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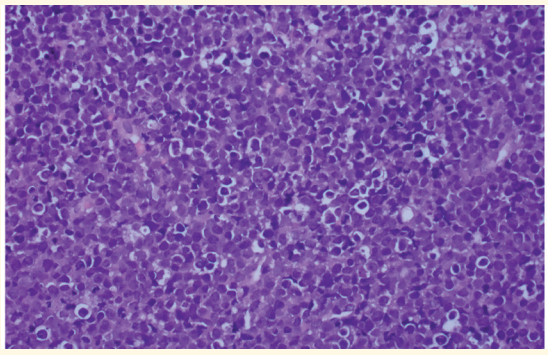

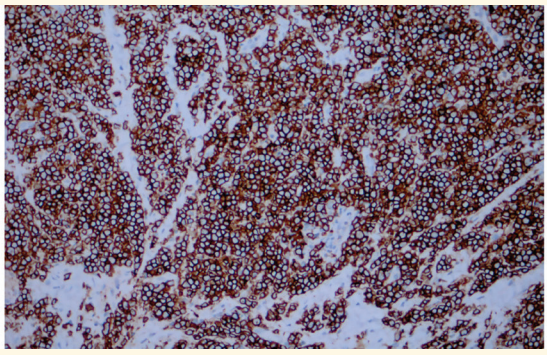

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PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med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

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Acknowledgements: Any technical or financial support or editorial contributions (statistical analysis, English evaluation) towards the study should appear at the end of the article.

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Editorial

"When I had all the answers, the questions changed..."
Paulo Coelho

Dear Readers,

The Coronavirus disease-2019 (COVID-19) outbreak has caused major changes in daily life and routine activities in societies all over the world. Different problems came up for all physicians related to child health, both mentally and physically. Multisystem inflammatory syndrome in children (MIS-C) and MIS-N in the newborn is a rare but severe condition resulting in excessive response of the immune system after SARS-CoV-2 infection. Fortunately, SARS-CoV-2 often causes mild illness in children. MIS-C is characterized by multisystemic symptoms similar to previously known diseases such as Kawasaki disease, toxic shock syndrome and macrophage activation syndrome. Another situation that pediatricians burdening in this period are the delay in the admission of patients to hospitals due to Lockdown rules and parental concerns about COVID-19 exposure during the pandemic period, causing diagnosis delays in children and inadequate treatment approaches.

We are proud to present the second issue of 2021, which includes valuable scientific studies on childhood diseases. In this issue, we present 14 original articles, three case reports and a letter to editor, which were meticulously written from different disciplines on both mental and physical (from tooth to toe) child health.

We would like to send our respects to all healthcare professionals who lost their lives due to the COVID-19 pandemic.

Sincerely
Prof. Dr. Betül Sözeri

J Pediatr Res 2021;8(2):102-9
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Original Article

A Novel Molecular Indicator for Inhibitor Development in Haemophilia A

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ABSTRACT

Aim: Previous studies have reported inhibitor development (ID) risk in those patients who have hemophilia A (HA) with missense mutations to be 3-10%. We investigated the association between ID risk and various features of missense mutations; including the impact directly related to amino acid group change.

Materials and Methods: Missense mutations in the *F8* gene, clinical findings of the patients including severity of HA, and ID status were obtained from the *F8* gene variant database (http://www.factorviii-db.org/). Twenty amino acids were then classified into groups according to their side chains. All information regarding each specific mutation, as well as any impact of the mutation on the amino acid group change, was recorded. Additionally, localization (at which domain) of any changed amino acid in the F8 protein was noted. Combined Annotation Dependent Depletion (CADD), Rare Exome Variant Ensemble Learner (REVEL), Mendelian Clinically Applicable Pathogenicity and Deleterious Annotation using Neural Networks scores were applied to identify a significant cut-off value indicative of ID.

Results: Three variations were identified that could be considered as useful in the prediction of ID in mild HA. The first being that among mild HA patients, 7.9% (n=70/883) with mutations causing no amino acid group changes showed ID. This rate, however, was only 2.9% in patients with mutations leading to amino acid group changes. Secondly; in patients with mutations causing no amino acid group changes affecting A2, A3 and C2 domains, ID risk was found to be higher than in patients with mutations leading to amino acid group changes. Thirdly; an association between ID and CADD and REVEL scores was observed.

Conclusion: In mild HA patients, the characteristics of missense mutations in terms of amino acid group changes, and CADD and REVEL scores could be of considerable utility in the prediction of ID risk.

Keywords: Hemophilia A, inhibitor, *F8* gene, mutation, missense, interpretation

Introduction

The development of neutralizing antibodies against Factor VIII (FVIII) is a serious complication to the early stages of replacement therapy in hemophilia A (HA). This is known as inhibitor development (ID). The overall incidence of ID is 20-30% (1). The mechanisms underlying ID are very complex and full understanding remains elusive. Risk factors that carry the potential for ID are classified simply

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into two groups: modifiable and unmodifiable. The most critical unmodifiable risk factors relate to the type of the causative mutation in the *F8* gene, and the clinical severity (2-4). The major modifiable risk factors tend to be age at first treatment and the source of FVIII concentrate (5,6).

To date, a number of studies have investigated the complex pathophysiological mechanisms leading to the development of FVIII inhibitors. Relationships between the type of F8 mutation and the risk of inhibitor formation have been extensively discussed in recent years. In 1995, Schwaab et al. (2) reported that cumulative incidences of ID in severe HA patients carrying large deletions, non-sense mutations, and intron 22 inversions were 35.7%, 38.4% and 34.4% respectively. However, in those patients with missense F8 mutations, this incidence was 4.3% (2). Several other studies on ID risk for missense F8 mutations confirmed these findings with reports of prevalence between 3 and 10% (7,8).

It is thought that the position and type of substitution of missense mutations may influence the risk of ID. The INSIGHT study analyzed the association between F8 mutation and ID in 1.112 patients with non-severe HA. From a total of 214 different F8 missense mutations identified, 19 were reported to be associated with ID (p.Leu412Phe, p.Arg531Cys, p.Arg593Cys, p.Asn618Ser, p.Pro1761Gln, p.Phe1775Val, p.Arg1781Gly, p.Pro1854Leu, p.Arg1997Trp, p.Asp2074Gly, p.Phe2101Cys, p.Tyr2105Cys, p.Arg2150His, p.Arg2159Cys, p.Glu2228Asp, p.Trp2229Cys, p.Val2232Ala, p.His2309Asp, p.Ter2333Cys) (9). It remains unclear why these specific missense mutations carry a higher risk for ID.

In this study, we investigated the association between ID risk and various features of missense mutations including those caused by amino acid group change.

Materials and Methods

Missense mutations in the *F8* gene, along with the patients' clinical findings (including severity of HA and ID status) were obtained from the *F8* gene variant database (http://www.factorviii-db.org/) via The European Association for Haemophilia and Allied Disorders. Any cases with mutations corelating to those previously found to be associated with ID in the INSIGHT study (19 mutations) were excluded (9).

Twenty amino acids make up structural proteins. They can be classified into two major groups based on their side chains: non-polar hydrophobic and polar. The polar group is then further classified into 3 subgroups: basic, acidic and polar uncharged (Table I). All information regarding each mutation, including whether the mutation caused an amino acid group change, was recorded; this included the localization (at which domain) of the changed amino acid in the F8 protein.

In silico protein modelling programs were used to interpret the effects of a mutation at the protein level. Almost all such programs give probability scoring ranging from benign to pathogenic for the effect of a certain mutation. Each have a unique algorithm and cut-off value. In this study, we used Combined Annotation Dependent Depletion (CADD), Rare Exome Variant Ensemble Learner (REVEL), Mendelian Clinically Applicable Pathogenicity (M-CAP) and Deleterious Annotation using Neural Networks (DANN) scores to find a significant cut-off value and specific markers indicative of ID (10-13).

Statistical Analysis

Statistical package IBM SPSS version 25 (IBM Corp., Armonk, NY, USA) was used to analyze the data. The chi-squared test was used to compare differences in the categorical data between groups and $p<0.05$ was regarded as statistically significant. The two-sample independent t-test was used for the numeric data; and then to define a cut-off value ROC analysis was performed in data found statistically significant. All hypothesis tests were carried out based on a 0.05 significance level.

Results

All recorded cases prior to May 2019 in the *F8* gene variation database (http://www.factorviii-db.org/) were screened and 3,248 different cases with 954 different missense mutations possessing the necessary study criteria were recruited. The missense mutations recruited were in 607 different points in the cDNA of the *F8* gene. Of the total 3,248 cases, 3,078 contained information regarding the clinical severity of the disease, and 2,251 had information regarding ID. When cases were evaluated based on clinical severity, 1.717 (55.8%) had mild HA, 639 (20.8%) moderate, and 722 (23.5%) severe HA. Thus, of all 3,078 cases, 76.5% (n=2,356) were considered mild or moderate HA. There were 2,251 cases possessing information regarding inhibitor status. From among these, 157 (7%) cases were recorded as being positive for ID against the FVIII protein. There were 2,207 cases in which information about both clinical phenotype and inhibitor information was present. Of these, 153 (6.9%) had been reported as being inhibitor positive. Using a 4-group classification (including subgroups of amino acids) (Table I), evaluation according to whether substitution caused changes in the amino acid class was

Table I. Amino acid classification according to their side chains	
Amino acid class	**Amino acids**
Class 1: Non-polar Hydrophobic	Ala, Val, Leu, Ile, Met, Pro, Phe
Class 2a: Polar uncharged	Ser, Tyr, Asn, Gln, Cys, Thr, His, Gly
Class 2b: Acidic	Asp, Glu
Class 2c: Basic	Lys, Arg

made. Results showed that mutations causing no amino acid group changes had a higher association with ID (p=0.012). The ID rate was 8.9% (n=67/755) in cases with mutations resulting in no changes in amino acid groups, while it was 6.0% (n=90/1,496) in cases with mutations causing amino acid group changes. The difference between these two groups increased when the cases differed in terms of clinical severity. In mild HA, 9.8% (n=44/451) of patients with mutations causing no amino acid group changes had inhibitors while inhibitors were seen in only 4.8% (n=34/709) of patients with mutations causing amino acid group changes (p=0.001). In moderate and severe HA patients, no significant difference between these two groups was observed (Table II).

Using 2 group classifications for the evaluation of missense mutations according to whether substitution caused changes in the amino acid class, it was observed that mutations causing no amino acid changes had a higher relationship with ID in mild HA cases than those mutations resulting in amino acid changes (p=0.001). Among mild HA patients, 7.9% (n=70/883) with mutations causing no amino acid group changes showed ID. This rate, however, was only 2.9% in those patients with mutations leading to amino acid group changes. In moderate and severe HA, no statistical difference was detected between the groups, similar to the 4-group classification (Table II).

In this study, associations between affected domains and ID were also investigated. There was no ID in patients having mutations affecting A1, A2, A3 and SP domains of the protein. Those patients with mutations affecting C1 (11.4%) and C2 (10.8%) domains had the highest risk. The rates were 7.3%, 7.2%, and 3.1% in patients with mutations affecting A2, A3 and A1 domains respectively.

After combining the information in this study; including clinical severity and amino acid group changes, associations between affected domains and IDs were reevaluated. Using the 4-group classification, in mild HA patients with mutations causing no amino acid group changes affecting A2, A3 and C2 domains, ID risk was found to be higher than for those patients with mutations leading to amino acid group changes; ID rates were 10.4% (n=18/173) versus 3.7% (n=7/188) for the A2 domain (p=0.012), 7.6% (n=7/92) versus 1.4% (n=2/142) for the A3 domain (p=0.016) and 20.8% (n=11/53) versus 7.3% (n=7/96) for the C2 domain (p=0.016) (Table III).

To identify a significant cut-off value and a specific marker indicative of ID, we used CADD, REVEL, M-CAP, and DANN scores; breaking down the cases according to clinical phenotype. No significant association between M-CAP or DANN scores and ID was noted. However, in mild/moderate HA cases, CADD and REVEL scores were found to be associated with ID. To establish a cut-off value indicative of ID in mild/moderate HA cases, ROC analysis was applied. For CADD and REVEL scores, the area under the curve (Table IV) (Figure 1) was found to be significant.

Discussion

Herein, we showed for the first time in the literature that, in mild HA, there could be significant relationship between whether a mutation causes amino acid group change and its impact on ID.

It is commonly regarded that the degree of severity of the disease represents an important factor for the occurrence of ID; and in severe hemophilia, factors affecting ID are well-known. These include familial predisposition, mutation type, human leucocyte antigen class II polymorphism, immunological factors, and environmental factors such as surgery and trauma (14-16). However, it is considered that genetics play the most crucial role when it comes to mutation type. The treatment protocols for patients are designed only after considering all these factors.

It has been reported that there is approximately a 3-10% risk of ID in mild hemophilia cases. Most of the mild HA cases with inhibitors have found to be missense mutations. However, in these cases, no predictive data related to features of the mutations in the *F8* gene in terms of ID has been reported (17). In this study, based on information obtained from the F8 variant database, the risk of ID in mild HA cases was determined to be approximately 7%.

Table II. Relationship between inhibitor development and changes in amino acid class due to missense mutation. In this table, statistics in the four-group classification indicate changes between non-polar hydrophobic, basic, acidic and polar uncharged amino acids and statistics in the two-group classification indicate changes between non-polar hydrophobic and polar amino acids.

All patients (n=2,251)		Changes in amino acid class (Four-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	688 (91.1)	1,406 (94)	0.012
	Yes	67 (8.9)	90 (6.0)	
In mild cases (n=1,160)		Changes in amino acid class (Four-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	407 (90.2)	675 (95.2)	0.001
	Yes	44 (9.8)	34 (4.8)	
In mild cases (n=1,160)		Changes in amino acid class (Two-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	813 (92.1)	269 (97.1)	0.001
	Yes	70 (7.9)	8 (2.9)	
In moderate cases (n=496)		Changes in amino acid class (Four-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	123 (96.1)	345 (93.8)	0.322
	Yes	5 (3.9)	23 (6.3)	
In moderate cases (n=496)		Changes in amino acid class (Two-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	326 (94.8)	142 (93.4)	0.549
	Yes	18 (5.2)	10 (6.6)	
In severe cases (n=551)		Changes in amino acid class (Four-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	135 (89.4)	369 (92.3)	0.286
	Yes	16 (10.6)	31 (7.8)	
In severe cases (n=551)		Changes in amino acid class (Two-group classification)		**p-value**
		No (%)	Yes (%)	
Inhibitor development	No	352 (91.9)	152 (90.5)	0.322
	Yes	31 (8.1)	16 (9.5)	

Table III. Relationship between inhibitor development and changes in amino acid class due to missense mutation in specific domains (in this table, A2, A3 and C2 domains are shown)

Domain A2		**Changes in amino acid class (Four-group classification)**		
In mild cases (n=361)		No (%)	Yes (%)	**p-value**
Inhibitor development	No	155 (89.6)	181 (96.3)	0.012
	Yes	18 (10.4)	7 (3.7)	
In moderate cases (n=98)		No	Yes	**p-value**
Inhibitor development	No	31 (93.9)	62 (95.4)	0.759
	Yes	2 (6.1)	3 (4.6)	
In severe cases (n=112)		No	Yes	**p-value**
Inhibitor development	No	38 (88.4)	62 (89.9)	0.805
	Yes	5 (11.6)	7 (10.1)	
Domain A3		**Changes in aminoacid class (Four groups classification)**		
In mild cases (n=234)		No	Yes	**p-value**
Inhibitor development	No	85 (92.4)	140 (98.6)	0.016
	Yes	7 (7.6)	2 (1.4)	
In moderate cases (n=110)		No	Yes	**p-value**
Inhibitor development	No	32 (97.0)	74 (96.1)	0.824
	Yes	1 (3)	3 (3.9)	
In severe cases (n=101)		No	Yes	**p-value**
Inhibitor development	No	16 (84.2)	67 (81.7)	0.797
	Yes	3 (15.8)	15 (18.3)	
Domain C2		**Changes in aminoacid class (Four groups classification)**		
In mild cases (n=149)		No	Yes	**p-value**

Table III. contiuned

Inhibitor development	No	42 (79.2)	89 (92.7)	0.016
	Yes	11 (20.8)	7 (7.3)	
In moderate cases (n=62)		No	Yes	**p-value**
Inhibitor development	No	5 (83.3)	49 (87.5)	0.772
	Yes	1 (16.7)	7 (12.5)	
In severe cases (n=64)		No	Yes	**p-value**
Inhibitor development	No	8 (100)	52 (92.9)	0.435
	Yes	0 (0)	4 (6.3)	

Table IV. (A) The comparison between interpretation scores and inhibitor development and (B) cut-off values of interpretation scores and their sensitivity/specificity in those patients with mild/moderate HA.

(A) Scores	**Inhibitor development**	**n**	**Mean**	**Standard deviation**		**p-value**	
CADD phred	No	1,550	27.87	±5.23		0.000	
	Yes	106	29.80	±4.05			
CADD raw	No	1,550	5.74	±1.51		0.000	
	Yes	106	6.41	±1.15			
REVEL score	No	1,550	0.84	±0.11		0.000	
	Yes	106	0.89	±0.08			
(B) Scores	**Susceptible to inhibitor development if greater than or equal to**	**Sensitivity**	**Specificity**	**LR+**	**LR-**	**Area under the curve**	**p-value**
CADD phred	28.05	0.65	0.60	1,599	0.585	0.634	0.000
CADD raw	6.11	0.65	0.61	1,647	0.573	0.640	0.000
REVEL score	0.87	0.65	0.62	1,681	0.566	0.642	0.000

LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, CADD: Combined Annotation Dependent Depletion, REVEL: Rare Exome Variant Ensemble Learner

The risk factors for ID in severe hemophilia are well-known; however in mild hemophilia, these influencing factors remain unclear. Although a family history of ID and treatment-related factors have been reported as risk factors, the specific mutation features have not been closely investigated (18). Certain *F8* gene missense mutations contribute to the development of inhibitors in patients with mild hemophilia, sometimes up to levels observed in patients with the severe form of HA disease (7,14). This association with F8 mutations was first demonstrated in a cohort study by Eckhardt et al. (16). In that study of 128 patients with mild HA and 10 patients with moderate HA, of the ten subjects that developed inhibitors, eight carried the Arg593Cys mutation. Eckhardt et al. (9), further investigated the results of the INSIGHT study, cross referencing a registry involving 34 hemophilia treatment centers across 11 countries. In their study, from among a total of 214 different F8 missense mutations, 19 were found to be associated with

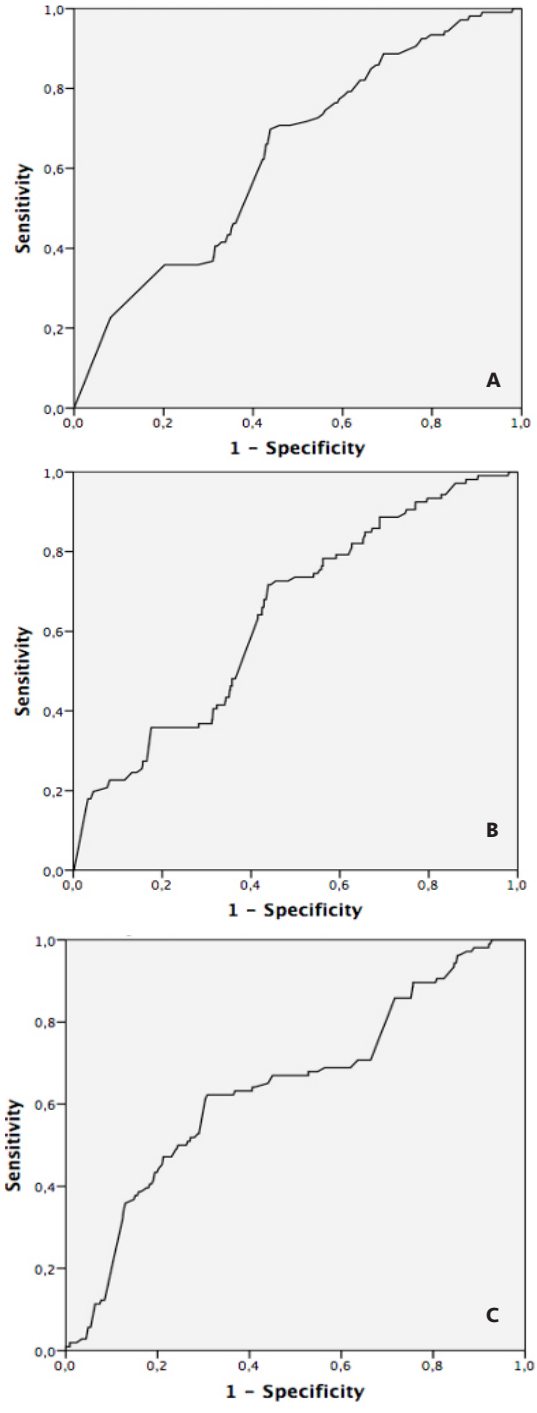

Figure 1. ROC analysis of A) CADD phred score B) CADD raw score C) REVEL score.

CADD: Combined Annotation Dependent Depletion, REVEL: Rare Exome Variant Ensemble Learner

ID (The INSIGHT Study). However, to date, no association between the different features of missense mutations and ID risk had been studied.

In this study, we detected 2 features offering potential for the prediction of inhibitor risk development in mild HA cases caused by missense mutation. Firstly; if the missense mutation found in a mild HA patient does not cause amino acid group change, ID risk increases. Secondly; missense mutations causing no amino acid group change which also affect the A2, A3 and C2 domains of the F8 protein lead to a statistically significant higher risk of ID.

A number of previous studies have confirmed the relationships between 19 missense mutations reported in the INSIGHT study and ID. In the study by Kempton et al. (19), 3 different missense mutations (R593C, R2150H, and N1922S) were found to be causative for ID in a cohort that included 18 mild/moderate HA patients. Two (R593C and R2150H) of these three mutations were also reported in the INSIGHT study, while one mutation (N1922S) was not. We consider that as the N1922S mutation does not cause amino acid group change, it could, therefore, be a factor for ID (19). Iioka et al. (20) reported a mild HA patient developing high titers of inhibitors. This patient had a missense mutation, c.3780C>G (p.D1241E), and required a long-term treatment protocol. They argued that the high titers of ID was directly related to the long treatment course. Regarding our study results, we consider that in Iioka et al.'s (20) patient, ID possibly resulted from a mutation (c.3780C>G) that did not cause amino acid group change.

In this study, we also investigated predictive cut-off values of several mutation pathogenicity scoring systems for ID risk. It is considered that that CADD and REVEL scores can be used for this purpose (Table IV).

Conclusion

In mild HA patients, the variations of the amino acid group changes in missense mutations, as well as CADD and REVEL scores could offer some utility for the prediction of ID risk. Additionally, missense mutations causing no amino acid group change, and also involving A2, A3 and C2 domains of the FVIII protein are considered to lead to the highest risk of ID. Due to the retrospective design of our study, information regarding the treatment regimen of patients presented was severely limited. It is considered that further prospective studies, including treatment regimens, are required to fully evaluate the utility of the ID prediction hypotheses put forward in this study.

Ethics

Ethics Committee Approval: The article was made using data pulled from a database (openaccess).

Informed Consent: The article was made using data pulled from a database (openaccess).

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.I., Design: K.K., F.Ö., T.A., Data Collection or Processing: H.M., B.A., Analysis or Interpretation: T.K., Reviewing of Manuscript: F.Ö., T.A.

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

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Original Article

Long-term Outcome of Infants with Spina Bifida Through Assessment of the Prognostic Value of Hostile Bladder Parameters

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ABSTRACT

Aim: In 2016, the Centers for Disease Control and Prevention published a management algorithm for Spina Bifida (SB) cases from birth and started collecting data prospectively. They designated risk factors from urodynamic studies as end filling pressure or detrusor leak point pressure (DLPP) ≥40 cmH_2O or neurogenic detrusor overactivity (NDO) with detrusor sphincter dyssynergia (DSD), and categorized this type of bladder dysfunction as "hostile bladder" (HB). They recommended the immediate start of clean intermittent catheterization and anticholinergics in these patients. Having similar concerns regarding this patient population, we designed a retrospective study to identify and reveal the long-term outcomes of SB patients with HB.

Materials and Methods: All urodynamic studies and hospital records of SB patients admitted and followed between 1994-2014 were reviewed retrospectively. The demographic data, the presence of DLPP, DSD and NDO in the first urodynamic examination, bladder compliance, first and last radiologic and scintigraphic imagings, and surgical interventions were evaluated. Upper tract damage was defined as new scars in dimercaptosuccinic acid scans.

Results: A total of 58 patients were included in this study. The mean follow-up was 12.17±5.17 years. The presence of a scar in the first scintigraphy (p=0.01) and the presence of hydronephrosis in the first and last ultrasonography (p=0.03) were found to be independent risk factors for new scar development. When DLPP values were evaluated with receiver operating characteristic curve analysis, 50 cmH_2O was observed as a significant threshold value with 73% sensitivity and 60% specificity.

Conclusion: Our study confirmed the detrimental effects of high pressure and detrusor-sphincter dyssynergia; however, HB parameters were not sufficient to distinguish high-risk group patients. The presence of scars in the first scintigraphic evaluation, DLPP above 50 cmH_2O, and the presence of hydronephrosis in the first ultrasound were found to be risk factors for renal deterioration. More frequent monitoring and detailed evaluation may be necessary for patients with these risk factors.

Keywords: Spina Bifida, hostile bladder, neuropathic bladder, detrusor leak point pressure, neurogenic detrusor overactivity

Introduction

Spina Bifida (SB) is a condition that may result in chronic kidney disease secondary to bladder dysfunction. Despite a worldwide varying incidence, 1-6 cases are seen in every 1,000 live births (1). Several studies reported better results with pro-active management strategies (2,3). Different urodynamic parameters or scoring systems were suggested to detect patients who are at risk of upper urinary tract damage (4-6). In 2016, the Centers for Disease Control and Prevention (CDC) published a management algorithm for SB

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cases from birth and started collecting data prospectively (7). They designated risk factors from urodynamic studies as end filling pressure or detrusor leak point pressure (DLPP) 40 cmH_2O or greater, or neurogenic detrusor overactivity (NDO) with detrusor sphincter dyssynergia (DSD), and categorized this type of bladder dysfunction as "hostile bladder". They recommended the immediate start of clean intermittent catheterization and anticholinergics in these patients. Having similar concerns regarding this patient population in our region, we designed a retrospective study with an aim to identify and reveal the long-term outcomes of SB patients with "hostile bladders".

Materials and Methods

All urodynamic studies and hospital records of SB patients admitted and followed between 1994 and 2014 were reviewed retrospectively to identify patients with "hostile" bladders according to the above-mentioned criteria. Patients with the first admission after infancy, those who did not comply with the SB follow-up protocol of our department, and those patients followed for less than five years were excluded from this study.Urodynamic tests (pressure-flow study) were performed according to our protocols in all cases using a double-lumen 6 Fr urodynamic catheter by Dyno (AYMED® Istanbul, Turkey). Pelvic floor activity was evaluated with a total of 3 electromyography electrodes, 2 of them placed on the perianal skin (right and left of the anus), and one for reference on the thigh. Intraabdominal pressure was measured using an 8 Fr rectal balloon catheter. At least two fillings were done for each case. The lowest leakage pressure was described as DLPP. DSD was defined as involuntary pelvic floor activation during voiding. The involuntary phasic or terminal detrusor contractions during filling were described as NDO. The demographic data of the cases, the presence of DLPP, DSD and NDO in the first urodynamic examination, bladder compliance, first and last dimercaptosuccinic acid (DMSA) scans, first and last voiding cystourethrograms, surgical interventions, first and last kidney ultrasound, and the medications they received were evaluated. All patients were followed according to the standardized program of our department (Table I). Urodynamic and clinical data, along with the above-defined hostile bladder parameters, were evaluated for predicting upper tract damage. Upper tract damage was defined as new scars in DMSA scans.

