a water-soluble, red colored vitamin that is essentially synthesized by microorganisms at the end of nearly twenty different enzymatic stages. Inadequate dietary intake of vitamin B12 is a fundamental reason for cobalamin deficiency because the vitamin B12 that is required for humans is totally supplied by the diet from animal food. The other causes of vitamin B12 deficiency are autoimmune diseases, environmental pollution, malabsorption and non-separating of cobalamin bound to food (3). Vitamin B12 is an essential cofactor of enzymes which play a role in the synthesis of DNA, fatty acids and myelin. For this reason, symptoms of hematological, neurological diseases and neuropsychiatric diseases may occur. Vitamin B12

Address for Correspondence
Selime Özen Bölük, Ege University Faculty of Medicine, Department of Pediatrics, İzmir, Turkey
Phone: +90 507 380 51 56 E-mail: yarenseli85@hotmail.com ORCID: orcid.org/0000-0002-7156-7480
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deficiency predisposes neurodevelopmental diseases and megaloblastic anemia. Folate is a form of water-soluble vitamin B9. It has different missions for different body cells, especially in purine and pyrimidine synthesis. Pregnant women have a high risk of folate deficiency because of an increased requirement and the catabolism of folate. Folate deficiency is related with ablatio placenta, spontaneous abortion, prematurity, congenital defects such as neural tube defects and perinatal mortality. The Centers for Disease Control and Prevention advices 400 µg/day folic acid replacement during the period starting from preconception until the 20th week of pregnancy (4). Both folate and vitamin B12 are essential cofactors for homocysteine metabolism (5). In addition, high levels of plasma homocysteine are associated with vascular endothelial damage and occlusive vascular problems. Therefore, this situation poses a high risk for renal and cardiovascular diseases. Increased plasma homocysteine concentration is related with vascular placental thrombosis and may cause preeclampsia and ablasio placenta which result in early pregnancy losses (6).

The aim of this study was to evaluate the ratio of maternal vitamin B12 and folate deficiency and to analyze maternal and cord blood vitamin B12, folate and homocysteine levels. For this reason, we evaluated the relationships between maternal and cord blood vitamin B12 and folate status.

Materials and Methods

Study Population

Those pregnant women who met the inclusion criteria and agreed to take part in this study were included in this study. This study took place during the month of December 2012 in İzmir Ege Maternity Hospital. The inclusion criteria for the study were having a single, term birth (38-42 weeks). Preterm birth (≤37 week), birth after the 42nd week and multiple births were exclusion criteria. Both normal and cesarean deliveries were included. Written consent was received from the pregnant women who took part in this study. The sample size was calculated to be 97 with 95% confidence interval.

Study Design and Ethical Approval

This retrospective cross-sectional study was approved by the Clinical Research Ethics Committee of Ege University, Faculty of Medicine Hospital on 19.10.2012 (decision no: 12-9.1/10).

Data Collection

The study group consisted of 117 pregnant women and their single, term babies in İzmir Ege Maternity Hospital. A questionnaire via a face-to-face interview method was performed with the pregnant women. Socio-demographic features (mother's age, economic status, mother and father's education and profession, nutritional status during pregnancy, usage of vitamin/mineral supplements, maternal illness during pregnancy, use of cigarettes, alcohol or substances during pregnancy) were investigated. Birth weight-height, gender, gestational age, and congenital anomaly presence were recorded at birth. Analysis of vitamin B12, folate and homocysteine levels were carried out on venous blood samples which were obtained from the mother and the cord at birth. The blood samples were protected by centrifugation at -20 °C degree until analysis. A chemiluminescence immunoassay technique with Immulite 2000 was used for analysis.

Data Analysis

One hundred thirty pg/mL was accepted as the deficiency limit for serum B12 vitamin levels of both the pregnant women and their babies. Measured serum cobalamin levels do not reflect metabolic active vitamin B12 status exactly. There is no correlation between the occurrence of clinical findings and serum vitamin B12 levels. The lowest level seen in studies was taken as the limit for serum vitamin B12 levels as there is no clear cut-off limit in the literature. Four ng/mL has been determined as the deficiency level of folate according to the criteria of NHANES 3 classification in 2005 (7). Homocysteine is used as a metabolic indicator for this classification. Hyperhomocysteinemia during pregnancy is defined as ≥8 µmol/L based on the literature (8). A hemoglobin value below 11 gr/dL is accepted as the anemia level for pregnant women.

Statistical Analysis

Data was evaluated using the SPSS 18.0 (Statistical Package for Social Sciences) statistical package software. Categorical variables were expressed as number (n) and percentage (%). Comparisons between groups of qualitative variables were determined using the chi-square test, numerical variables were compared using Student's t-test and One-Way ANOVA. Multiple logistic regression analysis was used to investigate potential independent risk factors which may affect vitamin B12, folate or homocysteine levels. Correlation analysis was used to determine the relationship between numerical variables such as the maternal and cord blood vitamin B12, folate and homocysteine levels. Statistically significance was considered for $p<0.05$.

Results

During the one month period of the study, there were totally 177 births. Six births were multiple pregnancy, 19 births were <37 weeks and 5 births were >42 weeks, 24 pregnant women did not agree to participate in this study and 6 of the samples were insufficient. As a result, the study was carried out with 117 pregnant women and their babies. The average gestational week was 39.1±0.89. Fifty-three percent of babies were male. The mean birth weight was 3,402±47.5 gr, and the mean birth height was 50.24±1.22 cm. There was no congenital anomaly in 97.4% of babies. Two babies had antenatal bilateral hydronephrosis and one baby had antenatal sacral teratoma pre-diagnosis. The socio-demographic and some laboratory characteristics of the mothers and babies are given in Table I and the descriptive characteristics of pregnant women are given in Table II.

According to the classifications of the education status of the mothers, 59.8% of the mothers had primary school level or lower and 9.3% of them had high school or higher educational status. There was no vitamin/mineral supplementation for 12.8% of mothers, 52.9% of them had 3 months or less, 32.4% of them had between 3-6 months, and 14.7% of them had more than 6 months vitamin/mineral supplementation. However, detailed information about the name of the preparation and its contents could not be obtained from those pregnant women who stated that they had taken vitamin/mineral supplementation during pregnancy.

The nutritional habits of the mothers during their pregnancy were investigated. It was observed that 2.6% of them had a vegetarian diet and 83.8% of them consumed red meat every 2 weeks or less (Table III).

The mean maternal and cord blood vitamin B12 levels were determined to be 73±40.6 pg/mL and 121.7±68.36 pg/mL respectively in this study.

The maternal and cord blood B12 deficiency rates were determined to be 88.9% and 59% respectively. The mean maternal and cord blood folate levels were 12.43±7.31 ng/mL and 22.2±4.39 ng/mL respectively in our study. The maternal folate deficiency rate was 6.8%, but all of the babies had normal folate levels. It was found that the babies of those pregnant women with low serum vitamin B12 levels had more vitamin B12 deficiency ($p<0.01$) (Table IV). When the limit for hyperhomocysteinemia is accepted as 8 µmol/L; high serum homocysteine levels were seen in 58.1% of the mothers, while this ratio was 63.2% in cord blood. The mean maternal and cord blood homocysteine levels were 8.98±3.2 µmol/L and 9.2±3.03 µmol/L respectively.

When the relationship between serum vitamin B12 deficiency and hyperhomocysteinemia was examined, no statistically significant difference was found for maternal serum homocysteine levels between those mothers with vitamin B12 deficiency and those without ($p>0.05$). Homocysteine levels were high in 59.6% of those mothers with low serum vitamin B12 levels and this ratio was 46.2% for those mothers who had normal vitamin B12 levels. The relationship with folate deficiency, which is one of the reason of hyperhomocysteinemia, was also examined. Fifty percent of the 8 mothers with low serum folate levels had normal serum homocysteine levels. Ninety-four percent of the 68 mothers who had high serum homocysteine levels had normal serum folate levels ($p>0.05$). Gender was not related with vitamin B12 and folate levels, however homocysteine levels were statistically higher for the male babies ($p=0.026$) (Table IV). There was no statistical

Table I. Socio-demographic and laboratory characteristics of mothers and babies

	Mean	Standard deviation	Minimum value	Maximum value
Birth weigth (gr)	3402.0	474.5	1750	4500
Birth height (cm)	50.24	1.22	47	53
Gestational age (week)	39.15	0.89	38	42
Mother age (year)	28.2	6.2	18	42
Maternal folate (ng/mL)	12.43	7.31	2.00	25.5
Maternal vitamin B12 (pg/mL)	73.0	40.6	30.00	208.0
Maternal homocysteine (µmol/L)	8.98	3.2	1.32	21.6
Cord blood folate (ng/mL)	22.2	4.39	8.1	25.0
Cord blood vitamin B12 (pg/mL)	121.7	68.36	30.0	339.0
Cord blood homocysteine (µmol/L)	9.2	3.03	4.21	19.14

difference between cord blood vitamin B12 deficiency and homocysteine levels. All babies had normal cord blood folate levels so its relation with homocysteine levels was not analyzed. The relation between the concentration of maternal and cord blood vitamin B12, folate and homocysteine was analyzed by Spearman correlation analysis (Table V). There was a strong relationship between maternal and cord blood folate levels (p=0.306, p<0.01), between maternal and cord blood vitamin B12 levels (p=0.499, p<0.01) and between maternal and cord blood homocysteine levels (p=0.483, p<0.01). There was also a strong correlation between maternal vitamin B12 and hemoglobin levels (p=0.53; p<0.01). The relation between the concentration of maternal and cord blood vitamin B12, folate and homocysteine were analyzed with Spearman correlation analysis. There was a strong relationship between maternal and cord blood folate levels (p=0.306, p<0.01), between maternal and cord blood vitamin B12 levels (p=0.499, p<0.01) and between maternal and cord blood homocysteine levels (p=0.483, p<0.01). There was also a strong correlation between maternal vitamin B12 and hemoglobin levels (p=0.53, p<0.01). There was a negative correlation between cord blood vitamin B12 and homocysteine levels (p=-0.236, p<0.05).

Anemia was found in the analysis of 38.8% of mothers with vitamin B12 deficiency and 7.7% of mothers with normal vitamin B12 levels (p<0.05). When we examined relationship between maternal folate levels and anemia, 85.7% of mothers who had folate deficiency had anemia (p<0.01) but 31.7% of mothers had anemia despite having normal serum folate levels. There was no relation between the maternal educational status and maternal vitamin B12 or folate levels (respectively p=0.082, 0.325). It was determined that there was no statistical difference between anemia in pregnant women and the number of parity, maternal educational status, maternal smoking during pregnancy or the economic status of the families. However, it was found that the frequency of anemia was lower in those mothers who used vitamin-mineral supplementation during pregnancy (p<0.01). None of the independent variables had a meaningful effect on the dependent variable when independent variables were defined as the parity number of mothers, vitamin/mineral supplementation during pregnancy, maternal education status, or maternal smoking in pregnancy and the dependent variable was defined as serum vitamin B12. Smoking, which is one of the reasons of hyperhomocysteinemia, was not related with maternal and cord blood homocysteine levels in this study.

Table II. Descriptive characteristics of pregnant women

Characteristics	Number (n)	%
Parity of mother		
1	42	35.9
2	44	37.6
≥3	31	26.5
Education status of mother		
Uneducated	17	14.5
Primary school	53	45.3
Middle school	35	29.9
High school and upper	12	9.3
Vitamin/mineral supplements during pregnancy		
Yes	102	87.2
No	15	12.8
Smoking during pregnancy		
Yes	26	22.2
No	91	77.8
Alcohol consumption during pregnancy		
Yes	0	0
No	117	100

Table III. Analysis of mothers' nutrition during their pregnancy

Consumption frequency	Red meat (%)	Poultry (%)	Fish (%)	Egg (%)	Milk (%)	Dairy product (%)	Green vegetables (%)
Daily	0.9	0.9	0.0	41.0	27.4	89.7	82.1
4-5 times/week	1.7	1.7	1.7	19.7	7.7	6.0	6.8
1-2 times/week	13.7	59.8	47.9	19.7	18.8	3.4	9.4
1 times/2 weeks	36.8	19.7	13.7	4.3	1.7	0.0	0.0
1 times/month	39.3	10.3	19.7	0.9	1.7	0.0	0.0
Never	7.7	7.7	17.1	14.5	39.3	0.9	0.9

Discussion

In this study, it was shown that the babies of pregnant women with low serum vitamin B12 levels had more severe vitamin B12 deficiency (p<0.01) and there was a strong correlation between maternal and cord blood vitamin B12 levels (p=0.499, p<0.01). Studies conducted with similar population groups in developing countries and our country support the view that the vitamin B12 levels of mothers and their newborns are very closely correlated and show that vitamin B12 deficiency is severely high in mothers and infants. In a study carried out in Brazil, a developing country like our country, blood samples of 69 pregnant women and their babies were examined at birth in 2002 (9). The mean serum maternal vitamin B12 levels were 154.1±77.8 pmol/L (208.6±104.3 pg/mL), these levels were determined to be 256.8±198.9 pmol/L (346.8±268 pg/mL) for the babies.

Table IV. Relationship between maternal serum vitamin B12, folate levels, gender and maternal serum homocysteine level

	Cord blood vitamin B12 level		
	Low	**Normal**	**p-value**
Maternal vitamin B12 level			
Low, n (%)	66 (63.5)	38 (36.5)	0.005
Normal, n (%)	3 (23.1)	10 (76.9)	
	Maternal homocysteine level		
	Normal	**High**	**p-value**
Maternal vitamin B12 level			
Low, n (%)	42 (40.4)	62 (59.6)	0.354
Normal, n (%)	7 (53.8)	6 (46.2)	
Maternal folate level			
Low, n (%)	4 (50)	4 (50)	0.63
Normal, n (%)	45 (41.4)	64 (58.7)	
Gender			
Male, n (%)	17 (27.4)	45 (72.6)	0.026
Female, n (%)	26 (47.3)	29 (52.7)	

There was a strong correlation between maternal and neonatal B12 levels (r=0.68, p<0.01). Similar to our results, cord blood vitamin B12 levels were found to be 2-3 times higher than the maternal values. The vitamin B12 deficiency ratio in pregnant women was determined to be 74.1% in a study that was designed by Pathak et al. (10) in India. Vitamin B12 deficiency was found in early pregnancy (at 13-17 gestational weeks) to be 48.8%, in later pregnancy (at 28-32 gestational weeks) to be 80.9% and at postpartum 13-17 weeks to be 60%. Açkurt et al. (11) designed their study with prenatal and postnatal nutritional evaluation of 133 randomized pregnant women in our country, in Istanbul and Izmit. Onal et al. (12), evaluated 250 mothers and their term babies at postnatal 48th hour in 2010. The lowest limit of vitamin B12 was accepted to be 300 pg/mL for mothers and 200 pg/mL for babies in this study. Vitamin B12 deficiency was found in 81.6% of mothers and 41% of their babies. In a similar study which was designed in the northern region of Turkey between 2008 and 2009, the ratio of maternal vitamin B12 deficiency was 72% and this ratio was 56% for their babies (13). In another study in Urfa, values below 160 pg/mL were defined as deficiency and values below 120 pg/mL were defined as severe deficiency for both mothers and babies (14). The vitamin B12 deficiency ratio was 72% (severe deficiency 48%) for mothers and 41% for babies (severe deficiency 23%) (14). There was also a statistically significant correlation between maternal and cord blood vitamin B12 levels (r=0.395, p<0.001). In a study conducted with 72 pregnant women at the same time and in a similar region of Turkey as our study, it was observed that 70.8% of mothers and 83.9% of infants had vitamin B12 deficiency when 200 pg/mL was accepted as the cut-off point for vitamin B12 (15). It is considered that the dominance of the Mediterranean diet in the region might be responsible.

There is no clear limit value for defining serum vitamin B12 deficiency in the literature. Different reference ranges have been determined according to the methods and kits which are used. RDA reports the lowest limit of vitamin

Table V. Relationship between maternal and cord blood vitamin B12, folate and homocysteine concentrations (Spearman correlation analysis)

	Cord folate	**Cord B12**	**Cord Hcy**	**Maternal Hcy**	**Hb**
Maternal folate	0.306**	0.152	-0.168	-0.126	0.164
Maternal B12	0.101	0.499**	-0.119	-0.016	0.53**
Maternal Hcy	-0.129	-0.105	0.483**		-0.014
Cord Hcy	-0.098	-0.236*		0.483**	

*p<0.05, **p<0.01

B12 as 120-180 pmol/L (170-250 pg/mL) for adults (16). In addition, serum total vitamin B12 concentration starts to decrease during the early processes of the first trimester of pregnancy. This decrease becomes more significant at 6 months. For this reason, in our study, 130 pg/mL was accepted as the limit of vitamin B12 deficiency despite this value being one of the lowest values for vitamin B12 deficiency. The rates of vitamin B12 deficiency in mothers and babies were found to be slightly higher in this study. The mean vitamin B12 levels were also lower compared to similar studies. It is considered that the reason for this might be related with this study group which represented a poor socio-economic region. Our findings may be related with the mothers' nutritional insufficiencies which continue during their pregnancy and so their newborn babies were born with insufficient vitamin levels. When the nutritional status of the mothers during pregnancy was investigated, they were seen to be inadequate. Waldmann et al. (17) compared strict and mild vegetarians in their study. Strict vegetarians had significantly lower cobalamin levels and higher homocysteine levels. However, there was no difference found with folate levels.

It was observed that the number of births and pregnancies of the mother did not affect the serum and cord blood vitamin B12, folate and homocysteine levels when evaluated by logistic regression analysis. In addition, there was no relationship between whether the mother used vitamin-mineral support during pregnancy and maternal serum and cord blood vitamin B12 and folate levels. However, 65.7% of the mothers had used vitamin-mineral support for less than 3 months during pregnancy. This period is quite short and it is thought that the relationship could not be clearly determined since the content of the preparations used is not known.

Monagle and Tauro (18) determined that the most frequent reason of infantile megaloblastosis was maternal vitamin B12 deficiency and they stated that fifty percent of these mothers were asymptomatic. All of these data support the view that babies who are born to mothers who have insufficient vitamin B12 stores, substantially also have insufficient vitamin B12 stores at birth (19). Normally, babies are born with 25-50 mcg vitamin B12 storage. The essential quantity for growth is 0.1 mcg/day. This vitamin B12 store which a newborn has at birth is enough for 6-12 months. The development of infants with insufficient vitamin B12 storage is generally normal for the first months. If the initiation of complementary nutrition is delayed, clinical features may present due to vitamin B12 deficiency after sixth months (20,21). Prenatal 3rd trimester and postnatal 3-6 months is the period of life in which brain development and myelination are fastest. If the mother has insufficient vitamin B12, the baby's vitamin B12 deficiency develops earlier (19). Cerebral atrophy and hypoplasia may develop at birth because of the effects of myelinization during the last trimester. If vitamin B12 deficiency is not detected and treated early, it may result in irreversible neurological damage in infants (22-25). The most common symptoms are lethargy, hypotonia and convulsions, and sometimes encephalopathy may present. Severe pancytopenia may develop in addition to megaloblastic anemia due to vitamin B12 deficiency because of impairment of DNA synthesis. Hypogammaglobulinemia may accompany vitamin B12 deficiency and reach normal levels with vitamin B12 treatment (26). In a study with a large sample size designed in Holland in 2010, it was claimed that maternal vitamin B12 deficiency might be an etiological reason for excessive crying in babies (27). In a comparison of two groups of pregnant women in India, oral vitamin B12 (50 μgr) was given to one group during pregnancy and early lactation and a placebo were given to the other group (28). Vitamin B12 levels were statistically higher in the group taking vitamin supplements in an evaluation of the breast milk, the maternal serum and the babies' serum at postnatal 6th week. In a systematic review, the importance of vitamin B12 supplementation during pregnancy is emphasized according to results which state that having maternal vitamin B12 levels <148 pg/L is a risk factor for low birth weight for newborns and there is a linear relationship between maternal B12 vitamin levels and preterm birth (29). In addition, in a study designed as premarital screening of women, vitamin B12 and iron deficiency were seen to be an important health problem for women of reproductive age in our country (30).

There was also a significant relation between maternal and cord blood folate levels ($p=0.306$; $p<0.01$) in our study. The mean maternal folate levels were 13.9±5.6 nmol/L (5.74±2.21 ng/mL) and the mean serum folate levels for babies were 6.6±2.8 nmol/L (2.65±0.88 ng/mL) at birth in a study designed by Guerra-Shinohara et al. (9) in Brazil. All of the mothers had normal folate levels and 4% of babies had folate deficiency in Sayar et al.'s (13) study. The results of the Koc et al.'s (14) study in Urfa overlapped with our results. In their study, maternal folate deficiency was found to be 12% (severe deficiency was 9%) and serum folate levels that were below 4 ng/mL were defined as deficient, and below 2 ng/mL were defined as severely deficient. All of the babies had normal folate levels as in

our study. It is considered that folic acid supplementation plays a big role in these results.

Cord blood homocysteine levels were higher in males than females in this study. There have been different results in recent studies, but generally, it is accepted that there is no relation to gender (29). There was a strong correlation between maternal and cord blood homocysteine levels ($p=0.483$, $p<0.01$) and also a negative correlation between cord blood vitamin B12 and homocysteine levels ($p=-0.236$, $p<0.05$) in this study. In the study of Guerra-Shinohara et al. (9), there was a strong correlation between maternal and neonatal homocysteine levels and a negative correlation between neonatal vitamin B12 and homocysteine levels, as in our study. This indicates that there is a weak negative correlation between neonatal folate and homocysteine levels (9).

Plasma homocysteine levels decrease physiologically in normal pregnancies. It is argued that this is a physiological adaptation (31). No significant correlation was found between maternal vitamin B12 and homocysteine levels, and it is thought that homocysteine levels are not a significant indicator in revealing vitamin B12 deficiency in pregnancy due to the physiological decrease in homocysteine levels during pregnancy. As emphasized by Wallace et al. (32), vitamin B12 is not a good marker of homocysteine levels in pregnancy. There was no significant relation between smoking during pregnancy and cord blood homocysteine levels in our study. In a study in Oslo, babies whose mothers smoked 10 or more per day had significantly higher homocysteine levels (33).

Study Limitations

The daily consumption of cigarettes was not investigated in our study, and this can be considered to be an incomplete point of our study. It was observed that the frequency of anemia was 3.9 times higher in those who did not use vitamins and minerals during pregnancy. One of the limitations of this study is that we could not fully investigate at what dose and for how long vitamin-mineral support was taken, because the pregnant women did not know the exact contents of the preparations they used. Due to the fact that the region in which this study was conducted has a low socioeconomic level, it is not possible to generalize the results to the whole society. However, our findings are important in terms of emphasizing that babies are born with vitamin deficiencies in cases of maternal nutritional deficiency.

Conclusion

Maternal vitamin B12, folate and homocysteine levels are significantly correlated with cord blood levels. This study reveals that vitamin B12 deficiency which can occur from the neonatal period is a preventable public health problem. Babies may be born with adequate vitamin B12 stores by supporting their mothers' intake of vitamin B12 during and/or before pregnancy. Pregnant women and physicians should be made aware of the importance of vitamin B12 intake during pregnancy.

Ethics

Ethics Committee Approval: This retrospective cross-sectional study was approved by Clinical Research Ethics Committee of Ege University Faculty of Medicine Hospital on 19.10.2012 (decision no: 12-9.1/10).

Informed Consent: Written consent was received from the pregnant women that included to study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: S.Ö.B., E.Ş., O.B.H., F.K., S.A. Design: S.Ö.B., E.Ş., O.B.H., F.K., S.A. Data Collection and/or Processing: E.Ş., Analysis or Interpretation: E.Ş., Supervision: S.Ö.B., F.K., Writing: S.Ö.B., E.Ş., O.B.H., F.K., S.A.

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Original Article

Effect of Prone Position, Pacifier and Smelling Breast Milk on Pain and Stress Parameters Among Term Neonates Undergoing Venipuncture: A Randomized Controlled Trail

Fuat Özdemir[1], Derya Evgin[2], Nalan Gördeles Beşer[3]

[1]Niğde Training and Research Hospital, Niğde, Turkey
[2]Akdeniz University Kumluca Faculty of Health Sciences, Department of Nursing, Pediatric Nursing, Antalya, Turkey
[3]Niğde Ömer Halisdemir University Niğde Zübeyde Hanım Faculty of Health, Department of Nursing, Psychiatry Nursing, Niğde, Turkey

ABSTRACT

Aim: This study was conducted to examine the effect of three different methods for reducing pain and stress among term neonates undergoing venipuncture.

Materials and Methods: The research sample comprised 80 term neonates with hyperbilirubinemia who were being treated at the Neonatal Intensive Care Unit. The term neonates were allocated, according to the randomization method, into a smelling breast milk group (n=20), a pacifier/dummy group (n=20), a prone position group (n=20), and a control group (n=20).