Statistical Analysis

Statistical analysis was done with the IBM SPSS 21 program. Kolmogorov-Smirnov test was used to evaluate the distribution, Pearson chi-square, Mann-Whitney U, Kruskal-Wallis, regression analysis, and receiver operator characteristics curve (ROC) tests were used for analysis where appropriate. Institutional Ethical approval was obtained for the retrospective patient chart review (20-8T/26).

Results

The flow-chart describes patient selection for the study (Figure 1). A total of 58 patients (33 boys and 25 girls) with "hostile" bladder were included in this study. The mean follow-up was 12.17±5.17 years. Hydronephrosis was present in the first ultrasonography (US) in eight cases. It resolved in five cases and was present in a total of 5 cases with two being new-onset cases. Renal scars were observed initially in the first DMSA scans of 10 cases. Nine patients had developed new scars at the last DMSA, making a total of 19 cases with renal parenchymal scars. The mean DLPP value at the first urodynamic study was 54.88±30.43 cmH_2O. Of the 58 patients, DSD was present in 48 (82.7%) in the first and 49 (84.5%) in the last urodynamic study. NDO was present

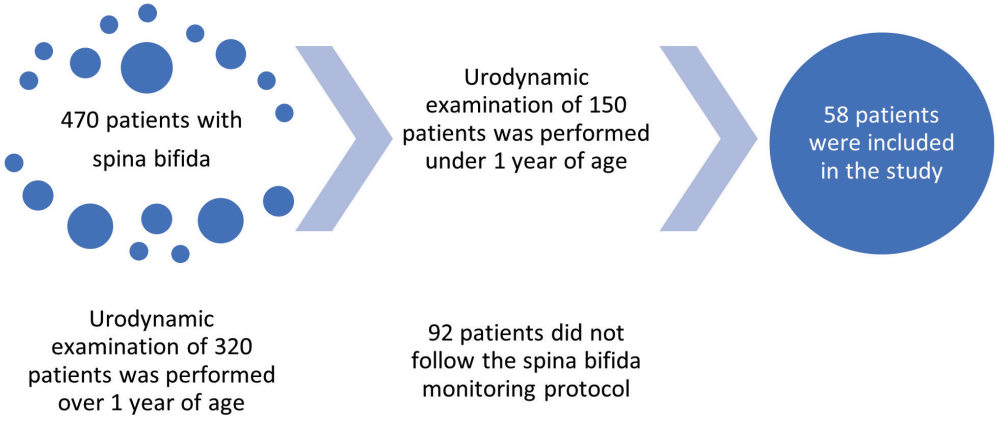

Figure 1. Patient admission flow chart

in 38 cases (65.5%) in the first and last urodynamics (with nine of these being new-onset cases in the last and nine others having resolved after the first).

The presence of a scar in the first scintigraphy (p=0.01) and the presence of hydronephrosis in the first and last US (p=0.03) were found to be independent risk factors for new scar development (Table II). When 40 cmH_2O was accepted as the threshold value, no significant relation was found between DLPP and new scar development (p=0.05). When DLPP values were evaluated with ROC analysis for new scar development, 50 cmH_2O was observed as a significant threshold value with 73% sensitivity and 60% specificity. A significant correlation was found between this threshold of DLPP and the development of a new scar (p=0.03).Kidney failure was observed in only one case. Bladder augmentation was performed in 7 (12.1%) of the cases. All patients who underwent augmentation cystoplasty had NDO (p=0.041).

Table I. Urological follow-up protocol in Spina Bifida patients

Age	UA & UC	Urinary US	UD	Scintigraphy	VCUG
Preoperative	X	X			
Postoperative 1 week	X	X			
6 week	X	X	X		
6 months	X	X	X	X	X
1 year	X	X	X	(X)	
1.5 year	X				(X)
2 year	X	X	X	X	
3 year	X	(X)	(X)		(X)
4 year	X	X	X		
5 year	X	(X)	(X)		(X)
6 year	X	X	X		
7 year	X	(X)	(X)		(X)
8 year	X	X	X		
9 year	X	(X)	(X)		
10 year	X	X	X		(X)

X: These are mandatory examinations.
(X): In patients without infection, reflux and urodynamic examination within normal limits, these examinations are optional depending on the clinical situation.
CIC: CIC training is given to families of all newborn patients. If there is no hydronephrosis and if there is no residual urine in the urodynamic examination at Week 6, CIC is discontinued.
Anticholinergic therapy is started in patients with the following features:
-NDO
-DSD
-DLPP>40 cmH_2O
-Low compliance
Prophylactic antibiotic is started (amoxicillin or trimethoprim-sulfamethoxazole, depending on the age) in patients with the following features:
-3-6 months after initiation of CIC
-VUR
-Recurrent UTI
UA and UC: Urinalysis and urine culture, US: Ultrasound, UD: Urodynamics, VCUG: Voiding cystourethrogram, CIC: Clean intermittent catheterization, NDO: Neurogenic detrusor overactivity, DSD: Detrusor-sphincter dyssynergia, DLPP: Detrusor leak point pressure, VUR: Vesicoureteral reflux, UTI: Urinary tract infection

Table II. Patient characteristics according to the presence of new-onset scar in the last DMSA

New-onset scar in the last DMSA	First urodynamic DLPP value (mean)	HN presence in the first RBUS	Scar presence in the first DMSA	DSD Presence in the first UD	NDO presence in the first UD	DLPP>50 cmH_2O in the first UD
Positive	65.40±40.03*	6**	9**	15*	11*	11**
Negative	51.21±25.88	2	1	33	27	17

*p>0.05
**p<0.05
DMSA: Dimercaptosuccinic acid scintigraphy, DLPP: Detrusor leak point pressure, RBUS: Renal bladder ultrasound, DSD: Detrusor sphincter dyssynergia, UD: Urodynamics, NDO: Neurogenic detrusor overactivity

There was no relation to other variables, such as the presence of vesicoureteral reflux or DSD. The mean DLPP values (63.86±24.98 cmH_2O) in the first urodynamic studies of the patients who required bladder augmentation were higher than in other cases (53.65±31.12 cmH_2O), but it was not significant (p=0.41).

Discussion

The necessity of urological follow-up is not questioned for patients with SB, but the frequency and design of follow-up are not well-defined or standardized. Close monitoring and pro-active patient management are shown to reduce upper urinary tract damage and bladder surgery (2,3). The follow-up protocol of our department involves outpatient visits every 6 months until the age of 2, followed by annual visits even in uneventful cases. Long-term follow-up of all cases is recommended due to the risk of tethered cord and its urological consequences (8). However, our study revealed that only a small number of patients were compliant with this protocol, and only a small group of the patients we followed could be included in the study (flow-chart/Figure 1). Many factors, such as mobility restrictions of the patients, or socio-economic problems, may hinder attending the outpatient visits regularly. Identifying patients at risk and arranging follow-up intervals according to these risk factors may lead to better adherence of the families to the protocols and decrease the burden on those centers caring for these patients.

International Children's Continence Society classifies detrusor anomalies in the voiding phase as overactive and underactive (8). In the voiding phase, the inability of the urethral sphincter to relax due to neuropathic reasons has been defined as DSD. By the addition of cases with no sphincter activity, basically, four types of neuropathic bladder-sphincter disorders are revealed (9,10). Various studies were conducted showing the possible negative effects of high detrusor pressure on kidney function. Steinhardt et al. (11) showed that in patients with SB, the glomerular filtration rate decreased when the bladder filling pressure was 35-40 cmH_2O (11). Austin et al. (12) highlighted that DLPP over 40 cmH_2O is a high value that can cause upper urinary system damage. The cases with overactive bladder and DSD would be the most vulnerable patients who have higher bladder pressure (13). For these reasons, DLPP over 40 cmH_2O, DSD, and high-pressure overactivity are defined as urodynamic "hostile bladder" parameters by the CDC (7).

There have been different studies to determine the threshold DLPP value that causes urinary tract damage. The threshold value that generally indicates the hostility of the bladder has been determined to be 40-50 cmH_2O (11,12). Intravesical pressure reaching high values during the filling phase will likely affect the upper urinary tract. Determining this threshold may help to guide treatment. Although this threshold value appeared to be 50 cmH_2O in our study, it might be safer to accept a lower threshold value as different results were found in different studies, and some of these were as low as 20 cmH_2O (14). Prospective large series are needed in order to find the answer to this question.

Tanaka et al. (4) found the renal scar rate in the first scintigraphic examination of children with SB to be 7.5%. This rate seems to be lower than in our study. This may be due to unintentional sampling bias in our study as patients with more problems tend to comply better with longer follow-up periods.

Vesicoureteral reflux studies revealed existing renal scars to be a risk factor for developing new scars (15,16). Our study showed similar results with having a scar in the first scan being an independent risk factor for new scar development. Hydronephrosis was observed in 8 (13.7%) of our cases; six of these were high grade. Tanaka et al. (4) detected hydronephrosis in 44.1% of cases with SB, and 3.7% had high-grade hydronephrosis in the initial US examinations. This rate is quite high compared to the rate in our study. In our series, 6 of 8 cases with hydronephrosis in the first US developed new scars ($p<0.05$). A high degree of HN was present in 5 of these six cases. High-grade HN was found to be a significant risk factor in terms of new scar development. Due to the sequelae dilatations observed in some patients, hydronephrosis in SB patients can sometimes be considered insignificant. Our study emphasizes the importance of high-grade hydronephrosis as a warning sign for further evaluation to prevent kidney damage.

Bladder augmentation is performed in cases with high bladder pressure, low capacity, incontinence, and upper tract damage despite appropriate management (17). The rate of patients undergoing bladder augmentation in pre-treated patients varies between 5-17%, while in untreated cases, this rate can rise to 41% (3,17-19). In our study, the bladder augmentation rate was 12.1%, similar to these, and similar to that of American SB Centers (12.7%) (19). In a study by Corona et al. (20), VUR and a DLPP of 40 cmH_2O or above at the end of the filling phase were defined as the factors that increase the need for bladder augmentation. In studies conducted by different researchers, VUR incidence in patients with SB was between 27-34% (6,21). The VUR incidence in our study was similar to other studies at

24.1%. The mean first DLPP of patients who underwent augmentation cystoplasty was higher than those patients who did not require this procedure; however, the difference was not significant. The presence of NDO in the first urodynamic examination emerged as an independent risk factor for the need for augmentation.

Study Limitations

The most important limitation of our study is its retrospective nature. Also, the lack of compliance with protocols caused many patients to be excluded from this study. This is mostly secondary to being a tertiary center with patients referred from other provinces and centers, to re-evaluate their management.

Conclusion

Loss of low-pressure reservoir function of the bladder in cases with SB can cause upper urinary tract damage. Therefore, "hostile bladder" was defined by CDC to predict patients at risk of kidney damage. Our study confirmed the detrimental effects of high pressure and detrusor-sphincter dyssynergia; however, the hostile bladder parameters were not sufficient to distinguish high-risk group patients. The presence of scars in the first scintigraphic evaluation, DLPP above 50 cmH_2O, and the presence of hydronephrosis in the first ultrasound were found to be risk factors for renal deterioration. More frequent monitoring and detailed evaluation may be necessary for those patients with these risk factors. Prospective studies are required to design better management strategies individualized for each patient.

Ethics

Ethics Committee Approval: Institutional Ethical approval was obtained for the retrospective patient chart review (20-8T/26).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.T., S.T., İ.U., Design: A.T., S.T., İ.U., Data Collection or Processing: A.T., E.A.T., Analysis or Interpretation: A.T., Supervision: S.T., İ.U., Writing: A.T., S.T., E.A.T., İ.U.

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Original Article

The Effects of Game Intervention on Postoperative Anxiety and Pain Levels in Children: A Randomized Controlled Study

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ABSTRACT

Aim: Surgery is a stressful and painful experience for children and it is important to control postoperative anxiety and pain. The aim of this study is to evaluate the effects of game intervention on postoperative anxiety and pain levels in children.

Materials and Methods: A randomized controlled trial design was employed in the current study. Seventeen children in the intervention group started to play a game at their bedside with their parents at 15 minutes after their arrival at the service from the recovery room (pre-intervention period); while the twenty children in the control group only obtained the routing service protocol without any game intervention. The effectiveness of the game intervention was assessed at 60 minutes after arrival at the service from the recovery room (post-intervention period) using the facial affective scale for anxiety and the visual analog scale for pain. The analgesic needs of children after the surgery were recorded.

Results: In both groups, the pre-intervention anxiety and pain were significantly decreased in the post-intervention period ($p<0.05$). The reduction of anxiety in the control group was significantly higher than the intervention group ($p=0.006$) and there was no significant difference between the post-intervention pain levels of the groups. The rate of analgesic need in the control group was significantly higher than the rate in the intervention group ($p=0.048$).

Conclusion: The results indicate that children who took part in the game intervention with their parents did not have lower levels of anxiety or pain than children in the control group; however, the intervention was effective in decreasing both anxiety and pain levels after surgery. Based on the decreased rates of analgesic needs, it is recommended that nurses encourage parents to play with their children after surgery.

Keywords: Anxiety, children, game intervention, pain, postoperative period

Introduction

Day surgery is being increasingly used for pediatric patients around the world (1,2), and there is a significant rate of use in Turkey, ranging from 21.1% to 67.1% (3,4). Surgery is a stressful and painful experience, and day surgeries are associated with inadequately managed postoperative pain (5,6). Many study results have indicated that more than 30% of children experience moderate to severe pain after day surgery (7,8), and this can lead to negative psychological outcomes such as anxiety (9). According to a systematic review, a positive relationship exists between postoperative

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anxiety and pain among children who have undergone surgery (10). Hence, to reduce and alleviate pain, it is important and necessary to control postoperative anxiety.

According to the postoperative pain management panel, analgesics in combination with non-pharmacological interventions are recommended to manage postoperative pain in children (11). Given that playing a game takes an important place in the lives of children, games can also be important for helping children cope with anxiety and painful procedures (12,13). In a study conducted by Vagnoli et al. (14), relaxation through guided imagery was found to reduce postoperative pain among children aged 6-12 years. Based on the results of a qualitative exploratory study that examined children's surgical pain experiences, non-pharmacological pain management interventions (such as distraction, playing games on tablets or phones, and watching films) were reported as useful and helpful in dealing with children's pain (8). According to a randomized controlled study by He et al. (15), an hour-long play intervention was found to be effective in reducing postoperative pain in children who had undergone elective inpatient surgery. Similarly, children took part in a playful intervention involving toys and video games in the study by Kumar et al. (16), where play was found to be effective in reducing postoperative anxiety and pain levels in children who had undergone heart surgery.

Among the available postoperative pain management interventions, play is not always applied with a high priority due to time restrictions and the lack of people who are available to work with pediatric patients (12,13). It has been reported in the literature that nurses frequently insufficiently use non-pharmacological pain management methods for postoperative pain management in children (17). Along with the nurses, the children's parents have also been reported to have limited involvement in play interventions (18). As children mostly need the presence and acceptance of their parents when playing games (19), parents joining in the play is a vital part when using non-pharmacological postoperative pain management strategies such as playing games with school-aged children (5). In the literature, it is recommended that parents be actively involved in play interventions in order to increase the positive effects on perioperative anxiety and the relative effects on postoperative pain (18). In order to cope better with postoperative pain, there is a need to develop a collaborative non-pharmacological intervention between the child and their parents. Unfortunately, several previous studies have focused on the impact of preoperative therapeutic play activities on children's perioperative anxiety and postoperative pain levels, but the effects of game interaction between children and parents on postoperative anxiety and pain levels remain unknown. Therefore, this study aimed to evaluate the effects of game intervention on postoperative anxiety and pain levels in children. Eventually, the results of the game interventions between children and their parents might shed light on areas for further research in the field of pediatric surgery.

Study Questions

In this study, two questions were addressed:

Q1: Is playing a game effective in decreasing children's postoperative anxiety levels?

Q2: Is playing a game effective in decreasing children's postoperative pain levels?

Materials and Methods

Study Design and Sample

This study was designed as a randomized controlled trial consisting of 37 children (intervention group n=17; control group n=20) and conducted at the pediatric surgery service of a university hospital in the eastern region of Turkey between January 7th and May 4th, 2018.

The sample size was calculated using power analysis. In a study carried out by William Li et al. (20), where the visual analog scale (VAS) group score standard deviations were 1.18 and 1.24, with the optimal effect size of 0.03 and 95% power, the sample number was calculated to be 16 children in each group. In the present study, the sample size was 40, with a total of 20 children in each group, allowing for an estimated attrition rate of 25%. Randomization was performed using Random Allocation Software (Version 1.0.0) for parallel group randomized studies, and the children followed through with the allowed order. To eliminate any contamination between the groups, the two groups were allocated to different rooms after the randomization.

In this study, the age range was selected dependent on the children's developmental age and because school-aged children are able to cooperate in game interventions and have cognitive ability (21), children for both groups were included if they were between 7 and 12 years old. The other inclusion criteria were; having undergone minor elective day surgery (e.g., circumcision, hernia, cyst resection), being able to converse, a willingness to play the game, having no previous surgical experience, being able to be mobile in the bed, and having a parent who was willing to play the game during the postoperative period.

Outcome Measurements

Data were collected using a data collection form, the facial affective scale (FAS), and the VAS.

The data collection form was prepared by researchers to collect demographic data from the children, and it contained three questions, which were the age, gender, and analgesic needs of the child, including the analgesic's name and time given, to record the child's analgesic needs after surgery.

The FAS was used to evaluate the children's postoperative anxiety in this study. It was previously validated by Quiles et al. (22), and had been used to evaluate anxiety levels in children aged 7-12 years after surgical procedures previously (23). The findings of previous studies indicated that FAS scores were significantly correlated with the scores for anxiety, with concurrent validity (24,25). This self-reported scale has five graded faces where 0 means "I have no anxiety"; 1 means "I have a little anxiety"; 2 means "I have some anxiety"; 3 means "I have high levels of anxiety"; and 4 means "I have extremely high levels of anxiety." In this study, the children were asked to select one of these faces that best described their current level of anxiety.

The VAS was used to evaluate the children's postoperative pain in this study. It is a widely used pain assessment scale, and in many studies, it has been reported to be a valid scale for measuring postoperative pain levels in children over 6 years old, who know how to rate the numbers (8,20,26-28). It is a scale numbered from 1 to 10 and placed horizontally on the paper, with descriptive words between the 0 point and the 10 point. Here, 0 means "I have no pain," and 10 means "I have the worst pain I've ever had." In this study, children were asked to select the number that best described their current level of pain.

Study Procedure and Data Collection

In this study, 37 children who met the study's inclusion criteria were enrolled.

Before the Procedure

At this stage, the staff registered nurse researcher (Ö.G.) and the registered nurse researcher (S.A.) were informed by the principal researcher (S.Ü.) about the study procedure and the usage of the FAS and VAS scales. On the morning of the day surgery, the children and their parents were admitted to the service room and visited by Ö.G. and S.A., who introduced themselves and helped the children and their parents prepare for the surgery. After the children were gowned, they were told by Ö.G. about the purpose of the study and about the scales, and if they were willing to participate in the study, written permission was obtained from the parents; with verbal permission being obtained from the children by S.A. The possibility of withdrawing from the study was explained whenever the children and the parents wanted. In the intervention group, the children and their parents were informed about the strategy game intervention by Ö.G. that they would play after they arrived at the service area. Findings from the researchers' previous study showed that playing this game was effective at decreasing children's and parents' preoperative anxiety levels (29). Also, after consulting with the pediatric surgeon of the department, this strategy game was selected as the game intervention in this study. This game is suitable for children over 3 years of age and can be played in pairs (30). The game and rules were presented by Ö.G. to the children and their parents on an over-bed table at the child's bedside. According to the rules; the child must first build an 18-layer tower using 54 wooden blocks, then remove one block from any layer of the tower with one hand and place it on the top layer. Then, a parent would do the same for their turn, and the game would continue until the tower falls. The player whose turn it is when the tower falls is the loser, but if they wanted, the game would be restarted, and game duration would not be limited. The children and their parents were allowed to play for one hour before the children were transferred to the operating room. This stage took nearly 15 minutes.

As a routine procedure after the surgery, children were kept in the recovery room for approximately 1 hour before being transferred to the service area until they were awake, able to communicate, and their vital signs were stable.

During the Procedure

In this stage, the children and their parents were visited by Ö.G. and S.A. after arrival in the service area. The children in both groups received routine service postoperative protocol, including vital signs monitoring, awakeness, pain assessment, and analgesic administration (paracetamol 15 mg/kg PRN as needed). After completing these checks, which took approximately 15 minutes, if all the checks were normal, S.A. collected data from the willing participants and asked children to select a face on the FAS that best described their current level of anxiety. Then, she asked them to select a number on the VAS that best described their current level of pain. According to the random assignment by Ö.G., the children in the intervention group were asked if they were ready for the game intervention so that they received both the routine service postoperative protocol and the game intervention with their parents.

After the Procedure

Children in both groups were asked 60 minutes after arrival to the service area from the recovery room to rate their anxiety levels on the FAS and their pain levels on the VAS. Figure 1 shows the CONSORT flow diagram of this study.

Ethical Considerations

Before the study was conducted, ethical permission was obtained from the Ethics Committee of the Trakya University Faculty of Medicine (protocol number: 2017/344), and official permission was obtained from the directorate of the university hospital (number: 54542207-600). Children and their parents were also informed about this study, after which verbal consent was obtained from the children and written informed consent was obtained from the parents. They were also told that it was possible to withdraw from the study if they wanted at any time.

Statistical Analysis

All data were analyzed using the Statistical Package for the Social Sciences version 22.0 statistics software package (IBM, Armonk, NY, USA). The gender of the children is presented as percentages, and the ages of the children are presented both as medians with minimum-maximum (min-max) values and as means with standard deviations (SD).

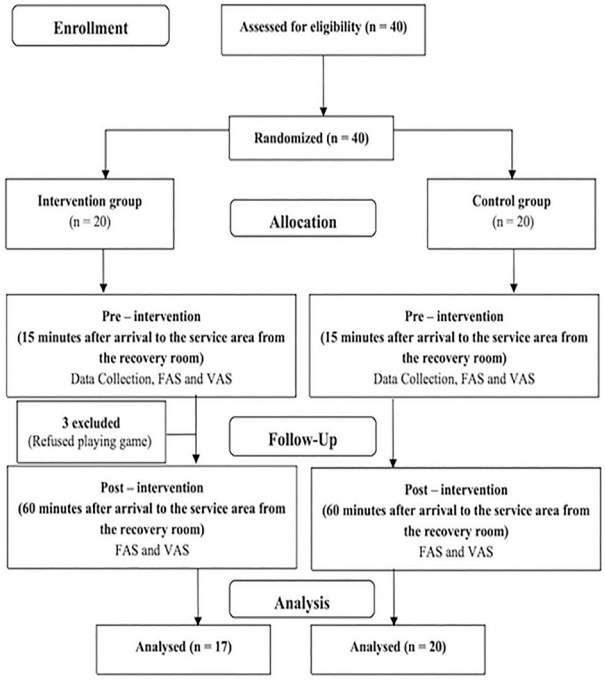

Figure 1. The CONSORT flow diagram of this study
FAS: Facial affective scale, VAS: Visual analog scale

Anxiety and pain level scores are also presented as both medians with min-max and means with SD. The data were not normally distributed, and non-parametric tests were performed. To analyze whether the characteristics of the children were comparable in the two groups, Fisher's exact test and the Mann-Whitney U test were used. To compare the pre- and post-intervention anxiety and pain levels of the children within each group, the Wilcoxon test was used, and to compare the children's anxiety and pain levels between groups, the Mann-Whitney U test was used. To compare the analgesic needs of children after surgery between groups, Fisher's exact test was used; $p<0.05$ was considered as significant.

Results

After the group allocation and the pre-intervention evaluation (at 15 minutes after arrival at the service from the recovery room), three children were excluded from the study as they became bored with the game and refused to play the game after building the tower. In total, data were analyzed for 37 children (intervention group=17; control group=20).

The mean age of all the children was 8.54±1.77 years, 86.5% of them were male and 21.6% of them needed analgesic after the surgery. There were no significant gender or age differences between the groups (p=0.348, p=0.104, respectively). Up to the end of the post-intervention period (at 60 minutes after arrival at the service from the recovery room), 35% of the children in the control group needed to use an analgesic, and this rate was found to be statistically significantly higher than the rate for the intervention group (5.9%; p=0.048) (Table I).

In the intervention group, the median pre-intervention anxiety level of the children was 2 (min-max 1-4) and significantly decreased to 1 (min-max 0-2) in the post-intervention period (z=-3,169; p=0.002). The median pre-intervention pain level of the children was 3 (min-max 0-9) and significantly decreased to 2 (min-max 0-5) in the post-intervention period (z=-2,896; p=0.004) (Table II).

In the control group, the median pre-intervention anxiety level of the children was 2 (min-max 0-4), and it significantly decreased to 0 (min-max 0-2) in the post-intervention period (z=-3,097; p=0.002). The median pre-intervention pain level of the children was 4 (min-max 0-9) and significantly decreased to 1 (min-max 0-5) in the post-intervention period (z=-3,843; p=0.000) (Table II).

In the post-intervention period, the median anxiety level of the children was 0 (min-max 0-2) in the control

group and 1 (min-max 0-2) in the intervention group. The difference between groups was found to be statistically significant (z=-2,947; p=0.006). The median pain level of the children was 1 (min-max 0-5) in the control group and 2 (min-max 0-5) in the intervention group. There was no statistically significant difference between groups (z=-1,567; p=0.133) (Table II).

For the post hoc power analysis, the G Power Program (G Power 3.1 9.2, Kiel, Germany) was used. With the sample size of 37 and the alpha level p<0.05, the statistical power was calculated as 0.93 for anxiety with the effect size of 1.08 (Cohen's d≥0.5), and as 0.42 for pain with the effect size of 0.50 (Cohen's d≥0.5).

Discussion

Play is reported to be an important resource for helping children overcome stressful and painful procedures such as hospitalization, burn dressing change, venipuncture, and of course surgery, in addition to others (31,32), but little is known about the effects of play intervention on anxiety and pain relief during the postoperative period. This study primarily focused on the effects of the game intervention on children's postoperative anxiety levels and results showed that the children in both groups experienced significantly decreased levels of anxiety. However, the anxiety reduction was significantly improved in the control group and this difference may be explained by the winning feeling when

Table I. Demographic characteristics of children

Characteristics	**Control (n=20)**		**Intervention (n=17)**		**Total (n=37)**		**Statistical test**
	n	**%**	**n**	**%**	**n**	**%**	
Gender of child							
Male	16	80	16	94.1	32	86.5	p=0.348*
Female	4	20	1	5.9	5	13.5	
Analgesic need							
Yes	7	35	1	5.9	8	21.6	**p=0.048***
No	13	65	16	94.1	29	78.4	
	Median (min-max) Mean ± SD		**Median (min-max) Mean ± SD**		**Median (min-max) Mean ± SD**		
Age of child	8.00 (7-12)		7.00 (7-11)		8 (7-12)		z=-1.713 p=0.104**
	9.00±1.94		8.00±1.41		8.54±1.77		

*: Fisher's exact test; **: Mann-Whitney U test; SD: Standard deviation, min-max: Minimum-maksimum

Table II. Comparison of children's mean anxiety and pain levels between groups

Variables		**Control (n=20)**		**Intervention (n=17)**		**Test**	
		Median (min-max)	**Mean ± SD**	**Median (min-max)**	**Mean ± SD**	**Z****	**p-value**
Anxiety							
Pre-intervention		2 (0-4)	1.70±1.34	2 (1-4)	2.17±0.88	-0.979	0.357
Post-intervention		0 (0-2)	0.50±0.60	1 (0-2)	1.17±0.63	-2.947	**0.006**
Test	**Z*** **p**		-3,097 **0.002**		-3,169 **0.002**		
Pain							
Pre-intervention		4 (0-9)	4.65±2.36	3 (0-9)	3.70±1.99	-1.242	0.232
Post-intervention		1 (0-5)	1.30±1.52	2 (0-5)	2.05±1.47	-1.567	0.133
Test	**Z*** **p**		-3,843 **0.000**		-2,896 **0.004**		

Z*: Wilcoxon test; Z**: Mann-Whitney U test; SD: Standard deviation, min-max: Minimum-maksimum

playing a strategy game. As reported in a previous study, children might expect to win the game so that the game play can positively affect their anxiety levels (33). In the literature, many studies have supported the beneficial effects of playing a game for decreasing anxiety in child patients during hospitalization and painful interventions (34-36). In a randomized controlled study by Al-Yateem et al. (37), the efficacy of play interventions (including coloring activities, pictures, and storytelling) for reducing anxiety among children undergoing day surgery were compared with a pharmacological premedication technique, and the results showed that play interventions were very effective at reducing anxiety as an alternative to pharmacological premedication. Moreover, involving parents in game interventions with their children is reported to have positive effects on reducing the anxiety of both the child and their parents during hospitalization and surgical periods (29,34,38). In a prospective and randomized controlled trial conducted by Fincher et al. (34) in North America, parental presence through play was maintained in a preoperative preparation program with children aged between 3 and 12 years. Although there was no significant difference between groups, this intervention was effective in decreasing the children's and parents' anxiety postoperatively. According to these results, playing a game with parents is beneficial for children in reducing their postoperative anxiety.