Results: It was determined that there was a statistically significant correlation between the pain and stress score averages of the breast milk, prone position, and pacifier/dummy groups according to all measurements taken before, during, and after the procedure ($p<0.001$). It was discovered that there was a difference between the Premature Infant Pain Profile-Revised form (PIPP-R) and the mean stress scores of the control group and the breast milk, prone position, and pacifier/dummy groups after the procedure ($p<0.001$).

Conclusion: In study, it was concluded that the breast milk smell, prone position, and giving a pacifier made of sterile gloves are effective in reducing the pain and stress of newborns during the venipuncture procedure. Non-pharmacological methods such as smelling breast milk, prone position, and giving a pacifier/dummy are recommended to reduce pain and stress among term neonates during interventional procedures.

Keywords: Breast milk smell, giving pacifier/dummy, pain, prone position, stress, term neonates

Introduction

Newborn infants who encounter health problems in the first days of their life are referred to a Neonatal Intensive Care Unit (NICU) service in order to receive the necessary medical care and treatment (1). Neonatal hyperbilirubinemia is a common cause of readmission for newborn infants to hospital after early discharge (2,3). A high frequency of neonatal hyperbilirubinemia continues to be seen, both globally as well as in Turkey, and therefore remains an important public health problem (4). Neonatal hyperbilirubinemia is defined as a general yellow coloring of the sclera and skin in newborn infants. About 60% of infants born in the term period and around 80% of pre-term infants develop hyperbilirubinemia in the first days of life; hyperbilirubinemia requiring

Address for Correspondence
Derya Evgin, Akdeniz University Kumluca Faculty of Health Sciences, Department of Nursing, Pediatric Nursing, Antalya, Turkey
Phone: +90 506 736 13 92 E-mail: evginderya@gmail.com ORCID: orcid.org/0000-0001-6460-3124
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treatment develops among just 5-6% of these infants (5,6).

Newborn infants undergo many painful and stressful practices during hospitalization in intensive care units (heel prick, arterial catheter application, venous intervention, newborn examination, lumbar puncture, dressing change, intermuscular (IM) injection, gavage tube insertion, postural drainage, stitch removal, circumcision etc.) (7-9). Such painful interventions faced by newborns and being in this environment cause stress and this can negatively affect the sensitivity, experiences, behaviors, and clinics of these infants (9,10). Newborn infants can experience physiological, metabolic, and behavioral problems as a result of suffering from such pain. These problems include excessive protein consumption, fluid-electrolyte imbalance, sepsis due to a weakened immune system, metabolic acidosis, pulmonary-cardiac failure, and death (1). Reducing or stopping pain and stress plays an important role in increasing the quality of life, decreasing any side effects that might occur, and shortening the duration of the hospital stay (11).

Although the absence of any verbal response among these newborns makes it difficult to identify pain when it is being assessed, the presence of behavioral responses for the expression of pain makes it easier for clinicians to understand when the newborn is experiencing pain (12). In addition, the degree of pain and response to treatment in the newborn can be determined by observing physiological, hormonal, and metabolic changes (9). The purpose of pain management of newborn infants is to reduce the pain felt by newborns during painful interventions such as vaccination and venipuncture. Various methods are used by nurses to reduce pain, including family centered care, and individualized developmental care, as well as pharmacological and non-pharmacological methods (13).

When conducting pain-reduction methods in infants using a pain scale in interventional applications for newborns, a care plan must first be created by each unit using non-pharmacological and pharmacological methods and the necessary applications for reducing pain should be applied to each newborn (14). It has been discovered that non-pharmacological methods, such as changing the infant's lying position (if there is no reflux or surgical contraindication), a noiseless environment, massage, giving breast milk, giving a pacifier/dummy, listening to classical music, kangaroo care, cuddling, swaying, giving sucrose, or speaking in a calm tone of voice to the infants are effective in reducing pain (15-21). In those studies conducted by nurses, it was determined that non-pharmacological methods were effective in reducing pain and stress among newborns (14-16,19-23). In addition, non-pharmacological methods are advantageous because they are easier to apply, inexpensive, and entail no side effects (1,11,24,25).

Neonatal nurses who spend the most time with newborns, and who take care of these newborns, should be able to identify and take steps to reduce the stress and pain of the infant, minimize stimuli, and independently apply appropriate non-pharmacological methods to each infant. Reducing and eliminating pain is one of the goals of nursing care. Nurses have an important role in relieving pain by making infants comfortable (9,20,23). It is important that nurses have sufficient knowledge, skills, and experience of pain among infants and children (20,23).

This study was conducted via as a randomized controlled trial in order to determine the effect of non-pharmacological methods (giving a pacifier/dummy, breast milk smell, prone position) used during venipuncture on the reduction of neonatal stress and pain among term neonates who were being monitored after being diagnosed with hyperbilirubinemia.

Materials and Methods

Design

This study was conducted as a randomized controlled trial.

Participants

The study population comprised 264 term neonates who had been diagnosed with hyperbilirubinemia and who were hospitalized at the NICU of a university education and research hospital. In consideration of previous studies in the literature, it was planned that the number of term neonates that should comprise the study sample was 20 infants per group, with a total number of participants in the sample being determined as 80. Power analysis was then performed on the sample number, and the power was found to be 0.99 with α=0.05. Within the study sample, the hospitalized term neonates were randomly distributed to the different groups by writing the initials of their mother's name and surname on a piece of paper and drawing lots. An equal number of term neonates were selected for each group.

Inclusion criteria: Term neonates with a birth weight over 1,800 grams, a gestational age of 37 weeks or longer, less than 28 postnatal days, who could tolerate nutrients given in an external way, who had been admitted to the NICU with a diagnosis of hyperbilirubinemia, and who had

not received medical treatment other than the appropriate vitamin supplements were included in this study.

Instruments

Mother-Infant Assessment Form (MIAF)

This form, prepared by the researcher using the literature, consists of 28 questions containing descriptive information about the term neonates and their parents. The answers to these questions were collected from each patient's file, by using the nurse observation form, and by conducting face-to-face interviews with the patient's parents.

Interference Follow-up Form (IFF)

This form was prepared by the researchers after reviewing the literature and was completed using the patient information file, the nurse observation form, and this study's data collection tools.

Neonatal Stress Assessment Form (NSAF)

The Neonatal Stress Scale was developed by Ceylan and Bolışık (26) with the consideration that it would help the assessment of stress among those infants hospitalized at an NICU, and in consideration of the care plans nurses might make regarding stress in infants. The form items can be subdivided into eight subgroups. These subgroups comprise a total of 24 items to which three different responses are given according to a Likert-type including facial expression, body color, respiration, activity level, consolability, muscle tone, extremities, and posture. In regard to scoring, each subgroup is rated between 0-2 points; a minimum of 0 points and a maximum of 16 points are obtainable from all the scale responses. The assessment of the scale is carried out through observation. The fact that the infant exhibits only one of the behaviors in each field involved in the scale is sufficient for scoring. Higher scores indicate higher stress levels (16).

Premature Infant Pain Profile-Revised (PIPP-R)

The infants' pain levels were assessed based on the total score obtained from the PIPP-R. The highest possible score obtainable from the scale is 21 for preterm infants and 18 for term infants. As stated by Stevens et al. (27), if the overall PIPP-R score is 0-6 points, the pain level is determined as mild; if it is 7-12 points, the pain level is moderate; and if it is 13-21 points, the pain level is severe. The validity and reliability study of the Turkish version of the PIPP-R was conducted by Taplak and Bayat (28). An important revision was made to the scale so that it could also be applied to term neonates (28).

Pre-application

Pre-application of the data collection tools was performed with term neonates having a gestational age of 37-40 weeks, having been assigned to one of the four following groups: breast milk smell group, prone position group, false pacifier/dummy group, or the control group in the NICU. These neonates were not included the research sample.

Application

The data used in the present study were collected within a period of 7 months between 18.10.2018 and 10.05.2019. The study data and infants who met the criteria for inclusion in this study were selected, and the MIAF was filled out. The information in this form was obtained through face-to-face interviews, the nurse observation form, and patients' files. After the parents of the term neonates had given their written informed consent, a nurse working at the clinic assigned each term neonate into the aforementioned four groups using a simple randomization method.

At least half an hour before the procedure, all the term neonates' diapers were changed so that they were clean. All the term neonates were fed. In order to prevent heat loss in the term neonates and to ensure all the term neonates were subject to equal conditions, the procedure was carried out under a radiant heat source and without clothes.

On the day of the application, no painful procedure was applied to the term neonates at least half an hour before and half an hour after the blood-collection procedure. Five minutes before the procedure, the physical findings relating to the term neonates whose blood was to be collected were measured using the same instruments, and their vital signs [Heart rate (HR), oxygen saturation (SpO_2), body temperature, and respiratory rate] were measured using a pulse oximeter.

Venous venipuncture procedure was conducted on those term neonates who had been hospitalized with a diagnosis of hyperbilirubinemia; this procedure was conducted every morning, regardless of the present study.

- The term neonates involved in the breast milk group were made to smell 2 mL of breast milk dropped on a sterile sponge during the procedure.
- The term neonates involved in the prone position group were moved to the prone position during the procedure.
- The term neonates involved in the pacifier/dummy group were given a false pacifier/dummy made from sterile gloves.

• The term neonates in the control group were not subject to any application, and were subjected to the routine venipuncture procedure.

During venipuncture, attention was paid to ensure that the needle thickness (green: 0.80x38 mm) used was the same for all term neonates. Blood was collected from the right hand of all the term neonates. Before, during, and after the procedure, the blood-collection process was video-recorded so that it could be assessed using the PIPP-R scale and the neonatal stress assessment scale. During this process, the camera view covered the entire body. Pain and stress assessments of the term neonates were performed 5 minutes before, during, and 5 minutes after the procedure. Physical and vital signs were measured again 5 minutes after the venipuncture procedure.

Analysis of Video Camera Recordings of the Blood-collection Process

The process of venipuncture from the term neonates was recorded with a video camera. These images were then deciphered by three blind observers, and the pain and stress assessments of the infants at the time of the procedure were made. The video camera images of the term neonates were assessed by three specialists (two academic doctors in the field of Pediatric Nursing and a neonatology specialist) according to the PIPP-R scale and NSAF.

Ethical rules were followed during this research process. For this research, approval was obtained from the Interventional Clinical Research Ethics Committee of Nevşehir Hacı Bektaş Veli University, and written permission was obtained by the Provincial Health Directorate. The parents of the term neonates who were planned for inclusion in this study were verbally informed about the purpose of the study and the methods that were to be applied within the scope of this research. Written consent was obtained from the parents of all those term neonates who participated in this study.

Data Analysis

Data obtained from the present study were assessed using IBM SPSS Statistics 22 (Statistical Package for the Social Sciences for Windows) and Med Calc statistical package programs. The post-study power analysis of the PIPP-R scale used in this study was α=0.05 and its power was 0.99. While assessing those data collected in accordance with the purpose of this research, variance homogeneity was tested by the Levene test; One-Way analysis of variance (ANOVA) was used to compare the means of quantitative variables, for comparisons among more than two groups, Tukey's test for multiple comparisons; and chi-square test was used for the analyses of the relationships between categorical variables. The differences of HR, SpO_2, respiratory rate, body temperature, stress scale, and pain scale variables between the study groups (the breast milk smell, prone position, pacifier/dummy, and control groups) and the measurement times (before, during, and after the procedure) were tested by two-way analysis of variance (Figure 1).

Results

The research findings were then interpreted and the statistical results were calculated. It was determined that the groups were homogeneous in terms of the descriptive characteristics of the families of those infants included in this study. No statistically significant relationship was found between the variables of the infants in the breast milk smell, pacifier/dummy, prone position, and control groups (Table I).

When the HR and SpO_2 of the term infants included in this study were compared before, during and after the procedure, it was found that average heart rate increased the most among members of the control group during the procedure. While the average heart rate after the procedure decreased in all groups, compared with pre-procedure average heart rate, the heart rate increased in the control group.

When comparing the oxygen saturation measurements during the procedure, a statistically significant difference was found between the pacifier/dummy group and the control group ($p<0.05$), and the highest average saturation was found for the pacifier/dummy group (95.15±4.04), while the lowest average saturation was found for the control group (85.70±9.96). On comparison of the oxygen saturation measurement after the procedure, a statistically significant difference was found between the control group (96.85±1.18) and the breast milk smell (98.10±0.97), pacifier/dummy (98.00±0.92) and prone position (98.05±0.83) groups ($p<0.05$) (Table II).

Upon comparison of the average respiratory rate and body temperature of the term infants involved in this study, a statistically significant difference was found between the average respiratory rates of the neonates who were given pacifiers/dummies ($p=0.018$) and who were in the prone position, and those in the control group, according to the multiple comparison test, depending on the groups ($p=0.006$). The average respiratory rate of those neonates who were given a pacifier/dummy or who were moved into

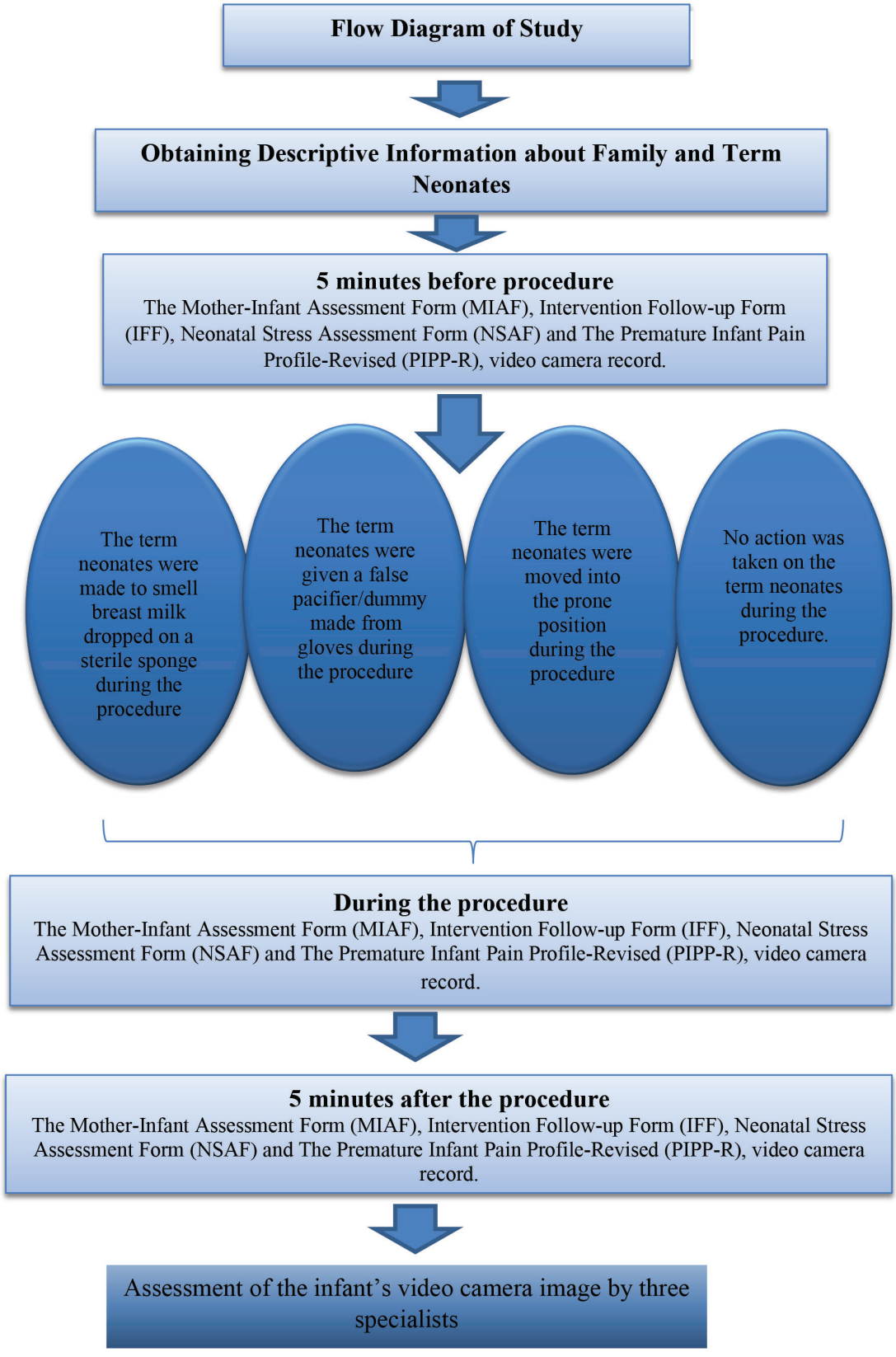

Figure 1. CONSORT flow diagram

the prone position was lower than that of the control group (Table III).

It was seen that the average pain scores of those term neonates to whom the methods of breast milk smell, prone position, and pacifier/dummy were applied were less than that of the term neonates in the control group during and after the procedure ($p<0.001$). A statistically significant difference was found between the average stress scores of the control group and the breast milk smell ($p=0.018$), pacifier/dummy ($p=0.001$), and prone position ($p<0.001$) groups during the procedure (Table IV).

Discussion

Newborn infants who are referred to the NICU in order to receive the necessary medical care and treatment due to health problems during the first days following their birth undergo many painful and stressful practices (7,10). The developmental stage is crucial for non-pharmacologic treatment in children (29). Pediatric nurses should use effective methods to reduce the negative effects of pain in children (13). The level of pain and stress experienced by infants should be determined by using valid and reliable scales, and necessary procedures should be performed in order to reduce pain and stress (25). Nurses play the most important role in regard to these applications.

In this study, the effects of the prone position, pacifier/dummy, and breast milk smell on the stress and pain experienced among term neonates were investigated. In the present study, the mean heart rate scores of the term infants in all groups (breast milk smell, pacifier/dummy, prone position, and control groups) that were determined during the procedure were higher than those determined

Table I. Descriptive characteristics of infants

Descriptive characteristics	Breast milk group (n=20)		Pacifier/dummy group (n=20)		Prone position group (n=20)		Control group (n=20)		χ^2	p-value
	n	%	n	%	n	%	n	%		
Gender										
Female Male	7 13	35.0 65.0	10 10	50.0 50.0	11 9	55.0 45.0	11 9	55.0 45.0	2.151	0.542
Height at birth										
Below 46 cm Above 46 cm	0 20	0 100.0	2 18	10.0 90.0	3 17	15.0 85.0	0 20	0 100.0	5.760	0.124
Height at the time of blood collection										
Below 46 cm Above 46 cm	0 20	0 100.0	2 18	10.0 90.0	3 17	15.0 85.0	0 20	0 100.0	5.760	0.124
Head circumference at birth										
Below 33 cm 33-37 37 cm or above	1 18 1	5.0 90.0 5.0	2 18 0	10.0 90.0 0	0 20 0	0 100.0 0	0 20 0	0 100.0 0	6.877	0.332
Head circumference at the time of blood collection										
Below 33 cm 33-37 37 cm or above	1 18 1	5.0 90.0 5.0	2 18 0	10.0 90.0 0	0 20 0	0 100.0 0	0 20 0	0 100.0 0	6.877	0.332
Nutritional status										
Breast milk Formula TPN	20 0 0	100.0 0 0	17 3 0	85.0 15.0 0	19 1 0	95.0 5.0 0	20 0 0	100.0 0 0	6.316	0.097
Length of hospital stay										
1 day 2 days More than 2 days	14 6 0	70.0 30.0 0	16 2 2	80.0 10.0 10.0	17 1 2	85.0 5.0 10.0	17 3 0	85.0 15.0 0	11.708	0.230

χ^2: Chi square test

before and after the procedure ($p<0.05$). Furthermore, while the heart rate means of the term infants in the breast milk smell, pacifier/dummy, and prone position groups that were determined after the procedure decreased, they were found to increase in the control group. During the procedure, the greatest increase in mean heart rate was

Table II. HR and SpO_2 values of term infants participating in the study before, during, and after the procedure

Groups	Time	HR		SPO_2	
		X ± SD	Med. (Min.-Max.)	X ± SD	Med. (Min.-Max.)
Breast milk smell	Before the procedure	137.45±14.44	136 (113-169)	96.90±1.48	97 (94-99)
	During the procedure	175.05±25.18	170 (132-224)	88.70±8.65	92 (74-99)
	After the procedure	130.15±24.38	124 (110-228)	98.10±0.97	98 (96-99)
Pacifier/ dummy	Before the procedure	147.30±15.61	150 (120-169)	97.05±1.23	97 (94-99)
	During the procedure	163.30±21.61	159 (135-208)	95.15±4.04	96 (85-99)
	After the procedure	124.85±9.77	120 (113-144)	98.00±0.92	98 (96-99)
Prone position	Before the procedure	141.95±17.98	139.50 (116-168)	96.70±1.75	97 (92-99)
	During the procedure	161.70±20.23	162.50 (119-190)	91.15±8.16	94.50 (72-99)
	After the procedure	123.25±11.58	120 (110-148)	98.05±0.83	98 (96-99)
Control group	Before the procedure	130.45±13.45	126.5 (112-156)	97.80±0.77	98 (96-99)
	During the procedure	178.70±21.90	174 (146-224)	85.70±9.96	88 (69-99)
	After the procedure	138.60±14.25	133 (120-174)	96.85±1.18	97 (94-99)
Test	**Time** **Time + group** **Group**	F 129.006 5.196 1.236	p-value **<0.001** **<0.001** 0.302	F 64.082 4.935 4.939	p-value **<0.001** **<0.001** 0.003

HR: Hazard ratio, SD: Standard deviation, Min.: Minimum, Max.: Maximum, Med.: Median

Table III. Respiratory rate and body temperature of the term infants participating in the study before, during and after the procedure

Groups	Time	Respiratory rate		Body temperature	
		X ± SD	Med. (Min.-Max.)	X ± SD	Med. (Min.-Max.)
Breast milk smell	Before the procedure	48.65±2.39	48 (44-56)	36.65±0.14	36.65 (36.40-36.90)
	During the procedure	49.60±2.48	48 (46-54)	36.64±0.14	36.70 (36.10-36.80)
	After the procedure	47.10±1.89	48 (44-52)	36.66±0.11	36.70 (36.40-36.80)
Pacifier/ dummy	Before the procedure	48.10 2.38	48 (44-52)	36.64±0.19	36.65 (36.30-36.90)
	During the procedure	48.10±3.14	48 (40-52)	36.63±0.15	36.60 (36.30-36.90)
	After the procedure	47.00±1.15	48 (44-48)	36.59±0.13	36.60 (36.30-36.80)
Prone position	Before the procedure	47.10±1.77	48 (44-50)	36.62±0.17	36.70 (36.30-36.90)
	During the procedure	49.30±3.51	48 (44-56)	36.63±0.13	36.60 (36.40-36.90)
	After the procedure	46.20±2.33	46 (44-50)	36.59±0.15	36.50 (36.30-36.90)
Control group	Before the procedure	47.20±2.46	48 (44-52)	36.62±0.15	36.60 (36.40-37.00)
	During the procedure	51.80±3.37	52 (48-58)	36.71±0.13	36.70 (36.40-37.00)
	After the procedure	49.20±2.28	49 (44-54)	36.72±0.17	36.75 (36.40-37.00)
Test	**Time** **Time + group** **Group**	F 24.118 4.731 4.802	p-value **<0.001** **<0.001** 0.004	F 0.720 1.982 1.586	p-value **0.482** **0.075** 0.200

SD: Standard deviation, Min.: Minimum, Max.: Maximum, Med.: Median

seen in the control group. Peyrovi et al. (30) determined that the HR of their study's fetal-position group during a painful procedure applied to a newborn was significantly lower than that of the control group. Likewise, in the study by Bayat et al. (16), where the researchers examined the effects of aromatherapy, music therapy, and vibration applications on the stress and behaviors of term neonates, the authors concluded that the newborns' heart rates decreased during the aromatherapy, music therapy and vibration applications, while it increased for those in the control group. Liaw et al. (31) investigated the effect of the use of pacifiers/dummies or sucrose on physiological parameters among term neonates who were subject to a hepatitis vaccination; they concluded that the increase in heart rate and respiratory rate was less in both groups compared with those of the control group. Similar to that study, the present research revealed a statistically significant difference ($p<0.05$) between the pacifier/dummy and the control group when comparing SpO_2 measurements during the procedure; the highest average SpO_2 (95.15±4.04) was obtained in the pacifier/dummy group, and the lowest SpO_2 (85.70±9.96) was obtained in the control group. When SpO_2 measurements after the procedure were compared, a statistically significant difference was found between the control group (96.85±1.18) and the breast milk smell (98.10±0.97), pacifier/dummy (98.00±0.92), and prone position (98.05±0.83) groups ($p<0.05$). Çakı's (32) study, which examined the effects of massage and music therapy on neonatal stress and behavior, found that the term neonates in their study's massage, white noise, and control groups had a decreased peak heart rate and increased SpO_2 after the study.