As reported previously, anxiety is related with pain and decreasing the anxiety levels of children is important for the reduction of pain levels (10,34). This study was secondly focused on the effects of game intervention on children's postoperative pain levels and results showed that it was not effective in decreasing their pain levels significantly. On the other hand, children in both groups experienced significantly decreased levels of pain at 60 minutes after arrival at the service area from the recovery room. A randomized clinical trial from Spain, Ullán et al. (39) examined the effects of a program that included stuffed toys on the postoperative pain of children between 1 and 7 years of age. Children in the experimental group were allowed to play with their parents postoperatively, and their pain scores were significantly lower than those children who had only received the standard protocol and did not play with any toys. Although the children's ages were between 1 and 7, the results support the effect of playing with toys alongside parents in decreasing postoperative pain. Yayan et al. (40) determined the effects of therapeutic play by including distracting games (such as computer games, puzzles, toy cars, baby dolls) on reducing acute postoperative pain among children 6 to 12 years old. These activities were applied during the postoperative period, and were effective in relieving children's postoperative pain. In the present study, although no statistically significant difference was found between the groups' post-intervention pain levels, the analgesic needs of those children in the control group was meaningfully higher than in the intervention group. This finding suggests that the game intervention had a positive effect on the pain levels of children after surgery. In a randomized trial from Singapore, He et al. (15) examined the effect of therapeutic play intervention on the postoperative pain levels of children who had undergone elective surgery. Children who received therapeutic play intervention for one hour before surgery had 1.5 points lower pain severity scores (2.11 vs. 3.60) than those children in the control group who did not receive play intervention. Consequently, using non-pharmacological pain management methods such as playing games with children preoperatively or postoperatively is helpful in decreasing pain levels after surgery, so it is recommended to encourage parents to play game interventions with their children.

Study Limitations

There were some limitations with the present study. Firstly, blinding the patients and the researchers was not possible because of the nature of the game intervention, and the lack of blinding may have caused some thought bias. Secondly, all of the children's participating parents were their mothers, which may be considered a parental limitation.

Conclusion

The results of this study indicate that children who take part in game intervention with their parents do not have lower levels of anxiety or pain than those children in the control group; however, it was effective in decreasing both anxiety and pain levels after surgery. Children in the game intervention group needed significantly less analgesic than those children in the control group. It is suggested that game intervention be employed with children and their parents as an effective intervention strategy for reducing postoperative anxiety and pain. Based on the decreased rates of analgesic needs, it is also recommended that nurses encourage parents to play with their children after surgery. It supports a basis for a low-cost and easy game intervention that can be used as a non-pharmacologic pain management method to reduce both anxiety and pain levels in children between 7 and 12 years of age who undergo minor elective day surgery. Although one child in the present study's intervention group needed analgesics, the study

results are meaningful and promote the effects of non-pharmacological methods of reducing pain after surgery. Parental involvement in postoperative childcare is also a meaningful aspect of this study for promoting holistic care, so it is recommended that nurses encourage parents to play games with their children after surgery. For future studies, it would be beneficial to generalize the effects of this game intervention on other age groups of children and examine its effects on other major surgical procedures.

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Ethics

Ethics Committee Approval: Ethical permission was obtained from the Ethics Committee of the Trakya University Faculty of Medicine (protocol number: 2017/344), and official permission was obtained from the directorate of the university hospital (number: 54542207-600).

Informed Consent: Verbal consent was obtained from the children and written informed consent was obtained from the parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.Ü., Ö.G., S.A., Design: S.Ü., Ö.G., S.A., Data Collection or Processing: S.Ü., Ö.G., S.A., Analysis or Interpretation: S.Ü., Ö.G., S.A., Literature Search: S.Ü., Ö.G., S.A., Writing: S.Ü., Ö.G., S.A.

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Original Article

Comparison of the Efficacy of Three Natural Surfactants in Preterm Turkish Newborns with Respiratory Distress Syndrome

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ABSTRACT

Aim: To determine the efficacy of three natural surfactant preparations in our community and the short- and long-term results of these on preterm infants.

Materials and Methods: This was a retrospective research on 193 premature babies with respiratory distress syndrome (RDS). The patients were divided into three groups, each of which received one of three surfactants: Group 1; beractant (100 mg/kg); group 2; poractant alfa (first dose of 200 mg/kg, recurrent doses of 100 mg/kg); group 3; calfactant (100 mg/kg). The groups were compared according to demographic characteristics, 1- and 5-minute Apgar scores, weight percentiles by gestational week, presence of pulmonary hemorrhage, surfactant dose repetition, air leak, bronchopulmonary dysplasia (BPD), stage of intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), hemodynamically significant-patent ductus arteriosus (hs-PDA) and its medical or surgical treatment, retinopathy of premature (ROP) and its treatment, sepsis, ventilation time (both non-invasive and invasive), free oxygen need time, time to start full enteral feeding, discharge time, and mortality.

Results: A total of 193 preterm infants with a mean gestational age of 28.9±3.1 weeks and mean birth weight of 1,190.4±504.3 grams were included in this study. The neonates were allocated into three different groups randomly, namely group-1 (n=77), group-2 (n=59), and group-3 (n=57). There were no differences in the clinical and demographic features of the groups. The incidence of pulmonary hemorrhage, surfactant dose repetition, air leak, ventilation time for both non-invasive and invasive, free oxygen need time, hs-PDA and surgical treatment of PDA, BPD, NEC (≥stage II), IVH (>stage III), ROP, time to start full enteral feeding, and discharge time were similar between the study groups. The sepsis and mortality rates were lower in group 3 compared to groups 1 and 2 (p=0.015, p=0.001).

Conclusion: In this study, beractant, proctant alfa and calfactant had clinically similar efficacy in patients with RDS.

Keywords: Respiratory distress, neonate, surfactant, Turkey

Introduction

Respiratory distress syndrome (RDS) continues to be one of the main causes of morbidity and mortality in preterm newborns admitted to neonatal intensive care units (NICU). Atelectasis and progressive respiratory failure develop due to a lack of surfactant in RDS (1). Preterm infants with RDS have inadequate surfactant pools, as well as immature surfactant composition and function. Surfactant is a substance produced by type II pneumocytes which form a lipid bi-layer over the inner surface of the

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alveoli. This lipid bilayer reduces the interaction between molecules at the alveolar air-liquid interface, lowering alveolar surface tension and stabilizing alveoli and the end of expiration (2). Surfactant deficiency and dysfunction leads to increased alveolar surface tension, which results in alveolar acinar collapse and decreased lung aeration. Surfactant replacement therapy forms the cornerstone of the management of moderate to severe RDS among preterm neonates and it is associated with reduced mortality and pulmonary air leak (3,4). Clinically, RDS occurs in the early postnatal period with signs of tachypnea, retraction, groaning and respiratory distress accompanied by cyanosis. Respiratory insufficiency can be detected by blood gas values and its diagnosis is supported by classical ground glass and air bronchograms on chest X-ray. Transient tachypnea of the newborn, pneumonia, air leak syndromes, cyanotic congenital heart diseases and other extrapulmonary systemic diseases should be considered in the differential diagnosis (5).

Currently, there are three animal-derived lung surfactant replacement products - calfactant (Infasurf®), beractant (Survanta®), and poractant alfa (Curosurf®)- in Turkey that are Food and Drug Administration approved for the treatment of newborn infants with RDS. Until May 2017 in our country, poractant alfa and beractant were the only available animal-derived natural surfactants. Therefore, there are not enough objective studies comparing the efficacy of these three preparations. The aim of this study was to determine the efficacy of these three natural surfactant preparations in our community and to determine the short- and long-term outcomes of these on preterm infants.

Materials and Methods

Design

This research was performed as a retrospective study between May 2017 and September 2019 in the tertiary NICU of Necmettin Erbakan University Meram Medical Faculty.

Ethics Committee approval for this study was obtained from Necmettin Erbakan University with the Ethics Committee decision number of 2019/2105.

Patients

During the study period, 223 patients were diagnosed with RDS in the NICU between 22th and 34th weeks of gestation. Exclusion criteria included congenital lung diseases, chromosomal abnormalities or major congenital malformations, congenital cyanotic cardiac disease and meconium aspiration syndrome. One hundred and ninety-three of the patients met the study criteria (Figure 1). Patients were evaluated clinically and by chest radiography for RDS diagnostic criteria. RDS was diagnosed clinically with early respiratory distress manifesting by symptoms of groaning, cyanosis, retractions and tachypnea. The diagnosis was confirmed by a blood gas analysis and chest X-ray with the classical "ground glass" appearance and air bronchograms. Surfactant therapy was administered prophylactically or as a rescue therapy, depending on the clinical presentation, in accordance with the European Consensus Guidelines (5) and the prospectus for each drug. The surfactant selection for each patient was determined by drawing lots. In the delivery room, babies were treated according to European guidelines (5). Nasal continuous positive airway pressure (CPAP) was started in the delivery room immediately after stabilization or while being admitted to the NICU. In the NICU, appropriately sized nasal canulas or masks (Fisher & Paykel Healthcare, Auckland) and Leoni Plus ventilator (HeinenþL € owenstein, Bad Ems, Germany) were placed in the nasal CPAP mode. When required, mechanical ventilation was set to be PaO_2>50 mmHg, $PaCO_2$ 55-65 mmHg and SpO_2 90-95%. Surfactants were given by the InSURE (intubation-surfactant-extubation) method when the inhaled oxygen fraction (FiO_2) was higher than 0.4. Babies who were administered surfactant were then switched to nasal CPAP mode as soon as possible, depending on their respiratory conditions. Repeated surfactant doses were administered if babies had MAP>7 and FiO_2>0.3. When intubated in the delivery room or NICU, the surfactant was given through an endotracheal tube. Surfactants were injected into the

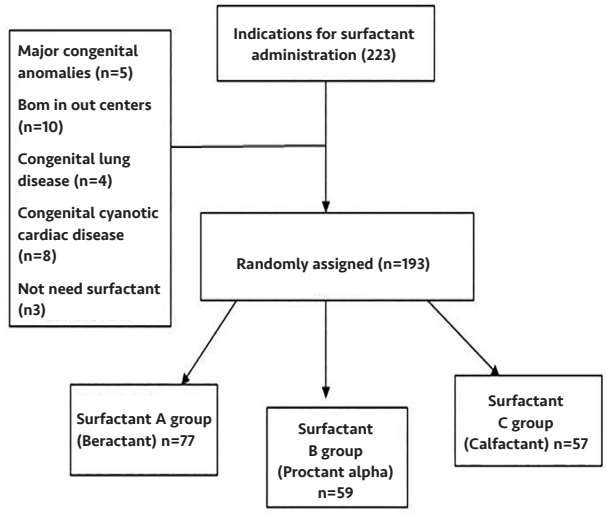

Figure 1. Flow chart of the study group

trachea via an orogastric tube through an endotracheal tube.

The patients were divided into three groups; Group 1; beractant (Survanta, AbbVie Inc, North Chicago, IL, USA; 100 mg/kg, 4mL/kg); group 2; poractant alfa (CurosurfVR, Chiesi, Parma, Italy; first dose of 200 mg/kg, 2.5 mL/kg, recurrent doses of 100 mg/kg, 1.25 mL/kg); group 3; calfactant (Infasurf, ONY Biotech, NY, USA; 100 mg/kg, 3mL/kg).

The groups were compared according to demographic characteristics, 1- and 5-minute Apgar scores, weight percentiles according to gestational week, pulmonary hemorrhage, surfactant dose repetition, air leak, BPD, stage of IVH, NEC, hs-PDA and its medical or surgical treatment, ROP and its treatment, sepsis, ventilation time (both non-invasive and invasive), free oxygen need time, time to start full enteral feeding, discharge time, and mortality.

Hemodynamically-significant PDA (hs-PDA) was defined with echocardiographic evidence of ductal lumen over 1.5 mm and left atrium diameter/aortic root diameter ratio of over 1.4 and documentation of left to right shunt. Bronchopulmonary dysplasia was defined as the need for oxygen at 36 weeks postmenstrual age or at the time of discharge or transfer to Level 2 NICUs (6); NEC≥Stage 2 (7); and severe brain injury, which included IVH≥Stage 3.

The examined maternal variables included the type of delivery, maternal age, antenatal steroid treatment, maternal gestational diabetes, maternal preeclampsia and clinical chorioamnionitis (defined as the presence of fever with one or more of the following: maternal leukocytosis >15,000/mm^3, fetal tachycardia, uterine tenderness or foul-smelling amniotic fluid).

Statistical Analysis

The data related to the research were transferred to computer and the analyses were performed with SPSS. The mean ± standard deviation and median (minimum-maksimum) were summarized in the summary of the numerical data; Numbers and percentages were used to summarize the categorical data. One-Way analysis of variance (ANOVA) and Welch's ANOVA were used for numerical data to investigate the relationships between the groups; chi-square test was used for categorical data. After the analysis of variance, Tukey HSD was used as a post-hoc test when deemed necessary. P<0.05 was accepted for statistical significance.

Results

A total of 193 preterm infants with a mean gestational age of 28.9±3.1 weeks and mean birth weight of 1,190.4±504.3 grams were enrolled and allocated into three groups. Beractant (group 1) was administrated to 77 (39.8%) neonates, poractant alfa (group 2) to 59 (30.5%), and calfactant (group 3) to 57 (29.5%). The gestational age of group 1 was 29.16±2.9 weeks, group 2 was 28.4±3.5 weeks and group 3 was 29.0±2.8; and the birth weight of group 1 was 1,236.38±563.6g, group 2 was 1,222.4±519.4g and group 3 was 1,198.7±391.9g. There were no differences between the groups with regard to gestational age, birth weight, maternal age, 1- and 5-minute Apgar scores, antenatal corticosteroids, antenatal antibiotics, maternal gestational

Table I. Demographic and clinical characteristics of the subjects by groups

Characteristics	Group 1 (Beractant) (n=77)	Group 2 (Poractant alfa) (n=59)	Group 3 (Calfactant) (n=57)	p-value
Birth weight (g), mean ± SD	1,236.38±563.6	1,222.4±519.4	1,198.7±391.9	0.42
Gestational age (w), mean ± SD	29.16±2.9	28.4±3.5	29.0±2.8	0.32
Maternal age, mean (SD)	28.97±5.7	28.9±6.5	29.1±6.2	0.9
5-min Apgar, mean (SD)	5.56±1.46	5.78±1.69	5.37±1.44	0.35
1-min Apgar, mean (SD)	4.45±1.6	4.27±1.82	4.21±1.69	0.68
Maternal gestational diabetes n (%)	3 (3.9)	5 (8.5)	6 (10.5)	0.31
Maternal preeclampsia, n (%)	17 (22.1)	19 (32.2)	11 (19.3)	0.22
Antenatal antibiotics	13 (16.9)	8 (13.6)	11 (19.3)	0.7
Chorioamnionitis, n (%)	4 (5.2)	9 (15.3)	10 (17.5)	0.59
Antenatal steroid use, n (%)	41 (53.2)	34 (57.6)	33 (57.9)	0.82
SGA, n (%)	18 (23.4)	8 (13.6)	8 (14)	0.23

SD: Standard deviation

diabetes mellitus, maternal preeclampsia, chorioamnionitis or SGA (Table I).

Table II shows a comparison of the groups in terms of RDS-related results respiratory outcomes. Pulmonary hemorrhage was lower in group 3, but there was no statistically significant difference (p=0.07). Although the number of cases with air leakage was higher in group 2, there was no statistically significant difference (p=0.13). There were no differences with regard to surfactant redosing, invasive mechanical ventilation, non-invasive mechanical ventilation and free oxygen need time.

The incidence of hs-PDA and surgical treatment of PDA, BPD, NEC (≥stage 2), high grade IVH (>stage 3), ROP, and time to start full enteral feeding were similar between the study groups, as was discharge time. Sepsis and mortality were lower in group 3 compared to the groups 1 and 2 (p=0.015, p=0.001 respectively) (Table III). Treatment of ROP was higher in group 2 compared to the other groups but

Table II. Comparison of groups in terms of RDS-related results

Characteristics	Group 1 (Beractant) (n=77)	Group 2 (Poractant alfa) (n=59)	Group 3 (Calfactant) (n=57)	p-value
Surfactant redosing, n (%)	28 (36.4)	17 (28.8)	15 (26.3)	0.41
Pulmonary hemorrhage, n (%)	14 (18.4)	10 (17.2)	3 (5.3)	0.07
Pulmonary air leak, n (%)	2 (2.6)	5 (8.5)	1 (1.8)	0.13
Invasive mechanical ventilation time (days), mean (SD)	8.4±13.2	8±11.7	9±10.7	0.9
Non-invasive mechanical ventilation time (days), mean (SD)	12.0±15.4	10.4±10.8	15.2±16.7	0.25
Duration of free oxygen need (days), mean (SD)	18.3±18.03	16.7±14.3	15.2±17.4	0.15

RDS: Respiratory distress syndrome, SD: Standard deviation

Table III. Comparison of secondary outcomes of patients

Characteristics	Group 1 (Beractant) (n=77)	Group 2 (Poractant alfa) (n=59)	Group 3 (Calfactant) (n=57)	p-value
hs-PDA, n (%)	Positive 19 (25.3) Negative 56 (74.7)	Positive 15 (27.3) Negative 40 (72.7)	Positive 10 (18.2) Negative 45 (81.8)	0.49
Surgical treatment of PDA, n (%)	Positive 3 (15.8) Negative 16 (84.2)	Positive 1 (6.7) Negative 14 (93.3)	Positive 2 (20) Negative 8 (80)	0.6
BPD, n (%)	Positive 16 (27.1) Negative 43 (72.9)	Positive 7 (18.4) Negative 31 (81.6)	Positive 15 (30) Negative 35 (70)	0.45
NEC, n (%)	Positive 8 (11.3) Negative 63 (88.7)	Positive 7 (14) Negative 43 (86)	Positive 3 (5.7) Negative 50 (94.3)	0.36
IVH, n (%)	Positive 21 (28.4) Negative 53 (71.6)	Positive 14 (24.5) Negative 41 (75.5)	Positive 13 (22.8) Negative 44 (77.2)	0.82
ROP, n (%)	Positive 22 (36.7) Negative 38 (63.3)	Positive 12 (30.8) Negative 27 (69.2)	Positive 15 (29.4) Negative 36 (70.6)	0.68
Treatment of ROP, n (%)	Positive 7 (29.2) Negative 17 (70.8)	Positive 6 (85.7) Negative 1 (14.3)	Positive 4 (40) Negative 6 (60)	.....
Time to enter full enteral feeding, (days), mean (SD)	18±11.8	14.2±7.1	17.2±11	0.19
Discharge time, (day), mean (SD)	52±35.2	49.3±28.5	52.5±32.4	0.9
Sepsis, n (%)	Positive 23 (31) Negative 51 (68.9)	Positive 21 (38.2) Negative 34 (61.8)	Positive 8 (14.3) Negative 48 (85.7)	0.015
Mortality, n (%)	Positive 16 (20.8) Negative 61 (79.2)	Positive 18 (30.5) Negative 41 (69.5)	Positive 8 (14) Negative 49 (86)	0.001

hs-PDA: Hemodynamically significant-patent ductus arteriosus, BPD: Bronchopulmonary dysplasia, NEC: Necrotizing enterocolitis, IVH: Intraventricular hemorrhage, SD: Standard deviation, ROP: Retinopathy of premature

statistical significance could not be shown, as the number of patients in the groups was insufficient.

Discussion

In this study, we compared the clinical efficacy of calfactant, which has recently become available in our country, beractant and poractant alfa among preterm infants with RDS. In patients with respiratory distress, the surfactant, a phospholipid mixture, reduces surface tension to keep the alveoli open. The efficacy of surfactant treatment for the treatment of RDS has been fully specified; however, it is not clear which type of natural surfactant should be employed. There are studies in the literature comparing these three natural surfactants. However, there are not enough studies in Turkey making this comparison.

We found that calfactant, beractant and poractant alfa had similar relative effectiveness on pulmonary hemorrhage, surfactant redosing, prevention of air leak syndromes, duration of invasive mechanical ventilation, duration of non-invasive mechanical ventilation and length of free oxygen need, hs-PDA, surgical treatment of PDA, NEC, IVH, BPD, ROP, treatment of ROP, time to start full enteral feeding and discharge time. Only sepsis and mortality were lower in group 3 than the others. In a multi-center study by Trembath et al. (8), in which they compared beractant, calfactant and poractant, there were no differences in outcomes obtained including air leak syndromes, NEC, IVH (grade 3 or 4), BPD and mortality. The authors concluded that the differences between the surfactants included in previous studies with regard to mortality and outcomes did not show the actual differences in effectiveness of surfactants and that it was related to variations in outcomes attributed to different institutions. In this retrospective study, the median gestational age, below the 37 weeks of gestation, was 30 weeks and the infants had a median birth weight of 1,435 grams. In contrast, our study had a lower mean birth weight and gestational week; we thus think we produced similar but more objective data. It is known that, with surfactant administration, the incidence of air leak reduces in patients with RDS. This, in turn, means a reduction in associated morbidities including hypoxia, hypotension and IVH (9). Although it was determined in our study that the incidence of patients with air leak was higher in the poractant alfa group compared to the other two groups, this was not statistically significant. In a retrospective study, Jeon et al. (10) compared three natural surfactants in patients with a mean gestational age of 28th weeks and a birth weight of 1,130g. They found that all three surfactants were similarly effective in respiratory outcomes and secondary outcomes including complications of prematurity. Although they had largely similar results to our study, they could not randomize the patients because the study was retrospective and they left results of patients under 24 weeks of gestation unrecorded. In contrast to several studies on this issue in the literature, our study also included infants born at 24-22th weeks' gestation.

In the study of Karadag et al. (11), surfactant redosing was more frequent in the beractant group. However, the poractant alfa group had a higher perfusion index and better oxygenation index after surfactant. In the study by Dilli et al. (12), however, surfactant redosing was determined to be more frequent in the calfactant group. However, in neonates with RDS, poractant alfa and beractant lessened oxygen demand to a similar extent in accordance with lung ultrasonography findings. They seem to be superior to calfactant. In contrast to what Dilli et al. (12) expressed, in our study, there were no differences between the groups with regard to redosing. In addition, the patients' duration of ventilation support, which is an indicator of oxygenation, was similar in all three groups.

Similar studies have discussed the comparative efficacy of surfactants on mortality (13,14). Ramanathan (15) reported that poractant alfa was associated with decreased mortality rates compared to beractant or calfactant and it was suggested that the differences in mortality rates might be related to the composition of these surfactants, including higher amounts of phospholipids and plasmalogens and a smaller volume of poractant alfa compared to other animal-derived surfactants. Ramanathan et al. (16), conducted a retrospective observational cohort study in the US, which compared poractant alfa with calfactant and beractant. They also concluded that poractant alfa at a dose of 200 mg/kg was associated with reduced mortality rates compared with calfactant at a dose of 105 mg/kg or beractant at a dose of 100 mg/kg. The same dose of poractant alfa and beractant at a dose of 100 mg/kg resulted in no difference in mortality rates, and mortality rates were also similar to those of calfactant and beractant. In our study, causes of death in groups I and II included particularly sepsis, as well as causes including pneumothorax and hypoxic respiratory failure. Mortality and sepsis rates were determined to be significantly lower in group III compared to the other two groups. We therefore directly correlate mortality with sepsis, not with RDS. The low mortality rate in the calfactant group was attributed to the low sepsis rate. Low sepsis in this group is a completely random result. We know that there is no statistically significant risk factor (e.g., birth week, birth weight, RDS-

related respiratory outcomes) that increase mortality other than sepsis among the groups. Furthermore, we could not directly correlate the effectiveness of surfactant molecules with mortality and sepsis rates, as we are of the opinion that mortality and sepsis are related to several factors other than the severity of RDS.

The need for cost-effectiveness studies has increased with the increased number of alternatives in the surfactant market. Sekar et al. (17), compared length of stay, costs, mechanical ventilation, and mortality in preterm infants with beractant, calfactant, and poractant alfa for RDS. They found adjusted NICU length of stay and costs were similar among the three groups. Infants receiving poractant alfa were less likely to be on mechanical ventilation at days 3 and 7, and poractant alfa treatment was associated with lower odds of NICU mortality when compared to calfactant. In this study, there was no association between the duration of respiratory support and mortality results. Additionally, we did not investigate cost-effectiveness in this study. Nonetheless, we examined the clinical efficacy of three natural surfactants used in our ethnicity and our own medical practice. Hence, this study, as an important tool for comparative effectiveness research, helps clinicians and buyers reach a decision on product preference (18).

Conclusion

In this study, beractant, poractant alfa, and calfactant had clinically similar efficacy in patients with RDS. Therefore, the decision regarding which surfactant preparation to use should be based on factors other than effectiveness.

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Ethics

Ethics Committee Approval: Ethics Committee approval for this study was obtained from Necmettin Erbakan University with the Ethics Committee decision number of 2019/2105.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.H.Y., Design: F.H.Y., Data Collection or Processing: N.D.G., M.Y., Analysis or Interpretation: R.K., E.N.Y.Ö., Literature Search: N.T., Writing: H.A.

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Original Article

The Effect of Web-based Diabetes Education on the Metabolic Control, Self-efficacy and Quality of Life of Adolescents with Type 1 Diabetes Mellitus in Turkey

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ABSTRACT

Aim: The purpose of this study was to examine whether web-based diabetes education is effective in improving metabolic control, self-efficacy for diabetes self-management, and quality of life in adolescents with type 1 diabetes mellitus in Turkey.

Materials and Methods: This study was conducted with adolescents with type 1 diabetes mellitus who were registered in the pediatric endocrinology polyclinic of a university hospital in the western region of Turkey. A total of 32 were included in the control group who received diabetes education in a clinical setting, and 30 were included in the intervention group who received web-based diabetes education. Although the adolescents in the control group received standard medical care as usual, with no participation on the website, those in the intervention group were also educated on diabetes management by using the web site.

Results: For self-efficacy, a statistically significant difference between the groups was found. Regarding the group, time, and group-time interaction for quality of life, a statistically significant difference was found between the mean scores of the groups. Web-based education was found to be effective in increasing the quality-of-life mean scores when compared with the standard care provided for the adolescents with type 1 diabetes mellitus, with the intervention group having a higher quality of life than the control group.

Conclusion: We found that the web-based diabetes education program had no effect on A1C levels, but the education model effectively increased the self-efficacy and quality of life of adolescents with diabetes.

Keywords: Adolescent with diabetes mellitus, web-based education, web-based diabetes education

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic metabolic disease commonly seen in childhood; it occurs as a result of the destruction of the pancreatic beta cells, accompanied by T-cells, for reasons relevant or irrelevant to autoimmunity (1). In Turkey, approximately 17,000 children have TIDM, and about 2,000 children are diagnosed with it per year (2).

Adolescence is the transitional phase of development between childhood and emerging adulthood and it is marked by the biological and psychosocial changes of puberty (3).

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Adolescence is a period in which these young patients can learn the required knowledge and self-care methods related to T1DM; nevertheless, management of the disease is most difficult during this developmental stage. Management of T1DM requires frequent blood glucose monitoring, multiple insulin injections or the use of an insulin pump, frequent alterations in insulin dose to match changing diet and activity patterns, and regular visits to health care providers (4).

Diabetes management requires frequent and high levels of education at diagnosis and afterward to support children and adolescents. Education is necessary for its successful management (5). Education programs aimed at diabetes have been revealed to be a very cost-effective intervention, due to the savings to be obtained via low levels of hospital admissions and emergency presentations. To maintain blood glucose of those adolescent with T1DM at recommended levels and prevent complications, these patients need to be able to contact the diabetes team frequently and easily and have access to health services at all times (6). Adolescents not receiving sufficient levels of education or not continuing to have educational support exhibit more possibility of developing diabetes-related complications (5). Diabetes education needs to be an ongoing process and be repeated in order for it to be more effective. In addition, the failure to increase the number of health professionals for diabetes care in line with the increase in the number of patients with diabetes has led to lower access to education for patient self-management (7). In addition, evidence obtained through group discussions conducted with young people points out that the education programs using web-based applications are attractive for them, and there are data to back up its frequent use (8). Web-based education also provides an accessible and permanent record of information for patients that enables fast and effective communication with health professionals. Web-based education has been shown to improve patient satisfaction, improve self-management of diabetes and clinical results (9). Web-based applications that include individualized evaluations, supervision and skills development by feedback are reported to be more effective in the improvement of glycemic control than didactic teaching (10). Studies have indicated that web-based education provided to adolescents with diabetes is effective in enhancing self-efficacy and quality of life (QOL) (11,12).

Higher self-efficacy is believed to have an impact on the adolescents' life, self-management, and outcomes related to diabetes. This concept has also been revealed to be significantly associated with the likelihood of obtaining positive results and acquiring the means of self-management for diabetes as well as QOL (13,14). A higher QOL, higher levels of self-efficacy and better diabetes education have positive effects on metabolic control (15,16). QOL is lower among children and adolescents with T1DM compared to children without diabetes; previous studies have shown that better QOL in adolescents is associated with higher self-efficacy and improved self-management. In addition, glycemic control may improve (17,18). Therefore, health professionals need to understand the importance of self-efficacy for diabetes self-management and QOL in diabetes, and they should evaluate adolescent self-efficacy for diabetes self-management (18,19). No studies have so far been conducted in Turkey to examine the influence of web-based diabetes education on metabolic control, self-efficacy for diabetes self-management, and QOL of adolescents with T1DM. In Turkey, there is limited access to pediatric endocrinology and pediatric endocrine nurses in every region. Most of the patients enrolled in the clinic where the study was conducted live six-to-ten hours away. Thus, it is very difficult for them to reach diabetes providers when they need to and it is very important that they have the right source of information. Thus, the aim of this study was to examine whether web-based diabetes education was effective in improving metabolic control, self-efficacy for diabetes self-management, and QOL in adolescents with T1DM in Turkey.

Hypotheses

Our hypotheses were:

H_1: A1C levels of adolescents with T1DM who were provided with web-based education will be lower than those who were educated about diabetes in a polyclinic environment.

H_2: Self-efficacy for diabetes self-management scores of adolescents with T1DM who receive web-based education will be lower than those who are educated about diabetes in a polyclinic environment.

H_3: QOL scores of adolescents with T1DM who were provided with web-based education will be higher than those who are educated about diabetes in a polyclinic environment.

Materials and Methods

This study was carried out with adolescents who had T1DM and who were registered in the pediatric endocrinology polyclinic of a university hospital in the western region of Turkey. Their ages ranged between 11 and 18 years. The design was a pre-test/post-test quasi-experimental approach.

The Study Population and Sample

The required study sample size was estimated based on an anticipated p-value, power level, and effect size, with G*Power 3.0. Researchers used the analysis of variance and accepted an effect size of 0.5, and type 1 and 2 error levels of 0.05 and 0.20 (80% power), so that sample size was estimated based on A1C with 19 adolescents; self-efficacy with 14 adolescents; and QOL with 29 adolescents. To account for potential losses due to the long-term follow-up, it was decided to include 36 adolescents in each group. We randomly assigned individuals into groups.

Data Collection

This study was conducted from September 2015 to March 2016. Before collecting pretest data, the researchers assigned participants to the intervention and control groups based on the order that they came to the polyclinic. The first patient was allocated to the control group and the second patient to the intervention group and so on. The researchers introduced the web site on IPads™ to the intervention group and explained how to use them. When the target sample size was achieved, the adolescents included in the intervention group were informed via text message that the web site was activated, and they should begin using it. A total of 34 were included in the control group who received diabetes education in the clinical setting, and 36 were included in the intervention group who received the web-based diabetes education. Following the initial data collection, data loss occurred in the intervention group at the end of the third month, because four of the adolescents' use of internet was limited at home, and two adolescents did not use the site actively. The researchers could not contact two control group adolescents at the end of the third month. Consequently, we completed the study with 32 adolescents in the control group and 30 in the intervention group (Figure 1).

Intervention

The standard of care at the Pediatric Endocrinology Outpatient Clinic at the University Hospital is the provision of basic diabetes education. A diabetes nurse, who has a Diabetes Training Nursing Certificate, provides basic diabetes education to adolescents with diabetes and their families. At diagnosis, they are told to come to the outpatient clinic when they need to, and patients are expected to come to the clinic every three months. The diabetes nurse meets with each child in the education room and provides T1DM education using various pamphlets and/or posters as needed. Children with newly diagnosed T1DM and their families receive basic diabetes education at the time of clinical admission. Information about diabetes, acute complications (hypoglycemia, hyperglycemia), treatment, insulin injection, and issues that they should pay attention to are provided at initial diagnosis.

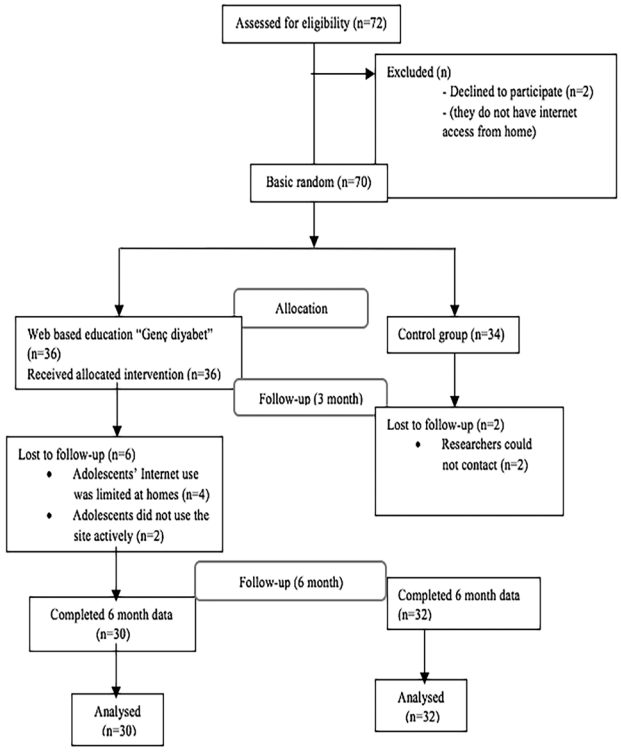

Figure 1. Flowchart for enrollment and follow-up of study participants

An experimental website was created using the open-source code WordPress Content Management System and was structured using PHP Script language. Two user groups use the website-administrators and members. The administrator panel includes the elements required for the performance, organization, and implementation of the operations that are necessary for the entire system. It has menu elements, user operations, and unanswered survey forms as well as the content display. It can also be used to add and delete users and update the website. The member panel user homepage includes a valid username and password that is given to the member by the researcher to enable signing in to the system. Users can login to the web page when they enter their usernames and passwords correctly. Both groups received standard medical care, while those in the intervention group were educated on diabetes management by using the web site. The web site includes a series of learning objectives. Where appropriate, the material uses multimedia learning

tools such as tutorials using Powerpoint™ files about diabetes management, quizzes, and blogs. The blog was designed as a platform so that adolescents included in the intervention group could chat and share their experiences. The adolescents were asked to write comments and share their experiences on issues suggested by the researchers. The appropriateness and sufficiency of the educational topics uploaded on the web site were examined by relevant experts (pediatric endocrinologists, professors working on pediatric nursing, diabetes clinic nurses, and the head of the diabetes association in Turkey-11 experts in all). The content validity index regarding educational content was found to be 0.96, suggesting high agreement among experts with this conclusion being higher than 0.80.

Data Collection Procedures

The intervention was delivered over a six-month period. Participants were encouraged to log in at least two times each week to update their blogs. The adolescents used the blog site actively. We used Counterize™ plugins to collect data on use. Counterize is a complete counter and statistics plugin with no external library dependency, and these data and site traffic and each users' usage were recorded. An active learning process was ensured where the adolescents received support from their peers via blog use. The researcher was notified by short message and e-mail when the adolescents posted a comment on the blog site. Comments were published on the website under the supervision of the researcher. The following are examples of issues included in the blog site: "What is diabetes in your opinion?"; "Is exercise important in the management of type 1 diabetes?"; "Let's share our experiences: how do you know when you have high blood glucose?"; "How do you know when you have low blood glucose?" The topics were selected from the literature. New topics (e.g., definition of the disease, nutritional aspects, carbohydrate counting, management of acute and chronic complications, insulin regimen, physical exercise, and self-management) and relevant blog texts were added to the site every week. A reminder message about new materials added to the web site was sent. The adolescents were not expected to respond to this message.

We introduced the website, "youth diabetes", to those adolescents included in the intervention group and informed them about its use as part of their polyclinic appointments. Adolescents included in the intervention group could log in at any time day or night.

Three-and-six-month follow-up data were collected by the researchers from the adolescents in the control group when they arrived for their polyclinic appointments. Adolescents included in the intervention group were asked to fill out the scale-based forms on the web. The A1C values of the adolescents in both groups were collected from the polyclinic records at 3 months and 6 months after the start of the intervention.

Measures

Psychosocial data were collected using the pediatric QOL Inventory™ 3.0 diabetes module and the diabetes management self-efficacy scale in adolescents with type 1 diabetes mellitus.

The Pediatric QOL Inventory™ 3.0 Diabetes Module

Varni et al. (20) developed the pediatric QOL Inventory™ 3.0 diabetes module, which was adapted for Turkish by Ayar and Ozturk (21). The scale comprises of 28 items. A five-point Likert scale was used in which 0=never a problem, and 4=almost always a problem. Items were linearly transformed to a 0-100 score. The score was 100 if the items were rated "never a problem" and 0 if the items were rated "almost always a problem". Thus, higher scores indicate higher health-related QOL. The Cronbach alpha for the scale in this population was 0.86. The split-half reliability was 0.71 for the first and second half of the child report. The correlation coefficient between the first and second halves was 0.55 ($p<0.001$). The Kaiser-Meyer-Olkin coefficients were found to be 0.80 and $\chi^2=15,275$, $p<0.001$, respectively. Item-total correlations for the scale varied between 0.32 and 0.86 ($p<0.001$). The model fit indicators were: CFI=0.87, incremental fit index (IFI)=0.87, GFI=0.78, $\chi^2=432.34$, df=337, and root mean square error of approximation (RMSEA)=0.051, $p<0.001$. Thus, this scale is a reliable and valid instrument that can be used by diabetes teams to measure QOL in Turkish children with T1DM (21).

The Diabetes Management Self-efficacy Scale in Adolescents with Type 1 Diabetes Mellitus

The diabetes management self-efficacy scale in adolescents with type 1 diabetes mellitus was developed by Moens (22) and its Turkish validity and reliability was determined by Ozturk et al. (23). The scale involves 26 single-choice items that are scored on a 5-point scale which range between 1 (definitely yes) and 5 (definitely not). The scores obtained are summed up and divided by the total number of items in order to show the magnitude of perceived self-efficacy of different self-management performance levels. High scores represent lower self-efficacy. The CFA showed respective factor-loading ranges of factor 1 (medical treatment and nourishment) as 0.41-0.86; factor 2

(evaluation of glycaemia) as 0.42-0.89; factor 3 (mentioning your diabetes) as 0.75-0.77; and factor 4 (honesty with yourself and others) as 0.41-0.72. The model fit indicators were as follows; the GFI=0.90, non-normed fit index=0.93, CFI=0.93, and IFI=0.93, with $\chi 2$=470.15 for df=290, p<0.001, and the RMSEA was 0.056. The total Cronbach's alpha internal consistency reliability coefficient value was 0.85. The item-total correlations changed between 0.40 and 0.59 and were seen to be statistically significant (p<0.001). This scale is designed as a disease-specific one in order to evaluate the level of self-efficacy in Turkish adolescents with T1DM (23).

Ethical Considerations

Written permission was obtained from the owners of the scales used. To conduct this study, the researchers obtained permission (with protocol no: 779-GOA, 2012/36-02) from the institution and University Non-Invasive Research Ethics Board. Since participants of this study were adolescents, the researchers obtained written consent from participants' parents, and verbal assent from the adolescents.

Statistical Analysis

Multivariate analysis of variance was employed in order to compare the total mean of A1C levels and mean scores on the diabetes management self-efficacy scale and QOL of children with diabetes scale according to grouping, timing, and the group-time interaction. One-Way analysis of variance was used for analysis of the difference between the total mean scores of diabetes management self-efficacy scale for the adolescents with T1DM and QOL in children with diabetes scale. It was also employed in an attempt to compare the mean scores of groups. The Bonferroni correction t-test for dependent groups was employed in an attempt to compare the measurements. The Bonferroni significance test of the difference between two corrected averages was used for the analysis of the difference between the groups over time.

Results

Patients' Characteristics

We completed our study with 62 adolescents who had T1DM with 30 in the intervention group and 32 in the control group. The mean ages of those who participated in the intervention group were seen to range between 11 and 18 years [mean, 14.60 years; standard deviation (SD), 1.90 years] and that of the control group ranged in age from 11 to 18 years (mean, 13.96 years; SD, 1.61 years). The duration of diabetes of the intervention group participants was 5.66±4.22 years and that of the control group was 6.34±3.17 years. The mean A1C value observed in the intervention group was found to be 8.10±1.30%, while the same value in the control was 8.36±1.82% at the start of the study. The mean diabetes related QOL scores of those included in the intervention group was 37.06±6.91 and the same score in the control group was 38.79±10.15 at the start of the study. The diabetes management self-efficacy scores of those in the intervention group was 108.40±15.30, while the same in the control group was 108.48±13.27 at the start of the study. The number of adolescents using insulin injection in the intervention group was 8 (26.66%), and 22 were using insulin pumps (73.33%). The socio-demographic characteristics of the participants are presented in Table I.

Analyses indicated that the experimental and control groups did not differ in terms of age, sex, diabetes history, initial A1C levels, and initial mean scores of self-management and self-efficacy in the diabetes scale for the adolescents with type 1 diabetes and the QOL in children with diabetes scale. While there were no statistically

Table I. Descriptive characteristics of the adolescents with type 1 diabetes (n=62)

Characteristic	Intervention group (n=30) (mean ± SD)	Control group (n=32) (mean ± SD)	p-value
Mean age	14.60±1.90	13.96±1.61	0.129
Duration diabetes (years)	5.66±4.22	6.34±3.17	0.069
Onset level of HbA1c	8.10±1.30	8.36±1.82	0.068
Onset diabetes related quality of life scores	37.06±6.91	38.79±10.15	0.354
Onset diabetes management self-efficacy scores	108.40±15.30	108.48±13.27	0.620
Using insulin injection (%)	8 (26.66)	10 (31.25)	
Using insulin pump (%)	22 (73.33)	22 (68.75)	

SD: Standard deviation

significant differences between the two groups (p>0.05), A1C levels and diabetes duration showed trends toward significance, with the intervention group having lower A1C and shorter diabetes duration at baseline (Table I).

Multiple analysis of variance was used to determine whether there was a difference between the A1C mean scores of the study groups. Regarding the group (F=0.212, p=0.647), time (F=1.225, p<0.297) and group-time interactions (F=1.393, p<0.252), no statistically significant differences were found between the mean scores of the groups (Table II).

Multiple analysis of variance was used to determine whether there was a difference between the QOL mean scores of the study groups. Regarding the group (F=7.862, p=0.007), time (F=7.555, p<0.001) and group-time interactions (F=12.747, p<0.001), statistically significant differences were found between the mean scores of the groups (Table II).

Multiple analysis of variance was used to determine whether there was a difference between the self-efficacy mean scores of the study groups. Regarding the group (F=44.058, p=0.001), time (F=37.715, p<0.001) and group-time interactions (F=45.115, p<0.001), statistically significant differences were found between the mean scores of the groups (Table II). Further analysis for the mean scores of the self-efficacy scale and QOL for the adolescents with T1DM in group, time, group-time interaction revealed statistically significant differences between the mean scores of the groups (Table III).

Discussion

In this study, we found no significant difference between the intervention and control groups on A1C levels. In previous studies conducted by a multi-disciplinary team, it was reported that web-based educational programs were effective in improving A1C levels of adolescents with T1DM (15,24,25). While our study group was not multidisciplinary, we found no significant difference between the A1C mean scores of the two groups. This finding suggests that this difference may be related to differences in methods. Self-management is necessary to prevent short and long-term

Table II. Comparing A1C, quality of life and self-efficacy mean scores of the adolescents with type 1 diabetes in groups, at time and at group*time points (n=62)

Time/group	A1C		Quality of life		Self-efficacy	
Time	**Intervention group (mean ± SD)**	**Control group (mean ± SD)**	**Intervention group (mean ± SD)**	**Control group (mean ± SD)**	**Intervention group (mean ± SD)**	**Control group (mean ± SD)**
Baseline	8.10±1.30	8.36±1.82	108.40±15.30	108.48±13.27	37.06±6.91	38.79±10.15
Three months	8.38±1.47	8.33±1.89	116.53±14.49	108.84±11.98	34.40±7.81	41.75±10.30
Six months	8.19±1.39	8.63±1.58	122.23±13.24	106.53±7.78	35.13±6.70	59.28±8.74
	F	**p**	**F**	**p**	**F**	**p**
Group	0.212	0.647	7,862	0.007	44,058	0.001
Time	1,225	0.297	7,555	0.001	37,715	0.001
Group*time	1,393	0.252	12,747	0.001	45,115	0.001

SD: Standard deviation, p: Significance level, F: Analysis of variance

Table III. Further analysis for the mean scores of self-management and self-efficacy in diabetes scale and quality of life for the adolescents with type 1 diabetes in groups, time, group*time (n=62)

Time/group	Self-efficacy				Quality of life			
Time	**Intervention group (mean ± SD)**	**Control group (mean ± SD)**	**t**	**p**	**Intervention group (mean ± SD)**	**Control group (mean ± SD)**	**t**	**p**
Baseline	37.06±6.91	38.79±10.15	0.793	0.431	108.40±15.30	108.48±13.27	0.024	0.981
Three months	34.40±7.81	41.75±10.30	3,170	0.002	116.53±14.49	108.84±10.98	2,301	0.025
Six months	35.13±6.70	59.28±8.74	12,244	0.000	122.23±13.24	106.53±7.78	5,644	0.000
F	1,381	74,154			16,956	0.846		
p	0.260	0.000			0.000	0.434		

SD: Standard deviation, p: Significance level, t: Significance test of the difference between two means/t-test for dependent groups, F: Analysis of variance

complications such as seizures, nephropathy, diabetic ketoacidosis, neuropathy and retinopathy (15).

Self-management requires frequent blood glucose monitoring and insulin injections or the use of pump therapy, both of which require frequent adjustment depending on food intake and physical activity. Diabetes self-management is intensive, constant, complex and visible, and it contributes to feelings of stress and social awkwardness in adolescents. Any acute or chronic stress in this period will directly affect diabetes management (26). General emotional stress in adolescents negatively affects both their blood sugar and glycemic control levels and may limit their ability to perform diabetes self-management tasks. Feeling stress due to diabetes management and a stressful living situation may lead to poorer metabolic control for those adolescents with T1DM and also to problems with adaptation to the condition (13,27). Among our intervention group adolescents, 33% were studying for the high school or university admission exams, which are important and often stressful periods. Even the presence of a factor such as an exam causes great stress for adolescents. In addition, with the hormonal changes in the approach to maturity, metabolic control of adolescents is negatively affected. Consequently, in the follow-up assessments, no statistically significant difference was found between the A1C mean scores of the adolescents with diabetes in the intervention or in control groups.

Self-efficacy levels of those in the intervention group were found to increase compared with those provided only with standard care. Therefore, hypothesis H_2 was accepted. Increasing self-efficacy in adolescents with T1DM is important for healthy self-management behavior change. The findings of this study are similar to those of previous studies (13,14,27) that examined the impact of Web-based diabetes education on self-efficacy. Web-based diabetes education was found to be an effective method for increasing self-efficacy for diabetes self-management in adolescents with diabetes. It is believed that web-based educational programs for those adolescents with T1DM should be made more commonly available because the number of adolescents using the Internet is increasing in Turkey, and web access allows for information to be sought whenever they need and wherever they are.

In this study, we found a significant difference between the QOL Mean Scores in the adolescents with T1DM in the intervention group and also those in the control group. Our web-based education program was found to be effective in increasing QOL mean scores when compared with the standard care provided for the adolescents with T1DM. Therefore, the third hypothesis was accepted. This finding is similar to that of some previous studies (14,27) that examined the impact of diabetes education on QOL of adolescents with diabetes. Thus, web-based diabetes education was found to be an effective method for enhancing the QOL of the adolescents with T1DM.

Study Limitations

This study has several limitations that must be considered in the interpretation of the findings. Initially, there were 36 adolescents in the experimental group, and at the end of the third month, four adolescents could not access the internet from their homes, and two adolescents did not actively use the site. In addition, we did not look at long-term effects. The initiation of the website by the group of entrants in June created difficulties for the researcher in reaching the adolescents due to the summer holiday. Also, the Pediatric QOL Inventory sub-scale which we used in our study had low Cronbach alpha and so limits our results.

Conclusion

We found that our web-based diabetes education program effectively increased the self-efficacy and quality of life of adolescents with diabetes, but it had no effect on A1C levels. Thus, we recommend web-based programs for adolescents with T1DM to enhance their QOL and self-efficacy and to perform observations over a longer time period to see the impact on A1C levels more clearly. We did not examine cost-effectiveness. It is also recommended that cost-effectiveness should be evaluated in new studies for better understanding of the effectiveness of the web-based education.

On the basis of this study, we believe that the use of web-based diabetes education will help to ensure that both adolescents with diabetes and health care providers will use their time together more effectively. It is also possible that diabetes education may be more accessible by using Web-based approaches. Our study contributes to the diabetes education literature because there was no web-based education program for those adolescents with T1DM set up and available to diabetes nurses in Turkey.

Ethics

Ethics Committee Approval: To conduct this study, the researchers obtained permission (with protocol no: 779-GOA, 2012/36-02) from the Institution and University Non-Invasive Research Ethics Board.

Informed Consent: Since participants of this study were adolescents, the researchers obtained written consent

from participants' parents, and verbal assent from the adolescents.

Authorship Contributions

Concept: D.A., C.Ö., Design: D.A., C.Ö., Data Collection or Processing: D.A., Analysis or Interpretation: D.A., C.Ö., M.G., Literature Search: D.A., C.Ö., M.G., Writing: D.A., C.Ö., M.G.

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

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Original Article

Is the Winograd Technique an Effective Method in the Treatment of Ingrown Toenails among the Pediatric Population?

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ABSTRACT

Aim: This study aimed to examine the results of the Winograd technique applied in the treatment of ingrown toenail cases, which are frequently encountered in childhood, especially in adolescence. The recurrence, satisfaction, and complication rates in pediatric age groups were evaluated with the surgical matrixectomy application of the Winograd technique, a frequently used one in adult age groups.

Materials and Methods: Ingrown toenail cases that had undergone surgical matrixectomy with the Winograd technique between September 2016 and December 2018 were evaluated. Those detected to be stage 2-3 according to the Heifetz classification were operated on. Demographic information was recorded. The cases were divided into three groups as 3-7, 7-12, and ≥12 years as age groups. Such data as recurrence, post-operative infection, osteomyelitis, long term complication and return to regular activity were evaluated. The visual analog scale was used for the satisfaction scale.

Results: A total of 162 operated ingrown toenails from 142 children were followed for an average of 21 months (12-38). The mean age of patients was 13.8 years. When the ingrown toenail location was evaluated, the lateral sides (98, 61.2%) of the patients were seen to have been affected more. According to age groups, 65% (105) of the cases were in the group aged 12 years and over. While the frequency of all complications was 8.1% (13), no chronic complications or osteomyelitis were encountered. Recurrence was observed in 3.1% of cases and also early infection (<15 days) was observed in 10 cases, while late infection (<15 day) was observed in 3 cases. According to the visual analog scale scores, 135 (95.0%) cases were found to be satisfactory or very satisfactory.

Conclusion: The Winograd technique (surgical matrixectomy) has low recurrence, low complication and high satisfaction rates in all pediatric age groups even with advanced ingrown toenail complaints. Complete excision of the affected matrix with magnification increases the success of the Winograd technique.

Keywords: Pediatric, ingrown toenail, Winograd, matrixectomy, recurrences

Introduction

Ingrown toenail (onychocryptosis) can be seen frequently in the pediatric population as well as in the adult population (1,2). Especially in the adolescent age group, the failure of the nail bed to fit properly in the nail groove causes inflammation, pain and swelling, leading to lateral and/or medial margin penetration. Wearing tight-fitting shoes, foot plantar pressure disorders (pes planovalgus), foot deformities (clubfoot, hallux valgus) and incorrect short or straight trimming of the nail can lead to cases with this condition. Different from the adult age groups,

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this situation in the pediatric population creates discomfort while conducting school work and sports activities, thus affecting the suffers' success rates (2,3). Psychologically, it affects the relationships of children with their friends. Many children try to treat their ingrown nail on their own, causing the formation of acute infection, suppuration, and granulation tissue.

Recurrence rates have been reported in studies comprising adult age groups in ingrown nail cases, where many means of surgical treatments other than conservative treatments are applied (4). Similar to adults, Heifetz stage 1 cases also benefit from conservative measures (5). In advanced cases where infection, drainage and granulation tissues are encountered, avulsion of the ingrown nail by a surgical procedure and debridement is required. Various surgical techniques are applied in the adult age groups, ranging from simple nail avulsion to germinal matrix excision. Germinal matrixectomy is performed with a sharp excision, while chemicals such as phenol, NaOH or electrocoagulation can also be applied for matrixectomy (2,4). The pediatric age group, on the other hand, has an immature structure different from that of adults, and results regarding the consequences that such procedures may have in the pediatric group who are still growing are insufficient (1-3).

The Winograd technique was first described in 1929 and is still often the preferred technique (6). The important thing in this technique is the complete avulsion of the germinal matrix in the excised part, which determines recurrence. For this purpose, auxiliary techniques such as phenol or electrocoagulation have been additionally used to disrupt this matrix.