In the present study, the respiratory rate of the term neonates in all groups other than the pacifier/dummy group increased during the procedure when compared with the respiratory rate 5 minutes before the procedure; the greatest increase was seen in the control group. In all groups (breast milk smell, pacifier/dummy, prone position, and control groups), the respiratory rate after the procedure decreased when compared with the average respiratory rate during the procedure. It was determined that the average respiratory rate per minute of those infants in the pacifier/dummy group ($p=0.018$) and those in the prone position group ($p=0.006$) was lower than the average respiratory rate per minute of those in the control group. This study concluded that most of the non-pharmacological methods used reduced crying and respiratory rate, and regulated SpO_2 after the application (25).

In this study, interventions for pain reduction did not have a significant effect on body temperature ($p>0.05$). Similarly, in the study in which Taplak and Erdem (19) examined the effect of breast milk and sucrose on reducing

Table IV. Average stress and pain scores, of the term infants participating in the study before, during and after the procedure

Groups	Time	Stress scale		Pain scale	
		X ± SD	Med. (Min.-Max.)	X ± SD	Med. (Min.-Max.)
Breast milk smell	Before the procedure	6.85±3.50	6 (2-13)	5.85±2.74	5.50 (2-11)
	During the procedure	5.80±3.99	5 (0-12)	6.80±4.66	5.50 (1-13)
	After the procedure	1.60±1.67	1 (0-7)	3.05±1.70	3 (0-7)
Pacifier/dummy	Before the procedure	5.80±4.09	5.50 (0-12)	4.80±2.84	4.50 (1-12)
	During the procedure	4.55±3.10	5 (0-10)	5.10±2.99	5 (0-10)
	After the procedure	2.35±2.25	2 (0-7)	2.75±1.55	2 (1-8)
Prone position	Before the procedure	4.95±3.86	5 (0-13)	5.05±1.99	5 (3-9)
	During the procedure	5.75±3.63	6 (0-12)	6.05±4.35	5.50 (0-15)
	After the procedure	0.60±1.19	0 (0-5)	3.30±1.13	3 (1-6)
Control group	Before the procedure	2.65±2.81	2 (0-11)	5.20±2.57	5 (1-11)
	During the procedure	10.80±3.71	12 (1-14)	12.00±3.91	14 (1-15)
	After the procedure	6.40±4.83	7.50 (0-14)	8.55±4.11	8 (2-15)
Test	**Time** **Time + group** **Group**	F 27.816 11.004 8.421	p-value **<0.001** **<0.001** **<0.001**	F 19.633 5.657 29.185	p-value **<0.001** **<0.001** **<0.001**

SD: Standard deviation, Min.: Minimum, Max.: Maximum, Med.: Median

pain in retinopathy of prematurity (ROP) examination, it was found that there was no change in body temperature before and after the procedure among the infants in all three groups.

As stated by Stevens et al. (27), the average PIPP-R scores indicate the pain levels of newborns as follows: 0-6 points as mild, 7-12 as moderate, and 13-21 as severe. While there was no significant relationship between the pain levels of the study groups (breast milk smell, pacifier/dummy, and prone position groups) compared to the control group before the procedure ($p=0.699$), a statistically significant relationship was found between these both during ($p<0.001$) and after the procedure ($p<0.001$). Although there was no significant difference in pain intensity between all groups before the procedure, the least pain intensity during the procedure was seen among those infants in the pacifier/dummy group, followed by those in the prone position group, those in the breast milk smell group, and finally those in the control group. The lowest pain intensity after the procedure was seen among those infants in the prone position group, which was followed by the breast milk smell group, the pacifier/dummy group, and finally the control group. Rosali et al. (33) found that administering expressed breast milk to premature infants during ROP screening reduced pain both during and after the procedure. Alemdar and Tüfekci (34) found that the intervention of smelling amniotic fluid is one that can be used in order to reduce pain and stress of preterm infants during peripheral cannulation.

In the present study, the infants' average pain scores during the procedure increased in all groups. The highest increase was seen in the control group, which was found to be higher than the average pain scores of all the other study groups at all times (before, during and after the procedure). In all the study groups, the average pain score after the procedure was found to be lower than the average pain score during the procedure, with only the average pain score in the control group being higher after the procedure was applied. According to this result, it can be deduced that the pain of infants in the control group continued after the procedure. It can be seen that these non-pharmacological methods applied to infants in the study groups are effective in reducing pain. Nishitani et al. (35) examined pain in infants by having the infants smell their mother's milk, formula, and another individual's breast milk in term infants during a heel prick procedure. They found that the breast milk smell of the infant's own mother was more effective than that of another mother's milk, and that the infants felt less pain than others as a result (35). It was reported in a study carried out by Akcan and Polat (22) that using the smells of lavender, breast milk and amniotic fluid is an effective method in reducing pain during invasive procedures in term neonates. Likewise, a study conducted by Jebreili et al. (36) comparing the effect of breast milk smell and the smell of vanilla on reducing the response of premature infants to pain during and after venous venipuncture collection revealed that both smells calmed infants. In addition, it was found that breast milk smell still calmed infants at the end of sampling, and therefore it was concluded that it was more effective than the smell of vanilla.

In this study, the mean stress scores of those infants who were made to smell breast milk and given a pacifier/dummy decreased both during and after the procedure. Although the mean stress scores increased during the procedure among those infants in the prone position and control groups, the increase in the control group was higher than that of the prone position group. Among those infants in the prone position group, the mean stress score after the procedure decreased to almost zero. It was seen that the stress level of term neonates on whom the methods of smelling breast milk, being moved into the prone position, and being given a pacifier/dummy were applied was lower than that of the term neonates in the control group both during and after the procedure. Furthermore, it is stated that early kangaroo care was also effective on newborns' comfort behavior during invasive interventions, and that kangaroo care reduced pain and stress (37). In their study investigating the effect of certain positions of the newborn without any invasive procedure on the salivary cortisol level, Cândia et al. (38) propounded that the prone position significantly reduced salivary cortisol levels and respiratory rates and that, as a result, there was a link between the prone position and decreased stress among preterm neonates. Bayat et al. (16) examined the effects of aromatherapy, music therapy, and vibration applications on the stress and behaviors of newborns and concluded that these practices positively affect the behavior of preterm newborns by reducing their stress.

According to the findings of this study, non-pharmacological methods, rather than pharmacological methods, should be used in order to reduce pain and stress in infants. In pain management, non-pharmacological methods can be effective in simple invasive procedures because they are practical, inexpensive, simple and time saving without involving complications (15,39,40). When used together with pharmacological methods, they also increase the effect of these pharmacological methods (25).

In addition, the involvement of the infant's family when using non-pharmacological methods as part of this care strengthens the bond between the infant and their family (41,42).

Conclusion

It can be seen that non-pharmacological methods applied in the response groups are effective in reducing pain. Furthermore, the stress levels of term neonates on whom the methods of smelling breast milk, being moved into the prone position, and being given a pacifier/dummy were applied were lower than those of the term neonates in the control group during and after the procedure. Additionally, no statistically significant difference was found between these methods in terms of reducing the pain and stress of infants during the venipuncture process ($p>0.05$).

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the Nevşehir Hacı Bektaş Veli University (date and number: 23.09.2018 and 20814).

Informed Consent: Informed written consent was obtained from the mothers participating in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: D.E., Design: D.E., Supervision: D.E., N.G.B., Resources: F.Ö., Data Collection and/or Processing: F.Ö., Analysis and/or Interpretation: F.Ö., D.E., Literature Search: F.Ö., D.E., Writing: D.E., Critical Review: D.E., N.G.B.

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Original Article

Distinguishing Kawasaki Disease from Other Febrile Illnesses in Infants

Rumeysa Yalçınkaya[1], Fatma Nur Öz[1], Türkan Aydın Teke[1], Ayşe Kaman[1], Sevgi Yaşar Durmuş[1], Utku Arman Örün[2], Gönül Tanır[1]

[1]University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynecology, Child Health and Diseases Training and Research Hospital, Clinic of Pediatric Infectious Diseases, Ankara, Turkey
[2]University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynecology, Child Health and Diseases Training and Research Hospital, Clinic of Pediatric Cardiology, Ankara, Turkey

ABSTRACT

Aim: Kawasaki disease (KD) is difficult to diagnose in infants, since the disease course is subtle. We aimed to identify whether infants with KD demonstrate significant alterations in laboratory parameters that can be used to distinguish them from other febrile infants.

Materials and Methods: In this retrospective case-control study, infants diagnosed with KD between January 2010 and December 2019 were evaluated and compared to a cohort of febrile infants admitted with prolonged fever during the same period. Demographic, clinical, and laboratory features were recorded and compared between these two groups.

Results: A total of 42 infants (27 male) with KD (32 incomplete KD) and 84 age-matched febrile infants (57 male) were evaluated. Coronary artery involvement was identified in 20 (47.6%) infants of whom 5 (25%) had coronary aneurysms. All infants with KD were treated with IVIG and high dose acetylsalicylic acid, and 38 (90.5%) responded to treatment. The duration of fever and hospitalization were longer in infants with KD compared to the controls ($p<0.001$). White blood cell (WBC), eosinophil, platelet counts, platelet distribution width, acute phase reactants, alanine aminotransferase, and gamma glutamyl transferase were significantly higher; whereas, mean platelet volume (MPV), hemoglobin, and albumin levels were lower in the KD group compared to the controls. Lower MPV and albumin values were found to be independently associated with a higher likelihood of having a KD diagnosis.

Conclusion: It may be difficult to diagnose KD in infants. Our data shows that MPV and albumin may be used as supportive parameters to differentiate KD from other febrile conditions in infants.

Keywords: Kawasaki disease, infant, mean platelet volume, albumin

Introduction

Kawasaki disease (KD) is an acute febrile childhood illness with unknown etiology characterized by systemic inflammation of predominantly the medium arteries. Approximately 80% of patients are younger than five years of age. Classical KD diagnosis is based on clinical criteria in the presence of at least five days of fever. Some infants might only present with an unexplained fever in the absence of other manifestations, which leads to diagnostic difficulties and delays in treatment (1). It is challenging to distinguish KD from other febrile conditions in the infant age group since the incomplete

Address for Correspondence
Rumeysa Yalçınkaya, University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynecology, Child Health and Diseases Training and Research Hospital, Clinic of Pediatric Infectious Diseases, Ankara, Turkey
Phone: +90 312 317 03 53 E-mail: rumeysa_ra@hotmail.com ORCID: orcid.org/0000-0002-0083-0587
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form of the disease [incomplete KD (IKD)] is common, and findings are exceedingly similar to those of other febrile diseases. It is well established that the 'typical' clinical criteria are mostly absent or infrequent in infants with KD (2).

On the other hand, KD is one of the important causes of acquired heart disease in childhood, leading to cardiac and/or coronary abnormalities. It is known that infants with KD more frequently have coronary and cardiac complications, highlighting the importance of prompt diagnosis and treatment (3). Therefore, a well-defined laboratory parameter (or a set of parameters) could help clinicians identify suspected cases of KD in the infant age group, even when typical criteria are not present.

This study aimed to investigate the clinical and laboratory features of infants with KD and to identify whether infants with KD demonstrate significant alterations in laboratory parameters that can be used to distinguish them from other febrile infants.

Materials and Methods

Patients and Clinical Data

In this retrospective case-control study, we reviewed the data of infants diagnosed with KD who were younger than 12 months at the time of disease onset between January 2010 and December 2019.

Ethics Committee approval was obtained from University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynecology, Child Health and Diseases Training and Research Hospital (date: 08.07.2019, approval no: 2019/7).

The control cohort comprised 84 age-matched febrile infants. The cohort was formed by evaluating the medical records of infants admitted with prolonged fever without a known source during the same period. Febrile infants diagnosed with any specific infection, such as tonsillitis, urinary tract infection, pneumonia, etc., or infants with any specific infectious etiology were excluded.

The following data were recorded: (i) Epidemiological and clinical features (gender, age, symptoms, number of positive main diagnostic criteria at admission, length of hospitalization, findings on physical examination, treatment modalities, echocardiography results), (ii) Complete blood count parameters, (iii) Biochemistry results, including serum sodium levels and acute phase reactants [C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)], and (iv) liver function test panel [alanine aminotransferase (ALT), aspartate amino transaminase (AST), albumin, gamma-glutamyl transferase (GGT)]. Comparisons were performed between these two groups (infants with KD vs febrile controls).

Definitions and Echocardiographic Analysis

According to American Heart Association guidelines, the patients in this study were classified as either complete KD (cKD) or IKD (2).

Echocardiogram results were classified as Z scores: (i) no involvement: Always <2; (ii) Dilation only: 2 to <2.5; or if initially <2, an increase of ≥1 in *Z* score during follow-up; (iii) Small aneurysm: ≥2.5 to <5; (iv) medium-sized aneurysm: ≥5 to <10 with an absolute dimension of <8 mm; (v) Large or giant aneurysm: ≥10 or absolute dimension of ≥8 mm (2).

Cardiac involvement was defined as the presence of cardiac abnormalities such as coronary artery anomalies, valvular regurgitation, or pericardial effusion.

Treatment

All infants with KD were treated with intravenous immunoglobulin (IVIG, 2 gr/kg) as a single infusion over 12 hours as soon as possible after diagnosis was confirmed. Resistance to IVIG treatment was defined when persistent or recrudescent fever occurred 36 hours after the first IVIG infusion. Acetylsalicylic acid (ASA) was commenced with a dose of 60 mg/kg/day. Once the fever was absent for 48-72 hours, patients were switched to a low dose of aspirin, 3-5 mg/kg per day, for its antiplatelet effect. The low-dose ASA therapy was terminated 6-8 weeks after diagnosis if the infant had no coronary artery involvement. If coronary artery involvement persisted, ASA was continued as long as the involvement continued. Infants with a positive polymerase chain reaction or rapid antigen test for influenza virus received dipyridamole (2-6 mg/kg/day) instead of ASA.

Follow-up

The routine echocardiogram follow-up of infants with KD was conducted according to the following schedule: at diagnosis, two weeks after disease onset, 4-6 weeks after disease onset, and in the 3rd and 6th months. After the first year, examinations were scheduled yearly.

Statistical Analysis

All data obtained in this study were transferred to SPSS (version 25.0) software (SPSS Inc., Chicago, IL, USA). Descriptive results of all variables were determined. Categorical variables are given as frequency (n) and percentage (%). Continuous variables are presented as mean ± SD or median (min-max) depending on their conformity

with normal distribution -which was checked with the Shapiro-Wilk test. Categorical variable analyses were conducted via chi-square tests. Receiver operating curve (ROC) analysis was used to analyze the capability of various parameters in identifying KD according to area under curve (AUC) values. The Youden J Index was calculated for the determination of cut-off values. Multivariable regression with the backward conditional method was performed to determine factors that were independently related to the diagnosis of KD. Only variables that demonstrated significant difference in univariate analyses were included in the model. P-values less than 0.05 were considered to demonstrate statistical significance.

Results

We included a total of 42 infants [27 (65%) male] with KD and 84 age-matched febrile infants [57 (67.8%) male] as a control group. The median age was nine months (min-max: 1-12 months) in both groups. In the KD group, four (9%) infants were under the age of three months, eight (19%) were between 3-6 months old, and 30 (72%) were older than six months of age. The youngest KD patient was a 1-month-old girl.

Thirty-two (76%) infants were diagnosed with IKD, while 10 (24%) were diagnosed with cKD. Coronary artery involvement was identified in 20 (47.6%) infants, 5 (25%) had coronary aneurysms. All of the infants were treated with IVIG and high dose ASA; 38/42 were responsive to treatment. Four (9%) infants received more than one IVIG infusion, and two received steroid therapy due to IVIG resistance. Five infants (12%) were administered enoxaparin therapy because of coronary aneurysms. The demographic and clinical features of those infants with KD are shown in Table I.

Except for one infant, all of the infants with coronary artery involvement attended follow-up examinations (n=19). Among these infants, five suffered from aneurysms; however, during follow-up, even though coronary artery involvement persisted, the dimensions of the aneurysms reduced to within normal dilatation limits. The longitudinal evaluation showed that 14 infants who had coronary dilatation at the initial assessment demonstrated a return to normal. The time elapsed until the regression of coronary dilatation ranged from 1 month (minimum) to 19 months (maximum) after diagnosis.

A comparison of the KD group to the control group showed that the duration of fever and hospitalization was longer in infants with KD ($p<0.001$). Significantly higher levels of white blood cell (WBC), eosinophil, platelet counts, platelet distribution width, ESR, CRP, ALT, and GGT were identified in infants with KD. Additionally, significantly lower MPV levels, hemoglobin, and albumin were detected in infants with KD in comparison to the febrile controls. Also, echocardiography was performed in 65 of the 82 (79.2%) patients in the control group, and the results were normal. The comparison of demographic features and laboratory findings between infants with KD and the febrile controls is shown in Table II.

Multivariable logistic regression was performed with KD diagnosis as the dependent variable. The parameters found to be statistically significant in univariate analysis

Table I. Demographic, clinical, laboratory data, and echocardiographic findings of infants with KD

Infants with KD (n=42)	
Male : female ratio	1.8 : 1
Full diagnostic criteria, n (%)	10 (23.8%)
Incomplete KD, n (%)	32 (76.1%)
Duration of hospitalization, days, median (min-max)	7 (2-22)
Major criteria, n (%)	
Rash	26 (61.9%)
Conjunctivitis	25 (59.5%)
Lymphadenopathy	15 (35.7%)
Extremity changes	10 (23.8%)
Mucosal changes	25 (59.5%)
Other clinical findings, n (%)	
Irritability	30 (71.4%)
Diarrhea	15 (35.7%)
Vomiting	17 (40.4%)
BCG induration	8 (19%)
Arthritis	1 (0.2%)
Aseptic meningitis	10 (23.8%)
Gallbladder hydrops	5 (11.9%)
Sterile pyuria	7 (16.6%)
Echocardiography findings, n (%)	
Coronary artery involvement	20 (47.6%)
Coronary artery aneurysm	5 (11.9%)
Giant coronary artery aneurysm	0
Ascendant aortic dilatation	1 (0.2%)
Mitral regurgitation	3 (0.7%)
IVIG resistance, n (%)	4 (9.5%)

BCG: Bacillus Calmette-Guarin, IVIG: Intravenous immunoglobulin, KD: Kawasaki disease, min: Minimum, max: Maximum

were included in the model. Albumin and MPV values were categorized with regards to the cut-off values obtained via ROC analysis. Lower MPV and albumin values (based on the cut-off values) were independently associated with a higher likelihood of having KD diagnosis. Following this, we used ROC curve data to assess the diagnostic abilities of albumin and MPV to distinguish KD patients from the febrile controls (Figure 1). AUC values, cut-off points determined by the Youden J Index, and sensitivity, specificity, accuracy, and positive and negative predictive values for the parameters are given in Table III.

Discussion

It is well known that infant KD presents a diagnostic challenge due to its subtle signs and symptoms, often resulting in a diagnosis of IKD. However, in this age group, the cardiac and coronary complications of the disease are also more common, which makes accurate diagnosis crucial (4). The literature shows that infants with KD may present with only two symptoms, namely, irritability and prolonged fever (5). Distinctive laboratory or clinical features that can differentiate KD from other febrile illnesses have not yet been identified in this age group. Therefore, we aimed to evaluate the role of laboratory parameters in distinguishing KD from other febrile cases.

In our study, 76% of patients had IKD. Although there are different frequencies in previous reports, such as 56.6% (6), 68% (7), 88% (8), and 71% (9), it seems that our results are in agreement with the majority of studies showing that IKD is more common among infants. Infants younger than three months of age often present with the incomplete form of the disease (10). This situation is thought to be associated with the immaturity of the immune system in infants. Researchers have argued that the neutralization of superantigens by maternal antibodies transferred through the placenta may be related to the

Table II. Comparison of clinical and laboratory features of infants with KD and febrile controls

	Infants with KD (n=42)	**Febrile controls (n=84)**	**p-value**
Age, months	9 (1-12)	9 (2-12)	0.565
Male : female ratio	1.8 : 1	2 : 1	0.688
Duration of fever (days)	7 (1-20)	5 (2-10)	**<0.001**
Duration of hospitalization (days)	7 (2-22)	5 (3-10)	**<0.001**
Laboratory parameters			
WBC (x10^3/mm^3)	15.8 (3.9-36.2)	13.8 (4.7-28.3)	**0.023**
Neutrophil (x10^3/mm^3)	8.2 (1.4-20.5)	7.1 (1.3-17.1)	0.077
Lymphocyte (x10^3/mm^3)	5.1 (1.8-12.4)	1.05 (4.1-11.1)	0.084
Monocyte (x10^3/mm^3)	1.3 (0.2-3.5)	1.1 (0.1-3.07)	0.688
Eosinophil (x10^3/mm^3)	0.2 (0.03-2.02)	0.1 (0-6.3)	**<0.001**
Platelet count (x10^3/mm^3)	527 (72-1394)	356 (155-672)	**<0.001**
MPV (fL)	7.8 (6.6-10.7)	8.7 (6.6-11.5)	**<0.001**
Hemoglobin (g/dL)	10.2 (7.5-12)	11 (8.8-15.5)	**<0.001**
PDW (%)	39.2 (11.5-60.7)	16.6 (8.2-66)	**0.005**
CRP (mg/L)	88.1 (3.4-289)	36.5 (3-179)	**0.002**
ESR (mm/h)	72.5 (1.3-130)	47.5 (7-108)	**0.002**
Aspartate aminotransferase (units/L)	36 (16-170)	39 (18-140)	0.625
Alanine aminotransferase (units/L)	30.5 (10-184)	17 (7-120)	**0.001**
Gamma-glutamyl transferase (units/L)	45.5 (10-233)	23.5 (8-100)	**0.002**
Albumin (g/dL)	3.3 (2-4.8)	3.8 (3.3-4.6)	**<0.001**
Sodium (mEq/L)	136 (128-139)	135 (131-143)	0.851

*All values are given as median (min-max)
CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, KD: Kawasaki Disease, MPV: Mean platelet volume, PDW: Platelet distribution width ratio, WBC: White blood cell, min: Minimum, max: Maximum

higher likelihood of IKD among infants when compared to older children (11).

The possibility of KD should be considered when an infant has prolonged fever, even without other signs (5). In our study, four patients (9%) were admitted with fever and did not initially present with any other criteria. Published case reports also show that it is not uncommon to have an infant present with only fever but subsequently receive a diagnosis of KD after the detection of coronary artery anomalies or aneurysms (1). Classically, it is suggested that fever duration must be at least five days to suspect KD; however, in a recently published revision of KD criteria, it was stated that the requirement for a specific duration of fever should not be considered as a basis for the diagnosis of KD. The Japan Nationwide Surveillance study showed that approximately 9%, 25%, and 35% of KD patients received their first IVIG treatment on the 3rd, 4th, and 5th days of illness. It was noted that the prevalence of coronary artery lesions was low (12).

As KD is a systemic inflammatory disease, laboratory findings include increased levels of acute-phase reactants, thrombocytosis, leukocytosis, and a left shift in WBC; however, these results are not specific to KD. There are currently no laboratory markers that can be used to distinguish KD from other febrile illnesses. In our study, albumin and MPV levels were identified as parameters that could help to differentiate KD from other febrile illnesses. In a study that compared 64 children with KD (of whom 20 were infants) and 16 infants who had at least 5 days of fever, the authors found that ESR, CRP, and the N-terminal prohormone of brain natriuretic peptide were significantly higher in the KD group (13). Another study compared 72 infants with KD who were younger than six months against 50 cases of adenovirus-infected infants; WBC, platelet count, CRP, and neutrophil levels were significantly higher; whereas hemoglobin and serum albumin levels were significantly lower in those infants with KD (14). Our analysis showed that albumin levels below 3.35 g/dL were associated with a KD diagnosis in infants (Odds ratio: 112.073, 95% confidence interval 13.237-948.895). In a relatively large study conducted with 309 KD patients and 160 healthy controls, it was found that those patients with KD had lower MPV than the control subjects (15). Another study supported this by comparing changes in platelet parameters between KD patients and febrile and afebrile controls; the authors found that MPV was significantly lower in those patients with KD than the febrile controls (16). Our analysis found an MPV cut-off value of <7.97, which demonstrated a sensitivity and specificity of 59.5% and 77.4%, respectively, in distinguishing KD from febrile infants. The mechanism causing lower MPV in KD has not been clarified yet; however, it is known that MPV levels are affected by inflammation which is a characteristic of KD. It is thought that parameters that increase during the acute phase of KD, such as interleukin-6, granulocyte colony-stimulating factor, and macrophage colony-stimulating factor, might lead to decreased platelet volume (17). Also, the regulation of thrombopoiesis may be defective in inflammatory diseases, and consumption of activated platelets may cause reduced MPV (18). Since MPV is a component of the routinely performed complete blood count test, its measurement will not lead to any additional costs. Therefore, it is feasible to use this almost-ubiquitous parameter in order to differentiate between KD and other febrile diseases.