We hypothesized that the use of the Winograd technique without using any matrixectomy tools in addition to surgical matrixectomy might result in better outcomes in the surgical treatment of ingrown toenails. This study aims to evaluate the results of the Winograd technique in the treatment of ingrown toenails among children.

Materials and Methods

Ingrown toenail cases who were operated on between September 2016 and December 2018 were included in this study. We included children with Heifetz stage 2 or 3 ingrown toenails that had not responded to conservative management. Patients who were followed up for at least 12 months were included. We excluded patients with Heifetz stage 1 ingrown toenails, traumatic nail deformity, dystrophic nail, hemophilia, onycholysis, onychomycosis, immune deficiency, and previously operated recurrent cases (except complete nail avulsion). The patients were divided into 3 groups according to Heifetz classification (5) and those listed in stage 2-3 were operated on.

Data such as age, gender, limb (left or right), ingrown edge (medial or lateral), infection and disease time were recorded. The cases were divided into three groups by age, namely as 3-7 years, 7-12 years and ≥12 years. All procedures and assessments were performed by the same surgeon (MCS).

All cases underwent conservative therapy with local rifampicin dressing for 3 weeks and first-generation cephalosporin for 7 days before the procedure in the hope that the infection would regress and the short nail or the nail aimed to be removed would grow back (Figure 1).

Surgical procedures (6) were performed by applying digital anesthetic block (2% lidocaine without epinephrine) and also a digital tourniquet was used. A longitudinal vertical incision was made in the distal of the nail with a sharp-pointed scalpel, starting from 3 mm proximal to the eponychium, removing 1/4-1/5 of the total nail in the medial or lateral edge of the nail. The application of incision at once advancing down to the matrix prevents additional damage to the nail bed. With a clamp advanced proximal to the germinal matrix from the nail, the involved toenail and germinal matrix were separated and exposed. In order to prevent recurrence, the nail plate and germinal matrix were completely excised and gently scraped with a fine-tipped curette. Following this, the hypertrophic granulation tissue was excised. After abundant irrigation, with sharp tip 2-0 prolene suture (Ethicon™), by first passing the needle from the nail and soft tissue together, then without tying a knot, just passing the needle from the nail in the inferior superior direction, the lip-like soft tissue was ensured to remain under the nail and the knot was left on the nail (Figure 2). Non-compressive dressings were applied and a daily change of the dressing was recommended. Oral antibiotics and non-steroidal anti-inflammatory drugs were given and the stitches were removed after an average of 3 weeks in parallel with the wound healing (Figure 3).

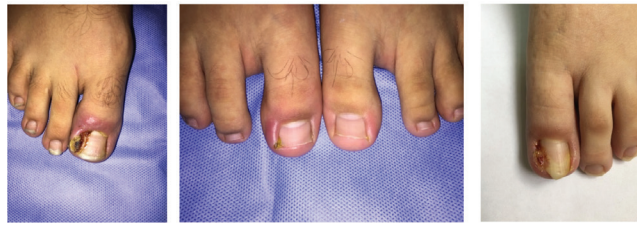

Figure 1. Samples of the severe ingrown toenail. The causes in these samples are including wearing tight-fitting shoes, cutting nails too short, or not cutting the nails straight

Post-operative local recurrence, complications (including infection), and satisfaction rates were evaluated. While evaluating satisfaction rates after surgical treatment, the patients were asked to respond by considering factors such as feeling of stinging or pain, the ability to wear comfortable shoes, cosmetic appearance, and the ability to keep up with daily activities. For this purpose, with a visual analog scale out of 10 points, a score ≥7 was found to be very satisfactory, 6 or 7 as satisfactory, 4 or 5 as unsatisfactory and if their score was <4, the result was found to be very unsatisfactory (1,4). The satisfaction scale was filled in during examinations in follow-ups. This study was approved by the ethics committee of Dr. Behçet Uz Child's Disease and Surgery Training and Research Hospital, Local Ethics Committee. Informed consent was obtained from the parents/care givers of the patients.

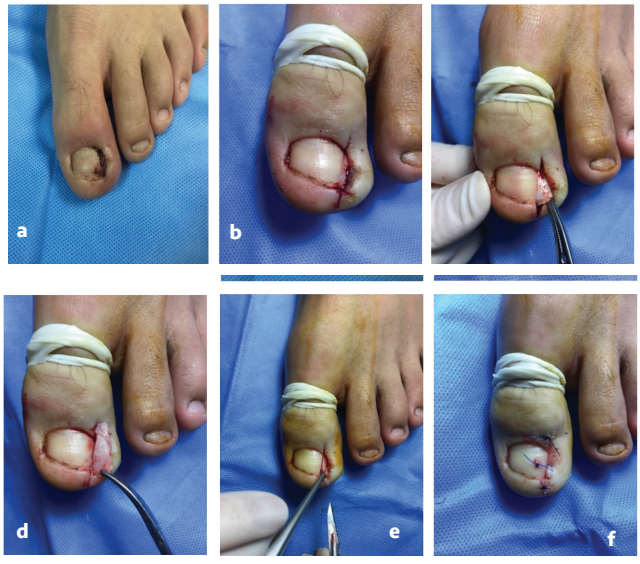

Figure 2. a) 14 years old male lateral edge ingrown toenail, b) Longitudinal incisions were used to perform a wedge resection of the involved lateral edge of the toenail. The incision extended into the eponychium. c) The involved toenail were removed with a clamp placed under the toenail
d) The removed ingrown toenail part is shown in place before operation
e) The involved germinal matrix was excised using a surgical lancet
f) The external fold of the toe was then approximated to the nail plate with 2-0 Prolene suture

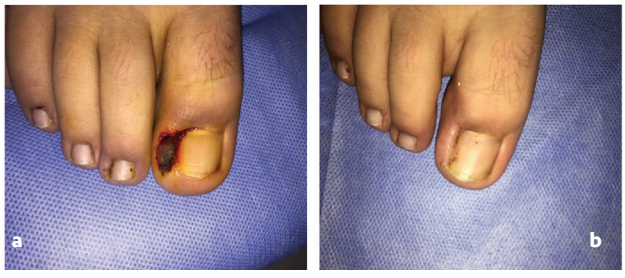

Figure 3. a) 13 years old male Heifetz grade 3 lateral edge ingrown toenail. b) 1 month later from Winograd operation. Lateral edge healed no symptom exist

Results

The mean age of the 162 ingrown toenail cases of the 142 pediatric patients evaluated was 13.8 years (3-18) and the mean follow-up was 21 months (12-38). The number of cases as well as their demographic data are summarized in Table I. When the ingrown toenail location was evaluated, the lateral side (98, 61.2%) of the patients were seen to be more affected on both sides. Location features are also given in Table I.

When the cases were analyzed by age groups, it was seen that 65% (105) of the cases were in the group over ≥12 years of age. While the frequency of all complications was 8.1% (13), no chronic complications or osteomyelitis were encountered. Recurrence was observed in 3.1% of cases. Early infection (<15 days) was observed in 10 cases, and late infection (≥15 day) in only 3 cases. The process characteristics and results according to age groups are given in Table II.

According to the evaluated visual analogue scale scores of the 142 patients, 135 (95.0%) cases were found to be satisfactory or very satisfactory, while it was also observed that 7 (5.0%) patients were unsatisfactory or very unsatisfactory. While 4 of them were the ones who experienced recurrence, the remaining 3 were patients whose regular activity return was delayed due to infection (Table III).

Table I. Patient descriptive features and location variables

		n (patients)	%
Gender	Female	54	28.0
	Male	88	61.9
Age in years	13.8±2.8	(3.00-18.00)	
Follow-up in months	21	(12.00-38.00)	
Heifetz group	Stage 3	108	67.5
	Stage 2	52	32.5

	Side			
		Right	Left	Bilateral
Ingrowing edge	Lateral	47 (29.3%)	39 (24.3%)	12 (7.5%)
	Medial	32 (20%)	24 (15%)	6 (3.75%)

Table II. Heifetz classification by age groups, complications, recurrence and regular activity return results

	Age groups			
	3-7		**7-12**	**≥12**
n (patients)	11		44	105
Heifetz	Stage 3	3 (1.8%)	31 (19.3%)	69 (43.1%)
	Stage 2	8 (5%)	13 (8.1%)	36 (22.5%)
Recurrence		0 (0%)	1 (2.27%)	4 (4.59%)
Infection	Early (<15 days)	1 (0.6%)	3 (1.8%)	6 (3.7%)
	Late (≥15 days)	0 (0%)	1 (0.6%)	2 (1.2%)
Long term complication		0 (0%)	0 (0%)	0 (0%)
Osteomyelitis		0 (0%)	0 (0%)	0 (0%)
Return to regular activity		6.8 days	14.5 days	17.4 days

Table III. Patient satisfaction results evaluated by visual analog scale by age groups

	Age groups			
	3-7	**7-12**	**≥12**	**Total**
Very satisfied (8-10)	9	32	51	92
Satisfied (6-7)	2	11	30	43
Dissatisfied (4-5)	0	1	2	3
Very dissatisfied (1-3)	0	0	4	4

Discussion

We determined that in the pediatric population of our study, the Winograd method for ingrown toenail results in high satisfaction rates, low recurrence rates, and low complication rates. As we hypothesized, this study indicates that the use of the Winograd technique without using any matrixectomy tools in addition to surgical matrixectomy results in better outcomes and can be considered an excellent modality of treatment of ingrown toenails in the pediatric population.

Ingrown toenail often results in walking difficulties, problems while wearing shoes, and restricted daily activities at work, school, and in the normal course of life (2). It is frequently seen not only in adults but also in the pediatric age group, particularly during the adolescent age. Unlike adults, the age, school status, and psychological status of the child are to be taken into account while deciding the treatment plan. It is important in this sense that the time to return to school and to wear shoes is minimal. While preparing the treatment plan for pediatric groups, Heifetz staging is as important as it is in adults.

In our study, conservative treatment alone was preferred for Heifetz stage 1 patients, whereas recurrence and complications were observed at a low rate in stage 2-3 patients in whom surgery was performed. Heifetz classification has an important place in deciding the treatment method in pediatric age group. Of the 210 cases treated, the stage 1 group of 150 cases were seen to have successfully healed with a conservative treatment plan. In conservative treatment, daily dressing, topical/oral antibiotics, education on proper nail trimming, and manipulations to separate the corner of the nail from the adjacent soft tissue were applied (7). The timing of conservative treatment is important. If conservative treatment is initiated in advanced cases, a dead-end cycle is expected to occur, the periungual tissue will emerge at the skin corner, thus forming granulation tissue, which can result in inflammation, infection, and foul-smelling discharge (4). This will affect children socially and psychologically as well as leading to a physical strain, all of which will cause failure in school and daily activities (3).

The Winograd method is an effective surgical method based on removing the affected nail edge using wedge resection and removing the partial matrix underneath it and the procedure has low complication rates (8). However, Acar (4) reported 6% recurrence in his study, while in another study, Aydin et al. (9) reported similarly in 62 patients (6.5%), Gerristma-Bleeker et al. (10) reported 20.6%, and Kose et al. (11) reported a 13.2% recurrence rate. In these studies, the high recurrence rates raise questions about the insufficiency of the Winograd technique alone. Traditionally, matrixectomies are performed with a surgical (sharp) excision of the germinal matrix. In spite of the application of matrixectomy, the reason for recurrence is thought to be due to insufficient excision. It was reported that additional procedures such as phenol administration, electrocoagulation or bipolar diathermy reduced recurrence in studies (2,4,12,13). Islam et al. (12), in one of their studies conducted in pediatric cases, reported that 4% recurrence was observed in chemical matrixectomy with phenol, while a 42% recurrence rate was seen in surgical matrixectomy. However, in their study, a total of 69 patients were evaluated and the recurrence rate was determined by telephone survey (12). Yang et al. (2), on the other hand, in their study conducted only in the pediatric population, reported a 12% recurrence rate in 112 children when applying chemical phenolisation. The fact that chemical matrixectomy is

less dependent on a physician's experience and ability compared to surgical matrixectomy may explain the lower recurrence rate encountered (14). However, Kayalar et al. (15) encountered a 9.8% recurrence rate with surgical matrixectomy in 224 cases of advanced stage where they did not perform any additional procedures. They emphasized that the procedures should be performed under surgical loupe magnification and that the matrixectomy should be performed completely. In our study, matrixectomy was performed with surgical loupe magnification in the evaluated cases and a 3.1% recurrence rate was observed. The use of a loupe magnification helps the surgeon work more comfortably and provides a more detailed view especially during matrix excision. Unlike other studies, the evaluation of the pediatric age group alone can also be considered to be effective while achieving this result in terms of the speed and advantage of wound healing observed in the pediatric age group. In addition, chemical matrixectomy methods such as phenol or 10% NaOH have some disadvantages. Tissue destruction, apparent drainage, prolonged wound healing, and post-operative infection can be counted as the side effects of these chemicals (2). In recent comparative studies, it has been determined that surgical matrixectomy has lower recurrence rates compared to chemical ones. Akkus et al. (16) reported 3.6% recurrence in cases where wedge resection was applied, while 5.4% in chemical matrixectomy with NaOH and no statistically significant difference was observed. On the other hand, Romero-Pérez et al. (17) reported a recurrence rate of 17.8% in phenol and matrixectomy in 520 procedures, while the same was 8.2% in surgical matrixectomy, and no difference between overall satisfaction rates was reported to have been seen (17).

In our study, while a total of 13 (8.1%) infections were observed, 10 of them were seen in the early period, and 3 in the late period. All of the cases were observed to heal. In studies conducted in adults, Peyvandi et al. (18) reported a 6% infection rate in 50 patients. Yang et al. (2), on the other hand, reported a 9% infection rate in the pediatric population in the early period. In our study, the rates show similarity in terms of rates of infection with the rates of those undergoing surgical matrixectomy (2,18). Also, similar infection rates have been reported in studies in which matrixectomy was performed with chemical or electrocoagulation (source electrochemical). Infection treatment response was seen to be good in children and osteomyelitis was not observed. The fact that these infections were not complicated can be explained by routine pre-and post-operative oral antibiotic therapy provided and dressings applied twice a day, which was recommended to the patients. This situation can also be explained by the fact that children have an advantage in terms of wound healing. We are also of the opinion that detailed granuloma extraction and infective tissue debridement performed with the use of loupe magnification mentioned by Kayalar et al. (15) have a positive effect on the process.

It was witnessed that the satisfaction rates pertaining to the procedure were very high for patients. 95% of the patients were found to be very satisfied or satisfied. This situation of our cases was similar to the satisfaction rates expressed in similar studies (4,11). This determining factor of basic satisfaction obtained in pediatric age groups was associated with the desire of children to return to activity quickly and to be able to wear shoes as soon as possible. It was observed that the main reason of almost all of the cases indicating dissatisfaction was in those cases where recurrence was reported. The early recovery period of cases is also what determines the satisfaction level of the sufferers. For this reason, pre-and post-operative detailed information about the process ensures that the expectations are not higher than what is to be expected and the level of subsequent satisfaction may increase afterward.

Study Limitations

The limitations of this study were the absence of a control group; however, our aim was to evaluate the results of the Winograd technique in children and adolescents. Our analysis is not therefore necessarily affected by the lack of a control group. Nevertheless, a control group would have provided an opportunity to observe the differences between chemical or electrocoagulation matrixectomy and the Winograd technique. Secondly, this is a retrospective single-center study with all the restrictions associated with such studies. All procedures and assessments were performed by a single surgeon so that could be a potential source of bias.

Conclusion

The Winograd technique has low recurrence, low complication, and high satisfaction rates in pediatric age groups with advanced (Heifetz 2-3) ingrown toenails. According to our results, it may be suggested that the use of the Winograd technique with detailed surgical matrixectomy using magnification and meticulous wound care results in better outcomes and can be considered an effective modality of treatment of ingrown toenails in the pediatric population.

Ethics

Ethics Committee Approval: This study was approved by the ethics committee of Dr. Behçet Uz Child's Disease and Surgery Training and Research Hospital Local Ethics Committee (date: 11.03.2021, number: 2021/05-13).

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

Peer-review: Externally peer-reviewed.

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Original Article

Psycholinguistic and Psychometric Measurements of the Turkish Pediatric Epilepsy Medication Self-management Questionnaire

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ABSTRACT

Aim: The aim of this study was to examine the psychometric and psycholinguistic characteristics of "The Pediatric Epilepsy Medication Self-management Questionnaire" which was adapted into Turkish.

Materials and Methods: The sample of this methodological study consisted of 540 parents whose children (between the ages of 2-17 years) were followed up at Akdeniz University Hospital Pediatric Neurology Polyclinic between April 2015 and June 2016 with a diagnosis of epilepsy. The data of the study was grouped into 4 sub-dimensions. A face-to-face interview method was conducted by the researcher using "The Child and Parent Introduction Form" and "The Pediatric Epilepsy Medication Self-management Questionnaire". Written informed consent was obtained from Akdeniz University Clinical Research Ethics Committee and the parents.

Results: Scoring received from 10 specialists was assessed to evaluate the scales scope validation and it was determined that there was agreement between the specialists (content validity index=0.89). The Kaiser-Meyer-Olkin value of 0.656 and Barlett's test value of (df=210, p=0.000) indicated that the data were sufficient in amount and suitable for factor analysis. As a result of exploratory factor analysis, 6 items for which factor loads were below 0.40 were removed from the scale. The factor structure of the newly formed 4 sub-dimension scale was tested by confirmatory factor analysis and the structure was confirmed (χ^2/df=2.372, root mean square error of approximation=0.079, comparative fit index=0.901, goodness of fit index=0.927 and adjusted goodness of fit index=0.851). The Cronbach Alpha coefficient was found to be 0.71 for the total score of the scale. The sub-dimension correlations of the scale were determined to vary between 0.829-0.690. The testing correlations for test-retest were found to be significant (p<0.001) and high (0.91).

Conclusion: The Turkish adapted version of "The Pediatric Epilepsy Medication Self-management Questionnaire" was determined to band reliably for Turkish society.

Keywords: Epilepsy, self-management, psycholinguistics and psychometrics measurements, pediatric nursing

Introduction

Epilepsy is the most common disorder in childhood for which significant negative impacts are known in terms of the development of the child and the child's life quality (1,2). Although different statistics showing the prevalence of epilepsy are available, 4-10% of children worldwide experience a sort of attack before 20-years-old and 1% of them are diagnosed with epilepsy (3).

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The self-management of epilepsy involves the participation of the child and their parents in daily activities, controlling symptoms generated by the illness and epilepsy itself, mitigation of the generated symptoms' effects on the quality of life and health status, regular utilization of medicines, development of a healthy lifestyle (sleep hygiene), participation in healthcare professionals' team decisions and coping with epilepsy (2,4-8). Based on current theories of self-management; parents whose children are diagnosed with epilepsy do not have the necessary knowledge about the self-management of epilepsy (9,10), encounter difficulties in accessing healthcare professionals when they want to receive information (11), and so are not able to cope with epilepsy. Consequently, they experience a decrease in the quality of life, along with high levels of depression, anxiety and stress (12-14).

Epilepsy and Cultural Characteristics

Cultural characteristics play an important role towards the parents' self-management abilities of chronic diseases (3). Seizures cannot be controlled with antiepileptic drugs, especially resistant epilepsies, during childhood and parents use different traditional methods depending on their cultural beliefs (15,16). When the literature is examined, it is seen that the knowledge and attitude of a society towards epilepsy differ with respect to cultural backgrounds (17-22). Religious and socio-cultural beliefs affect the care and treatment processes of individuals diagnosed with epilepsy. In many developing societies, it is believed that epilepsy arises from witchcraft or possession by evil spirits. For this reason, it is not unheard of for treatment using healing herbs suggested by traditional doctors, priests, and religious leaders to be applied. Such practices can lead to a worsening of the symptoms and the development of complications (23,24).

Keeping seizures under control by ensuring regular and recommended dosages of antiepileptic drugs and therefore improving the quality of life of the patient are key elements of the self-management of epilepsy. However, approximately 60% of children who receive antiepileptic treatment do not take their drugs as prescribed due to a lack of information about epilepsy and culture-specific reasons (23,24). Providing accurate, up-to-date information about epilepsy to the individual diagnosed with epilepsy and their parents, and the development of self-management skills for both sides are among the most important components of the family-centred healthcare philosophy (25).

The care and maintenance process of individuals diagnosed with epilepsy and their parents is carried out under the guidance of a nurse and therefore nurses have a central role in the self-management of epilepsy (7). Therefore, it is very important for nurses to be knowledgeable about the different culture-specific practices used by parents in epilepsy self-management. When the literature is reviewed, only one scale is used to evaluate the self-management skills of the parents of children diagnosed with epilepsy in the international platform (5). In our country, there was no scale which could be used to determine the self-management of the parents of children diagnosed with epilepsy and a such scale is a prerequisite for research in this area. The aim of this study is the Turkish adaptation of "The Paediatric Epilepsy Medication Self-management Questionnaire (PEMSQ)", the determination of its validity-reliability, the evaluation of its suitability for Turkish culture, and a comparison of the psycholinguistic and psychometric properties of the scale with respect to different cultures.

Materials and Methods

Participants and Setting

The parents of all children who applied to Akdeniz University Hospital Paediatric Neurology Outpatient Clinic between April 2015 and June 2016 were the population of the study which is described as a methodological type. The sample group consisted of 540 parents of children aged between 2 and 17 years who had been diagnosed with epilepsy and who had been receiving antiepileptic treatment for at least six months. The size of the sample was calculated by taking into consideration the number indicated in the literature in order to enable the implementation of factor analysis. Based on the literature, a sample size corresponding to 50 is indicated as very weak, 100 as weak, 200 as a medium, 300 as good and 1,000 as excellent (26,27).

Data Collection

After obtaining the necessary permissions to conduct the research, the data were collected by the researcher using the Description Form of the Child and Parent and the PEMSQ by means of face-to-face interviews with the parents in a quiet interview room at the Akdeniz University Paediatric Neurology Clinic. In order to determine the comprehensibility of the PEMSQ, after its translation processes, pre-application was carried out with 10 parents. As no problems were encountered in the preliminary application, the data collection process of the study was initiated.

Data Collection Tools

The Description Form of the Child and Parent: It consists of 21 questions prepared in conformity with the related literature comprising information regarding the child (age, gender, duration of illness, frequency of seizure, regular drug usage, side effects of the drugs), their parents (ages of the mother and the father, education level) and knowledge on epilepsy.

PEMSQ: It is a 5-point Likert-type scale consisting of 27 items and 4 sub-dimensions developed by Modi et al. (5) to evaluate the self-management level of the parents of children diagnosed with epilepsy. The score from the scale varies between 27-135 and it is interpreted that self-management skill increases as the score increases (5).

Statistical Analysis

The data of the study were evaluated in the SPSS (SPSS Inc., Chicago, IL) 23.0 package program. Since the data showed normal distribution after the normality test, the impact of independent variables (age, gender, etc.) on dependent variables (PEMSQ) were tested by application of t-test in pairwise groups and the One-Way ANOVA test was applied in groups of three or more to test the significance of the difference. The percentage and mean values were used to determine the descriptive characteristics of the parents and children who participated in the study. Adaptation and translation processes of the scales proposed by the World Health Organization for language validity were followed in the Turkish adaptation study of the PEMSQ (28) (Table I).

Psycholinguistic and Psychometric Measurements: The scale was translated and re-translated by six independent translators who have knowledge of the language, who are familiar with the culture, who are experts in scale studies, and who know how the scales are used and interpreted based on the methodological part of the research report. Four translators translated the original scale into the target language, and the two translators retranslated the scale in the target language back to the original language.

Table I. Psycholinguistic and psychometric measurements of PEMSQ-TR

Psycholinguistic measurements	Psychometric measurement
Language validity	Validity methods
Advanced translation Expert panel Re-translation Pre-test Final draft of the scale Documentation	Content validity (content validity index) Structural validity [exploratory factor analysis (Kaiser-Meyer-Olkin, Barlett test, confirmatory factor analysis]
	Reliability methods
	Internal consistency reliability coefficient (Cronbach Alpha) Test-retest method

PEMSQ-TR: Turkish Pediatric Epilepsy Medication Self-Management Questionnaire

The translations from the translators were reviewed by the researchers and, to obtain an expert opinion, the translations were sent to 10 faculty members in total; two from the child neurology field and eight from the field of paediatric nursing. Experts scored each item of the scale from 1 to 4 (1 point=not appropriate, 2 points=must be optimized, 3 points=appropriate but some small change required, 4 points=very appropriate) and these numerical values were evaluated by content validity index (CVI) analysis. As a result of the suggestions and evaluations from the experts, the final version of PEMSQ was created by the researchers. In order to determine the scale's comprehensibility, before the study, 10 parents completed the questionnaire in the outpatient clinic and no problems relating to comprehension were encountered.

In order to test the constructive validity of the scale, exploratory factor analysis (EFA) [Kaiser-Meyer-Olkin (KMO), Barlett's test] and confirmatory factor analysis were used (Table I). LISREL v8.8 (LISREL v8.8, Scientific Software International, Inc., Lincolnwood, IL) package program was used for confirmatory factor analysis (CFA). The time-invariance test was evaluated by the test-retest method and the scale was reapplied to 50 parents at two-week intervals. The Cronbach alpha coefficient, which is commonly used in Likert type scales, was calculated to determine the internal consistency of the scale.

Ethical Considerations

In order to use the pre-research scale, permission was obtained from the author who originally developed the scale by e-mail. Ethical approval of the study was obtained from Akdeniz University Clinical Research Ethics Committee. Written informed consent was obtained from the parents who volunteered to participate in the study.

Results

Sample Characteristics

The majority of the children who participated in the study were in the 7-12-year age group, the mean age was 10.33 (±4.02) years and 52.2% of them were males. When their epilepsy and treatment characteristics were examined,

it was determined that 44.4% of the children had been diagnosed with epilepsy more than 49 months prior, 50.3% had one or more seizures per year, 61.5% of them had forgotten their drug intake, 73.7% of them encountered side effects of the drugs and 86.9% came for check-ups on a regular basis. When the characteristics of the parents were examined, 49.1% of their mothers were between the ages of 31-40 and 65.7% of them were primary school graduates. 48.5% of the fathers were aged 41 or over and 65.2% were primary school graduates. The mean age and gender of the children, the level of knowledge of their parents about epilepsy, the maternal age and the level of education of the parents were found to be related to the self-management of paediatric epilepsy on part of the parents (Table II).