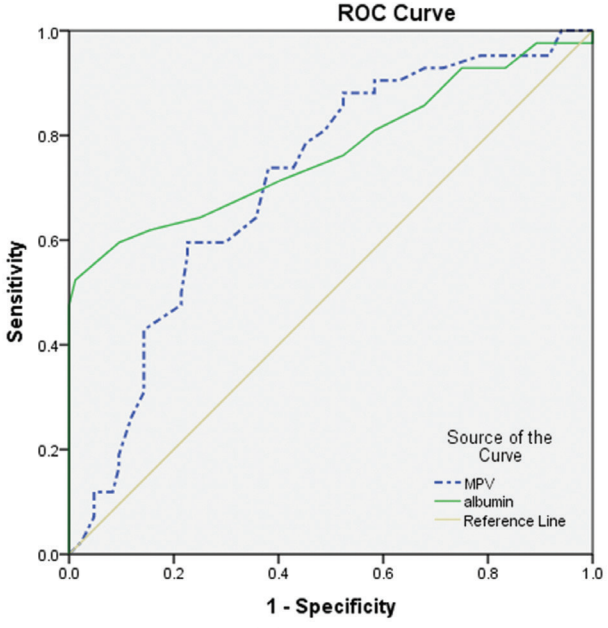

Figure 1. ROC curves for the diagnosis of Kawasaki disease with MPV and albumin

ROC: Receiver operating characteristic, MPV: Mean platelet volume

In our study, 4 of the 42 infants (9.5%) were not responsive to initial treatment with IVIG. In a study from Italy that analyzed the characteristics of 32 infants with KD, 6 (18%) infants were unresponsive to initial IVIG treatment (7). Furthermore, a study from India reported 3 of their 17 infants (17%) were resistant to initial IVIG and required additional treatment. In support of these findings, a previous study observed that younger children had a relatively higher frequency (19%) of requiring a second IVIG dose than older children (14%), even though the difference between groups was not significant (19). Additionally, it has been noted

that infants with KD might be more resistant to treatment overall (20). However, the frequency of resistance to IVIG was much lower in our group of patients, possibly indicating a difference caused by genetic factors.

The PubMed database was searched using terms such as "infant Kawasaki disease," "Kawasaki disease aged below 1 year" and "Kawasaki disease age". Only studies in the English language and only ones published after January 2000 were included. Eight publications were found in which the characteristics of infants with KD who were younger than 12 months old were compared to those in other age groups. The data from these published studies regarding infants with KD (3,4,7,13,21-24) are summarized in Table IV. Although various case series and studies focusing on infants with KD have been published, our study is among the few that evaluated whether a distinctive laboratory marker can be used to distinguish KD from other febrile conditions in the infant age group.

Study Limitations

One of the limitations of our study is the limited number of infants included. All evaluated data were drawn from a single center, which may be a limitation even though this center is a tertiary referral center that receives patients from all regions throughout Turkey. The retrospective design is another limitation. Additionally, low albumin is a known diagnostic parameter of IKD, and any results should be interpreted with this in mind.

Conclusion

It may be difficult to diagnose KD in infants since the disease course is subtle and difficult to differentiate from other febrile diseases. Our data shows that MPV and albumin may be used as supportive parameters to differentiate KD from other febrile conditions in the infant age group. However, more prospective studies are needed to identify specific clinical features, laboratory parameters, and specific criteria in the infant age group.

Table III. Parameters associated with the diagnosis of KD with multivariable logistic regression analysis and diagnostic accuracy analysis of parameters

	OR (95% CI)	p-value	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
Albumin <3.35 g/dL	112.073 (13.237-948.895)	**<0.001**	0.770	52.4%	98.8%	83.3%	95.7%	80.6%
MPV <7.97 fL	0.153 (0.054-0.432)	**<0.001**	0.713	59.5%	77.4%	71.4%	56.8%	79.3%

AUC: Area under curve, CI: Confidence interval, MPV: Mean platelet volume, NPV: Negative predictive value, OR: Odds ratio, PPV: Positive predictive value

Table IV. A short literature review of studies including infant KD cases aged below 12 months

Reference number	Study year, country	Number of KD cases <12 months/ total cohort	Age of infant KD group (months)	M:F ratio	Fever duration (days)	iKD n (%)	IVIG resistance n (%)	Coronary artery involvement n (%)	Coronary aneurysm n (%)	Albumin (g/dL)
4	2012, Korea	52/242	8.3§	37:15	6.7§	35 (67)	N/A	Z score: 5.83±1.39†	N/A	N/A
13	2015, Korea	20/64	5.65±2.76†	15:5	6.45±1.88†	13 (65)	4 (20)	3 (15)	N/A	4.2±0.4†
3	2018, USA	80/250	7 (1-11)*	43:37	N/A	30 (38)	15 (16)	Max Z score: 3.37±3.38†	n (48)	3.11±0.57†
21	2019, Korea	192/859	7 (5-9)	115:77	6 (5-7)‡	61 (31.7)	11 (6)	40 (21)	4 (2)	N/A
7	2019, Italy	32/113	5.7±2.7†	20:12	>5 days in all patients	22 (68.7)	6 (18.7)	16 (50)	N/A	19 patients had hypoalbuminemia
22	2020, China	64/213	7 (5-11)*	N/A	N/A	4 (6.1)	5 (7.8)	5 (7.69)	N/A	N/A
23	2020, Brazil	23/301	N/A	18:5	>5 days in all patients	9 (39.1)	31 (11.4)	12 (52.2)	N/A	N/A
24	2021, China	62/398	8.48±1.12†	40:22	7.4±2.4†	28 (45.2)	15 (24.2)	15 (24.2)	N/A	N/A

F: Female, IVIG: Intravenous immunoglobulin, IKD: Incomplete Kawasaki disease, KD: Kawasaki disease, M: Male, USA: United States of America
*Median (minimum-maximum), †Mean ± standard deviation, ‡Median (interquartile range), §Mean

Ethics

Ethics Committee Approval: Ethics Committee approval was obtained from University of Health Sciences Turkey, Ankara Dr. Sami Ulus Gynecology, Child Health and Diseases Training and Research Hospital (date: 08.07.2019, approval no: 2019/7).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: R.Y., F.N.Ö., T.A.T., A.K., S.Y.D., U.A.Ö., G.T., Design: R.Y., F.N.Ö., T.A.T., A.K., S.Y.D., U.A.Ö., G.T., Data Collection and/or Processing: R.Y., Analysis or Interpretation: R.Y., Literature Review: R.Y., F.N.Ö., T.A.T., A.K., S.Y.D., U.A.Ö., G.T., Edited Manuscript: F.N.Ö., G.T., Writing: R.Y.

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Original Article

Impact of the COVID-19 Pandemic on Pediatric Intensive Care Unit Admissions: A Single Centre Experience

Pınar Yazıcı Özkaya[1], Hatice Feray Arı[1], İlke Baş[2], Eşe Eda Turanlı[1], Bülent Karapınar[1]

[1]Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Intensive Care, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Pediatrics, İzmir, Turkey

ABSTRACT

Aim: During the coronavirus disease-2019 (COVID-19) pandemic, pediatric emergency department visits and pediatric intensive care unit (PICU) admissions were significantly decreased. This study aimed to evaluate the impact of prevention strategies on PICU admissions during the COVID-19 outbreak.

Materials and Methods: We included all patients admitted to a PICU from March 15th to September 15th, 2020, and those admitted in the same period in 2019. Incidence rates ratios (IRR) between the 2019 and 2020 cohorts (IRR-1) and restriction periods (March 2020-June 2020) and post-restriction periods (July 2020-September 2020) (IRR-2) were computed using Poisson modelling according to the data distribution. We analyzed the number of PICU admissions and the patient characteristics.

Results: A total of 437 patients from 465 admissions were included. In 2020, the number of PICU admissions significantly decreased by 65% compared to 2019 [0.355 (0.287-0.440)]. During the restriction period, the number of PICU admissions was significantly lower by 40.3% compared to the post-restriction period [0.597 (0.493-0.722)]. Lower respiratory tract infections (LRTIs) significantly decreased by 19% in the post-restriction period compared to the restriction period [0.811 (0.684-0.960)]. Cardiopulmonary arrests before PICU admission were significantly higher during the restriction period compared to the post-restriction period (15.5% vs 4.8%, p<0.015).

Conclusion: It was shown that there was a significant decrease in all PICU admissions, especially those due to LRTIs during the COVID-19 period. During the restriction period, cardiopulmonary arrest prior PICU admission was higher compared to the post restriction period and 2019. Our study does not reveal an increase in illnesses severity scores but the significant increase in cardiopulmonary arrest may be explained by a delay in health care access and fear of COVID-19 transmission.

Keywords: COVID-19, children, pediatric critical care, quarantine

Introduction

The coronavirus disease-2019 (COVID-19) started as an epidemic in Wuhan in 2019, and the World Health Organization announced on March 11th 2020 that the outbreak of COVID-19 had become a pandemic. After the first attack of COVID-19, most countries went under strict quarantine in March 2020. In Turkey, restrictions on mobility at the population level and regional quarantine applications were introduced. Schools and sport activities were shut down in March 16th 2020 and going out was restricted to 3 hours and then to 4 hours for children.

In addition to these restrictions, with the protective effect of social distancing and masks, a decrease in infections

Address for Correspondence
Hatice Feray Arı, Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Intensive Care, İzmir, Turkey
Phone: +90 232 390 15 63 E-mail: dr.hferayyavas@gmail.com ORCID: orcid.org/0000-0002-2208-2524
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due to other viral causes was detected in children during this period (1,2). Studies showed that trauma patients and pediatric emergency department admissions decreased, especially during the periods of curfews (3,4). During the pandemic, parents avoided emergency department admission due to the risk of COVID-19 transmission. This caused children to be admitted to the hospital with a higher risk of severe illnesses.

With this study, we aimed to evaluate the influence of the COVID-19 measures on the rates, types and severities of pediatric intensive care unit (PICU) admissions.

Materials and Methods

Patients

We performed a retrospective single center study comparing the periods March 15th - September 15th in 2019 and in 2020. We included all admission to a PICU which is a 17-bed medical and surgical unit. All of the available medical records of the patients were reviewed. Their demographic characteristics, medical history, comorbidity, discharge diagnosis, need for mechanical ventilation support, duration of positive pressure ventilation, and length of stay in the PICU were recorded. The severity of illness was measured by means of the Pediatric Risk of Mortality-3 (PRISM3) score at PICU admission. We excluded "early re-admissions" which are defined as re-admission within 48 hours of PICU discharge and those patients who were discharged within 24 hours.

Diagnoses at admission were categorized into 8 subgroups: (1) Respiratory failure including lower respiratory tract infections (LRTIs) and asthma; (2) Heart failure including congenital and acquired cardiovascular disease; (3) Encephalopathy/seizure including convulsion, meningitis/encephalitis; (4) Sepsis; (5) Trauma and intoxication; (6) Surgery including planned and unplanned surgery; (7) Metabolic/dehydration including acute gastroenteritis, renal failure, hepatic failure, diabetic ketoacidosis; and (8) Other including acute leukemia, left ventricular assist device thrombosis and others.

Ethical approval was obtained from Ege University Faculty of Medicine Clinical Research Ethics Committee for this study (ethics committee number: 21-2T/65).

Statistical Analysis

Statistical analysis was performed with SPSS statistical package for Windows 22.0. Descriptive data were reported in terms of absolute frequencies for categorical variables, and in terms of medians and interquartile ranges (IQR) for continuous variables. For the analysis of independent continuous variables, Student's t-test was used under parametric, and Mann-Whitney U test under non-parametric conditions. Chi-square analysis was used for categorical variables. Differences were considered significant at $p<0.05$. The difference in incidence rates ratios (IRR) and their 95% confidence intervals (CIs) between the 2019 and 2020 cohorts (IRR-1), and the restriction (March 15th, 2020-June 15th, 2020) and post-restriction (July 15th, 2020-September 15th, 2020) (IRR-2) periods were computed using Poisson modelling according to the data distribution. The relative variation in the number of cases between the two periods were calculated as the difference between the two periods divided by the number of cases in the 2019 for the 2020 cohort and, the number of cases in the restriction cohort for the post-restriction cohort. Statistical significance was set with a two-sided p-value<0.05.

Results

We evaluated 437 patients who had 465 PICU admissions. During the study period in 2019, the number of PICU admission was 289. In 2020, the number of PICU admissions significantly decreased by 65% compared to 2019 [IRR-1 0.355 (0.287-0.440)]. Patient characteristics, types and frequencies of PICU admissions, and estimates of IRRs are shown in Table I. During the restriction period, the number of PICU admissions was significantly lower by 40.3% compared to the post-restriction period [IRR-2 0.597 (0.493-0.722)].

The median age was 35 months in the 2019 cohort (IQR 9-106), and 43 months (IQR 10-142.7) in the 2020 cohort. There were no statistically significant differences in the age groups (<1 year, 1-6 years, >6 years) between 2019 and 2020. PRISM score, length of PICU stay and length of positive pressure ventilation were similar in 2019 and 2020. The demographic and clinical data of PICU admissions are shown in Table II. Cardiopulmonary arrest before PICU admission was significantly higher during the restriction period compared to the post-restriction period (15.5% vs 4.8%, $p<0.015$). Cardiopulmonary arrest before PICU admission did not significantly change between 2019 and 2020 (6.9%, 9.1% respectively, $p=0.396$).

In the comparison of the admission diagnosis groups, there was no statistically significant difference between 2019 and 2020 and between the restriction and post-restriction periods. LRTIs in the post-restriction period significantly decreased by 19% compared to the restriction period [0.811 (0.684-0.960)]. Intra-hospital ward, planned

and unplanned surgical admissions did not significantly change. Patients admitted for diabetic ketoacidosis in the restriction period were lower by 25% compared to the post-restriction period [0.752 (0.562-1.005)], but this did not reach statistically significant levels (p=0.054).

Discussion

Since the COVID-19 outbreak, the rates of hospital admissions decreased in the pediatric patient group. Our study revealed that the rate of PICU admissions, especially due to LRTIs, decreased significantly. After the COVID-19 epidemic, it was seen that hospitalizations to both pediatric emergency departments and PICUs decreased and the patients' epidemiological characteristics changed. Although the decrease in emergency room admissions due to trauma and viral infections can be explained by the curfews, social distancing and the interruption of sports activities, the decreases in admissions to the pediatric intensive care unit cannot be explained by these factors alone.

During the peak period of the pandemic, mandatory quarantine and strict restrictions were imposed in many countries in order to control the spread of COVID-19. In Turkey, collective measures were implemented in the middle of March 2020, namely social distancing, mandatory mask wearing and school closures. In mid-April, a national mandatory quarantine application was initiated throughout the country during weekends. In June 2020, intercity travel restrictions ended and a normalization process started. Contrary to this situation, we found that those patients admitted for LRTIs decreased by 19% during the post-restriction period. We speculate that the measures such as mandatory masks, hand hygiene, social distancing and the closure of places where children have closer contact such as shopping malls and schools are more effective in reducing the number of patients with LRTIs than strict curfews. Previous studies reported a significant reduction in PICU admissions due to LRTIs (52-83%) between 2019 and 2020 (2,5). In our study, we did not see any difference between these periods.

In studies evaluating PICU admissions, a significant decrease was reported in hospitalization rates due to bronchiolitis, asthma, and community-acquired pneumonia, but no change was see in epilepsy, diarrhea, sepsis, bacterial meningitis, diabetic ketoacidosis and surgical patient groups (6,7). In our study, when the specific diagnosis subgroups were evaluated, no difference was found in the rates of admissions due to trauma and suicide. A statistically insignificant decrease in PICU admissions due to diabetic ketoacidosis was detected during the restriction period. Although it was reported that the number of patients diagnosed with traumatic brain injury decreased in the adult patient group during the period of full quarantine, no significant difference was found in our study in the number of pediatric patients who were admitted to PICU due to trauma (3). In Italy,

Table I. Patient characteristics, type and frequency of pediatric intensive care unit, and estimates of incidence rate ratios (IRR)

Variable	IRR-1 (95% CI) (2019 vs 2020)	p-value	IRR-2 (95% CI) (restriction vs post restriction 2020)	p-value
Admissions	0.355 (0.287-0.440)	**<0.001**	0.597 (0.493-0.722)	**<0.001**
Gender (female)	1.098 (0.814-1.481)	0.542	1.032 (0.700-1.521)	0.874
Age **0-1 year** **1-6 years** **>6 years**	 1.006 (0.101-10.024) 0.899 (0.092-8.810) 1.113 (0.114-10.908)	 0.996 0.927 0.927	 0.829 (0.522-1.314) 0.740 (0.463-1.181) 1	 0.424 0.207
Type of admission **Extra-hospital** **Intra-hospital, ward**	 0.875 (0.573-1.336) 1.202 (0.871-1.657)	 0.536 0.263	 1.032 (0.571-1.865) 1.293 (0.855-1.957)	 0.916 0.223
Diagnosis at discharge **LRTIs** **Trauma** **Planned surgery** **Unplanned surgery** **Diabetic ketoacidosis** **Suicide** **Comorbidity**	 1.225 (0.879-1.707) 1.040 (0.696-1.555) 1.116 (0.734-1.697) 0.720 (0.390-1.331) 1.47610 (0.818-2.664) 0.934 (0.047-0.461) 1.184 (0.878-1.597)	 0.231 0.847 0.607 0.295 0.196 0.894 0.267	 0.811 (0.684-0.960) 0.911 (0.739-1.122) 1.835 (0.493-6.831) 2.002 (0.459-8.739) 0.752 (0.562-1.005) 1.339 (0.400-4.480) 1.252 (0.763-2.054)	 **0.015** 0.379 0.366 0.356 0.054 0.636 0.374

IRR-1; March 15th - September 15th 2019 vs March 15th - September 15th 2020, IRR-2; restriction (March 15th, 2020-June 15th, 2020) vs post-restriction (July 15th, 2020-September 15th, 2020), CI: Confidence interval, LRTIs: Lower respiratory tract infections

following the national lockdown, a statistical significant decrease of ED visits due to accidents was observed for all types of injury groups except for injury in the domestic environment (8). This can be explained by the fact that the full quarantine application was not initiated in our country. Although it was determined that elective surgical operations were postponed and the total number of surgical procedures decreased during the COVID-19 outbreak, no significant decrease was shown in the number of patients admitted to PICU for planned or unplanned surgery.

The significant decrease in admissions to pediatric health care services due to a fear of COVID-19 has raised concerns that patients will access health care at a later date and so with a higher risk of severe illness. During the peak of the COVID-19 outbreak, pediatric ED visits significantly decreased especially for minor reasons (4,9). In a previous study, it was noted that the proportion of children presenting at an ED with high acuity was 25% greater and low-acuity was 15% lower in the peak lockdown period compared to the previous year (9). In a small series of 12 cases whose parents reported avoiding accessing hospital because of a fear of infection due to SARS-CoV-2, 50% of patients were admitted to PICU and 33.3% died (10). Our findings revealed that PRISM scores, mortality rates and comorbidities did not significantly change during the COVID-19 pandemic. However, during the restriction period, cardiopulmonary arrest before PICU admission significantly increased. Detailed evaluation revealed that 6 patients presented with respiratory failure and their symptoms continued for 3-6 days and 8 patients did not have any previous hospital admissions during their illness. The mortality rate of 11 patients with a history of cardiopulmonary arrest before admission was 63.4%, and the accompanying comorbidity rate was 45.4%. These findings are similar to the data that indicates that the fear of COVID-19 contact increases mortality by delaying hospital admissions.

Study Limitations

This study has several limitations, mainly as a result of its retrospective design. Although the patient data were collected from an electronic database, a small amount of data was missing. Statistical analysis was made on the complete data. Despite the center where the study was conducted serving a large region due to its status as a reference university hospital, our findings may not reflect all PICUs in Turkey due to the single center nature of our study data.

Table II. Demographic and clinical data of the study cohort

Variable	2019 n=289	2020 n=176	p-value	March-May 2020 n=71	June-August 2020 n=105	p-value
Age, months (median, IQR)	35 (9-106)	43 (10-142.7)	0.233	27.0 (10-98)	53 (10.5-154)	0.122
Gender (Female/Male)	113/176	75/101	0.454	30/41	45/60	0.937
PRISM	7 (3-11)	9.0 (2-16)	0.129	10.5 (2-18)	8 (1-16)	0.360
Death probability (%, median, IQR)	3.4 (1.5-7.6)	5.1 (1.3-18.7)	0.136	6.2 (1.3-25.8)	4.2 (1-18.7)	0.353
PICU length of stay (day, median, IQR)	4 (2-8)	5 (2-11.25)	0.207	4 (1.5-10.5)	5.0 (2-12)	0.179
PPV day (median, IQR)	3.0 (2-6.5)	4.0 (2-12)	0.201	3 (2-10)	6.5 (2-17)	0.063
Cardiopulmonary arrest before admission (n, %)	20.0 (6.9%)	16.0 (9.1%)	0.396	11 (15.5%)	5 (4.8%)	**0.015***
Diagnosis at admission (n, %)						
Respiratory failure	67 (23.2)	48 (27.8)	0.269	25 (35.2)	24 (22.9)	0.073
Heart failure	36 (12.5)	15 (8.5)	0.184	6 (8.5)	9 (8.6)	0.978
Encephalopathy, seizure	39 (13.5)	16 (9.1)	0.150	4 (5.6)	12 (11.4)	0.190
Sepsis	32 (11.1)	19 (10.8)	0.916	5 (7.0)	14 (13.3)	0.187
Trauma, intoxication	63 (21.8)	33 (18.8)	0.420	12 (16.9)	21 (20)	0.605
Surgical	22 (7.6)	16 (9.1)	0.580	5 (7)	11 (10.5)	0.437
Metabolic/dehydration	22 (7.6)	22 (12.5)	0.083	10 (14.1)	12 (11.4)	0.631
Others	7 (2.4)	6 (3.4)	0.570	4 (5.6)	2 (1.9)	0.181

*Odd ratio: 1.127 (95% CI 1.011-1.256)
IQR: Interquartile range, PRISM: Pediatric risk of mortality, PPV: Positive pressure ventilation, CI: Confidence interval

Conclusion

In conclusion, in our study, it was shown that there was a significant decrease in all PICU admissions especially due to LRTIs during the COVID-19 period. Although there was no difference in terms of disease severity, it is thought that the high cardiopulmonary arrest prior to PICU admission in the first 3 months of the pandemic was due to an avoidance of admission to the health center and consequent later admission.

Ethics

Ethics Committee Approval: Approval was obtained from Ege University Faculty of Medicine Clinical Research Ethics Committee for this study (ethics committee number: 21-2T/65).

Informed Consent: Informed consent was obtained from the families.

Peer-review: Extrenally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: P.Y.Ö., H.F.A., E.E.K., Concept: P.Y.Ö., B.K., Design: P.Y.Ö., B.K., Data Collection or Processing: E.E.K., İ.B., Analysis or Interpretation: P.Y.Ö., B.K., Literature Search: P.Y.Ö., H.F.A., Writing: P.Y.Ö., B.K.

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Original Article

How was Pediatric Flexible Bronchoscopy Implementation Affected During the COVID-19 Era? A Retrospective Study

Gizem Özcan[1], Özlem Selvi Can[2], Fazılcan Zirek[1], Merve Nur Tekin[1], Batuhan Bakırarar[3], Nazan Çobanoğlu[1]

[1]Ankara University Faculty of Medicine, Department of Pediatric Pulmonology, Ankara, Turkey
[2]Ankara University Faculty of Medicine, Department of Anesthesiology and Reanimation, Ankara, Turkey
[3]Ankara University Faculty of Medicine, Department of Biostatistics, Ankara, Turkey

ABSTRACT

Aim: As the application of flexible bronchoscopy (FB) has a high risk for infection transmission, it is recommended to postpone elective FB procedures during the coronavirus disease-2019 (COVID-19) pandemic. We aimed to determine how the COVID-19 pandemic affected pediatric FB implementation.

Materials and Methods: Medical records of patients who underwent FB from March 11, 2015, to September 11, 2020, were examined retrospectively. Records of the post-COVID-19 period (March 11, 2020, to September 11, 2020) were compared with records of pre-COVID-19 (consists of each six-month time window from March 11 to September 11 of each year from 2015 to 2019) period in terms of hospitalization status of the patients, with numbers and indications of FB. Some additional measures were taken during the FB procedure in the post-COVID-19 period. A number of health workers infected during FB procedures in the post-COVID-19 period were reviewed.

Results: Of the total of 182 procedures, the least FB was performed in the post-COVID-19 period (34, 30, 36, 36, 25, and 21 procedures respectively from 2015 to 2020). While microbiological sampling with bronchoalveolar lavage was the most common indication in the post-COVID-19 period, atelectasis was leading in the pre-COVID-19 period ($p<0.001$). In the post-COVID-19 period, most of the patients were inpatients while outpatient predominance was determined in the pre-COVID-19 period ($p<0.001$). None of the health workers was infected during the FB procedure.

Conclusion: Postponing elective FB procedures decreased the numbers and affected the indications of procedures during the COVID-19 era. Taking additional measures is of great importance and effective to prevent transmission of infection during FB.