Table II. Effect of demographic and disease related characteristics of participants on PEMSQ-TR Scores (n=540)

Parameters	n	%	Mean (SD)	Statistic	p-value
Characteristics of child					
Age					
0-6	116	21.5	2.98 (0.27)		
7-12	235	43.5	3.01 (0.29)		
13-18	189	35.0	3.32 (0.31)	0.027[a]	0.005*
Gender					
Female	258	47.8	3.25 (0.30)		
Male	282	52.2	3.01 (0.38)	0.414[b]	0.002*
Features of epilepsy disease					
How long has your child had epilepsy?					
6-12 months	81	15.0	3.18 (0.39)		
13-24 months	98	18.1	3.25 (0.34)		
25-36 months	69	12.8	3.24 (0.31)	1.052[c]	0.380
37-48 months	52	9.6	3.23 (0.36)		
49 months or over	240	44.4	3.27 (0.34)		
Seizure frequency					
Per week ≥1	117	21.6	3.18 (0.22)		
Per month ≥1	91	16.9	3.23 (0.31)		
Per year ≥1	271	50.3	3.20 (0.35)	1.427[c]	0.326
Per year ≤1	61	11.2	3.24 (0.33)		
Have you ever forgotten to give your child antiepileptic medicine?					
Yes	332	61.5	3.23 (0.35)		
No	208	38.5	3.27 (0.34)	-1.198[b]	0.973
Are there any side effects of antiepileptic drugs?					
Yes	142	26.3	3.21 (0.30)		
No	398	73.7	3.24 (0.27)	1.403[b]	0.258
Are you attending check-ups regularly?					
Yes	469	86.9	3.25 (0.35)		
No	71	13.1	3.19 (0.33)	1.422[b]	0.788
Have you been trained in epilepsy?					
Yes	113	20.9	3.40 (0.32)		
No	372	68.9	3.20 (0.35)	15,154[c]	0.000*
Somewhat	55	10.2	3.25 (0.29)		

Table II. contiuned

The features of the mother					
Age					
20-30	129	23.9	3.19 (0.25)	8,008[c]	0.001*
31-40	265	49.1	3.47 (0.30)		
41 years or older	146	27.0	3.22 (0.35)		
Educational level					
Literate	53	9.8	2.73 (0.30)	6,608[c]	0.000*
Primary education	355	65.7	3.23 (0.33)		
High school	115	21.3	3.34 (0.35)		
University	17	3.2	3.98 (0.30)		
The features of the father					
Age					
20-30	51	9.4	3.24 (0.27)	1.186[c]	0.751
31-40	227	42.1	3.03 (0.31)		
41 years or older	266	48.5	3.18 (0.34)		
Educational level					
Literate	22	4.1	2.96 (0.28)	8,640[c]	0.000*
Primary education	352	65.2	3.19 (0.32)		
High school	133	24.6	3.31 (0.40)		
University	33	6.1	3.70 (0.29)		

[a]Pearson correlation, [b]Independent samples t-test, [c]One-Way ANOVA
*Statistically significant at p<0.05.
PEMSQ-TR: Turkish Pediatric Epilepsy Medication Self-management Questionnaire, SD: Standard deviation

Validity Methods

CVI

In assessing the scope validity of the PEMSQ, scoring findings from 10 experts were evaluated and CVI of 0.86 was determined.

Structural Validity

EFA and CFA were used to test the structural/constructive validity of the scale. In order to perform factor analysis, the suitability of the data set was firstly evaluated by KMO and Barlett's test. KMO=0.656 and Barlett's test was found to be 4,691.86 (df=210, p=0.000). As a result of the EFA, it was determined that the factor loads of items 5, 10, 15, 19, 21 and 27 of the scale consisting totally of 27 items (PEMSQ) were below 0.4 and these items were excluded from the scale. After the analysis of the remaining 21 items, the four-factor structure was formed and this structure explained 53,423% of the total variance. The sub-dimensions of the newly formed structure are titled "information about epilepsy and treatment (F1)", "adaptation to treatment and clinical appointments (F2)", "treatment-related obstacles (F3)" and "treatment and social life (F4)" (Table III). In order to determine whether the 21-item structure of the 4 sub-dimensions of the scale was verified, CFA was applied.

The path diagram is shown in Figure 1. The fit indices were determined as follows: χ^2/df=2.372, root mean square error of approximation (RMSEA)=0.079, comparative fit index (CFI)=0.901, goodness of fit index (GFI)=0.927 and adjusted goodness of fit index (AGFI)=0.851. When the coefficients of the relationship between the observed variables and the factors showing the factor structure of this scale were examined, it was concluded that all coefficients were at sufficient levels. Considering the compliance statistics calculated by CFA, it has been decided that the previously determined structure of the scale conforms to the acceptable level with the collected data (Table IV).

Table III. Factor structure, loading and variance of PEMSQ-TR explained

Items	Factor			
	(F1)	(F2)	(F3)	(F4)
Information about epilepsy and treatment				
(1) Doctors/Nurses fully explained the diagnosis of seizures/epilepsy	0.580			
(2) I know which side effects I should follow in my child while the treatment is in progress	0.844			
(3) I know whom to contact when I encounter any problems	0.631			
(4) I believe that I can manage in cooperation with the health team when any side effects occur	0.588			
(5) I know the risks that my child may face if the drug is discontinued before a 2-year period without seizure	0.749			
(6) The medical team listens to my concerns about my child	0.600			
(7) It is easy for me to reach my child's healthcare providers and they will respond to my questions immediately	0.612			
(8) I am not having trouble bringing my child to appointments	0.593			
(9) Drug treatment is necessary for my child's disease	0.515			
(10) Medications given for treatment will control the seizures of my child	0.531			
Adaptation to treatment and clinical appointments				
(11) I check that my child has taken his medicine as prescribed		0.598		
(12) I usually follow the mentioned medical advice and treatment plans for myself and my child		0.693		
(13) I think it is important for my child to take his treatment as described		0.834		
(14) All members of the family comply with my child's treatment plan		0.581		
(15) I think it is important to provide the medication my child should take daily		0.558		
Treatment-related obstacles				
(16) My child does not like the taste of the drugs			0.750	
(17) My child has difficulty in taking the medicine			0.822	
(18) My child refuses to take his medicine			0.790	
(19) There are times when our medicine is depleted			0.449	
Treatment and social life				
(20) My child is ashamed of taking their medicine in front of others (friends, family, etc.)				0.813
(21) My child has some activities (sports, school activities, etc.) that may interfere with drug intake				0.867
Cumulative variance	4,187	2,812	2,364	1.856
Variance explained (%)	19,936	13,389	11,257	8.840
Cumulative explained variance (%)	19,936	33,326	44,583	53,423

Reliability Methods

Internal Consistency Reliability Coefficient

As a result of the factor analysis, the total scale and Cronbach Alpha values of each dimension were calculated. The overall Cronbach Alpha coefficient of the scale was 0.71 and the sub-dimensions were 0.829 (first sub-dimension: F1), 0.732 (second sub-dimension: F2), 0.726 (third sub-dimension: F3), and 0.692 (fourth sub-dimension: F4).

Test-Retest Method

Evaluation of invariance in the scale was carried out by the test-retest method and the scale was reapplied with 50 parents at two week-long intervals. The scores between the two applications were evaluated via the Pearson product-moment correlation test and the correlation coefficient was determined to be r=0.91.

Discussion

Epilepsy is the most common (0.5% to 1%) chronic neurological disease in childhood, and there are many problems related to antiepileptic treatment due to a lack of knowledge about the disease and also culture-specific reasons (29). When the literature is examined; it is seen that approximately 60% of children receiving antiepileptic therapy are not using their drugs as prescribed and they are not provided with regular polyclinic check-ups. Additionally, some of the parents whose children have resistant epilepsy think that antiepileptic treatment is ineffective and they use different traditional methods depending on their cultural beliefs (16,23,24,30). Taking all the above-mentioned reasons into consideration, it is very important to promote self-management for the effective management and treatment of epilepsy.

Self-management of chronic diseases such as epilepsy involves the control of the symptoms of the disease, compliance with the planned treatment regimen, interaction with health care professionals, making lifestyle changes to improve health, and coping with the disease based on feelings with a problem-focused approach (2,5,6,31). When the literature is examined, it is seen that the PEMSQ developed by Modi et al. (5) in English is the only measurement tool to evaluate the self-management of the parents of children diagnosed with epilepsy. In addition, the adaptation of the scale into different languages or cultures was not observed. For this reason, in our study, we aimed to determine the adaptation, validity and reliability of the Turkish PEMSQ (PEMSQ-TR) and the findings are discussed in three sections.

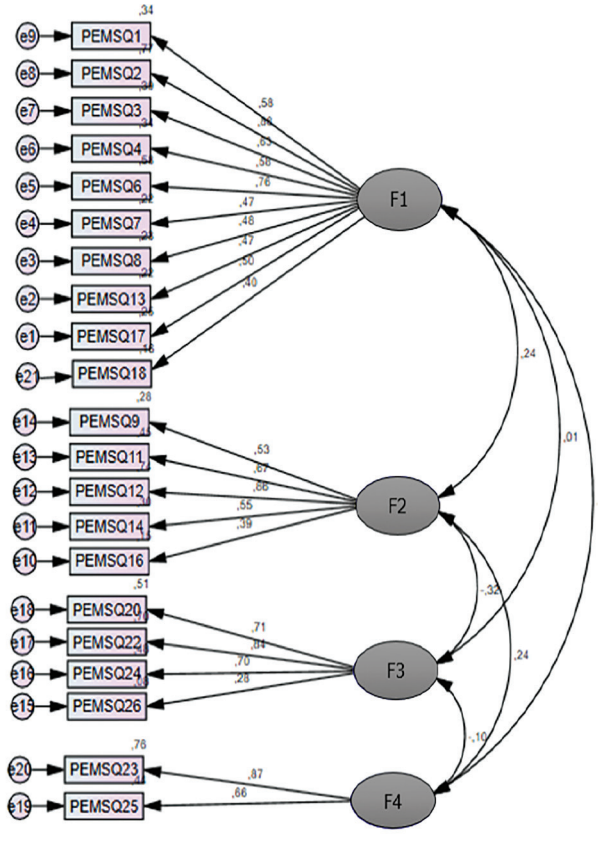

Figure 1. Path diagrams
PEMSQ: Pediatric Epilepsy Medication Self-management Questionnaire

Sample Characteristics

In our study, it was determined that the results of the "PEMSQ" were related to the average age of the children, maternal age, the knowledge of the participants about the disease and the level of education of the parents. It was determined that success in the self-management of epilepsy positively correlated with the age of the children, the maternal age, the knowledge level of the parents regarding the illness and the education backgrounds of the parents ($p<0.05$) (Table II). When the literature is examined, it is stated that there are similar findings with our study results, and as the level of knowledge about epilepsy increases, the levels of self-management and coping with the disease are specified to be better (6,32).

Validity Methods

CVI

CVI technique was used as a criterion for evaluating both the language and culture equivalence and content validity of the scale with numerical values and for evaluating the expert opinions. In this technique, the scale has to be 0.80 or above to indicate that the scale has scope validity (33). PEMSQ-TR's scoring scores from 10 experts were evaluated and it was determined that CVI=0.89. This value shows that the scope validity of the scale is at a good level.

Structural/Construct Validity

Before the construct validity is tested, the suitability of the data set for factor analysis is determined. For this purpose, the Barlett's test is applied to determine whether or not KMO and variables are correlated with each other. A KMO value of less than 0.50 indicates that the sample size is not sufficient for validity analysis (34). One of the commonly used methods to test the construct validity in scale studies is factor analysis. Factor analysis tests the integrity of the scale and helps to eliminate unrelated variables. Items that are highly related among themselves form factors. Factor analysis is done by two methods which are exploratory and confirmatory factor analysis (33,35).

Table IV. Goodness of fit indices of confirmatory factor analysis

Index of compliance	Excellent compliance limit	Acceptable compliance limit	PEMSQ-TR
RMSEA	0<RMSEA<0.05	0.05<RMSEA<0.10	0.079
GFI	0.95<GFI<1	0.90<GFI<0.95	0.927
AGFI	0.90<AGFI<1	0.85<AGFI<0.90	0.851
CFI	0.95<CFI<1	0.90<CFI<0.95	0.901
χ^2/df	χ2/df<3	3<χ2/df<5	2.372

*Civelek (35). Essentials of structural equation modelling. Lincoln, Nepraska: Zea Books, 2018.
PEMSQ-TR: Turkish Pediatric Epilepsy Medication Self-management Questionnaire, RMSEA: Root mean square error of approximation, GFI: Goodness fit index, AGFI: Adjusted goodness of fit index, CFI: Comparative fit index

In our study, it was determined that the sample size was sufficient (KMO=0.656) and the data set was suitable for factor analysis (df=210, p=0.000). According to Harrington (36), factor loads should not be less than 0.30. Factor loads of 0.71 or above are deemed to be excellent, 0.63 very good, 0.55 good, 0.45 acceptable and 0.32 or less is deemed to be weak. In our study, 6 items (5,10,15,19,21 and 27 items) with a factor load below 0.4 were excluded from the original scale. After the removal of these items, a 4-dimensional structure for 21 items was formed which explained 53,423% of the total variance. Henson and Roberts (37) stated that the variance explained in scale studies should be 52% or more. In our study, it was seen that the explained variance is above the stated value and the newly formed structure was approved.

In order to determine whether the 21-item structure of the 4 sub-dimensions of the scale was verified, DFA was applied. For the construct validity of a scale, the fit indices made in the confirmatory factor analysis should be at the desired level. For the model to be acceptable, the chi-square value should be 2 or less indicating that the model is a good model, and if it is 5 or less, it indicates that the model has an acceptable goodness of fit (33,35). If the RMSEA value is less than or equal to 0.80, it indicates that the compatibility is good, if it is equal to or less than 0.10, it indicates that the compatibility is low. When the CFI and GFI values are equal to or greater than 0.90, it indicates that there is compatibility (33). In our study, the fit index of the scale consisting of 4 sub-dimensions of 21 items was found to be at a good level and the new scale structure was approved.

Reliability Methods

Internal consistency (Cronbach Alpha)

It should be determined that each item of the scale measures the same attitude within itself. The most appropriate method for this is the calculation of the Cronbach Alpha reliability coefficient. A Cronbach Alpha coefficient between $0.00 \leq \alpha \leq 0.040$ indicates that the scale is not reliable, between $0.40 \leq \alpha \leq 0.60$ indicates that reliability of the scale is low, between $0.60 \leq \alpha \leq 0.80$ indicates that reliability of the scale is satisfactory and between $0.80 \leq \alpha \leq 1.00$ indicates that reliability of the scale is high (38). In parallel with the literature, it was concluded that the internal consistency coefficient of the scale was satisfactory and the scale was reliable.

Test-retest method

When the same measurement tool is applied to the same individuals at different times, the similarity of the responses given by the individuals to the measurement tool indicates the invariance of the measurement tool. The correlation coefficient (r-value) between the first and second application scores of this invariance is calculated by the Pearson Product Moment Correlation test. The closer this value is to 1, the better the reliability is, but the value should be at least 0.70 and a value above 0.80 is preferred (39). In our study, it was found that the value of "r" was very close to 1 (r=0.91). It was concluded that the scale was consistent with respect to time.

Conclusion

In our study, the psycholinguistic and psychometric measurements of the PEMSQ-TR were evaluated. As a result of the analyses, it was determined that PEMSQ-TR is a valid and reliable measurement tool for the evaluation of the disease self-management of those parents with epileptic children.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained by Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (approval no: 177, date: 15.04.2015).

Informed Consent: Written informed consent was obtained from the parents who volunteered to participate in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ş.T., A.İ.D., Design: Ş.T., Data Collection: Ş.T., Analysis or interpretation: Ş.T., Writing: Ş.T., A.İ.D.

Conflict of Interest: The authors declared no conflict of interest.

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Original Article

Outcomes of Dyslipidemia Screening Program in School-aged Children

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ABSTRACT

Aim: Screening of dyslipidemia in childhood has been controversial. While some guidelines recommend screening for dyslipidemia in children, others emphasize that there is insufficient evidence for screening for dyslipidemia in those less than 20 years of age. In this study, we aimed to evaluate the outcomes of a lipid screening program and reveal the pros and cons of this program.

Materials and Methods: All patients referred to a paediatric metabolism outpatient clinic by family physicians with the suspicion of dyslipidemia in a lipid screening program at schools were investigated. Demographic and physical examination findings, screening lipid profiles and fasting control lipid profiles of the patients were evaluated. The definitive diagnosis with fasting lipid profile and genetic analysis were recorded.

Results: Two hundred seventy-four patients suspected with dyslipidemia were enrolled in the study. The mean age of study group was 9.2±3.2 (5-17) years. While 158 (57.7%) patients were admitted with high total cholesterol (TC) and low-density lipoprotein cholesterol, high triglyceride level was detected in 58 (21.2%) patients via a paediatric lipid screening program. A high TC level was revealed in 26 (9.5%) patients.

With the control fasting lipid profile, 100 (36.5%) patients had a normal lipid profile. Fifty-nine (21.5%) patients were diagnosed with familial hypercholesterolemia (FH), and hyperchylomicronemia and hypobetalipoproteinemia were revealed in 5 (1.8%) and 4 (1.5%) patients, respectively. Eleven patients diagnosed with FH did not declare hyperlipidemia in parents. In a screening of these patients' immediate families, 11 parents and 3 siblings were diagnosed with familial hyperlipidemia.

Conclusion: This is the first study performed to date that evaluated the outcomes of a lipid screening program on school age children in Turkey. We found that this screening program is effective in diagnosing not only the patients but also asymptomatic parents and siblings. Evaluation and verification of dyslipidemia should be performed under fasting conditions to avoid false positive results.

Keywords: Screening of dyslipidemia, school-aged children, familial hypercholesterolemia

Introduction

It is well known that elevated total cholesterol (TC), especially low-density lipoprotein cholesterol (LDL-C), is the major preventable risk factor for atherosclerotic cardiovascular disease, which is the most common cause of death in developed countries. Early diagnosis of dyslipidemia and the initiation of lipid-lowering treatment is important to avoid atherosclerotic effects (1).

Screening for dyslipidemia in childhood has been controversial. While some guidelines recommend screening

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of dyslipidemia in children, the US Preventive Services Task Force emphasizes that there is insufficient evidence for the screening for dyslipidemia in those under 20 years of age (2,3). On the other hand, some authors recommend targeted screening based on a family history of premature cardiovascular disease or hypercholesterolemia or other risk factors (4). However, it has been pointed out that such an approach would miss a large number of children with dyslipidemia (5). No doubt, there is a need for clinical research to reach a consensus on this issue.

In the presence of such uncertainties, a dyslipidemia screening program in school-age children was initiated in Turkey in 2016 (6). The aim of this screening program was the evaluation of all school-aged children in terms of an abnormal lipid profile. Children with abnormal lipid profiles [TC ≥200 mg/dL, LDL-C ≥130 mg/dL, triglyceride ≥100 mg/dL for 5-9 years of age and ≥130 mg/dL for 10-18 years of age and high-density lipoprotein cholesterol (HDL-C) <40 mg/dL] are referred to paediatric metabolism outpatient clinics to be evaluated for dyslipidemia. To date, no study has analysed the efficacy and outcomes of this screening program in Turkey. In this study, we aimed to evaluate the outcomes and reveal the pros and cons of this lipid screening program.

Materials and Methods

Patients referred to paediatric metabolism outpatient clinics by family physicians with suspected dyslipidemia (TC ≥200 mg/dL, LDL-C ≥130 mg/dL, triglyceride ≥100 mg/dL for 5-9 years of age and ≥130 mg/dL for 10-18 years of age and HDL-C <40 mg/dL) during the lipid screening program at schools were investigated between July 2018 and October 2019.

Patients with results obtained during the screening program and a control fasting lipid profile (after fasting for at least 9 hours) were enrolled. Their demographics and physical examination findings [age, gender, family history of dyslipidemia, lipid-lowering treatment or premature symptomatic coronary artery disease, body mass index (BMI)], screening lipid profile (TC, LDL-C, triglycerides, HDL-C) and fasting control lipid profile were evaluated. Definitive diagnoses with fasting lipid profile and genetic analysis (*LDLR, APOB, LDLRAP1, PCSK9, APOC2, LPL,* and *MTP*) [normal lipid profile, familial hypercholesterolemia (FH), hypobetalipoproteinemia, hyperchylomicronemia, non-classified high total and LDL-cholesterol] were recorded. A BMI ≥95th percentile was defined as obesity (7). The cut-off points for TC and LDL-C were ≥200 mg/dL and ≥130 mg/dL, respectively. Furthermore, ≥100 mg/dL for 5-9 years of age and ≥130 mg/dL for 10-18 years of age were determined as the cut-off points of triglyceride level (8). Children with LDL cholesterol ≥190 mg/dL or LDL cholesterol ≥160 mg/dL with risk factors were evaluated for genetic mutation for FH. Also, children with LDL cholesterol ≥130 mg/dL with FH diagnosed parents were assessed with genetic analysis. All patients with high triglycerides levels (≥500 mg/dL and/or persistent triglyceride levels between 130-500 mg/dL without obesity) were investigated with genetic analysis for hyperchylomicronemia. In patients with LDL cholesterol ≤50 mg/dL, hypobetalipoproteinemia was genetically investigated (9). The lipid prolife of the parents and siblings of patients diagnosed with FH, hypobetalipoproteinemia or hyperchylomicronemia were investigated. Twelve patients were excluded from the study due to missing data. Informed assent from the participants (whenever appropriate) and written informed consent from their caregivers were obtained.

The study was initiated after the approval of the Ethics Committee of University of Health Sciences Turkey, Dr. Behçet Uz Children Training and Research Hospital (date: 12.09.2019, number: 2019/326).

Statistical Analysis

Categorical variables (gender, family history of hyperlipidemia, lipid-lowering treatment and premature coronary artery disease) were expressed as number and percentage, and continuous variables (age, TC, LDL-C, triglycerides and HDL-C in the screening program and the fasting control lipid profile) were expressed as mean ± standard deviation or median (min-max) according to the normality of variables. The normality of data was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparison of the frequency of hyperlipidemia and lipid-lowering treatment in family history across subgroups was analysed with chi-square test.

Data were analysed with the Statistical Package for Social Sciences (SPSS) computer software (version 21.0; SPSS, Chicago, IL, USA). A two-tailed p-value <0.05 was considered significant.

Results

Two hundred and seventy-four patients with suspected dyslipidemia were enrolled in the study. Of these, 145 (52.9%) were female and 129 (47.1%) were male. The mean age of the study group was 9.2±3.2 (5-17) years. In the family history, hyperlipidemia in parents was declared by 116 (42.3%) patients and 35 (12.8%) (father or mother) were receiving lipid-lowering treatment. Premature symptomatic

coronary artery disease was detected in 4 (1.5%) parents. Physical examination revealed obesity in 42 (15.3%) patients.

In physical examination, one of the patients diagnosed with familial hyperlipidemia had xanthoma in the knees. None of the children had ocular manifestations (corneal arcus, lipemia retinalis). Two out of 4 patients diagnosed with hypobetalipoproteinemia had mild hepatomegaly. Two patients with hypobetalipoproteinemia were asymptomatic.

The lipid profiles of the patients during screening are presented in Table I. While 158 (57.7%) patients were admitted with elevated TC (≥200 mg/dL) and LDL-C (≥130 mg/dL), elevated triglycerides (5-9 years of age: ≥100mg/dL, 10-17 years of age: ≥130 mg/dL) was detected in 58 (21.2%) patients by the paediatric lipid screening program. High TC level was noted in 26 (9.5%) patients.

In the assessment of patients via the control fasting lipid profile, 100 (36.5%) patients had a normal lipid profile. While 59 (21.5%) patients were diagnosed with FH, (patients with genetic mutation in *LDLR, PCSK9*), hyperchylomicronemia and hypobetalipoproteinemia were found in 5 (1.8%) and 4 (1.5%) patients, respectively. High TC and LDL-C without genetic mutations in *LDLR, APOB, LDLRAP1, PCSK9, APOC2, LPL,* and *MTP* was detected in 64 (23.4%) patients. The control fasting lipid profiles of the groups are presented in Table II.

Comparison of the lipid profile obtained during the screening program and the diagnosis of patients revealed that all patients admitted with a high TC level had a normal fasting lipid profile. Also, a normal fasting lipid profile was determined in 38 (65.5%) out of the 58 patients with elevated triglycerides. FH was diagnosed in 59 (37%) patients with high TC and high LDL-C. All patients (n=4) with low TC, low LDL-C and low triglyceride levels were diagnosed with hypobetalipoproteinemia (Table III).

In the evaluation of the family history of the patients, hyperlipidemia and receiving lipid-lowering treatment in parents were more frequent in patients with FH compared to the other groups ($p<0.0001$; $p<0.0001$) (Table IV). Eleven patients diagnosed with FH did not declare hyperlipidemia in their parents. An evaluation of the fasting lipid profile of these patients' families with cascade screening revealed that 11 parents (2.0% of the parents of patients enrolled in the study) and 3 siblings had familial hyperlipidemia. The mean TC and LDL-C levels of the parents were 297.4±76.3 (256-398) mg/dL and 234.0±64.1 (180-310) mg/dL, respectively.

Discussion

This is the first study performed to date to evaluate the outcomes of a dyslipidemia screening program in school-age children in Turkey. The most striking point of this study is that one third of the patients referred with suspected dyslipidemia had a normal fasting lipid profile. This finding may be explained by the fact that the lipid screening was conducted in a non-fasting state (10,11). It is well known that triglyceride level is affected by the fasting state. Consequently, most of our patients with normal lipid profiles were admitted with high triglyceride levels.

Another interesting point is those patients referred with elevated TC, all of whom had normal control fasting lipid profiles. Most studies indicate that the fasting or non-fasting state of patients does not affect TC and LD-C levels, advising that lipid screening be performed in a non-fasting state (12,13). However, a study recently performed in Turkey indicated that a fasting state may affect TC and non-HDL-C

Table I. Total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels of patients in the screening program

Lipid profile in screening (mg/dL)	Group 1 (n=26)	Group 2 (n=58)	Group 3 (n=158)	Group 4 (n=14)	Group 5 (n=14)	Group 6 (n=4)
TC, mean ± SD (min-max)	204.0±4.2 (200-214)	164.7±24.1 (87-199)	260.1±68.6 (200-834)	231.6±24.6 (201-298)	212.2±9.4 (200-230)	51.8±14.9 (32-68)
LDL-C, mean ± SD (min-max)	113.3±11.6 (86-128)	82.9±21.3 (32-127)	183.2±68.7 (130-752)	146.6±22.1 (131-219)	106.2±10.6 (89-125)	21.8±8.8 (11-32)
HDL-C, mean ± SD (min-max)	64.3±12.8 (42-94)	38.9±14.1 (12-81)	59.2±13.4 (33-100)	47.3±6.9 (39-52)	58.5±17.6 (30-80)	35.3±2.6 (28-39)
Triglycerides, median (min-max)	81 (39-126)	260 (145-1,939)	78 (41-127)	192 (142-312)	222 (110-343)	21.5 (19-35)

Group 1: High TC
Group 2: High triglycerides
Group 3: High TC, high LDL-C
Group 4: High TC, high LDL-C, high triglycerides
Group 5: High TC, high triglycerides
Group 6: Low TC, low LDL-C, low triglycerides
LDL-C: Low-density lipoprotein cholesterol, min: Minimum, max: Maximum, SD: Standard deviation, TC: Total cholesterol

Table II. Control fasting lipid profiles and diagnoses of patients

Control fasting lipid profile (mg/dL)	Normal lipid profile (n=100)	Familial hypercholesterolemia (n=59)	Obesity (n=42)	Non-classified high total and LDL-cholesterol (n=64)	Hyperchylomicronemia (n=5)	Hypobetalipoproteinemia (n=4)
TC, mean ± SD (min-max)	168.9±20.4 (107-198)	318.4±80.3 (234-798)	212.4±38.1 (144-297)	227.9±18.5 (200-279)	201.8±39.9 (157-252)	73.8±16.9 (52-91)
LDL-C, mean ± SD (min-max)	96.5±15.9 (52-124)	245.5±76.4 (170-702)	123.5±34.5 (63-186)	149.4±15.2 (130-187)	*	32.8±16.5 (13-53)
HDL-C, mean ± SD (min-max)	56.2±11.3 (40-90)	58.2±1361 (30-89)	47.9±14.1 (22-81)	59.8±12.9 (36-88)	15.8±5.7 (12-24)	43.8±1.9 (41-45)
Triglyceride, median (min-max)	78.5 (65-123)	79 (47-126)	204 (41-680)	82.3 (44-127)	984 (773-1,230)	32.5 (19-59)

*: Could not be calculated due to high triglyceride level.
HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, min: Minimum, max: Maximum, SD: Standard deviation, TC: Total cholesterol

Table III. Comparison of lipid profiles in screening and the diagnoses of patients

Diagnosis of patients with control fasting lipid profile and genetic investigations,	Lipid profile in screening n (%)					
	Group 1 (n=26)	Group 2 (n=58)	Group 3 (n=158)	Group 4 (n=14)	Group 5 (n=14)	Group 6 (n=4)
Normal lipid profile (n=100)	26 (100.0)	38 (65.5)	27 (17.0)	3 (21.4)	6 (41.9)	0 (0.0)
Familial hypercholesterolemia (n=59)	0 (0.0)	0 (0.0)	59 (37.0)	0 (0.0)	0 (0.0)	0 (0.0)
Obesity (n=42)	0 (0.0)	15 (25.9)	8 (5.1)	11 (78.6)	8 (57.1)	0 (0.0)
Non-classified high TC and LDL-C (n=64)	0 (0.0)	0 (0.0)	64 (41.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperchylomicronemia (n=4)	0 (0.0)	5 (8.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypobetalipoproteinemia (n=4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)

Group 1: High TC
Group2: High triglycerides
Group 3: High TC, high LDL-C
Group 4: High TC, high LDL-C, high triglycerides
Group 5: High TC, high triglycerides
Group 6: Low TC, low LDL-C, low triglycerides
LDL-C: Low-density lipoprotein cholesterol, TC: Total cholesterol

Table IV. Hyperlipidemia and lipid-lowering therapy in family history of subgroups

Medical history	Normal lipid profile (n=100) n (%)	Familial hypercholesterolemia (n=59) n (%)	Obesity (n=42) n (%)	Non-classified high total and LDL-cholesterol (n=64) n (%)	Hyperchylomicronemia (n=5) n (%)	Hypobetalipoproteinemia (n=4) n (%)	p
Hyperlipidemia in parents (n=116)	26 (26.0)	48 (81.4)	12 (28.6)	29 (45.3)	1 (20.0)	0 (0.0)	<0.0001
Lipid-lowering therapy in parents (n=35)	3 (3.0)	19 (32.0)	3 (7.1)	9 (14.0)	1 (20.0)	0 (0.0)	<0.0001

levels (14). It is difficult to comment on this subject within the method of our study. However, we determined that those patients admitted with elevated TC had slightly elevated HDL-C, which we believe is the reason for their high TC levels. This finding suggests that physicians should pay attention to elevated non-HDL-C instead of TC levels in lipid screening programs.