Keywords: Childhood, COVID-19 pandemic, flexible bronchoscopy

Introduction

In December 2019, a novel coronavirus identified in Wuhan, China led to a pandemic that quickly affected the whole of the world. World Health Organization named this illness coronavirus disease-2019 (COVID-19) on February 11, 2020, and then a global epidemic was declared on March 11, 2020 (1,2). The first case in Turkey was officially proclaimed on March 11, 2020 (3). The main transmission route of the disease is respiratory droplets (4). Therefore, aerosol-generating procedures (AGP) are the most hazardous medical procedures for disease spread. While there are differences between health centers and countries

Address for Correspondence
Gizem Özcan, Ankara University Faculty of Medicine, Department of Pediatric Pulmonology, Ankara, Turkey
Phone: +90 312 595 57 44 E-mail: gizemaltay87@hotmail.com ORCID: orcid.org/0000-0001-9063-4063
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during the COVID-19 outbreak, these procedures have been recommended to be delayed if they are elective. With the prolongation of the epidemic period, more knowledge was obtained about the disease, and the effectiveness of the measures to prevent its spread during these procedures was investigated. Thus, elective interventions in many centers began to be implemented in the 'new normal' order. Flexible bronchoscopy (FB), one of these procedures, is one of the methods used for the diagnosis and/or treatment of childhood respiratory diseases (5,6).

In this study, we aimed to determine how the COVID-19 pandemic affected pediatric FB implementation in terms of hospitalization status of the patients, with numbers and indications of FB, and measures taken during FB procedure of pre-and post-COVID-19 periods.

Materials and Methods

Study Design

Medical records of patients who underwent FB in a tertiary hospital from March 11, 2015, to September 11, 2020, were examined retrospectively. Patients' age, gender, hospitalization status, and indications of FB were reviewed.

Periods

Two study periods were formed according to the COVID-19 outbreak. The post-COVID-19 period is the period from March 11, 2020, to September 11, 2020, and the pre-COVID-19 period is the period from March 11, 2015, to March 10, 2020. To determine the characteristics of patients who underwent FB before the COVID-19 period, the procedures performed in the same months (March 11-September 11) of the 5 years before 2020 were retrospectively evaluated.

Comparison of Pre-and Post-COVID-19 Periods

The hospitalization status of the patients and indications of FB were compared for pre-and post-COVID-19 periods.

Flexible Bronchoscopy Procedure in the Pre-COVID-19 Period

Written informed consent was obtained from the parents of the patients in terms of the indications, purpose, reliability and possible complications of the procedure to be performed before bronchoscopy. Four hours of fasting for infants fed with breast milk only, four to six hours fasting for children between the ages of six months and three years, and six hours fasting for older children were required before the performance of the FB procedure. FB was performed in the operating room under general anesthesia with a team of bronchoscopists, assistant doctors, nurses, and anesthesiologists. Routine anesthesia monitoring included pulse oximetry, capnography, temperature, three-lead electrocardiogram, and non-invasive blood pressure monitoring performed on the patients taken to the operating room during the process. The patients were orally administered midazolam (0.1 mg/kg) and paracetamol (10 mg/kg). General anesthesia was induced with propofol (2-3 mg/kg), ketamine (0.5 mg/kg), and in patients if the use of muscle relaxants is not contraindicated (evaluation of upper airway or laryngomalacia/tracheomalacia), rocuronium bromide (0.6 mg/kg) was used. During induction, first of all, three minutes of positive pressure ventilation with the mask was applied to the patient, followed by laryngeal mask airway (LMA) ventilation, and then, the patient received propofol infusion (100-200 mcg/kg/min) or sevoflurane (1-1.3 MAC) with oxygen 50-100%. At the end of the procedure, the neuromuscular blockage was reversed with sugammadex (2-4 mg/kg) if rocuronium was used during induction. While the bronchoscope with an outer diameter of 3.8 mm was used in patients whose body weights were ≤15 kg, the bronchoscope with an outer diameter of 4.8 mm was used in heavier patients. During the procedure, 0.5-1 mL lidocaine with saline solution was given at the level of the vocal cord and the main carina. Bronchoalveolar lavage (BAL) was performed in patients with suspected respiratory infection to determine the offending microbe and cases of suspected pulmonary hemosiderosis, aspiration pneumonia, pulmonary alveolar proteinosis, and in situ cases of unclear diagnosis, in addition to therapeutic use to rechannelize airways. BAL was accomplished with the use of normal saline warmed to body temperature. A 3 mL/kg volume was calculated and administered in three divided doses in children <20 kg. In children weighing ≥20 kg, 20 mL volumes were injected using a syringe via the suction channel of the bronchoscope. Approximately 40-70% of fluids were recovered by suction using a pressure of 25-100 mmHg as recommended by the European Society for Clinical Respiratory Physiology (7). Samples were separated from the BAL fluid for cytological examination and microbiological evaluation under sterile conditions.

Measures were Taken During the FB Process During the Post-COVID-19 Period

All patients who were scheduled to undergo FB were tested for COVID-19 via oropharyngeal swab, using the real-time polymerase chain reaction (PCR) method 24-48 hours before the procedure. Once the test was confirmed

negative, the patients were processed. At the same time, the COVID-19 contact of all patients was questioned. Even if the test result was negative, patients with known COVID-19 contact were not included in the process.

All procedures were performed in a negative pressure operating room. Before the patient was admitted to the room, all drugs and equipment were available. Premedication was done for reducing crying and aerosol generation if necessary. The patient was taken to the operation room with disposable sterile gowns and a surgical mask. All doors of the room were closed before the procedure. The number of health care workers (HCW) involved in the procedure was kept to a minimum. All HCW were required to have full personal protective equipment (PPE); a minimum of an N95 mask, isolation gown, head cover/hood, shoe covers, goggles/face shield, disposable sterile gowns, and gloves. All of the PPE we used during the process are presented in Figure 1. During the procedure, we avoided applying mask induction as much as possible. We preferred to perform FB via an LMA or endotracheal tube with minimal opening or disconnection of the ventilatory circuit to prevent the spread of aerosol. A clear plastic barrier was utilized over the LMA. If the patient needed intubation before or after the procedure, he/she was intubated using a cuffed intubation tube using a video laryngoscope. Between the two procedures, the room was ventilated for at least 20 minutes and all the materials that the patient had contact with were renewed.

Review of a Number of Health Workers Infected in the Post-COVID-19 Period During FB Procedures

A number of health workers infected in the post-COVID-19 period during FB procedures were reviewed.

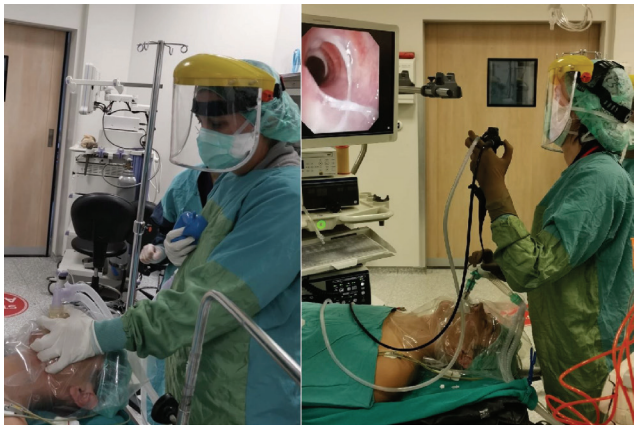

Figure 1. The bronchoscopist and anesthetist who worn PPE are performing FB to a 14-year-old patient

PPE: Personal protective equipment, FB: Flexible bronchoscopy

Statistical Analysis

SPSS 22.0 program was used to analyze the data. As descriptive, mean ± standard deviation and median (minimum-maximum) were used for quantitative variables, and the number of patients (percentage) for qualitative variables. Chi-square and Fisher's exact tests were used to compare categorical variables between the groups. The statistical significance level was taken as 0.05.

Ethics committee approval was received for this study from the Ethics Committee of Ankara University (date: November 12, 2020; no: i9-593-20).

Results

Evaluation of All Patients Included in the Study

A total of 182 patients were included in the study. The distribution of the number of patients who underwent FB by years is presented in Figure 2. Ninety-three (51.1%) patients were male and 89 (48.9%) were female. The median age of the patients was 48 months (range, 1- 204 months). Eighty-two (45.0%) patients had chronic diseases. While 62 (34.2%) patients were hospitalized for another reason before FB, 120 (65.9%) patients were outpatients who applied for FB. Three most common indications for FB were atelectasis (n=39, 21.4%), chronic cough (n=32, 17.5%) and stridor (n=27, 14.8%). All of the indications with their frequencies are given in Table I. Diagnostic findings were detected in 146 (80.2%) of the patients. The findings are presented in Table II.

Comparison of Pre-and Post-COVID-19 Periods

The least FB was performed in post-COVID-19 period. When we evaluated the post-COVID-19 period, 9 (42.8%) patients were male. The median age of the patients was 30 months (range, 1.5-204 months). Seventeen (81%) patients were inpatient. The indications of FB performed in post-

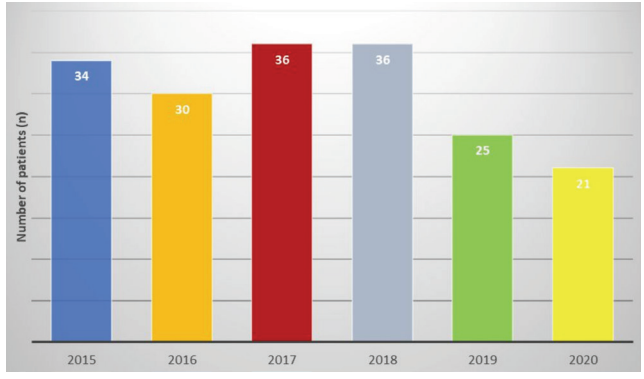

Figure 2. Number of patients undergoing FB by years

FB: Flexible bronchoscopy

COVID-19 period were microbiological sampling with BAL (n=6, 28.6%), extubation failure (n=4, 19.0%), atelectasis (n=3, 14.3%), stridor (n=2, 9.5%), persistent/recurrent pneumonia (n=1, 4.2%) and others (n=5, 23.8%).

One hundred and sixty-one (88.5%) underwent FB in pre-COVID-19 period. Eighty-four (52.1%) patients were male. The median age of patients was 49.5 months (range, 1-204 months). Sixty-two (38.5%) were inpatient. The indications of FB performed in pre-COVID-19 period were atelectasis (n=36, 22.3%), chronic cough (n=32, 19.8%), stridor (n=25, 15.5%), persistent/recurrent pneumonia (n=22, 13.6%), persistent/recurrent wheezing (n=15, 9.3%) extubation failure (n=3, 1.8%) and others (n=15, 9.3%).

While most of the patients in the post-COVID-19 period were inpatient, most of the patients in the pre-COVID-19 period were outpatient (p<0.001). There was also a statistically significant difference between the indications of FB in pre-and post-COVID-19 periods (p<0.001). Analysis of qualitative variables for the pre-and post-COVID-19 period is given in Table III.

Table I. Indications for flexible bronchoscopy of all patients

Indications	**n (%)**
Atelectasis	39 (21.4)
Chronic cough	32 (17.5)
Stridor	27 (14.8)
Persistent/recurrent pneumonia	23 (12.6)
Microbiological sampling	20 (10.9)
Persistent/recurrent wheezing	15 (8.2)
Extubation failure	7 (3.8)
Others*	19 (10.9)

*Other indications: Hemoptysis (n=8), localized air trapping (n=4), malignancy (n=2), evaluation before decannulation (n=2), interstitial lung disease (n=2), foreign body aspiration (n=1)

Table II. Bronchoscopic findings of all patients

Bronchoscopic findings	**n (%)**
Presence of purulent secretion suggesting active infection	46 (25.3)
Tracheo/bronchomalacia	46 (25.3)
Normal	36 (19.8)
Inferior airway anomalies (except tracheo/ bronchomalacia)	18 (9.9)
Mucus plugging	16 (8.8)
Upper airway anomalies	14 (7.7)
Mucosal inflammation/laceration/granulation	9 (4.9)
Airway pressure	4 (2.2)
Foreign body aspiration	2 (1.1)

Discussion

During the COVID-19 era, AGPs were the most affected among medical procedures due to their high risk for disease transmission. FB was also one of the risky procedures whose implementation had to decrease. In the post-COVID-19 period, we continued to apply FB without disease transmission to any HCW or patient with the measures we took in line with the information in the literature. When we compared the FB that were performed in pre and post COVID-19 periods, we found a statistical difference between the hospitalization status of patients and indications for FB.

Recommendations regarding the application of bronchoscopy during the COVID-19 period are presented as testing all patients before the procedure, postponing elective cases if possible, and taking the patient to the procedure after 2 weeks of strict isolation. Testing all patients for COVID-19 before the procedure is a recommendation given to detect asymptomatic patients (8). However, some asymptomatic patients may have false-negative results. Pre-analytical problems such as insufficient or inappropriate sampling, sample contamination, and analytical problems such as testing outside the diagnostic window, active viral recombination, use of poorly validated assays, device failure can be among the causes of false-negative results (9). Even if the patients to be processed are asymptomatic in an area where community transmission of COVID-19 infection is present, it is thought that the most important factor in preventing the spread of the disease is PPE. For this reason, it is recommended that PPE be worn by all HCW during the procedure, even if asymptomatic patients are tested before the procedure and found to be negative (10). We tested all patients we planned to undergo FB with or without COVID-19 disease symptoms using the PCR method. If the results were negative, we performed FB and continued the precautions during the procedure. Canadian Pediatric Anesthesia Society (11) recommended that all HCW should have appropriate PPE, all equipment must be ready before the patient's operative room is taken to shorten the procedure. It is also suggested to avoid mask induction during the procedure, performing all procedures in rooms with negative pressure if possible, and performing the patient's intubation with the help of a video-laryngoscope and by choosing a cuffed intubation tube. As aforementioned, we applied our FB processes with these recommendations in our center, and none of the HCWs were infected due to the FB procedure.

Table III. Analysis of qualitative variables for pre- and post-COVID-19 period

Variables		Post-COVID-19 period		Pre-COVID-19 period		p-value
		n	%	n	%	
Hospitalization of patients	Inpatient Outpatient	17 4	81.0 19.0	45 116	28.0 72.0	<0.001[a]
Indications for FB	Chronic cough Atelectasis Microbiological sampling Persistent/recurrent pneumonia Persistent/recurrent wheezing Stridor Extubation failure Others	0 3 7 1 0 2 4 4	0.0 14.3 33.3 4.8 0.0 9.5 19.0 19.0	32 36 13 22 15 25 3 15	19.9 22.4 8.1 13.7 9.3 15.5 1.9 9.3	<0.001[b]
Diagnostic findings	No Yes	5 16	23.8 76.2	31 130	19.3 80.7	0.572[b]

[a]Chi-square test, [b]Fisher's exact test
COVID-19: Coronavirus disease-2019, FB: Flexible bronchoscopy

In the literature, there are studies presented by pediatric otorhinolaryngologists and surgeons, in which the measures were taken by the centers during the bronchoscopy procedures performed during the COVID-19 era (12-14). However, to the best of our knowledge, there is no study evaluating FB through the eyes of a pediatric pulmonologist in the COVID-19 era.

When we compared the hospitalization status of patients who underwent FB in the pre-and post-COVID-19 period, we found that statistically significantly more inpatients were performed in the post-COVID-19 period ($p<0.001$). We think that decrease in outpatient admissions during the pandemic period caused the admission of patients with an indication for FB to be delayed. We also found that there was a statistical difference between the indications for FB in the pre-and post-COVID-19 period ($p<0.001$). The most common indications we found in the pre-COVID-19 period are the most common indications for pediatric FB, previously presented in many studies and these indications are atelectasis, chronic cough, and stridor (15-18). On the other hand, we think that the reason we found the frequency of microbiological sampling and extubation failure indications increased in the post-COVID-19 period is that we mostly apply the procedure among hospitalized patients. All four patients in whom we performed FB due to extubation failure were patients who were undergoing intensive care follow-up after an operation for congenital heart disease. Performing FB could not be delayed for withdrawal of respiratory support and discharge of these patients. Treatment of patients who underwent bronchoscopy due to microbiological sampling was adjusted with the microbiological data obtained. These examples allow us to predict that delaying FB procedure in children, even during the COVID-19 period, will have a negative effect on the morbidity and/or mortality of the patients.

The most important limitation of our study is that the number of procedures we performed in the post-COVID-19 period is much less compared to the other group. The purpose of our evaluation of the last 5 years before COVID-19 was to ensure the homogeneity of the data of previous years.

Conclusion

COVID-19 pandemic era has led to drawbacks in the application of pediatric FB, as the other AGP. Postponing elective FB procedures decreased the numbers and affected the indications of procedures in our center during the COVID-19 era. Taking additional measures is effective to prevent the transmission of infection during FB. Therefore, pediatric pulmonologists must continue to do FB, despite having to deal with AGP, in the 'new normal'. With the 'new normal', pediatric FB should be continued with pre-procedure contact/symptom questioning, PCR testing, and full use of PPE.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ankara University (date: November 12, 2020; No: i9-593-20).

Informed Consent: Written informed consent was obtained from the parents of the patients in terms of the indications, purpose, reliability and possible complications of the procedure to be performed before bronchoscopy.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Data Collection or Processing: G.Ö., F.Z., M.N.T., Analysis or Interpretation: G.Ö., B.B., Literature Search: Ö.S.C., N.Ç., Writing: G.Ö.

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

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Original Article

Education of Parents in Increasing Breastfeeding Rates, Success, and Self-Efficacy Levels

Gülsün Ayran[1], Ayda Çelebioğlu[2]

[1]Erzincan Binali Yıldırım University Faculty of Health Sciences, Department of Child Health and Disease Nursing, Erzincan, Turkey
[2]Mersin University Faculty of Nursing, Department of Child Health and Disease Nursing, Mersin, Turkey

ABSTRACT

Aim: This study aimed to determine the effect of breastfeeding education given to parents in the early postpartum period on the duration of exclusive breastfeeding for the first six months, breastfeeding success and breastfeeding self-efficacy levels of mothers.

Materials and Methods: This study had three groups including a control (n=49), intervention I (n=48) and intervention II group (n=48). As a nursing intervention, breastfeeding training using pre-structured training modules was given only to the mothers in intervention group 1 and to both the mothers and fathers in Intervention group 2. Routine nursing services were provided to the families in the control group. The infant feeding behaviors of the mothers in all three groups were monitored until the end of the sixth month. The parental introductory information form, infant follow-up form, LATCH diagnosis and evaluation scale and breastfeeding self-efficacy scale were used to collect the data.

Results: It was determined that the breastfeeding training given to mothers increased breastfeeding self-efficacy levels and breastfeeding success and this increase was statistically significant ($p<0.05$). It was determined that the difference between the supplemental nursing systems feeding rates for the intervention groups at the 1st, 2nd, 4th and 6th months were significantly higher than in the control group ($p<0.05$).

Conclusion: Breastfeeding training increased the mothers' breastfeeding self-efficacy and the duration of exclusive breastfeeding, but the fathers' support made no significant difference.

Keywords: Breastfeeding, parents, nursing, self-efficacy

Introduction

The neonatal period is one of the most sensitive periods of life. Newborns need to be fed sufficiently for healthy development (1). Breastfeeding is one of the most effective interventions that can benefit the child, the mother, and society (2). As a global public health proposal, babies should be exclusively breastfed for the first six months for optimal growth, health, and development (3). Exclusive breastfeeding (EBF) means not giving the baby any solids or liquids (including water) other than breast milk, with the exception of medicines and vitamins, for the first six months of life (4). The Global Breastfeeding Report, which assessed 194 countries, found that only 40% of infants under six months old were EBF and that only 23 countries had an EBF rate of above 60% (5).

Initiating and sustaining successful breastfeeding is a multidimensional process that includes not just the mother and her baby but also the family, community, and the health care system (6). While professional support is seen as an important element of breastfeeding success for mothers,

Address for Correspondence
Gülsün Ayran, Erzincan Binali Yıldırım University Faculty of Health Sciences, Department of Child Health and Disease Nursing, Erzincan, Turkey
Phone: +90 446 226 58 60 E-mail: gulsun_ayran@hotmail.com ORCID: orcid.org/0000-0002-1069-2683
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the role of the woman's spouse in the decision to initiate and sustain breastfeeding is considered more critical (7). Therefore, in addition to mothers, fathers should also be involved in breastfeeding training programs. Studies on the fathers' involvement in breastfeeding training programs reported that training increases the fathers' knowledge of breastfeeding and prolongs the duration of EBF (1,8).

Breastfeeding self-efficacy is one of the requirements for breastfeeding success (9). It refers to the mother's self-confidence to breastfeed her newborn or the adequacy she perceives in this regard. It affects the mother's desire and decision to breastfeed, efforts dedicated to breastfeeding, and her ability to deal with related difficulties (10). Perceived breastfeeding self-efficacy is a factor that can be influenced by health education (11). A longitudinal study in Singapore found that breastfeeding self-efficacy training increased breastfeeding self-efficacy and breastfeeding rates (12). Similarly, a study conducted in Iran showed that a self-efficacy intervention increased breastfeeding self-efficacy (13). Gümüşsoy et al. (14) reported that breastfeeding training given to mothers to increase their breastfeeding self-efficacy increased their perceived competence to breastfeed.

This study aimed to determine the effects of breastfeeding education given to parents in the early postpartum period on the duration of EBF for the first six months, breastfeeding success and the breastfeeding self-efficacy levels of the mothers.

Materials and Methods

This quasi-experimental study was conducted using pre- and post-tests in the obstetrics clinics of a research hospital in the province of Erzincan, Turkey, from November 2016 to September 2017. The hospital is not part of the "Baby Friendly Hospital Initiative". The study population consisted of fathers and mothers of infants who were born in the research hospital. The sample of the research is; in this study, "G. Using the "Power-3.1.9.2" program, it was calculated before the data collection phase whether the sample size was sufficient at the 80% confidence level. Accordingly, for the effect size t-test of the study, the minimum total number of samples was determined to be 40 by taking 0.05 as the alpha value, 0.46 as the effect size and 80% as the theoretical power. Ten percent was added to each group to account for data loss. The study was conducted with a total of 145 parents who had full-term, healthy babies, did not have any breastfeeding problems, had no communication barriers, were literate, resided in the city center, and agreed to participate in this study. The participants were divided into an intervention group 1 (where only the mothers received breastfeeding training, n=48), an intervention group 2 (where both parents were given breastfeeding training, n=48), and a control group (routine breastfeeding training group, n=49).

Data Collection Instruments

Parental introductory information form: This form, which was developed by the researcher, contains questions about the parents' socio-demographic characteristics, such as their age, education level, employment status, and income.

Infant follow-up form: This form developed by the researcher includes questions about the type of feeding.

LATCH diagnosis and evaluation scale: [Jensen et al. (15)] This scale was developed in 1993. The scale, whose Turkish validity was made by Yenal and Okumuş (16) in 2003 and whose Cronbach's Alpha value was 0.95, was recommended as a reliable tool. In this study, the Cronbach's Alpha value of the LATCH Breastfeeding Diagnosis and Evaluation scale was found to be 0.90. In this scale, which is similar to the apgar score system in terms of scoring, 0, 1, or 2 points are given for each criterion and breastfeeding is evaluated by adding the scores. The scores that can be obtained from the scale vary between 0 and 10. Increasing scores on the scale indicates breastfeeding success.

Breastfeeding self-efficacy scale: Originally developed by Dennis and Faux (17), this scale was revised to a 14-item short form in 2003. The validity and reliability of the scale in the Turkish setting was tested by Tokat (18). The Breastfeeding Self-Efficacy-Short Form uses a 5-point Likert-type scale (1= not at all confident, 5= very confident). The scores range from 14 to 70, with higher scores indicating higher breastfeeding self-efficacy (18).

Intervention Instruments

A breastfeeding training program, which was prepared separately for mothers and fathers, a breastfeeding training booklet, and a baby model were used in the study.

Breastfeeding training program and booklet for mothers: Mothers in the intervention groups were verbally taught the benefits of EBF (for babies, mothers, and society), when to start breastfeeding, breastfeeding techniques, when to start offering the baby additional foods, how to burp the baby, practices that should be avoided during the breastfeeding period (e.g. the use of bottles and pacifiers), and the role of fathers in breastfeeding. The training

materials, namely, a Power Point presentation and training booklet, were prepared in accordance with the literature.

Breastfeeding training program and booklet for fathers: Fathers in intervention group 2 were taught about the features of breast milk, the benefits of EBF (for babies, mothers, and society), the factors affecting the duration of EBF, the psychology of the baby and mother during breastfeeding, and the father's role in breastfeeding. A Power Point presentation and training booklet were prepared in accordance with the literature.

Data Collection

During the data collection stage, parents who met the research criteria were visited and informed of the research purpose, and their written informed consent was obtained. To prevent the groups from being influenced by each other, data were collected from the control group first, followed by intervention groups 1 and 2.