The primary aim of a lipid screening program is to determine elevated LDL-C, the most common and preventable factor for atherosclerotic cardiovascular

disease, in asymptomatic patients (15). In this study, we found that about 20% of patients were diagnosed with FH by means of the screening program. Moreover, the lipid screening program allowed for the diagnosis of FH not only in the children included in the screening, but also their asymptomatic parents and siblings, as is the case in the biotinidase deficiency screening program (16). These findings show that screening for common diseases in the community is effective for the diagnosis of asymptomatic patients and the initiation of appropriate treatment.

The family history of patients plays a key role in the diagnosis of dyslipidemia, especially FH, which is inherited in an autosomal codominant pattern (17). Another notable finding of the present study is that the history of dyslipidemia and its treatment with lipid-lowering drugs in parents were significantly higher in those patients diagnosed with FH compared to the other groups. This result highlights the importance of being aware of the family history of the patients enrolled in lipid screening.

In this study, we had no information about the number of children screened for dyslipidemia by family physicians, which prevented us from evaluating the frequency of dyslipidemia in school-aged children. Previous studies from different countries have diverse results. For instance, a study performed in Lebanon revealed high borderline dyslipidemia prevalence to be 77.3% in children 2-10 years old, while a Mexican study stated that the frequency of dyslipidemia in the same age group was 55.3% (18,19). A lower prevalence of dyslipidemia was reported from the USA at 8% for TC, 7% for LDL-C and 12% for triglyceride (20). We think that genetic variation and dietary habits play a key role in these differences. Furthermore, different rates of prevalence between countries suggest that every screening program should be revised in terms of national risk factors and screening age.

Previous studies about dyslipidemia screening in children did not determine hypobetalipoproteinemia (18-21). One of the striking finding of our study is that we revealed 4 children with hypobetalipoproteinemia. Although patients with hypobetalipoproteinemia are often asymptomatic, many develop fatty liver. In 2 out of the 4 patients with hypobetalipoproteinemia, we detected hepatomegaly, and hepatosteatosis. Two of them were asymptomatic. This finding emphasized that a screening program is effective in the detection of not only FH but also other types of dyslipidemias such as hypobetalipoproteinemia, or hyperchylomicronemia.

A study performed by Kalkan-Ucar et al. (22) in the same region as our study determined that the prevalence of obesity is 6.29% in children aged 2-15 years. However, this study was performed in 2009 and included children less than 5 years of age. It is well known that the number of obese children has significantly increased in developed and developing countries over the last decades (23). A recent study performed in Antalya located in southern Turkey reported that the prevalence of obesity was 9.8% in children aged 9-14 years in 2015 (24). The lipid profile screening program has provided an opportunity to determine the prevalence of obesity in children with abnormal lipid profile. We found the frequency of obesity to be 15%, which was higher than that estimated in previous studies. This study is the first in our country to evaluate the frequency of obesity in school-age children diagnosed with dyslipidemia.

There are several limitations of this study. First, we had no information about the number of children screened for dyslipidemia, which prevented us from evaluating the frequency of dyslipidemia in school-aged children. Secondly, this study relied only on the lipid screening results of one centre located in the western region of Turkey. Lastly, the low-number of subgroups limited our ability to analyse the effectivity of laboratory parameters in the diagnosis of dyslipidemia.

Conclusion

Screening programs are effective in diagnosing not only the patients but also their asymptomatic parents and siblings. Evaluation and verification of dyslipidemia should be performed in a fasting state to avoid false positive results. Analysis of multicentre screening results is warranted to further analyse the effectiveness of lipid profile screening programs in school-age children.

Ethics

Ethics Committee Approval: The study protocol was designed in compliance with the 1964 Declaration of Helsinki. The study was approved by the Ethics Committee of University of Health Sciences Turkey, Dr. Behçet Uz Children Training and Research Hospital (date: 12.09.2019, number: 2019/326).

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

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: E.K., M.K., B.Ö., Design: E.K., M.K., Data Collection or Processing: E.K., S.T., B.M., B.B., Analysis or Interpretation: E.K., Literature Search: E.K., Writing: E.K.

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Original Article

The Relation of Complete Blood Count Parameters with Metabolic and Clinical Parameters in Overweight and Obese Children

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ABSTRACT

Aim: This study aims to assess white blood cell count, platelet count, and platelet indices as a metabolic indicator in overweight, obese and morbidly obese children.

Materials and Methods: One-hundred and thirty overweight, 341 obese, 188 morbidly obese children and 110 controls were enrolled in the study. Anthropometric measurements, pubertal status, complete blood count parameters [white blood cells (WBC), platelet, mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW)], WBC differential (neutrophils, lymphocytes, and monocytes), neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR), and serum levels of glucose, lipids, aspartate transaminase (AST), and alanine transaminase (ALT), insulin and thyroid hormones were obtained from the hospital records. Insulin resistance was assessed according to the homeostasis model assessment-insulin resistance (HOMA-IR) index.

Results: WBC, neutrophil, lymphocyte, and monocyte counts were highest in the morbidly obese group followed by the obese, overweight, and healthy groups, respectively. Platelet count, PCT, and PDW were significantly higher in the morbidly obese, obese, and overweight groups compared to the healthy group. However, there was no significant difference between the groups in terms of MPV, NLR, and PLR. WBC, neutrophil, lymphocyte, platelet, PCT, ALT, and triglyceride levels were higher in children with insulin resistance than those without insulin resistance. There was a positive correlation with the neutrophil, lymphocyte, monocyte count, and PCT value, and a negative correlation with the PDW value. Moreover, there was a positive correlation between the HOMA-IR and WBC, neutrophil, lymphocyte count, and PCT.

Conclusion: WBC, neutrophils, lymphocytes, monocytes, platelets, and PCT values increase in childhood obesity, which could point towards low-grade chronic inflammation and this increase in WBC, neutrophils, lymphocytes, and PCT value may be associated with insulin resistance.

Keywords: Children, complete blood count, insulin resistance, obesity

Introduction

Obesity is a medical condition defined as abnormal or excessive fat accumulation in the body to the extent that it may cause a risk to health. Obesity has become a serious health problem across the world, and currently, one in three children in the United States are either overweight or obese. In parallel with the increase in childhood obesity, the frequency of comorbidities such as insulin resistance, type 2 diabetes mellitus, hypertension, non-alcoholic fatty liver, obstructive sleep apnea, and dyslipidemia have

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also increased (1). The close relationship between insulin resistance and obesity indicates that insulin resistance is an important public health issue (2). Cheap and useful markers that can show insulin resistance in the early period are important for the prevention of morbidities related to insulin resistance.

Complete blood count (CBC) is an easy-to-use and cheap test that is frequently used in clinical practice. Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) obtained by CBC are simple biomarkers showing inflammation (3-5). Moreover, platelet count, mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW) are other biomarkers that indicate systemic inflammation (6,7).

Metabolic syndrome can affect hematologic counts, and hematologic markers can be used for the early detection of individuals at risk of cardiovascular disease (8,9). According to studies conducted on the adult population, white blood cells (WBC), subtypes, and NLR were independently associated with insulin resistance (10,11). In Korean and Chinese adults, obesity, dyslipidemia, glucose intolerance, and hypertension were associated with changes in hematologic parameters (8,12,13). However, few studies have evaluated the relationship between hematological parameters and insulin resistance in childhood, and the results of these studies are inconsistent. Therefore, in this study, we aimed to examine the relationship between CBC parameters and clinical and metabolic parameters in overweight and obese children.

Materials and Methods

A total of 659 obese or overweight children between the ages of 5 and 18 years who were admitted to the outpatient general paediatrics clinic of our hospital between March 2018 and March 2020 were included in the study. One hundred and ten healthy children with a body mass index (BMI) below the 85th percentile who had a similar age and gender distribution were also enrolled as a control group in this retrospective study.

Those children with type 2 diabetes mellitus, secondary obesity (genetic syndromes, monogenic obesity, endocrine pathology), hypertension, chronic disease or history of acute infection, children with leukopenia (<4x10^3/mL), leucocytosis (>13.0x10^3/mL), anaemia or thrombocytopenia (<150x10^3/mL), a history of drug use that could affect the CBC parameters or bodyweight or incomplete data were excluded from the study.

The age, gender, anthropometric measurements, pubertal status, CBC parameters and biochemical test results of the subjects were obtained from the hospital records. Height was measured using a Harpenden stadiometer with a sensitivity of 0.1 cm, and weight was measured using a SECA scale with a sensitivity of 0.1 kg. BMI was calculated by dividing weight (kg) by height squared (m^2). Standard deviation scores (SDS) for weight, height, and BMI were calculated with the online calculator for paediatric endocrinologists (child metrics) using a reference created for the Turkish population by Neyzi et al. (14,15). Findings for pubertal development were evaluated according to Tanner and Whitehouse staging (16). A testicular volume of ≥4 mL in males and breast development ≥stage 2 in females were considered to be findings of puberty.

Subjects with a BMI over the 85th but less than the 95th percentile for age and gender are defined as overweight, and those with a BMI higher than the 95th percentile are defined as obese. Subjects with a BMI higher than the 99th percentile are defined as severely obese (17). Blood samples were taken after 10-12 hours of night fasting. Serum levels of glucose, lipids, aspartate transaminase (AST), and alanine transaminase (ALT) were measured by a spectrophotometric method; insulin and thyroid hormones were measured by chemiluminescent microparticle immunoassay (Abbot-Architect i2000SR). CBC parameters (WBC, platelet, PDW, MPV, PCT) and WBC differential (neutrophils, lymphocytes, monocytes) were measured by flow impedance, laser light scattering, and flow cytometry methods (Mindray, BC6800). NLR and PLR were obtained by dividing the number of neutrophils and platelets by lymphocytes, respectively. Insulin resistance was assessed according to the homeostasis model assessment-insulin resistance (HOMA-IR) index. Different cut-off values for prepubertal and pubertal stages were used to determine the status of insulin resistance (prepubertal >2.5, pubertal >4) (18).

The study was initiated upon approval form the local ethics committee of Aydın Adnan Menderes University Faculty of Medicine, in light of the Helsinki Declaration (2020/98).

Statistical Analysis

Statistical analysis of the data were conducted with SPSS 21.0 (SPSS Inc., Chicago, IL, USA). The distribution of data was evaluated with the Kolmogorov-Smirnov test and histograms. Clinical data were presented as number (%), mean ± standard deviation for normal distribution, and median (25p-75p-value) for data that were not distributed

normally. Categorical variables were compared using the chi-square test. Between study groups, the data obtained were compared by using Student's t-test (for normally distributed data), and Mann-Whitney U test (for non-normally distributed data). The correlations between the independent parameters were investigated with Pearson's correlation analysis or Spearman's rank correlation and partial correlation analysis with respect to the effect of age, gender, and pubertal status. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 110 healthy (45 males; median age 13.5 years), 130 overweight (58 males; median age 12.5 years), 341 obese (161 males; median age 12.5 years), and 188 morbidly obese (66 males; median age 14.0 years) subjects were included in this study. There were no significant differences between the groups in terms of age, gender, or pubertal status (Table I).

WBC, neutrophil, lymphocyte, and monocyte counts were highest in the morbidly obese group, followed by the obese, overweight, and healthy group, respectively (p<0.001). Platelet count, PCT, and PDW were significantly higher in the morbidly obese, obese, and overweight groups compared to the healthy group. However, there was no significant difference between the groups in terms of MPV, NLR, and PLR (Table II).

There was a significant difference in fasting serum insulin and HOMA-IR levels of the morbidly obese, obese, and overweight patients. Serum HDL levels were significantly lower in the morbidly obese children than in those of the other groups (Table III).

When obese children were compared according to insulin resistance status, WBC (p<0.001), neutrophil (p=0.002), lymphocyte (p=0.001), platelet (p=0.034), PCT (p=0.05), ALT (p<0.01), and triglyceride (p<0.001) levels were higher in those children with insulin resistance than in those without insulin resistance (Table IV).

There was a positive correlation between the BMI-SDS and WBC, neutrophil, lymphocyte, monocyte, platelet count, NLR, and PCT value (p<0.001), and a negative correlation with the PDW value (p=0.001). In partial correlation analysis, similar correlations were found between the BMI-SDS and blood count parameters except for platelets and NLR (Table V). Moreover, there was a positive correlation between the HOMA-IR and WBC (p=0.014), neutrophil (p=0.003), and NLR (p=0.02). Additionally, in partial correlation analysis, there was a positive correlation between the HOMA-IR and lymphocyte (p<0.001) and PCT (p=0.006) (Table VI).

Table I. The clinical characteristics of the study population

	Healthy (Group 1) (n=110)	**Overweight (Group 2) (n=130)**	**Obese (Group 3) (n=341)**	**Morbid obese (Group 4) (n=188)**	**p-value***
Age (year)	13.5 (10.2-15.3)	12.5 (10.6-14.0)	12.5 (10.0-14.5)	14.0 (10.0-15.7)	0.056
Gender					
Female (%)	65 (59)	72 (55)	180 (53)	122 (65)	0.055
Male (%)	45 (41)	58 (45)	161 (47)	66 (35)	
Puberty					
Prepubertal (%)	27 (25)	36 (28)	100 (29)	46 (24)	0.595
Pubertal (%)	83 (75)	94 (72)	241 (71)	142 (76)	
Weight-SDS	-0.20 (-0.74-0.36)	1.80 (1.30-2.13)	2.64 (2.18-3.07)	3.84 (3.43-4.37)	**<0.01[a]**
Height-SDS	0.09 (-0.89-0.75)	0.74 (-0.29-1.48)	0.65 (-0.09-1.37)	0.63 (-0.13-1.49)	**<0.01[b]**
BMI-SDS	-0.33 (-0.75-0.20)	1.76 (1.50-1.89)	2.53 (2.27-2.80)	3.34 (3.14-3.68)	**<0.01[a]**

Continuous variables are expressed as median (25-75p), while the categorical variables are expressed as number (percentage), *Kruskal-Wallis test
[a]Group 1 & Group 2, Group 1 & Group 3, Group 1 & Group 4, Group 2 & Group3, Group 2 & Group 4, Group 3 & Group 4, p<0.001.
[b]Group 1 & Group2, Group 1 & Group 3, Group 1 & Group 4, p<0.001.
BMI: Body mass index, SDS: Standard deviation score

Discussion

In this study, WBC, neutrophils, lymphocytes, monocytes, platelet count, MPV, and PCT values were significantly higher in the obese children. Also, there was a positive correlation between BMI, HOMA-IR and WBC, neutrophils, lymphocytes, and PCT.

White Blood Cells

Studies point towards low-grade chronic inflammation in adipose tissue as the initiating mechanism of obesity-related complications (19). Macrophage infiltration in adipose tissues plays a leading role in the development of chronic inflammation (20). It was shown that pro-inflammatory cytokines secreted from activated macrophages, such as tumour necrosis factor-alpha and interleukin 6, disrupt the autocrine and paracrine effect of insulin in adipocytes (21,22). As well as macrophages, the effects of other leucocyte subgroups in adipose tissue inflammation were shown (23). Experimental studies showed an increase in neutrophil count in adipose tissues even in the first few days of a high-fat diet, and this finding led to the idea that inflammation is seen in the early stages of obesity (24). On the other hand, studies investigating the relationship between childhood obesity and leucocyte subgroups and insulin resistance are limited. In this study, we found that WBC, neutrophil, and lymphocyte count were higher in obese children with insulin resistance compared to obese children without insulin resistance. Furthermore, a positive correlation was shown between HOMA-IR and WBC, neutrophil, and lymphocyte count. These findings seem to support the idea of the effect

Table II. Complete blood count parameters of the study population

	Healthy (Group 1) (n=110)	Overweight (Group 2) (n=130)	Obese (Group 3) (n=341)	Morbid obese (Group 4) (n=188)	p-value*
WBC (10^3/µL)	7,015 (5,867-8,140)	8,270 (7,223-9,725)	8,570 (7,375-9,810)	9,135 (7,805-10,288)	**<0.001[a]**
Neutrophil (10^3/µL)	3,745 (2,988-4,763)	4,295 (3,743-5,295)	4,530 (3,660-5,715)	5,135 (4,180-6,123)	**<0.001[b]**
Lymphocyte (10^3/µL)	2,375 (2,068-,2878)	2,960 (2,503-3,600)	3,060 (2,515-3,570)	3,085 (2,543-3,465)	**<0.001[c]**
Monocyte (10^3/µL)	440 (360-560)	520 (430-650)	520 (440-600)	535 (440-658)	**<0.001[d]**
N/L	1.57 (1.16-2.07)	1.44 (1.18-1.81)	1.48 (1.18-1.96)	1.76 (1.32-2.11)	0.370
Platelet (10^3/µL)	286.0 (249.5-336.5)	322.5 (289.5-365.5)	332.0 (287.0-383.0)	331.5 (289.0-388.8)	**<0.001[e]**
MPV	9.3 (8.7-9.8)	9.7 (8.9-10.2)	9.3 (8.7-9.9)	9.4 (8.8-10.0)	**0.045[f]**
PDW	15.9 (15.6-16.1)	15.8 (15.5-16.1)	15.7 (15.4-15.9)	15.7 (15.5-16.0)	**0.001[g]**
PCT	0.26 (0.23-0.31)	0.31 (0.27-0.35)	0.31 (0.27-0.35)	0.30 (0.27-0.36)	**<0.001[h]**
P/L	0.12 (0.09-0.14)	0.10 (0.08-0.12)	0.11 (0.09-0.13)	0.11 (0.10-0.13)	0.236

Data are expressed as median (25-75p)
*Dunn's test (non-parametric pairwise multiple comparison)
[a]Group 1 & Group 2, Group 1 & Group 3, Group 1 & Group 4, p<0.001; Group 2 & Group 4, p=0.014
[b]Group 1 & Group 2, p=0.012; Group 1 & Group 3, Group 1 & Group 4, Group 2 & Group 4, Group 3 & Group 4, p<0.001
[c]Group 1 & Group 2, Group 1 & Group 3, Group 1 & Group 4, p<0.001
[d]Group 1 & Group 2, p=0.001; Group 1 & Group 3, Group 1 & Group 4, p<0.001
[e]Group 1 & Group 2, p=0.002; Group 1 & Group 3, Group 1 & Group 4, p<0.001
[f]Group 1 & Group 2, p=0.045; Group 2 & Group 3, p=0.049
[g]Group 1 & Group 3, p<0.001; Group 1 & Group 4, p=0.001; Group 2 & Group 3, p=0.048
[h]Group 1 & Group 2, Group 1 & Group 3, Group 1 & Group 4, p<0.001
WBC: White blood cell, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, N/L: Neutrophil/lymphocyte, P/L: Platelet/lymphocyte

Table III. The laboratory characteristics of the study population

	Overweight (Group 2) (n=130)	Obese (Group 3) (n=341)	Morbid obese (Group 4) (n=188)	p-value*
Glucose (mg/dL)	88 (81-92)	87 (81-92)	86 (82-91)	0.776
Insulin (µIU/mL)	11.6 (8.7-15.8)	13.8 (9.9-19.5)	17.0 (10.8-24.9)	**<0.001[a]**
HOMA-IR	2.43 (1.84-3.40)	2.95 (2.09-4.30)	3.64 (2.24-5.33)	**<0.001[b]**
AST (U/L)	21 (18-26)	22 (18-22)	21 (17-26)	0.316
ALT (U/L)	18 (14-26)	21 (16-30)	21 (16-33)	**0.011[c]**
TC (mg/dL)	164 (149-186)	163 (145-182)	162 (144-179)	0.489
HDL (mg/dL)	50 (45-59)	47 (40-56)	45 (39-52)	**0.01[d]**
TG (mg/dL)	93 (66-123)	94 (70-130)	95 (67-137)	0.384
LDL (mg/dL)	93 (80-112)	92 (75-107)	92 (76-113)	0.668
fT4 (ng/dL)	0.97 (0.91-1.05)	1.0 (0.92-1.09)	0.98 (0.89-1.07)	0.075
TSH (mIU/L)	1.92 (1.24-2.75)	2.10 (1.47-2.79)	2.27 (1.60-3.03)	0.079

Data are expressed as median (25-75p), *Kruskal-Wallis test
[a]Group 2 & Group 3, p=0.001; Group 2 & Group 4, p<0.001; Group 3 & Group 4, p=0,040.
[b]Group 2 & Group 3, p=0.002; Group 2 & Group 4, p<0.001; Group 3 & Group 4, p=0,041.
[c]Group 2 & Group 3, p=0.044; Group 2 & Group 4, p=0.034.
[d]Group 2 & Group 3, p=0.041; Group 2 & Group 4, p=0.001.
HOMA-IR: Homeostatic model assessment for insulin resistance, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TC: Total cholesterol, HDL: High density cholesterol, TG: Triglyceride, LDL: Low density cholesterol, fT4: Free thyroxine, TSH: Thyroid stimulating hormone

of adipose tissue inflammation on insulin resistance and white blood cells.

In a study of 656 adults at high risk of developing type 2 diabetes mellitus, a positive correlation was found between leucocyte subgroups (neutrophils, lymphocytes, monocytes), NLR, and HOMA-IR (10). In contrast to that study, we did not see a correlation between monocyte count and NLR and HOMA-IR in our study; this difference was thought to be related to the difference in population and age group.

We could not find a significant relationship between BMI, HOMA-IR, and NLR, although it is well known that an increase in NLR and PLR in chronic inflammation is a marker of immune response (25,26). The lack of a significant relation between NLR and BMI in our study could be related to an increase in lymphocyte count together with a neutrophil count. Similar to our study, Marginean et al. (3) and Bahadir et al. (27) did not find a relation between NLR and obesity in children and adults. Nevertheless, it was shown that NLR is a marker suggesting inflammation in studies of obese patients with comorbidity. A positive correlation was found between NLR and carotid intima-media thickness in adult patients who were candidates for bariatric surgery (28). In another study, it was reported that NLR was higher in obese children who have obstructive sleep apnoea compared to those who do not (4). Vuong et al. (29) assessed the relation between blood count and waist circumference in 6,766 adults and showed that there is a positive correlation between neutrophil, lymphocyte, and monocyte count with waist circumference. Furthermore, in the same study, they saw that the percentage of neutrophils increased; however, lymphocyte and monocyte percentage decreased with increasing waist circumference (29). Similarly, in our study, the neutrophil percentage increased with increasing BMI;

Table IV. The clinical and laboratory characteristics of the overweight and obese patients with and without insulin resistance

	Insulin Resistance		
	No (n=424)	**Yes (n=227)**	**p-value***
Age (year)	12.1 (9.0-14.6)	13.0 (11.0-15.0)	0.002
Gender (%) Male Female	199 (47) 225 (53)	96 (42) 131 (58)	0.257
Puberty (%) Prepubertal Pubertal	129 (30) 295 (70)	50 (22) 177 (78)	0.022
BMI-SDS	2.53 (2.01-3.0)	2.83 (2.38-3.32)	<0.001
WBC (10^3/μL)	8,550 (7,200-9,770)	9,070 (7,980-10,420)	<0.001
Neutrophil (10^3/μL)	4,540 (3,680-5,550)	4,795 (3,990-5,970)	0.002
Lymphocyte (10^3/μL)	2,970 (2,460-3,490)	3,140 (2,680-2,670)	0.001
Monocyte (10^3/μL)	520 (430-610)	520 (450-640)	0.244
Platelet (10^3/μL)	327 (283-382)	341 (297-441)	0.034
PCT (%)	0.31 (0.27-0.35)	0.32 (0.28-0.35)	0.055
MPV (fL)	9.4 (8.8-10.1)	9.3 (8.7-10.0)	0.250
PDW	15.7 (15.5-16.0)	15.7 (15.4-15.9)	0.037
N/L	1.53 (1.20-2.01)	1.59 (1.22-1.99)	0.706
P/L	0.11 (0.09-0.13)	0.11 (0.09-0.13)	0.353
Glucose (mg/dL)	85 (80-90)	91 (85-95)	<0.001
Insulin (μIU/mL)	11.0 (8.2-14.4)	23.2 (18.9-28.6)	<0.001
HOMA-IR	2.3 (1.7-3.1)	5.1 (4.3-6.7)	<0.001
AST (U/L)	22 (17-16)	22 (17-28)	0.809
ALT (U/L)	19 (15-27)	24 (17-35)	<0.01
TC (mg/dL)	161 (145-181)	165 (147-182)	0.246
HDL (mg/dL)	49 (42-57)	44 (38-52)	<0.001
TG (mg/dL)	89 (66-120)	105 (79-146)	<0.001
LDL (mg/dL)	91 (77-109)	94 (77-110)	0.214

Continuous variables are expressed as median (25-75p), while the categorical variables are expressed as number (percentage)
*Mann-Whitney U test
BMI: Body mass index, WBC: White blood cell, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, N/L: Neutrophil/lymphocyte, P/L: Platelet/lymphocyte, HOMA-IR: Homeostatic model assessment for insulin resistance, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TC: Total cholesterol, HDL: High density cholesterol, TG: Triglyceride, LDL: Low density cholesterol

lymphocyte and monocyte count also increased in numbers, but their percentages in white blood cell distribution decreased, which means they did not increase as much as neutrophils. We saw that the percentage of neutrophils increased, and the percentage of lymphocytes decreased with increasing BMI, and NLR also increased; however, this increase did not reach statistical significance.

Platelets

Similar to an increase in WBC, platelet count also increases in acute and chronic inflammation due to secreted cytokines (30). Moreover, it is thought that thrombopoietin, one of the adipokines secreted from omental adipose tissue, may be responsible for both the increase in platelet count and insulin resistance (31). In addition, insulin resistance shortens platelet life, and that causes an increase in the number of platelets (32).