Pre-intervention data collection: After the mothers gave birth and breastfed their babies for the first time, the participants' consent was obtained as soon as the mothers were ready. The parental introductory information form and breastfeeding self-efficacy scale were administered to the control and intervention groups via face-to-face interviews. After the second breastfeeding session, information about the infants' nutritional status was recorded on the infant follow-up form and the LATCH breastfeeding diagnosis and evaluation scale was applied.

Intervention program: After the mother gave birth and the first breastfeeding was initiated, at the earliest time when the mother was resting and ready, a Breastfeeding Training Program was conducted only for the mothers in the first intervention group and for both mothers and fathers in the second intervention group. The training was given to the mothers in the first intervention group in an individual room in two 40-minute sessions. In the second experiment group, mothers and fathers were trained in their own individual rooms, which were single and seperate, in two 40-minute sessions. In addition, an additional 20-minute session was held for the fathers in the second intervention group. At the end of the training, the "Breastfeeding Education Booklet for Mothers" was given to the mothers and the "Breastfeeding Education Booklet for Fathers" was given to the fathers. No attempt was made by the researcher regarding the parents in the control group. These parents only benefited from the nursing services routinely provided in the hospital.

Post-intervention data collection: Four visits were made to the mothers (at the first, second, fourth, and sixth months), during which information on the infants' nutritional status was recorded in the infant follow-up form and then the LATCH breastfeeding diagnosis and evaluation scale was applied by observation made during breastfeeding. At the six-month home visit, in addition to the infant follow-up form, the breastfeeding self-efficacy scale was administered to the mothers. The mothers in the intervention groups were asked to answer the questions after being called twice in the first and second weeks after discharge and by making the necessary reminders during the home visits. The home visits to the participants in the intervention groups were limited to 30 minutes, and the visits to the control group participants were limited to 10 minutes.

Data Evaluation

Statistical analysis of the data was performed using IBM SPSS v. 22. The Shapiro-Wilk test was used to assess the normal distribution of the data. Medians, interquartile ranges, frequencies, and percentage distributions were calculated.

The Kruskal-Wallis test was used to evaluate the study data, and Dunn's pairwise test was used for post hoc evaluations. Pearson's chi-square test and the Fisher-Freeman-Halton exact test were used to evaluate the qualitative data. The level of significance was set at $p<0.005$.

Ethical approval was obtained from the Atatürk University Faculty of Health Sciences Ethical Committee on September 23rd, 2016 (no. 2016/09/04), before this study was conducted. Permission to conduct this study was obtained from the research hospital on October 26th, 2016 (no. 43527969/605.99). After the necessary explanations were made about the research purpose and method, the parents' verbal and written consent was obtained.

Results

The demographic characteristics of the mothers and fathers included in this study are given in Table I. In this study, 38.8% of the mothers in the control group were primary school graduates, 81.6% were not working, and 69.4% had income equal to their expenses; it was determined that the mean age of the mothers was 30.51±5.82 years and the average number of children they had was 2.16±0.92. It was determined that 35.4% of the mothers in intenvention group I were primary school graduates, 79.2% were not working, 70.8% of them had income equal to their expenses, the average age of the mothers was 29.19±4.56 years and their average number of children was 1.98±0.84. It was determined that 39.6% of the mothers in intervention

group II were university graduates, 77.1% were not working, 56.3% of them had income equal to their expenses, the average age of the mothers was 29.92±5.21 years and their average number of children was 2.19±0.91. In terms of maternal characteristics, the three groups were statistically similar to each other (p>0.05).

The demographic characteristics of the fathers included in the study are given in Table I. In the study, it was

Table I. Demographic characteristics of parents

		Control group		Experimental group 1		Experimental group 2		χ^2 and p
		n	%	n	%	n	%	
Mother's education level	Primary education	19	38.8	17	35.4	14	29.2	χ^2=1.562 p=0.816
	High school	16	32.7	15	31.3	15	31.3	
	University	14	28.6	16	33.3	19	39.6	
Father's education level	Primary education High school University	14 17 18	28.6 34.7 36.7	11 18 19	22.9 37.5 39.6	7 19 22	14.6 39.6 45.8	χ^2=2,845 p=0.584
Mother's employment status	Employed	9	18.4	10	20.8	11	22.9	χ^2=0.307 p=0.858
	Unemployed	40	81.6	38	79.2	37	77.1	
Father's employment status	Employed Unemployed	47 2	95.9 4.1	45 3	93.8 6.3	47 1	97.9 2.1	χ^2=1.051 p=0.591
Family type	Nuclear family	42	85.7	44	91.7	42	87.5	χ^2=0.872 p=0.647
	Extended family	7	14.3	4	8.3	6	12.5	
Income	Income<expenditure	13	26.5	12	25.0	17	35.4	χ^2=3,031 p=0.553
	Income=expense	34	69.4	34	70.8	27	56.3	
	Income>expenditure	2	4.1	2	4.2	4	8.3	
Gender	Female Male	17 32	34.7 65.3	24 24	50 50	28 20	58.3 41.7	χ^2=5,600 p=0.061
Breastfeeding experience	Yes	37	75.5	34	70.8	35	72.9	χ^2=0.271 p=0.873
	No	12	24.5	14	29.2	13	27.1	
Received breastfeeding education (mother)	Yes	5	10.2	1	2.1	5	10.4	χ^2=3,101 p=0.212
	No	44	89.8	47	97.9	43	89.6	
Received breastfeeding education (father)	Yes No	4 45	8.2 91.8	3 45	6.3 93.8	6 42	12.5 87.5	χ^2=1.207 p=0.547
First time the infant was breastfed	Within 30 minutes after birth	32	65.3	36	75.0	34	70.8	χ^2=2,065 p=0.724
	31-60 minutes after birth	6	12.2	6	12.5	7	14.6	
	61-120 minutes after birth	11	22.4	6	12.5	7	14.6	

	Control group			Experimental group 1			Experimental group 2			χ^2 and p
	n	Median	IQR	n	Median	IQR	n	Median	IQR	
Mother's age	49	31	25.50-36.00	48	29	25.25-32.00	48	30	26.00-33.00	χ^2_{KW}=1.525, p=0.467
Father's age	49	33	29.50-39.00	48	31.5	28.00-35.75	48	34	30.00-36.75	χ^2_{KW}=3,673, p=0.159
Number of children	49	2	1.50-3.00	48	2	1.00-2.00	48	2	1.25-3.00	χ^2_{KW}=1.616, p=0.446

IQR: Interquartile range

found that 36.7% of the fathers in the control group were university graduates, 95.9% were working, and the average age of the fathers was 34.16±6.11 years. It was determined that 39.6% of the fathers in intervention group I were university graduates, 93.8% were working, and the mean age of the fathers was 31.98±4.49 years. It was determined that 45.8% of the fathers in intervention group II were university graduates, 97.9% were working, and the average age of the fathers was 33.21±4.63 years. The demographic characteristics of fathers were statistically similar in all groups (p>0.05).

Table II shows the results of the pairwise comparisons of the EBF rates for the three groups. The EBF rates of the control group were lower than those of the two experimental groups in the first, second, and fourth months (p<0.05). There were no significant differences between the two experimental groups in terms of EBF rates at all follow-up periods (p>0.05).

As seen in Table III, the difference in the LATCH score average between the three groups is statistically insignificant at birth and at the 6th month (p>0.05). It was determined that the difference between the three groups at the 1st, 2nd and 4th months was statistically significant (p<0.05). In advanced analysis (U) used to determine which groups the differences come from, it was determined that the mean score of the control group was lower than the other groups at the 1st, 2nd and 4th months.

As seen in Table IV, the difference between the use of pacifier between the three groups at the 1st, 2nd, 4th and 6th months was statistically significant (p<0.05). In the advanced analysis (X^2) performed to determine the originating group of the difference, it was determined that the pacifier usage rates in the control group were higher than the 1st experiment and 2nd experiment groups in the 1st, 2nd, 4th and 6th months. At the 1st, 2nd, 4th and 6th months, the difference in the rates of use of feeding bottles between the three groups was found to be statistically significant (p<0.05). In the advanced analysis performed to determine the originating groups of the differences, it was determined that the baby bottle usage rates in the control group were higher than the 1st experiment and 2nd experiment groups in the 1st, 2nd, 4th and 6th months.

Table IV shows the results of Dunn's pairwise comparisons for the three groups. The control group had lower self-efficacy scores than the two experimental groups (p<0.001, adjusted using the Bonferroni correction). No significant differences were found between the two experimental groups with regard to self-efficacy scores (p>0.05; Table V).

Table II. Comparison of exclusive breastfeeding rates between the groups in six-month period

	Measurement time	Control group		1st experimental group		2nd experimental group		Test and significance
		n	%	n	%	n	%	
EBF	At birth	30	61.2	35	72.9	30	62.5	χ^2=1,756, p=0.416
	1st month	21	42.9	39	81.3	45	93.8	χ^2=34,243, **p=0.000**
	2nd month	19	38.8	37	77.1	41	85.4	χ^2=27,181, **p=0.000**
	4th month	19	38.8	36	75.0	36	75.0	χ^2=18,214, **p=0.000**
	6th month	19	38.8	36	75.0	36	75.0	χ^2=18,214, **p=0.000**

EBF: Exclusive breastfeeding

Table III. Comparison of LATCH scores between groups

	Measurement time	Control group		1st experimental group		2nd experimental group		Test and significance
		Mean	SD	Mean	SD	Mean	SD	
LATCH	At birth	5.18	1.79	5.21	1.66	5.08	1.41	F=0.079, p=0.924
	1st month	9.12	0.90	9.54	0.80	9.58	0.74	χ^2_{KW}=10.540, **p=0.005**
	2nd month	9.04	2.42	9.69	1.49	9.90	0.42	χ^2_{KW}=13.316, **p=0.001**
	4th month	8.47	3.51	9.17	2.79	9.58	2.02	χ^2_{KW}=8.061, **p=0.018**
	6th month	8.16	3.91	8.71	3.34	9.38	2.45	χ^2_{KW}=3.252, p=0.197

SD: Standard deviation

Table IV. Comparison of use of pacifier and feeding bottles between the groups in six-month period

	Measurement time	Control group		1st experimental group		2nd experimental group		Test and significance
		n	%	n	%	n	%	
Pacifier use	At birth	5	10.2	2	4.2	1	2.1	-
	1st month	23	46.9	10	20.8	4	8.5	χ^2=19,446, **p=0.000**
	2nd month	27	55.1	13	27.1	6	12.5	χ^2=20,031, **p=0.000**
	4th month	28	57.1	17	35.4	10	20.8	χ^2=13,771, **p=0.001**
	6th month	28	57.1	17	35.4	10	21.8	χ^2=13,771, **p=0.001**
Use of feeding bottle	At birth	4	8.2	1	2.1	-	-	-
	1st month	14	28.6	7	14.6	2	4.3	χ^2=10,672, **p=0.005**
	2nd month	19	38.8	10	20.8	5	10.4	χ^2=11,137, **p=0.004**
	4th month	21	42.9	10	20.8	10	20.8	χ^2=7,759, **p=0.021**
	6th month	22	44.9	10	20.8	10	20.8	χ^2=9,131, **p=0.010**

Table V. Comparison of the Self-Efficacy scale averages before and after training

	Measurement time	Control group		1st experimental group		2nd experimental group		Test and significance
		Mean	SD	Mean	SD	Mean	SD	
Self-Efficacy scale	Before the training	54.86	6.76	56.29	6.26	56.98	6.28	χ^2_{KW}=3,175, p=0.204
	6 months after	54.20	12.87	61.29	8.51	62.60	8.76	χ^2_{KW}=18,752, **p=0.000**

Discussion

Although the importance of breastfeeding in terms of infant and child health is a phenomenon that has been accepted in all countries of the world, UNICEF has reported the rate for infants fed with SAS for the first six months to be 39% (5). According to the 2018 data of TPHR (TNSA; Turkish Population and Health Research), the rate of babies breastfed for a certain period of time in our country is 98%, and the rate of babies who have supplemental nursing systems (SNS) in the first 2 months of their lives is 45%, and this rate decreases to 14% when the baby is 4-5 months old (19). There are various reasons affecting the gradual decrease in the rate of SNS during the first 6 months. One of the most remarkable of these reasons is the low level of knowledge and motivation of mothers towards breast milk and breastfeeding (6). It has been noticed when the literature is reviewed that the number of studies investigating the effects of paternal support and breastfeeding education in the early postpartum period upon breastfeeding outcomes has been increasing. In this sense, the research has provided target-driven and individualized guidance for breastfeeding after birth for mothers and fathers and maintained infant monitoring and counseling visiting homes during the postpartum first six months. When the findings of this research are evaluated, it can be seen that although the rates of SNS in the first sixth months of the baby's life are similar, there was remarkable information revealing the positive effects upon breastfeeding success, the breastfeeding self-efficacy levels of mothers and pacifier and bottle use.

It was found in this research that the participation of the fathers in the breastfeeding education process in the postpartum period and the breastfeeding support given in the first six months after birth increased the rate of EBF at the end of the 4th month. However, it did not create a difference at the end of the 6th month. The number of studies in the literature carried out on the fathers' participation in breastfeeding support has been increasing recently. Although there have been many research results indicating that the fathers' participation in the breastfeeding process increases the rate of EBF (20-24), there has also been evidence that support the view that the father does not change the rate of EBF, or even affects it negatively (25-27). It is believed that these differences in the results of the study could arise from varying income levels and cultural factors. There could also be significant differences in the role of fathers between high- and middle-income families. The roles of males and females in middle-income families are markedly different; males have culturally tended to be responsible for providing financial support for food, clothing, and health care.

Furthermore, unlike fathers in high-income countries, middle-income fathers rarely accompany their spouses to antenatal or postnatal appointments (28). In this sense, it has been considered that the similarity in the rates of SNS as of the sixth month in the research results could have arisen from cultural factors because the sample group was chosen from a province in the east of the country.

"Successful breastfeeding" is defined in different ways such as "the duration of breastfeeding is an indicator of breastfeeding success," "successful breastfeeding is the success felt by the mother" or "an interaction process that results in mutual satisfaction of mother and baby needs" (29). Various factors are efficient for initiating and maintaining successful breastfeeding, and the role of health professionals is significant. It has been noticed in the research that counseling services on breastfeeding starting in the hospital and continuing with home visits, and the participation of fathers in these services has increased the success of breastfeeding. Similarly, previous studies have revealed that breastfeeding success of mothers has increased with health education (29-31). In line with these results, it is considered that breastfeeding education for initiating and strengthening breastfeeding both prepares mothers for breastfeeding gradually and effectively and increases breastfeeding success and so enables mothers to better cope with the difficulties in the breastfeeding process.

Recommendations for pacifier use differ all around the world. The American Academy of Pediatrics recommends the use of pacifiers to prevent Sudden Infant Death Syndrome, and pacifiers can be introduced at about 3 to 4 weeks of age after breastfeeding is well established (32). In contrast, the WHO does not recommend using a pacifier in breastfed children as one of the "Ten Steps to Successful Breastfeeding" on which the "Baby-Friendly Hospital Initiative" is based (33). It was found in this study that the participation of fathers in the breastfeeding education process decreased the rates of pacifier and bottle use. Other studies that have been carried out similarly have reported that breastfeeding education decreased the rates of pacifier use (21,34,35).

It was seen in this study that both the fathers' participation in the breastfeeding education process in the postpartum period and breastfeeding support given in the first six months after birth increased the breastfeeding self-efficacy level of the mothers. The perception of breastfeeding self-efficacy is an important factor upon both initiating and maintaining breastfeeding. Breastfeeding behavior can be changed through health education, it is affected by spousal support, and it affects breastfeeding success (13,36,37). In this study and other studies based on this theory, it has been reported that paternal participation and breastfeeding education given in the early postpartum period both increase the level of breastfeeding self-efficacy and they are efficient at initiating and maintaining breastfeeding (38,39). These findings we obtained provide important data on the importance of the fathers' involvement in breastfeeding education and counseling services in the hospital and the importance of increasing the perception of breastfeeding self-efficacy.

Study Limitations

The research has several limitations. Firstly, there was no randomization between the groups. Secondly, the researchers were not blind to the study groups. In addition, the mothers were not put into multiparous or primiparous groups in this study, so the high number of mothers with breastfeeding experience in the groups may have affected the results of this study. Results for this study should not be generalized to other samples; instead, these findings are valuable for constructing theories and hypotheses about the issues that need to be explored in future qualitative and quantitative designs with a variety of samples.

Conclusion

Despite its limitations, this quasi-experimental design provides important evidence about the effects of the fathers' involvement in postpartum breastfeeding on EBF rates, breastfeeding self-efficacy levels, and breastfeeding success. To include fathers, future research should consider the socio-economic and cultural context when designing and implementing any intervention. In addition, randomized controlled studies should be included in the future to obtain stronger results. Furthermore, it is recommended to determine at risk groups and provide the necessary support by applying the postnatal breastfeeding self-efficacy scale in the first breastfeeding attempt to mothers who give birth in obstetrics clinics, by emphasizing the perception and importance of breastfeeding self-efficacy during in-service training programs for breastfeeding, and by providing information which mothers in the prenatal and breastfeeding process can reach at any time. It may be considered beneficial to establish breastfeeding service units within health institutions where mothers can receive support and to include fathers in breastfeeding education programs.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Atatürk University Faculty of Health Sciences Ethical Committee on September 23, 2016 (no. 2016/09/04).

Informed Consent: After the necessary explanations were made about the research purpose and method, the parents' verbal and written consent was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: G.A., A.Ç., Design: G.A., A.Ç., Data Collection and/or Processing: G.A., Writing: G.A., A.Ç.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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Case Report

Nasal Actinomyces in a 7-year-old Boy with Recurrent Nasal Bleeding: A Case Report

Mohammad Solduzian[1], Abbasali Hosseinpourfeizi[2], Amir Hossein Jafari-Rouhi[3], Fariba Pourkarim[1]

[1]Tabriz University of Medical Sciences Faculty of Pharmacy, Department of Clinical Pharmacy, Tabriz, Iran
[2]Tabriz University of Medical Sciences, Hematology Oncology Research Center, Tabriz, Iran
[3]Tabriz University of Medical Sciences, Tuberculosis and Lung Diseases Research Center, Tabriz Iran

ABSTRACT

Actinomyces rarely cause infections in nasal sinuses with local symptoms and its identification is difficult so the initiation of treatment might be delayed. A 7-year-old boy with recurrent nasal bleeding was found to be infected in the paranasal sinuses with actinomyces, which was confirmed by histopathological studies. This case was initially managed with surgical resection and systemic antibiotics and later discharged and referred to an out-patient clinic.

Keywords: Actinomyces, nasal sinus, recurrent bleeding

Introduction

The actinomyces species, fastidious gram-positive anaerobic bacteria, have been identified as an uncommon cause of infection in humans. These organisms remain in the host mucosa and invade the lower layers after the breakdown of protective barriers (1). They might consist of normal flora of the pharynx, but organisms might spread to the paranasal sinuses and other cervicofacial regions and cause infections in the gastrointestinal tract, thorax, head, and neck, or less commonly in female genitalia, the ophthalmic region and the oral cavity. Isolation of these organisms is difficult and histologic studies are required for their identification (2). The clinical presentation of actinomycosis might resemble malignancy and imaging studies are necessary for differentiation. Poor dental hygiene is the most frequently reported risk factor for cervicofacial actinomyces infection. Chronic gingivitis, dental procedures and other chronic disorders of the oral cavity could also increase the risk of cervicofacial actinomyces infection (3). A cervicofacial infection of actinomyces can lead to abscess formation, osteomyelitis or osteonecrosis. In cases of cervicofacial infection, treatment usually consists of surgical interventions for the drainage of abscesses and the debridement of necrotic bone tissue, in combination with prolonged courses of antibiotics (usually 6 to 12 months). Antibiotic options include amoxicillin, ceftriaxone, penicillin, clindamycin, macrolides and doxycycline. Some studies have reported successful treatment of cervicofacial actinomyces with 4 to 6 week courses of antibiotic therapy which is shorter compared to previously recommended durations (4). Actinomyces infections are rare and the incidence rate of these infections is 1 case in 300,000 (5). Pediatric actinomyces infections are rare and early

Address for Correspondence
Mohammad Solduzian, Tabriz University of Medical Sciences Faculty of Pharmacy, Department of Clinical Pharmacy, Tabriz, Iran
Phone: +989120920355 E-mail: dr.mohammadsolduzian@gmail.com ORCID: orcid.org/0000-0002-7504-5065
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epidemiological data indicate that in might account for less than 3% of all actinomyces infections (6). Some patterns of infection are even rarer such as nasal cavity involvement. In the current paper, we report a case of nasal cavity involvement with actinomyces, proven in histologic studies, who was managed with surgical debridement and intravenous antibiotics.

Case Presentation

A 7-year-old boy with poor oral hygiene was admitted to the emergency department of Tabriz Children's Hospital, a teaching hospital of Tabriz University of Medical Sciences, for the management of epistaxis. His medical history showed recurrent epistaxis during the previous three months leading to frequent emergency visits. He had previously undergone two nasal cautery for the treatment of epistaxis. The child was conscious but appeared pale. His vital signs were as follows; BP of 90/50 mmHg; body temperature of 37.5 °C; a pulse of 141 beats/minute; and a respiratory rate of 31 breaths/minute. On auscultation, his heart sounds were normal and his lungs were clear. Blood tests showed a high white blood cell count (lymphocyte predominance), and anemia (hemoglobin=8.1 g/dL). Other parameters including creatinine, platelet count, prothrombin time, partial thromboplastin time, and international normalized ratio were within the normal range (Table I). His erythrocyte sedimentation rate was 10 mm/hour (reference value: up to 12), while C-reactive protein was negative. A nasal tampon was inserted into the anterior of the nose. Active bleeding from the nose was stopped but the patient occasionally had bloody discharge from the pharynx. Other treatments included 800 mL of 10% dextrose solution intravenously, a transfusion of 350 mL fresh frozen plasma, a transfusion of 2 units of packed red blood cells, tranexamic acid (400 mg slow intravenous infusion 2 times daily), furosemide (15 mg intravenously), 3 drops of phenylephrine into each nostril every 8 hours, and 5 mL of syrup zinc sulfate orally per day.

Table I. Laboratory test results

Parameter	On admission	At discharge
WBC ($\times10^3$/mm^3)	14.69	14.3
Lymphocytes ($\times10^3$/mm^3)	4.19	6.4
Neutrophils ($\times10^3$/mm^3)	4.97	6.9
Hemoglobin (g/dL)	8.1	11.6
Platelet count ($\times10^3$/mm^3)	297	233
Creatinine (mg/dL)	0.5	0.4
PT (sec)	12.4	12
PTT (sec)	42	24
INR (sec)	1	1

WBC: White blood cells, PT: Prothrombin time, PTT: Partial thromboplastin time, INR: International normalized ratio

Computed tomography (CT) angiography of the neck vessels showed opacities in the paranasal sinuses. No evidence of space occupying mass, obvious vascular lesions, stenosis or blockage in the carotid artery or obvious vascular leakage in the nasopharynx or its surrounding spaces was observed.

A CT scan of the brain with radiocontrast media revealed mucosal thickening in the left maxillary sinus, suggesting a retention cyst or polyp measuring 17×15 mm. The nasal cuts can be seen in Figure 1. Osteomeatal complex obstruction and a mass in the nasal cavities was also identified. There

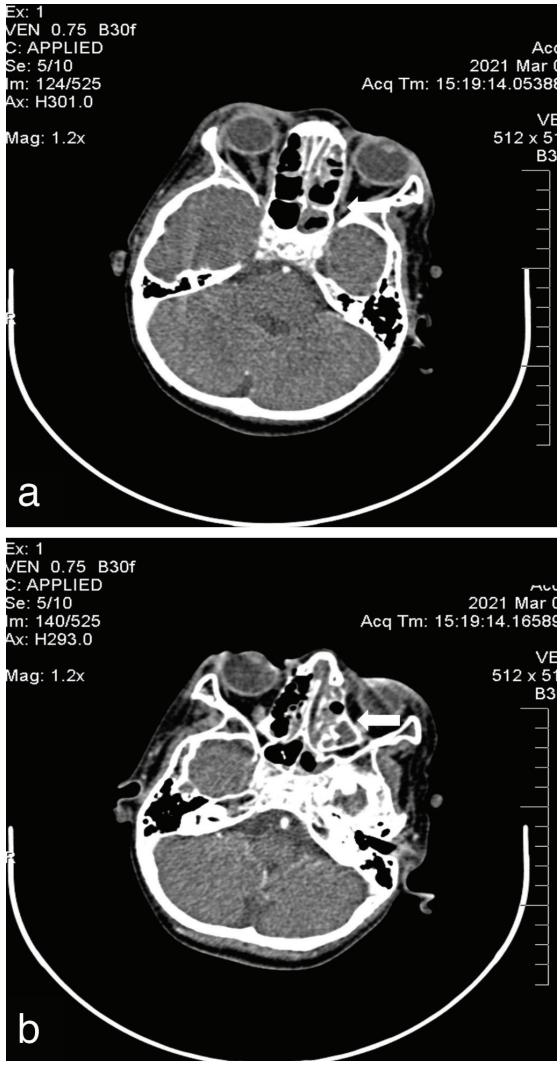

Figure 1. Brain computed tomography of the patient. Sinus involvement was noted

was no evidence of any brain space occupying mass in the brain parenchyma. There was no evidence of any nasopharyngeal mass or juvenile angiofibroma.

Surgical nasoscopy was performed to avoid bleeding. Following this, a specimen from the nasopharynx lesion was put into a fixative solution (formalin). The gross examination consisted of one dark-gray-colored tissue. One fragment of tube like tissue measuring 7.5×1.2 cm and another with a smooth surface measuring 5×2 cm was obtained for histopathology study. Histopathological investigation of the resected mass revealed actinomycotic colonies with fibrin leukocyte deposition and focal lymphoid aggregation.

Following the pathology report, the patient was initially treated with 500 mg of intravenous ceftriaxone every 12 hours for 6 days. After stabilization and control of the fever and bleeding, he was discharged and referred to an ear, nose and throat clinic for prolonged antibiotic therapy and follow-up.

Discussion

Overall, the number of cases with proven post nasal actinomyces is limited. The masses that are formed in patients with sinus actinomyces infection might look like malignant disease at first and require confirmatory work-up. The indolent nature of the organism can lead to a prolonged duration of the disease and several antibiotic trials. The masses that are formed in this type of involvement may be expansive and have bony erosions. Also, they might mimic fungal infections. The diagnosis of actinomyces should always be kept in mind when the patient has poor dental hygiene, which raises the suspicion of this infection (7).

As has been described in some cases, nasal actinomyces might present with a prolonged change in smell and purulent nasal discharge in patients without a known baseline medical condition. Also, it has been reported that nasal actinomyces can affect patients without any remarkable laboratory findings or lymphadenopathy and might look like a foreign body, although it may share radiological findings with fungal infections as well (8).

Although nasal actinomyces can be successfully managed with surgical resection and prolonged antibiotic therapy, death has also been reported following this infection. In a middle aged man with unilateral nasal obstruction, edema and pain, who had history of diabetes mellitus and hepatitis C, poor compliance with the antibiotic regimen and follow-up resulted in necrosis expansion, disseminated intravascular coagulation, multiple end organ failure and death (9).

That case highlights the importance of surgical intervention and the proper selection of the antibiotic regimen. The deceased patient did not undergo surgical debridement at first and this intervention was only performed following the dissemination of the infection and the rise of inflammatory markers. In addition, antibiotics were not given in a comprehensive way, with multiple discontinuation and adjustments.

In our case, the patient underwent surgical debridement and ceftriaxone was started and continued until discharge, at which point, the patient was referred to an out-patient clinic for further follow-up.

Dental procedures can result in an exposure to actinomyces embedded in the nasal cavity. One case described nasal actinomyces that were found following a tooth extraction which resulted in discharge and odor from the extraction site. Following these initial signs and symptoms and maxillary tenderness, the patient was evaluated with nasal endoscopy and surgical debridement followed by a prolonged course of oral antibiotics (10).

Considering that the sinus cavity is anatomically related to multiple sites and actinomyces could actually spread to it from junctional sites, it might be reasonable to rule out actinomyces infection in patients with extended signs and symptoms of sinusitis and no response to proper antibiotics. In our case, poor dental hygiene was noticeable and this may have contributed to nasal involvement.

Nasal sinus actinomyces infection may be observed following other infections that impair oxygenation of the area, such as nasal mucormycosis. In such cases, it might be difficult to distinguish between recurrent infections and new involvement with actinomyces, and so, pathologic evaluations should be done carefully as misdiagnosis can change the course of the therapy (11).

The patient presented in our case had several interesting features. All the cases discussed above were above middle age, while our patient was a 7-year-old boy. Also, all other cases had some type of risk factor which predisposed them to actinomycosis, while at the time of discharge, no confirmed immunologic or metabolic disease had been recorded for our patient and the only predisposing factor was poor oral hygiene.

In conclusion, it is worth remembering that actinomyces may infect children as well as adults and this organism should be suspected in those patients with nasal abscess, bleeding and a mass detected in radiologic studies. The process of isolation and culture of the organism can be

difficult and strong suspicion is needed in order to carry out proper laboratory investigations.

Ethics

Informed Consent: All patients are asked to sign an informed consent note at the time of hospital admission for general care.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.S., A.H., A.H.JR., F.P., Design: M.S., A.H., A.H.JR., F.P., Data Collection and/or Processing: M.S., A.H., A.H.JR., F.P., Analysis and/or Interpretation: M.S., A.H., A.H.JR., F.P., Literature Search: M.S., A.H., A.H.JR., F.P., Writing: M.S., A.H., A.H.JR., F.P.

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Case Report

An Astonishing Extrarenal Wilms Localisation; Spinal Cord

Ayşe Gülnur Tokuç[1], Nurşah Eker[1], Burcu Tufan Taş[2], Süheyla Uyar Bozkurt[3], Mustafa Sakar[4], Seda Aras[1], Kadriye Ebru Akar[3]

[1]Marmara University Faculty of Medicine, Department of Pediatric Hematology and Oncology, İstanbul, Turkey
[2]Marmara University Pendik Training and Research Hospital, Clinic of Pediatric Hematology and Oncology, İstanbul, Turkey
[3]Marmara University Faculty of Medicine, Department of Pathology, İstanbul, Turkey
[4]Marmara University Faculty of Medicine, Department of Brain and Neurosurgery, İstanbul, Turkey

ABSTRACT

Wilms' tumour is a renal tumour mostly seen during the first 5 years of life and it accounts for 95% of renal malignancies during childhood. Its origin is primitive metanephric cells and, very rarely, it may occur in places other than the kidneys. The estimated rate of nephroblastoma outside the kidneys is approximately 0.5 to 1% of Wilms' tumour cases. In this article, we report on a 3-year-old female patient who first presented with spinal dysraphism and a mass in the lumbar spinal cord with a histopathological diagnosis of nephrogenic rest, and after one year, a Wilms tumour arose in this location. This is a very rare extrarenal Wilms' tumour location. Here, we report on a case with immature renal cells located in the lumber spinal cord associated with spinal dysraphism and the development of Wilms' tumour there after one year.

Keywords: Children, Extrarenal, Wilms

Introduction

Wilms' tumour is a renal tumour mostly seen during first 5 years of life and it accounts 95% of renal malignancies during childhood. Its origin is primitive metanephric cells and, very rarely, it may occur in places other than the kidneys. The estimated rate of nephroblastoma outside the kidneys is approximately 0.5 to 1% of Wilms' tumour cases.

Wilms' tumour is one of the most common childhood solid malign tumours, mostly diagnosed during the first five years of life. 95% of renal malignancies are known to be Wilms' tumours in paediatrics, and they arise from primitive metanephric cells.

Very rarely, this tumour may occur in places other than the kidneys. Wilms' tumour located outside the kidneys without any primary tumour in the kidneys is called an extrarenal Wilms' tumour (ERWT). ERWT was first described by Moyson et al. (1) in 1961. The estimated ERWT rate is approximately 0.5 to 1% of Wilms' tumour cases. The prognosis is similar to that of renal Wilms' tumour. ERWT occurs mostly in childhood and rarely in adults (2).

Wilms' tumour may be observed outside the kidneys in two other situations: metastatic disease and Wilms' tumour arising in a teratoma. Therefore, for a diagnosis of ERWT, a metastatic lesion or teratoma should be ruled out. For this reason, it is necessary to evaluate the kidneys for primary tumour preoperatively and search the whole specimen for any teratoid element postoperatively (3).

In renal Wilms' tumours, it is known that, when the persistent intrarenal fetal nephrogenic blastemal tissue undergo oncogenic change, a tumour appears. It is thought

Address for Correspondence
Burcu Tufan Taş, Marmara University Pendik Training and Research Hospital, Clinic of Pediatric Hematology and Oncology, İstanbul, Turkey
Phone: +90 555 307 14 26 E-mail: drburcutufan@hotmail.com ORCID: orcid.org/0000-0003-4614-124X
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that, in ERWT cases, the ectopic nephrogenic rests may develop into a Wilms' tumour with the same mechanism. Several reports have described nephrogenic rests in the inguinal, retroperitoneal and lumbosacral regions (4).

In this article, we report a 3-year-old female patient who first presented with spinal dysraphism and a mass in the lumbar spinal cord with a histopathological diagnosis of nephrogenic rest, and after one year, a Wilms' tumour arose in this location. ERWT located in the spine is very rare. Thus, we report this case of a congenital Wilms' tumour associated with spinal dysraphism to increase awareness.

Case Report

A 3-year-old girl was admitted to our clinic with difficulty in walking beginning 1.5 months prior to admission. We learned that she had been operated on five times beginning at two months of age with dermal sinus and tethered cord diagnoses. However, no pathological examination was carried out on the operation specimen.

1.5 months previously, when she had difficulty in walking, she was admitted to our hospital, and an intradural mass (2.3x2x1.5 cm) was diagnosed in lumbosacral (L2-S2) spinal magnetic resonance imaging (MRI) (Figure 1). It was operated on and the mass was totally excised in our neurosurgical department with a diagnosis of intradural abscesses, but the pathological diagnosis was a solid mass containing immature renal cells. Forty percent of it was blastemal nodules. The proliferation index was 2.8%, and mitosis were rare (Figure 2).

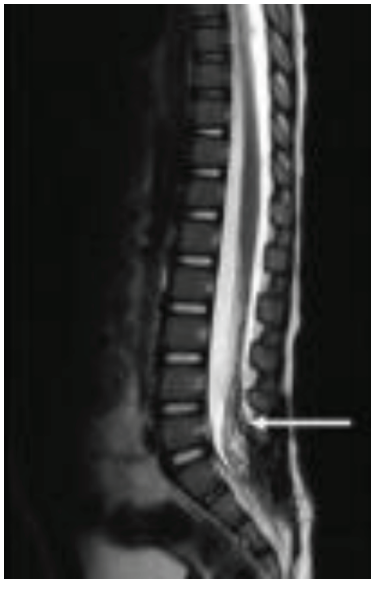

Figure 1. T2-weighted sagittal lumbosacral MR image shows a mass lesion within spinal canal between L2-S2 level (arrow)

After 1.5 months, the patient was readmitted with complaints of increased difficulty in walking, standing, and balance.

The patient was hospitalized. She had difficulty in walking, and she was incontinent for urine and stool. Physical examination revealed weakness in the lower extremities (muscle strength was 1/5 bilaterally), and deep tendon reflexes were absent. The plantar response was bilateral flexor. With these clinical findings, spinal cord compression was considered, and spinal magnetic resonance imaging was performed. Lumbar magnetic resonance imaging showed a mass at the L2-S2 level involving the spinal cord (Figure 3). Urinalysis, renal, and liver function tests were normal. Abdominal ultrasonography and MRI were normal. Surgical resection of the tumour was

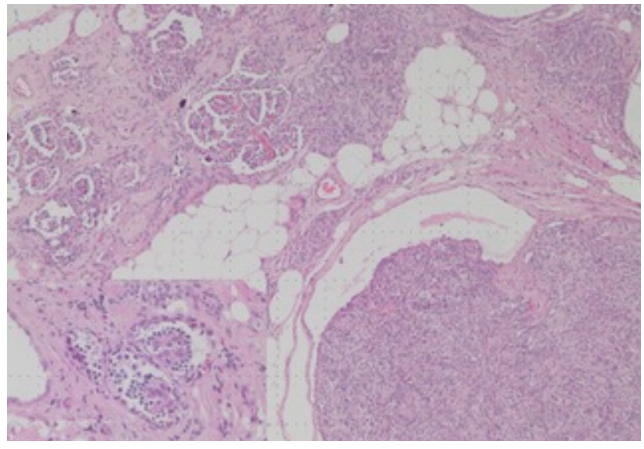

Figure 2. The lesion shows multiple immature glomeruli and tubules (upper left) and blastemal nodule (lower right) along with intervening fat tissue (H&E, X10). Immature glomeruli (H&E, X40, inside)

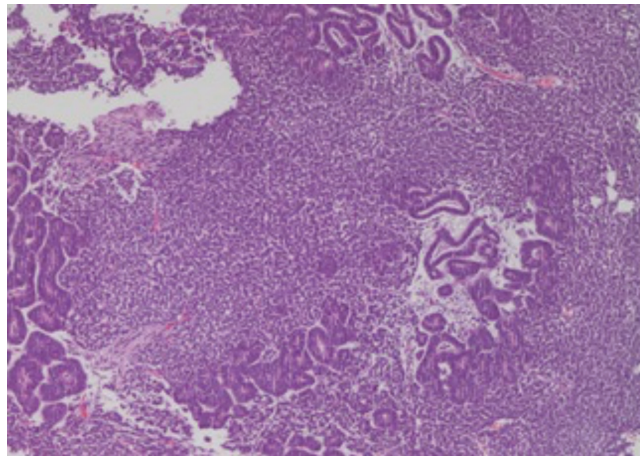

Figure 3. Contrast enhanced sagittal T1-weighted lumbosacral MR image shows the mass is enlarged, extending from L2 to S2 level (Arrow). The mass enhances markedly and there is also leptomeningeal enhancement around distal spinal cord (short arrows)

MR: Magnetic resonance

performed, but the tumour mass could not be completely removed (Figure 4). The histopathological examination of the mass revealed triphasic histology Wilms' tumour in which blastemal, stromal, and epithelial elements were present (favourable histology) (Figure 5). According to the National Wilms' Study Group protocol, the tumour was accepted as Stage III. She completed her chemotherapy and radiotherapy. She is being followed up in the oncology outpatient clinic. Physical examination reveals weakness in her right lower extremity (muscle strength 4/5). She has no incontinence.

Discussion

ERWT located at spinal cord is very rare. Wilms' tumour occurring within teratomas is another entity and it is diagnosed as teratoid Wilms' tumours pathologically (5-10). No elements of teratoma were found in our patient's specimen.

Posalaky et al. (11) described nephrogenic rests in the spine in two cases. There was no associated spinal dysraphism and the nephrogenic rests were benign with no evidence of ERWT. However, ten years later, Fahner et al. (12) described ERWT in the spine.

It is postulated that embryonic rests cause spinal dysraphism and ERWT (13). Some changes in the tissues around of these rests may cause them to proliferate and form masses that are like fetal tissues (14). Thus, according to this theory, ERWT arises from pluripotent mesenchymal cell rests which may transform into malignant masses (13,14). Fernbach et al. (15), and Grobstein (16) suggested that the embryonic central nervous system may induce nephrogenic differentiation in the embryonic mesenchyme from which the spine develops. Deshpande et al. (17) reported an ERWT within the dorsal lumbar spine's subcutaneous fat, and Horenstein et al. (18) reported nephrogenic rests in the same location.

The nephrogenic remnants in the spine, outside the kidneys, as in our case, supports the hypothesis that mesenchymal cells in the wrong place may develop into ERWT after an unknown stimulation (13).

In a review of 34 cases of ERWT, Coppes et al. (19) suggested that patients with ERWT receive postoperative chemotherapy, with the same protocol as for a renal Wilms' tumour. Sastri et al. (20) reviewed three additional cases and summarized a total of 48 cases of ERWT, and was in agreement with Coppes et al. (19).

Our patient was treated according to the latest guidelines for renal Wilms' tumour, following the National Wilms Tumour Study-V regimen for EE4A.

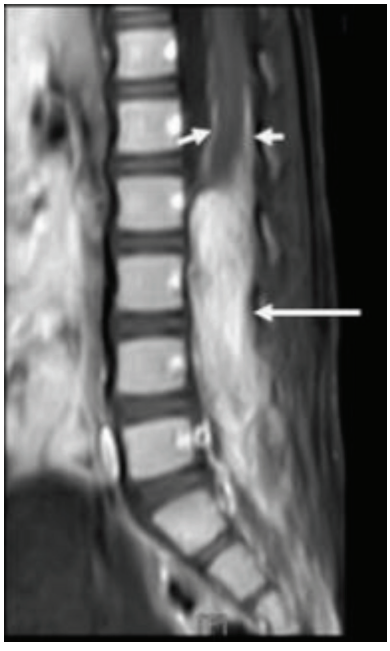

Figure 4. Contrast enhanced sagittal T1-weighted lumbosacral MR image shows post-operative defect within the mass lesion (arrow). The mass is slightly smaller than the previous MR examination. Leptomeningeal enhancement is still present (short arrows)

MR: Magnetic resonance

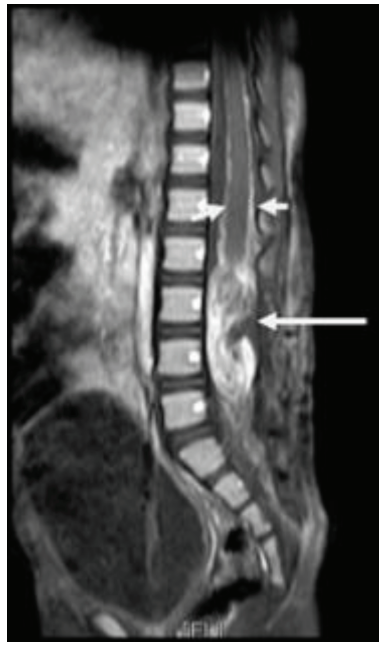

Figure 5. Diffuse blastemal component with small to medium-sized undifferentiated cells and epithelial component with tubule-like structures (H&E, X10)

Conclusion

In this article, we report a 3-year-old female patient who first presented with spinal dysraphism and a mass in the lumbar spinal cord with a histopathological diagnosis of nephrogenic rest, and after one year, a Wilms' tumour arose in this location. ERWT located in the spine is very rare. This report may explain the pathogenesis of ERWT with the embryonic rest theory.

Ethics

Informed Consent: Written informed consent was obtained from the patient's parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.G.T., N.E., B.T.T., S.U.B., M.S., S.A., K.E.A., Design: A.G.T., N.E., B.T.T., S.U.B., M.S., S.A., K.E.A., Data Collection and/or Processing: A.G.T., N.E., B.T.T., S.U.B., M.S., S.A., K.E.A., Analysis and/or Interpretation: A.G.T., N.E., B.T.T., S.U.B., M.S., S.A., K.E.A., Literature Search: A.G.T., N.E., B.T.T., S.U.B., M.S., S.A., K.E.A., Writing: A.G.T., N.E., B.T.T., S.U.B., M.S., S.A., K.E.A.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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Case Report

Asfotase Alfa Treatment in a 2-year-old Girl with Childhood Hypophosphatasia

Gönül Çatlı[1], Berna Eroğlu Filibeli[2], Handan Çelik[3], Özlem El[4], Bumin Dündar[5]

[1]İstinye University Faculty of Medicine, Department of Pediatric Endocrinology, İstanbul, Turkey
[2]University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Clinic of Pediatric Endocrinology, İzmir, Turkey,
[3]Ege University Faculty of Dentistry, Department of Pediatric Dentistry, İzmir, Turkey
[4]Dokuz Eylül University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, İzmir, Turkey
[5]İzmir Katip Çelebi University Faculty of Medicine, Department of Pediatric Endocrinology, İzmir, Turkey

ABSTRACT

Childhood hypophosphatasia (HPP) presents with bowing of the limbs, poor mobility, chronic pain, short stature, fractures, and motor impairment. Enzyme replacement therapy (ERT) provides improved pulmonary and physical function in life-threatening perinatal and infantile forms of HPP. However, treatment of those patients without life-threatening HPP is limited. This report describes the results of asfotase alfa (Strensiq®, Alexion Pharmaceuticals, Inc.) treatment in a 6-year-old girl with childhood HPP, who presented with premature loss of primary teeth, low mobility, and chronic pain in the legs. Sequence analysis of the *TNSALP* gene revealed three heterozygous variants; c.526G>A (reported previously), c.1051G>C (novel), c.787T>C (reported previously). After a four-year follow-up under ERT, a marked reduction in leg pain and restlessness was observed and physical therapy assessments showed remarkable improvements in motor function, pain score, and quality of life. The treatment decision in childhood HPP is not as clear as in infantile and perinatal forms and it is mostly based on the clinical and radiological condition of the patient. In patients with childhood HPP without severe skeletal involvement but accompanying motor retardation, ERT may improve quality of life, motor functions, and daily activities.

Keywords: Childhood hypophosphatasia, asfotase alfa, motor function

Introduction

Hypophosphatasia (HPP) is a rare inborn error of metabolism characterized by low serum alkaline phosphatase (ALP) activity (hypophosphatasaemia) due to loss-of-function mutations within the gene for the tissue-non-specific isoenzyme of ALP (*TNSALP*) (1). Inorganic pyrophosphate, pyridoxal 5'-phosphate (PLP), and phosphoethanolamine (PEA), which are natural substrates of *TNSALP*, accumulate and inhibit mineralization. HPP features remarkably broad-ranging manifestations, ranging from neonatal death due and severe skeletal hypomineralization to only dental problems in adults (1). Seven major forms have been defined according to the severity of the disease; perinatal HPP, infantile HPP, childhood HPP, adult HPP, odonto-HPP, pseudohypophosphatasia, and benign prenatal HPP (2). Childhood HPP presents with bowing of the limbs, poor mobility, chronic pain, short stature, fractures, motor impairment, and fatigue (3).

Before enzyme replacement therapy (ERT), HPP treatment was supportive and symptom-oriented (4). Asfotase alfa was first used in perinatal and infantile forms of the disease and was found to improve pulmonary and

Address for Correspondence
Gönül Çatlı, İstinye University Faculty of Medicine, Department of Pediatric Endocrinology, İstanbul, Turkey
Phone: +90 533 279 43 49 E-mail: gonulcatli@gmail.com ORCID: orcid.org/0000-0002-0488-6377
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physical functions in these patients (5). However, there is insufficient data regarding indications of ERT in childhood HPP. This report describes the results of a four-year asfotase alfa treatment in a 6-year-old girl with childhood HPP.

Case Description

A 26-month-old girl was admitted due to loss of primary teeth with intact roots, poor mobility, and chronic pain in the legs. She was born at term with a birth weight of 3,500 gr. Her teeth appeared at the age of 9 months, and the loss of teeth without caries started at two years old. She had started walking at 18 months of age and has suffered from gait disturbance and leg pain since then. She was taking non-steroidal anti-inflammatory drugs for leg pain almost every day and had no bone fractures. Her parents were unrelated. Her mother has suffered from severe tooth caries since she was nine years old. She had a history of an ankle fractures but had no skeletal problem. Several family members also had dental problems and fractures (pedigree is shown in Figure 1). At diagnosis; her weight was 9 kg [-2.57 standard deviation score (SDS)], height was 81.5 cm (-1.98 SDS), body mass index (BMI) was (-2.09 SDS), weight-for-height was 81% (-2.46 SDS) and mid-parental target height was 166.5 cm (0.51 SDS). Physical examination revealed scaphocephaly, frontal bossing, low-set ears, blue sclera, an open anterior fontanel, loss of primary teeth, and proximal muscle weakness in the lower limbs. She had difficulty in standing up, climbing stairs, and walking. Other physical examinations were normal. She was prepubertal. Repeated tests for serum ALP activity revealed low values ranging between 15 and 18 U/L (normal reference range for age and sex: 125-320 U/L). Levels of serum calcium, phosphate, thyroid, parathyroid hormones, and 25-hydroxyvitamin D were normal. Radiological examination showed tongue-like lucencies in the metaphyses of the distal femur and proximal fibula (Figure 2). Her bone age was compatible with chronological age (according to Greulich & Pyle). Bone mineral density was normal. Renal ultrasound showed no nephrocalcinosis. Ophthalmologic examination revealed normal findings. In cranial computed tomography, sagittal synostosis not requiring surgery was determined.

Results

The entire coding region of the *TNSALP* gene was sequenced using genomic DNA. Sequence analysis revealed a heterozygous variant (c.526G>A) in exon 6, a heterozygous variant (c.1051G>C) in exon 10, and a heterozygous variant (c.787T>C) in exon 7. Her asymptomatic father carried the first heterozygous variant. The mother carried both the second and third variants. The serum ALP, serum pyridoxal 5'-phosphate and urine PEA levels of the family are presented in Table I.

Physical therapy was applied; however, it did not achieve the desired benefit. In order to improve functional mobility, asfotase alfa (Strensiq®, 6 mg/kg/week) was initiated at the age of 31 months through a compassionate use program. The gross and fine motor functional assessments carried out by an expert physiatrist are presented in Table II. Enhancement in motor dexterity was observed. Complaints were markedly reduced, and analgesic medication was no longer required. No side effects were observed except for mild injection site reactions (erythema, pain, lipohypotrophy, and lipohypertrophy).

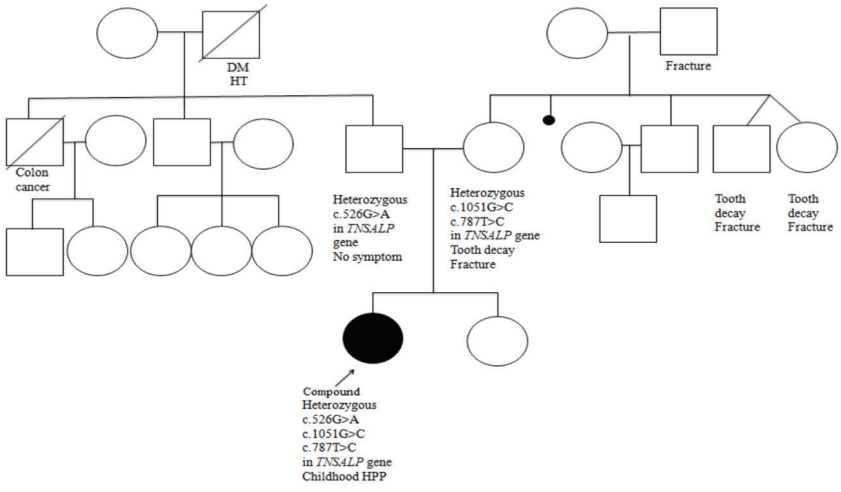

Figure 1. Pedigree of the patient

DM: Diabetes mellitus: HT: Hypertension TNSALP: Tissue-non-specific isoenzyme of alkaline phosphatase HPP: Hypophosphatasia

Pediatric dentistry treatment was given to the patient. The lower incisors were lost in the intraoral inspection (Figure 3). Preventive strategies were performed depending on the condition of the other teeth. The panoramic X-ray at the 4th year follow-up showed that the permanent teeth germs were present (Figure 3). On her last examination at six years old, her weight was 16.6 kg (-1.9 SDS), height was 108.6 cm (-1.82 SDS), BMI was (-1.07 SDS), and growth velocity was 6.3 cm/year. Similar to her peers, she could perform all physical activities such as climbing the stairs both up and down, rolling and playing games with friends.

Discussion

411 *TNSALP* mutations have been identified in HPP (http://alplmutationdatabase.hypophosphatasie.com/). Broad-ranging clinical heterogeneity is associated with the great variety of missense mutations and some mutations' dominant-negative effect. In severe forms of this disease (perinatal and infantile HPP), mutations are mostly homozygous (5) or compound heterozygous (2). Childhood HPP can either be inherited autosomal recessively or autosomal dominantly (1,6). Our patient had three

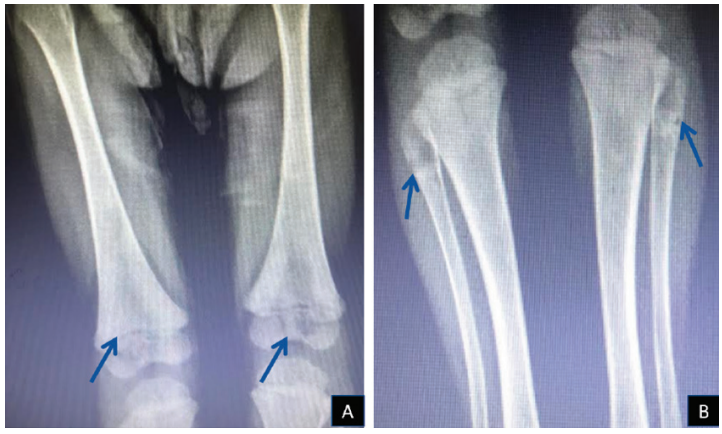

Figure 2. X-ray images of lower extremities (A, B). A. Characteristic "tongue" sign of metaphyseal radiolucencies are present in the femoral metaphysis (arrows). B. Oval radiolucent lesions and apparent physeal widening are present in the proximal fibula (arrows)

Table I. Biochemical test results

Biochemical tests	Proband	Mother	Father
Serum alkaline phosphatase (U/L)	15 U/L (N: 125-320)	12 U/L (N: 33-107)	34 U/L (N: 33-107)
Serum pyridoxal 5'-phosphate (µg/L)	740.1 µg/L (N: 5-50)	37 ug/L (N: 5-50)	66.5 ug/L (N: 5-50)
Urine phosphoethanolamine (µmol/g creatinine)	927.5 µmol/g kre (N: 33-342)	132 µmol/g kre (N: 0-48)	10.30 µmol/g kre (N: 0-48)

N: Normal range

Table II. Gross and fine motor functional assessment

Tests	Before treatment	At the six months of treatment
2-minute walk test	Uncooperative	Uncooperative
10-meter walk test	13 seconds	9.59 seconds
9-step stair climb test	With one hand support 10.44 seconds	Without support 5.07 seconds
Timed floor to stand-natural test	4.46 seconds	1.69 seconds
Nine-hole peg test **Insertion and removal times**	Right hand: 47.4/24.5 seconds Left hand: 40.3/26.3 seconds	Right hand: 23.69/26.3 seconds Left hand: 25.3/23.1 seconds
Child health assessment questionnaire	Pain score: 8.5 Disability index: 2.6	Pain score: 2.5 Disability index: 0.55
Toddler quality of life questionnaire **(Best possible score 180)**	27.7	73.6
Jumping	Absent	Present

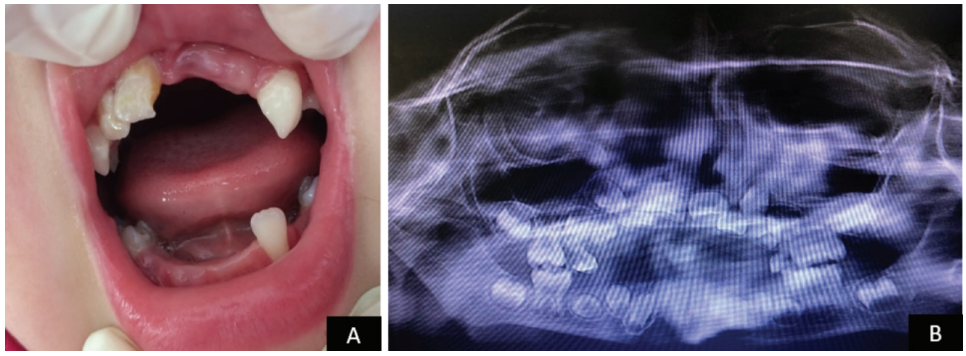

Figure 3. Intraoral appearance at diagnosis (A) and panoramic X-ray image at the 4th year of treatment (B)

heterozygous variants in the *TNSALP* gene; c.526G>A in exon 6, c.1051G>C in exon 10, c.787T>C in exon 7. The first variant, c.526G>A (p.A176T) in exon 6 was previously identified in infantile, mild, and severe childhood as well as adult and odonto-HPP, and it was shown by *in vitro* studies to possess a dominant-negative effect (7). In segregation analysis, the father of the patient carried the same heterozygous variant. This phenotypic difference could be explained by intra-familial variability in gene expression and incomplete penetrance of the variant as previously reported in HPP (8). The second variant (c.1051G>C, p.E351Q) is a novel variant and *in silico* analysis predicted it to be disease-causing (Mutation taster, http://www.mutationtaster.org) or likely pathogenic (Varsome, https://varsome.com/). The third variant (c.787T>C, p.Y263H) is a disease-associated polymorphism (9). The patient's mother was heterozygous for the second and third variants. She had suffered from multiple tooth decay and had undergone dental treatments; however, no skeletal disease was present. Based on *in silico* analyses and the mother's clinical findings, we suggest that c.1051G>C in exon 10 may be associated with odonto-HPP.

In a study evaluating 173 pediatric patients with HPP, researchers expanded the HPP classification; childhood HPP was divided into two subgroups, "mild" and "severe" according to radiological findings (2). Severe childhood HPP is characterized radiologically by diffuse osteopenia, calcification and osteosclerosis zones and clinically by muscle weakness, delayed walking, and waddling gait (2). Furthermore, lower height and weight z-scores, autosomal recessive inheritance, and the presence of two mutations in the *TNSALP* are more prominent in severe childhood HPP (2). According to this classification, our patient had severe childhood HPP due to gait disturbance, muscle weakness, low height and weight Z-scores, and compound heterozygous mutations, despite the absence of severe radiological findings.

The bone-targeted ERT, asfotase alfa (Strensiq®), was approved for pediatric-onset HPP in 2015. In life-threatening HPP (infantile and perinatal), ERT has shown improvements in skeletal findings as well as pulmonary and physical functions (5). In life-threatening HPP, it is relatively easy to make a treatment decision given the poor prognosis of the disease and the benefits of ERT. On the other hand, childhood HPP is usually not a life-threatening condition. The literature data regarding the decision on ERT in childhood HPP is not precise (3), despite significant improvements in growth, muscle strength, motor function, agility, and quality of life with five years of treatment (10). According to the clinical experience of Rush (3), ERT should be considered in the following conditions: No significant improvement in motor function with rehabilitation, pain resistant to conservative treatment, severe rickets, bone fractures, significant functional limitations without bone fracture, and short stature with insufficient growth velocity. ERT was initiated in our patient at 31 months of age owing to gross motor delay and the lack of adequate response to physical therapy. Six months after treatment had commenced, a marked reduction was observed in complaints and physical therapy assessments showed remarkable improvement in motor function, pain scores, and quality of life. She was able to participate in age-appropriate activities and keep up with her peers in the school environment. Considering the diagnosis and treatment process, we concluded that the patient benefited from this treatment.

Due to inadequately mineralized cementum anchoring the teeth to the periodontal ligament, early and painless tooth loss with intact root is one of the important findings in HPP (2). The primary dentition can be affected more than the permanent teeth. Therefore, dental problems should be followed up. There is insufficient data on the effectiveness of ERT on dental problems. Our patient had regular dental

examinations, and the permanent tooth roots were healthy in the last panoramic X-ray.

In conclusion, although HPP nosology is useful for classifying patients and using a common language, considering the disease's wide clinical presentation, individual evaluation may be better for treatment decision. Since impaired growth and developmental delay will result in a decreased quality of life and an inability to perform daily activities effectively, ERT should also be considered in patients without significant radiological findings, such as in our case.

Ethics

Informed Consent: Informed consent was obtained from the patient's parent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.Ç., B.E.F., H.Ç., Ö.E., B.D., Design: G.Ç., B.E.F., H.Ç., Ö.E., B.D., Data Collection and/or Processing: G.Ç., B.E.F., H.Ç., Ö.E., B.D., Analysis and/or Interpretation: G.Ç., B.E.F., H.Ç., Ö.E., B.D., Literature Search: G.Ç., B.E.F., H.Ç., Ö.E., B.D., Writing: G.Ç., B.E.F., H.Ç., Ö.E., B.D.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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Case Report

Successful Treatment of Refractory Graft-Versus-Host Disease with Ruxolitinib in a Child after Autologous Stem Cell Transplantation

Nurşah Eker[1], Burcu Tufan Taş[2], Ömer Doğru[1], Emel Şenay[2], Ayşe Gülnur Tokuç[1], Ahmet Koç[1]

[1]Marmara University Faculty of Medicine, Department of Pediatric Hematology Oncology, İstanbul, Turkey
[2]Marmara University, Pendik Training and Research Hospital, Clinic of Pediatric Hematology Oncology, İstanbul, Turkey

ABSTRACT

Autologous hematopoietic stem cell transplantation (AHSCT) is an increasingly used curative treatment for some solid tumors in children. Instead of allogeneic transplantation, the risk of developing graft-versus-host disease (GvHD) is much lower after AHSCT. Although the clinical findings of auto-GVHD are mild and self-limited in most cases, rare cases may be severe and need intensive immunosuppressive treatment. Here, we present a case who underwent autologous HSCT due to relapsed neuroblastoma, developed steroid-refractory GvHD after AHSCT, and achieved remission using ruxolitinib. A 12 years old female patient was diagnosed with relapsed neuroblastoma. After metaiodobenzylguanidine treatment, AHSCT was performed, and the status of the disease was a very good partial response at the time of transplantation. Our patient was diagnosed with severe and steroid-refractory GvHD with skin involvement after AHSCT. We used ruxolitinib with extracorporeal photopheresis because of the essential side effects of the other drugs and got a very good response. Over the following five months, there was no recurrence of GvHD. She was in complete remission of neuroblastoma after two years of AHSCT. It is crucial to keep in mind that GvHD may develop after AHSCT. Ruxolitinib is an effective treatment for GvHD also after AHSCT. Further studies and case reports are needed to understand the disease's pathogenesis and regulate appropriate treatment.

Keywords: Autologous stem cell transplantation, children, graft versus host disease, ruxolitinib, steroid-resistant

Introduction

Hematopoietic stem cell transplantation (HSCT) is an increasingly used curative treatment for many benign/malignant hematological diseases, some solid tumors, immunodeficiencies, and various metabolic and autoimmune diseases in childhood. One of the major complications of this curative treatment is graft-versus-host disease (GvHD). GvHD is an immune dysregulation condition caused by inflammatory cytokines resulting from the activation of donor T-cells (1). This complication, which can be fatal, is often expected after allogeneic HSCT. While it occurs in 50% of patients after allogenic transplantation (2), the risk of developing GvHD after autologous HSCT (AHSCT) is much lower. In the literature, AHSCT has been reported in adults, especially with multiple myeloma (1) and a few cases in pediatric patients who underwent autologous transplantation. Disruption of thymic-dependent immune reconstitution and failure of peripheral self-tolerance are considered in its pathophysiology (2). The main therapy of GvHD is immunosuppressive treatment.

Address for Correspondence
Burcu Tufan Taş, Marmara University, Pendik Training and Research Hospital, Clinic of Pediatric Hematology Oncology, İstanbul, Turkey
Phone: +90 555 307 14 26 E-mail: drburcutufan@hotmail.com ORCID: orcid.org/0000-0003-4614-124X
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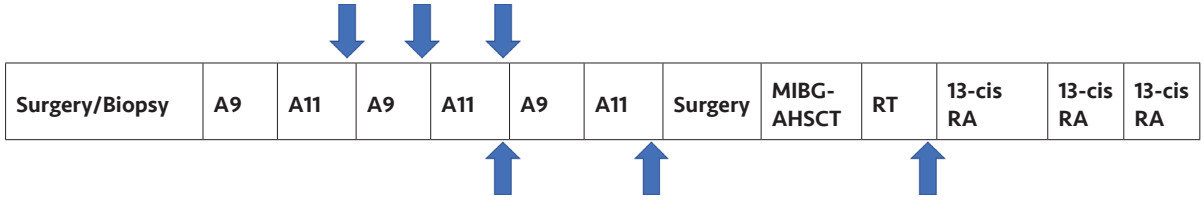
Although steroid is the first line therapy, cyclosporine A, tacrolimus, mycophenolate mofetil, photopheresis, and other immunosuppressive treatments can be used in steroid refractory cases. Another agent currently used in the treatment of steroid refractory GvHD after allogeneic HSCT is Ruxolitinib, which is an inhibitor of Janus kinases 1/2. Ruxolitinib was developed for the treatment of myeloproliferative disease, however, it has also been used successfully in the treatment of GvHD (3).

Here, we present a case who underwent AHSCT due to relapsed neuroblastoma, developed steroid refractory GvHD after AHSCT and achieved remission using Ruxolitinib. Written consent was obtained from patient's family for this case.

Case Report

A 12-year-old female was diagnosed with stage 1, low-risk neuroblastoma after total excision of a right suprarenal gland mass and was followed up without treatment according to our national treatment protocol; Turkish Pediatric Oncology Group Neuroblastoma 2009 (TPOG 2009). Eight months after diagnosis, she had a relapse due to multiple bone involvement of stage IV, high-risk group. Chemotherapy protocol was started according to the TPOG-2009 protocol (Table I), AHSCT was planned. During the treatment, the patient complained of a painless, immobile mass in the right parietal area. Cranial imaging showed bilateral, new bone metastasis. Her chemotherapy protocol was changed to ICE (Ifosfamide, Carboplatin, Etoposide). After metaiodobenzylguanidine treatment, AHSCT was performed (cell dose of $5.14x10^6$ CD34+ cells/kg), the status of the disease was very good partial response at the time of transplantation. The conditioning regimen for AHSCT were melphalan (140 mg/m^2), and busulfan (3.2 mg/kg/days-IV). Prophylactic defibrotide (25 mg/kg/g) for sinusoidal obstruction syndrome was used and prophylactic fluconazole, acyclovir, and ciprofloxacin were used. The patient was monitored weekly for Epstein-Barr virus (EBV) polymerase chain reaction, cytomegalovirus (CMV) pp65 antigen, and galactomannan antigen. She had neutrophil engraftment at day +12, platelet engraftment on day +13. On day 30, widespread, itchy erythematous macules, and lichenoid papular rashes developed in both cheek regions. There was no mucosal involvement or history of using new drugs in the prior two weeks. In laboratory tests, moderate thrombocytopenia was detected. Viral serological tests (EBV, CMV, TORCH, parvovirus, hepatitis markers) were negative. Her prophylactic antibiotics were changed to exclude possible adverse drug interactions at diagnosis, and antihistamine (H1 blocker) was initiated for the symptoms. Punch biopsy was performed because the skin rashes progressed to the hands and feet (Figure 1). Histopathological examination of skin biopsy showed

Table I. High risk neuroblastoma chemotherapy protocol in Turkey, TPOG 2009



Surgery/Biopsy	
A9-A11 (3 cycles chemotherapy)	A9: Vincristine, Dacarbazine, Ifosfamide, Adriamycin A11: Cyclophosphamide, Etoposide, Cisplatin
Surgery	
MIBG	Metaiodobenzylguanidine treatment
AHSCT	Autologous stem cell transplantation
RT	Radiotherapy
13- cis Retinoic acid treatment (3 cycles)	
Stem cell collection	Stem cell collection should be done after 1 cycle of A9- A11 chemotherapy, if necessary after other A9 or A11 chemotherapy
Evaluation	Evaluation should be done after 2 and 3 cycles of A9-A11 chemotherapy, and before the 13- cis retinoik acid treatment

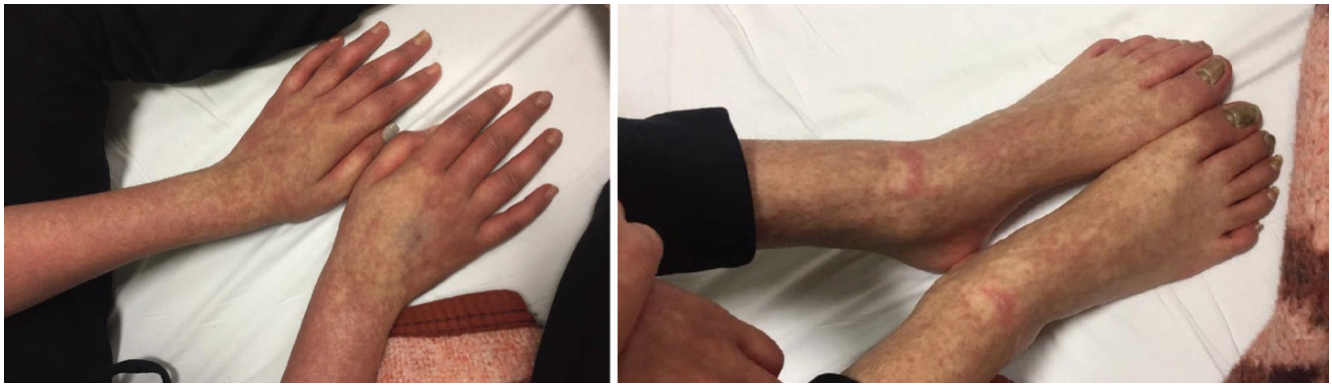


Figure 1. Images of the erythematous and edematous rash on the hands and feet

vacuolar degeneration in the epidermis, lymphocyte exocytosis, necrotic keratinocytes, and superficial perivascular mild-chronic inflammation in the dermis, compatible with acute GvHD. Eosinophil was not detected. Methylprednisolone was started (2 mg/kg/day). While receiving steroid therapy, the skin lesions progressed. The patient was diagnosed with steroid-refractory GvHD, and tacrolimus was added to the treatment. On the 15th day, tacrolimus was discontinued due to elevated liver enzymes and replaced by cyclosporine A. Liver enzyme levels went back to normal. In addition, extracorporeal photopheresis was applied on two consecutive days a week because the reactive skin lesions and sclerosis were compatible with chronic GvHD. The skin findings regressed but were not resolved under cyclosporine-extracorporeal photopheresis. After 8 weeks of photopheresis treatment, it was reduced to two consecutive days, biweekly. Cyclosporine treatment was discontinued due to a high creatinine level, and low glomerular filtration rate. However, we could not discontinue immunosuppressive therapy due to the progressive skin lesions. We added ruxolitinib to the treatment. The skin lesions of our patient regressed under ruxolitinib treatment. There was no severe side effect except for mild hyperlipidemia. The initial dose of ruxolitinib was 5 mg twice a day (B.I.D), and this was increased to 10 mg B.I.D after being well-tolerated. In the 9th month of treatment, the dosage was decreased to 5 mg B.I.D due to hyperlipidemia and it was stopped at the end of the first year. Photopheresis treatment was reduced to two consecutive days monthly after four months, and discontinued after 10 months of the treatment. Over the following 5 months, there was no recurrence of GvHD. She was in complete remission from neuroblastoma after the two years of AHSCT.

Discussion

GvHD is a rare complication of post-AHSCT which especially affects the skin, gastrointestinal tract, and liver (1). It can be spontaneous or induced for antitumor response. There are some reports stating that spontaneous GvHD can be seen after AHSCT, especially in adults with multiple myeloma (1), but only a few reports in children (4). To the best of our knowledge, our case is the third pediatric case in the literature with GvHD after auto-HSCT due to neuroblastoma.

One of the ideas regarding the pathogenesis of GvHD after AHSCT is that some chemotherapeutics, which are used before transplantation, induce changes in regulatory T cell functions, leading to a failure in the development of self-tolerance (4). It has been reported that GvHD can be seen especially after using melphalan in the conditioning regimen (4). Our patient had received melphalan prior to HSCT, and so, for our case, this may be one of the causes of auto-GvHD. The other hypothesis for auto-GvHD is the transfer of maternal cells during the fetal development period and the presence of these cells in circulation resulting in microchimerism. Microchimerism can also be caused by blood transfusions (5). Our patient received many blood transfusions but transfusion-related GvHD was not considered as the blood products were transfused after irradiation and leukocyte filter.

The most common involvement in acute GvHD is skin involvement but gastrointestinal and liver involvement can be part of auto-GvHD as well. In our patient, transaminase levels were elevated after tacrolimus treatment. These returned to normal levels after stopping the treatment, so we thought that this was a side effect of tacrolimus, not liver GvHD. In a study by Hood et al. (6), skin involvement was detected in 8% of patients. The majority of these patients

did not require any treatment and the skin lesions were self-limited. Despite being mostly mild and self-limited, auto-GvHD can be severe or life-threatening (1). In our case, GvHD was severe, and refractory to steroid treatment. As she had new skin lesions and sclerosis during the treatment, we opted for tacrolimus/cyclosporine. Photopheresis treatment was started. However, we had to change the treatment because of some side effects due to tacrolimus and cyclosporine. The other effective agent for GvHD is ruxolitinib. There are some reports in the literature. One of these reports described a response rate of 100% in eight patients with GvHD (7). In another study evaluating its effect on childhood GvHD, the overall response rate was found to be 77% in acute, and 89% in chronic GvHD (7). Uygun et al. (8) reported on 29 pediatric patients with steroid refractory acute or chronic GvHD treated with Ruxolitinib, resulting in 82% and 80% response rates respectively. In May 2019, the FDA approved Ruxolitinib for the treatment of adult patients and pediatric patients aged 12 years or older with steroid refractory acute GvHD (9). However, there were no reports associated with Ruxolitinib treatment of autologous GvHD in children. We used Ruxolitinib with extracorporeal photopheresis due to significant side effects from other drugs. A good response was achieved.

In terms of treatment response, our patient was the first pediatric patient who received Ruxolitinib treatment in addition to photopheresis for auto-GvHD. Ruxolitinib can be preferred, especially in steroid-resistant cases, and it shows a good treatment response. Although our patient had severe GvHD, we think that the graft versus tumor effect also contributed to the remission of her primary disease.

Conclusion

It is important to keep in mind that GvHD may develop after AHSCT. Although the clinical findings of auto-GVHD are mild, and self-limited in most of cases, they can be severe and require intensive immunosuppressive treatment in rare cases. Ruxolitinib is an effective treatment for GvHD after AHSCT. Further studies and case reports are needed to understand the pathogenesis of this disease and to determine appropriate treatment.

Ethics

Informed Consent: Written consent was obtained from patient's family for this case.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.E., B.T.T., Ö.D., E.Ş., A.G.T., A.K., Design: N.E., B.T.T., Ö.D., E.Ş., A.G.T., A.K., Data Collection and/or Processing: N.E., B.T.T., Ö.D., E.Ş., A.G.T., A.K., Analysis and/or Interpretation: N.E., B.T.T., Ö.D., E.Ş., A.G.T., A.K., Writing: N.E., B.T.T., E.Ş.

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