Similar to the results of our study, Arslan and Makay (33) reported that MPV was significantly higher in 128 obese children compared to normal-weight children. The different result from our study was that they reported a positive correlation between MPV and HOMA-IR (33). In 330 prepubertal children (125 normal weight, 205 overweight), platelet count was significantly higher in overweight children, and MPV was significantly lower in overweight children with metabolic syndrome (34). There was a negative correlation between MPV and HOMA-IR (34). There are also studies reporting higher platelet count, and lower MPV in adult obese subjects (35,36). In another study, in which children were assessed in three groups as normal weight, obese with insulin resistance and obese without insulin resistance, no difference was found between the groups in terms of platelet count, MPV and PDW values (37). Similar to our results, in an adult study, PDW decreased with increasing BMI, and it was shown that the risk of having lower PDW increased with an increase in BMI (38). In contrast, some studies report higher PDW in adults with metabolic syndrome (6). Also, some studies reported no significant difference in the platelet indices of obese and normal-weight children (37). Different results were reported in studies assessing the relation between obesity, metabolic syndrome, and platelet indices such as MPV and PDW (39-42). To the best of our knowledge, this is the first study assessing the relation between PCT and childhood obesity. PCT provides more accurate information about platelet mass (1). PCT is the volume occupied by platelets in the blood as a percentage and it is calculated according to the formula PCT=platelet count × MPV/10,000 (43). We

Table V. Correlation analysis of body mass index SDS with complete blood count parameters

	Spearman's rho	p*	Partial correlation	p**
WBC	0.260	<0.001	0.230	<0.001
Neutrophil	0.250	<0.001	0.196	<0.001
Lymphocyte	0.165	<0.001	0.135	<0.001
Monocyte	0.146	<0.001	0.147	<0.001
Platelet	0.125	<0.001	0.023	0.530
PCT	0.159	<0.001	0.206	<0.001
MPV	0.009	0.807	0.059	0.100
PDW	-0.137	<0.001	-0.125	0.001
N/L	0.097	0.007	0.048	0.186
P/L	-0.044	0.222	0.024	0.508

*Spearman's correlation analysis, **Partial correlation analysis (age, gender and pubertal status)
WBC: White blood cell, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, N/L: Neutrophil/lymphocyte, P/L: Platelet/lymphocyte

Table VI. Correlation analysis of HOMA-IR with complete blood count parameters

	Spearman's rho	p*	Partial correlation	p**
WBC	0.107	0.014	0.150	0.001
Neutrophil	0.130	0.003	0.089	0.042
Lymphocyte	0.024	0.582	0.164	<0.001
Monocyte	0.047	0.281	0.062	0.160
Platelet	-0.009	0.846	-0.056	0.206
PCT	0.045	0.303	0.121	0.006
MPV	0.066	0.133	-0.070	0.109
PDW	0.039	0.377	-0.061	0.163
N/L	0.102	0.020	-0.061	0.166
P/L	-0.019	0.672	-0.057	0.198

*Spearman's correlation analysis, **Partial correlation analysis (age, gender and pubertal status)
HOMA-IR: Homeostatic model assessment for insulin resistance, WBC: White blood cell, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, N/L: Neutrophil/lymphocyte, P/L: Platelet/lymphocyte

observed a positive correlation between PCT and BMI and HOMA-IR in our study. In a study of young adult morbidly obese patients, it was found that PCT was higher in obese patients compared to normal-weight patients, similar to our study, and no significant difference was found in MPV and PDW values (35). Han et al. (44) reported that platelet count and PCT were positively associated with BMI and body fat mass, but there were no significant associations between the other platelet indices (MPV, PDW), and body fat. There are also adult studies reporting similar results between PCT and BMI (45,46).

Although PLR, as well as NLR, are known as chronic inflammation-related markers, no relation was found between PLR with being overweight/obesity and insulin resistance in our study (47). PLR was found to be significantly higher in morbidly obese adult patients compared to normal-weight patients (35). Erdim et al. (4) found that PLR was significantly higher in 127 obese children with a hypopnea index of >5 compared to other children. Additionally, in another study, an increase in platelet and lymphocyte count was seen in obese children; however, no increase was seen in PLR, similar to our study (3). A study of 600 adult obese patients, assessing the relation between serum inflammatory markers and visceral adiposity, showed that there is a stronger correlation between WBC and high-sensitive C-reactive protein and visceral adipose tissue compared to PLR and NLR (48). These inconsistent results

were thought to be related to the different ages of the subjects included in the studies and the accompanying complications of obesity.

Obesity is an independent risk factor for having higher cell counts in children, specifically WBC, neutrophils, and platelets (49). In the present study, when subjects were compared according to their pubertal status, WBC, neutrophil, lymphocyte, and monocyte counts were higher in the obese groups than those of the healthy subjects in both prepubertal and pubertal children (data is not shown). However, there was no difference between the groups in terms of PLT, MPV, RDW, N/L and P/L in prepubertal children, and MPV and P/L in pubertal children. The lack of significance for these parameters is thought to be due to the relatively small sample size of the subgroups.

Study Limitations

Firstly, caution should be taken about causative comments regarding the observed findings due to the cross-sectional design. Secondly, infections cannot be strictly ruled out, although those patients with a suspicion of infection and WBC >13.5x10^3/mL were excluded from the study. Furthermore, despite the statistically significant p-values in correlation analysis, the correlation coefficients were low. We thought that the main reason for this situation was related to the large sample size. The strengths of the study were that the sampling size was large, the cases were compared in four different groups as normal, overweight, obese, and morbidly obese, and also, the study was performed in a single centre that has a standard protocol.

Conclusion

In this study, it was shown that WBC, neutrophils, lymphocytes, monocytes, platelets, and PCT values increase in childhood obesity, which could point towards low-grade chronic inflammation and also an increase in WBC, neutrophils, lymphocytes and PCT values might be associated with insulin resistance.

Ethics

Ethics Committee Approval: The study was initiated upon approval form the local ethics committee of Aydın Adnan Menderes University Faculty of Medicine, in light of the Helsinki Declaration (2020/98).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.A., Data Collection or Processing: Ay.A., E.Ç., Analysis or Interpretation: A.A., Writing: Ay.A., E.Ç.

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

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Original Article

Benign Early Repolarization Pattern: Is it Really Benign for Children?

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ABSTRACT

Aim: Early repolarization (ER) is a common electrocardiographic (ECG) finding. Although it is thought to be a benign phenomenon, recent studies have shown it to be an important marker of cardiac vulnerability, which can lead to sudden cardiac death. However, there are still conflicting data regarding the prognostic significance of ER in asymptomatic subjects, especially in children. The aim of this study was to investigate specific ECG markers that reflect ventricular repolarization in children with benign ER.

Materials and Methods: The study group included 56 healthy children with a benign ER pattern on ECG and a control group was formed of 81 children with normal ECG. Benign ER pattern was defined as terminal QRS notching or slurring accompanied by rapidly ascending ST elevation (>0.1 mV from baseline) in two or more leads. The ECG parameters of QT dis, QTc dis, Tpe dis, Tp-e/QT and Tp-e/QTc were evaluated at rest by a single experienced pediatric cardiologist blinded to the groups.

Results: Higher Tp-e, Tp-e dis, Tp-e/QT and Tp-e/QTc measurements were determined in the study group than in the control group. There were no significant differences in the studied ECG parameters with respect to ER location (inferior, lateral, inferolateral), or the ER type (slurring, notching or both).

Conclusion: These findings suggest that benign ER in children is associated with the risk of arrhythmogenesis through alterations in ventricular repolarization.

Keywords: Arrhythmia, children, sudden cardiac death

Introduction

Early repolarization (ER) is universally defined as 0.1 mV ST segment elevation in at least two adjoining leads (1). In the absence of symptoms, it has been considered as a normal state found mainly in teenagers, youngsters, and athletes (2,3). However, case reports, experimental and epidemiological studies with large samples have shown that ER could be related with arrhythmias leading sudden cardiac death (SCD) (4,5).

Therefore, recent studies have explained this phenomenon by classifying it into benign and malign patterns and have implied that clinical findings and electrocardiographic data are important for differential diagnosis. According to these studies, a benign ER pattern can be briefly defined as J wave distribution mainly in V3-V5 (precordial leads) and II, III, aVF (inferior leads) and rapidly ascending segment of ST (not descending or horizontal) with tall R waves in the absence of symptoms (4,6-8).

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However, this definition is still not consistent in adult studies, and is not known to be valid for the pediatric age group, where this ER pattern is ten times more prevalent than in adults (3).

Furthermore, although there are studies documenting ER patterns on the electrocardiograms (ECG) of healthy children with idiopathic ventricular fibrillation and SCD, data is still scarce about the clinical importance of benign ER patterns in children (3).

In this study, we wanted to examine if there is a risk of arrhythmogenesis in healthy children with a benign ER pattern. ECG markers; including T peak to end interval (Tp-e), QT interval (QT), corrected QT interval (QTc), dispersion of these intervals (Tp-e dis, QT dis, QTc dis), Tp-e/QT and Tp-e/QTc were calculated, as these are known to be non-invasive determiners of transmural dispersion of repolarization (TDR), which has been suggested to underlie arrhythmogenesis (9,10).

Materials and Methods

A retrospective analysis was made of pediatric subjects who presented at the pediatric cardiology department for miscellaneous reasons (chest pain, sports participation, murmur, etc.) and underwent a history and physical examination, baseline electrocardiogram and transthoracic echocardiogram.

History: The children recruited into the study were those with no history of arrhythmia and syncope, no known arrhythmia susceptibility syndrome, no known heart disease, and no family history of SCD. The subjects were asked about participation in regular sports activities and only those with no sports activities or less than one year of regular sports participation were included the study because of the well-known effect of sports on cardiac structure and ER pattern visualization (11).

Physical examination: Subjects with a good clinical condition with no significant findings of acquired or congenital heart disease, or any other infectious or chronic illness were eligible to participate.

Electrocardiography: An ECG recorder (Nihon Kohden, Tokyo, Japan) set at 25 mm/s paper speed and 10 mm/mV voltage was used to obtain 12 lead ECG recordings following the history and physical examination. Subjects were excluded from the study if the ECG demonstrated any pre-excitation syndrome, prolonged QTc, arrhythmogenic right ventricular cardiomyopathy or Brugada pattern.

Echocardiography: A Vivid 3 (General Electric, USA) echocardiography device with 3 MHz probes was used to perform echocardiography by a single pediatric cardiologist blinded to the ECG findings of the participants. Only those subjects without pathological valve insufficiency or structural heart disease, with normal systolic function and normal wall diameters according to age were included in the study.

Benign ER determination and analysis of ECG parameters: After primary analysis of the subjects as described above, the ECGs were then reviewed by a physician to avoid interobserver variability, with particular attention to benign ER pattern. The definitions by Tikkanen and Huikuri (6) and Perez-Riera et al. (7) were used to determine subjects with benign ER (12);

1- At least 1 mm (0.1 mV) but not more than 2 mm (0.2 mV) elevation of the J-point (the junction between the QRS complex terminal and the beginning of ST segment) in at least two consecutive leads.

2- J-point elevation (rapidly ascending-not descending or horizontal) manifestation could be QRS slurring (slow deflection of downslope R wave) or notching (a positive wave at the J-point)

3- These changes can be in II, III, aVF (inferior leads), I, aVL, and V4-V6 (antero-lateral leads) or both. In accordance with previous studies, the V1 to V3 leads were not interpreted to avoid confusion with the phenotype of Brugada syndrome (5,11).

The subjects were subdivided into two groups as those with benign ER pattern and those with no ER pattern. The QT interval was defined as being from the beginning of the QRS complex to the end of the T wave and Tp-e was defined as being from the peak of the T wave to the point at which the T wave returns to the baseline (Figure 1). At least three T waves, QT intervals were evaluated in each derivation and the mean was calculated as milliseconds (ms). QTc interval was calculated according to the Bazett formula.

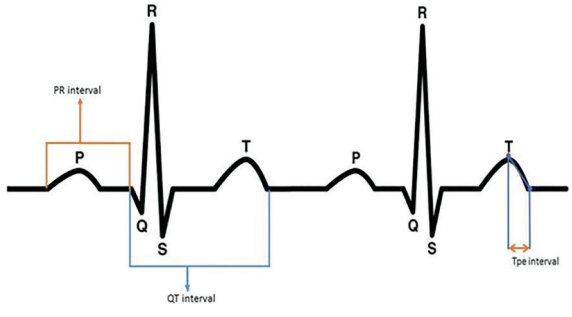

Figure 1. Parameters of ECG
ECG: Electrocardiography, Tpe: T-peak to T-end

The differences between the maximum and minimum intervals were defined as dispersions (QT dis, QTc dis and Tp-e dis).

As this is a retrospective study, an informed consent was not obtained from the parents' of the participants. Keçiören Training and Research Hospital's scientific committee approved the study and the study was performed in accordance with the principles of Helsinki Declaration (No: 43278876-929, dated: 2020).

Statistical Analysis

The Statistical package for social sciences program version 21 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses of the data. Shapiro-Wilk test was used to assess the normal distribution of variables. According to distribution, Student's t-test or Mann-Whitney U test were used to compare groups. The differences in median values between more than two independent groups were analyzed with the Kruskal-Wallis test. A p-value of under 0.05 was considered as the statistical significance level.

Results

A total of 137 subjects were examined during the study consisting of 56 children with benign ER pattern (Study group) and 81 children with no ER pattern (Control group). There were no differences between the groups in terms of age and gender (Table I). Heart rate was lower in the study group compared to the controls [75.8 (12.3) bpm vs. 81 (15.2) bpm, p=0.03] (Table II).

In the study group, benign ER was present in inferior derivations in 11 (19.6%) children, in lateral derivations in 35 (62.5%), and in inferolateral derivations in 10 (17.9%). QRS slurring was observed in 35 (62.5%), QRS notching in 18 (32.1%) and both in 3 (5.4%) in the study group.

The mean QT and QTc values were found to be higher in the study group, but not at a statistically significant level. The Tp-e interval was significantly higher in the study group [91.3 (11.9) ms, 82.9 (13.6) ms, respectively, p<0.001]. In terms of dispersion of these parameters, all were higher in the study group but only the Tp-e dis value was statistically

Table I. Demographic and clinical characteristics of the study groups

	Study group (with benign ER) (n=56)	Control group (without ER) (n=81)	p-value
Gender (Male/female)	48/8	59/22	0.07
Age (years)	13.5 (3.1)	13.8 (2.3)	0.49
Body mass index (kg/m^2)	21 (2)	21.3 (3.7)	0.77
Systolic blood pressure (mmHg)	118 (6.2)	116.1 (6.2)	0.52
Diastolic blood pressure (mmHg)	71.5 (3.9)	67.2 (8.7)	0.36

*Values are presented as mean (SD)
SD: Standard deviation, ER: Early repolarization

Table II. Comparison of groups according to ECG measurements

	Study group (with benign ER) (n=56)	Control group (without ER) (n=81)	p-value
Heart rate (bpm)	75.8 (12.3)	81 (15.2)	**0.03**
QT (ms)	365.3 (24)	359 (29.1)	0.18
QTc (ms)	415.7 (26.7)	408.9 (23)	0.12
Tpe (ms)	91.3 (11.9)	82.9 (13.6)	**<0.001**
QTdis (ms)	43.04 (14.7)	41.7 (14.2)	0.6
QTcdis (ms)	33.6 (18.9)	29.8 (17.6)	0.2
Tpedis (ms)	35.6 (15.8)	24.9 (12.5)	**0.001**
Tpe/QT (ms)	0.25 (0.3)	0.23 (0.3)	**0.03**
Tpe/QTc (ms)	0.22 (0.3)	0.20 (0.3)	**<0.001**

**Values are presented as mean (SD)
Bpm: Beat per minute, ms: Millisecond, QTdis: QT interval dispersion, QTc dis: Corrected QT interval dispersion, Tp-e: Interval between the peak and the end of T wave, Tp-e dis: Tp-e interval dispersion, SD: Standard deviation, ER: Early repolarization, ECG: Electrocardiographic

significant [35.6 (15.8) ms in study group, 24.9 (12.5) ms in control group, p=0.001].

A statistically significant difference was detected between the groups in the ratios of Tp-e/QT and Tp-e/QTc [0.25 (0.3) ms in study group, 0.23 (0.3) ms in control group, p=0.03; 0.22 (0.3) ms in study group, 0.20 (0.3) ms in control group, p<0.001 respectively]. The comparisons of the ECG measurements are summarized in Table II.

No significant differences were determined in the studied ECG measurements with respect to ER location (inferior, lateral, inferolateral) and the ER type (slurring, notching or both).

Discussion

The relationship between malignant arrhythmias and ER has not yet been fully clarified. TDR is one of the related underlying mechanisms. This phenomenon could be described as abnormalities in the ionic mechanism of cardiac cells creating myocardial heterogeneity. During ventricular depolarization, the transient outward potassium current mediated action potential notch is just in the ventricular epicardium and not in the endocardium, which results in a transmural voltage gradient and can be seen as J point elevation on ECG. As a result of this gradient, the phase 2 re-entry of action potential is propagated and/or adjacent cells activate ER cells for spontaneous activity starting arrhythmogenesis (13).

In previous studies, Tp-e has been reported as a marker of TDR and prolongation of Tp-e has been used to predict arrhythmias, including VT/VF (9). The QT and QTc intervals can also reflect repolarization of total myocardium on ECG. However, Tp-e and Tp-e dis are considered more useful markers of TDR than QT and QTdis (14). The measurements of Tp-e/QT and Tp-e/QTc are also used as a marker of ventricular arrhythmogenesis (10). In addition, these ratios are considered to be more sensitive in the prediction of arrhythmia compared to Tp-e as the constant index of arrhythmogenesis is not affected by heart rate.

In our study, children with benign ER were found to have increased Tp-e, Tp-e dis and Tp-e/QT, Tp-e/QTc ratios compared with the controls. However, there were no statistically significant differences between the groups with respect to QT, QTc and QTdis, QTc dis. In accordance with previous studies, the current study findings also suggest that repolarization changes could be local in children with ER and not affect QT and QTc intervals (15,16). Increased Tp-e, Tp-e dis in different J wave syndromes including Brugada, ER and long QT syndrome have been reported by several adult studies (16,17). However, to the best of our knowledge, these novel indexes of arrhythmogenesis in ER have not been previously investigated in the pediatric age group.

Different studies have pointed out that ER existence depends on autonomic tone and heart rate (16). J point amplitude increases under increased parasympathetic tone and in lower heart rates. In our study, although all the participants were in the same resting condition during ECG, the heart rate was observed to be lower in the study group. Although this result is consistent with the findings of previous studies, the participants were not asked about specific conditions that could affect autonomic tone such as the last time of eating or sleeping time.

The risk of SCD is minimally increased by the ER pattern according to adult studies (18,19). Those adults with an ER pattern in lateral leads, with less than 0.2 mV J wave amplitude, horizontal ST segment and QRS slurring are accepted as having a benign ER pattern and low risk for arrhythmias or SCD (20). In contrast to this absolute definition in adults, the definition and clinical implications of benign ER pattern remain unclear in the pediatric age group, in which the ER pattern is seen 5 to 10 times more often than in the adult population (3).

A previous study with a large sample of children reported that no patient characteristics such as gender, age, body mass index, blood pressure, or previous syncope were found to be markers of ER existence (3). However, there are no data about the risk stratification for arrhythmias and/or SCD in healthy children with ER. In the present study, no relationship was determined between the ER pattern location, QRS morphology and Tp-e dis, Tp-e/QT, Tp-e/QTc ratios which are known to be predictors of arrhythmia.

Study Limitations

We are aware of the limitations of this study, primarily the relatively small sample size of the study group. In addition, there was no evaluation of the correlation between ECG measurements and any ventricular arrhythmias (premature ventricular contractions, non-sustained ventricular tachycardia, etc.). Finally, it was not possible to prospectively follow up the study group for future arrhythmic events.

Conclusion

The findings of our study showed that children with benign ER may have an increased risk of SCD through alterations in ventricular repolarization parameters. This group of patients may be considered at risk for the

development of arrhythmias. For that reason, there is a need for further prospective and long-term follow-up studies including long term ECG recordings to be able to demonstrate the clinical and prognostic importance of these parameters in children.

Ethics

Ethics Committee Approval: Keçiören Training and Research Hospital's scientific committee approved the study and the study was performed in accordance with the principles of Helsinki Declaration (No: 43278876-929, dated: 2020).

Informed Consent: As this is a retrospective study, an informed consent was not obtained from the parents' of the participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.K., M.K., F.D., Design: S.K., M.K., C.K. Data Collection or Processing: S.K., M.K., C.K., F.D., Analysis or Interpretation: S.K., Literature Search: S.K., Writing: S.K.

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Original Article

Mean Platelet Volume and the Ratio of Mean Platelet Volume/Platelet Count in Acute Rheumatic Fever

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ABSTRACT

Aim: Acute rheumatic fever (ARF) is an endemic disease especially in developing countries. Due to an autoimmune response to group B streptococcus throat infection, ARF develops in susceptible children. Mean platelet volume (MPV) reflects the platelet size and the rate of platelet production. It is important in cardiovascular events and rheumatic diseases. The MPV/platelet count ratio was determined to be more sensitive than MPV alone in patients with hepatocellular carcinoma, deep vein thrombosis and myocardial infarction. The aim of this study was to investigate changes in MPV and the MPV/platelet count ratio during the active and remission periods of ARF compared with healthy subjects.

Materials and Methods: This study population consisted with 70 ARF patients and 70 age and gender matched healthy controls. In all subjects, complete blood count; including haemoglobin, white blood cell count (WBC), platelet count, MPV and C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were measured during the active stage and during the remission period and compared with healthy individuals.

Results: There was no statistically significant difference between the ARF and control groups in terms of the sex and age ($p>0.05$). Forty-one patients in the ARF group had carditis. The ARF patients in the active stage had significantly higher WBC, CRP and ESR values ($p<0.05$). Although no significant difference was observed in MPV between the groups ($p>0.05$); the MPV/platelet count ratio was low during the active stage but increased during the remission period and reached the same values as the healthy controls ($p<0.001$).

Conclusion: We did not find any relationship between MPV and ARF. However, a decreased MPV/platelet count ratio was detected during the active stage of ARF. The present findings emphasize the association between the MPV/platelet count ratio and ARF. The MPV/platelet count ratio may be used to determine the activity of ARF.

Keywords: Acute rheumatic fever, mean platelet volume, platelet count

Introduction

Acute rheumatic fever (ARF) results from an autoimmune response to group B streptococcus throat infection in genetically susceptible children (1). The most important complication of ARF is valvular insufficiency, known as rheumatic heart disease. Worldwide, it is the leading cause of cardiovascular death during the first five decades of life (2). In our country, the incidence of rheumatic heart disease has been found to be 8.9/100,000 (3).

In recent studies, mean platelet volume (MPV), which indicates platelet size (4), has been determined to be important in cardiovascular events, and in rheumatic

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diseases like familial Mediterranean fever, Behçet's disease, inflammatory bowel disease, and rheumatoid arthritis (5-11). In the literature, although there are a few studies showing the ARF and MPV association, (12-16) these studies do not put forward any conclusive results indicating the relationship between ARF and MPV. Additionally, in some studies that were done with patients who had hepatocellular carcinoma, deep vein thrombosis and non-ST elevation myocardial infarction, the MPV/platelet ratio was determined to be more sensitive than MPV alone (17-19).

In this study, our aim is to investigate MPV and the MPV/platelet count ratio in ARF during the acute phase and after treatment and to compare these results with healthy controls.

Materials and Methods

Study Population

The study was conducted in the Paediatric Cardiology Department of Pamukkale University. Seventy patients who were hospitalized with a diagnosis of ARF during the acute stage of the disease and an aged matched control group of patients who were diagnosed with innocent murmurs were included in this study. The clinical and laboratory data of the patients were collected retrospectively from hospital records. Although modified Jones criteria were introduced in 2015, our patients were diagnosed according to older criteria (20). The patients' complete blood count; including white blood cell count (WBC), MPV, platelet count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) during the acute stage and after eight weeks of treatment, and during remission were collected. Each patient was evaluated via echocardiographic examination and carditis was diagnosed when pathological valve regurgitation was detected.

All patients underwent 2D, M-mode and colour flow Doppler study using GE Vingmed Vivid 7 model echocardiography (GE Vingmed Ultrasound AS, Horten, Norway) and multi frequency transducer (2.5-4 Hz) images were taken with the children in the left lateral decubitus position. The patients did not receive sedation during these examinations. Pathological valve regurgitation and normal echocardiographic examination was differentiated by the criteria of the World Health Organization Expert Committee (2004), (21).

Those patients with chronic disease, using medication, or those having abnormal liver or renal function tests were excluded from the study. Forty-one patients of the seventy ARF patients had arthritis (34 of them had mild-moderate carditis and the remaining seven had severe carditis). Salicylate was given to the arthritis and mild carditis patients at 80-100 mg/kg/day (maximum dose was 3.5 g/day) and prednisolone was given to the moderate and severe carditis patients at 2 mg/kg/day (maximum dose was 60 mg/day). This was tapered after two weeks treatment and salicylates was initiated as the prednisolone dosage decreased.

CRP levels >0.5 mg/dL and ESR >20 /hour were considered as increased.

The Ethical Committee of our institution approved the study (with approval number: 60116787/020/28852 on 16/08/2013).

Statistical Analysis

All statistical analysis was performed using Sytat statistical software (version 17.0 for windows; SPSS Inc, Chicago, IL, USA). Data were tested for homogeneity of variance with Shapiro-Wilk test. Student's t-test, x^2 test and One-Way ANOVA were used for data analyses. Statistical significance was taken at $p<0.05$. All data are presented as mean and standard deviation.

Results

The study was performed on 70 patients (39 males and 31 females) diagnosed with ARF and 70 healthy subjects (39 males and 31 females) as a control group. The mean age of the ARF patients and healthy subjects were 10.8±3.1 and 11.1±3.5 years (range between 5-17 years) respectively. There was no significant difference between the groups in terms of their mean age and sex ($p>0.05$). Forty-one of the seventy (59%) ARF patients had carditis. All patients with carditis had mitral valve involvement. Additionally, in 15 of these patients, there was both mitral and aortic valve involvement (37%). Four of them (1.9%) had heart failure.

In patients during the active stage of ARF, CRP and ESR were significantly higher than for the ARF patients who were in remission ($p<0.001$). Mean CRP and ESR levels were 6.7±5.4 mg/dL and 61±28 mm/h respectively during the active stage of ARF and 0.36±0.25 mg/dL and 16.6±8 mm/h respectively in ARF patients who were in remission.

Those ARF patients who were in the active stage had significantly higher WBC count than both the control group and the ARF patients who were in the remission period ($p<0.001$). WBC values reduced to levels close to the control group values during remission ($p>0.05$) There was no significant difference between the groups for haemoglobin and platelet counts ($p>0.05$), (Table I).

In terms of MPV, although there was no significant difference between the ARF patients in the acute stage and those in remission; the MPV/platelet ratio was significantly lower in the ARF patients in the acute stage than for the healthy control subjects and for the ARF patients in remission ($p<0.05$). However, the decreased MPV/platelet count ratio in the active stage increased during the remission period and reached the same values as the healthy controls ($p<0.001$), (Table I).

There was no significant difference between ARF patients with or without carditis in terms of WBC, CRP, ESR, MPV, platelet count or the MPV/platelet ratio ($p>0.05$) (Table II).

Discussion

Rheumatic fever is caused by gram-positive bacteria Streptococcus pyogenes and follows untreated throat infections in susceptible children. It also depends on other environmental factors, such as poor living standards and poor access to medical care (20). It results from an autoimmune response to infection of *Streptococcus pyogenes*. The most important complication of this disease is rheumatic heart disease. While the mean incidence of ARF is 19/100,000 in school-aged children worldwide (22), it was found to be 8.9/100,000 in Turkey (3). The pathogenesis of rheumatic heart disease is complex and involves genetic factors that predispose a person to the development of autoimmune reactions (1).

Platelets are cytoplasmic fragments of megakaryocytes. MPV indicates platelet production and activation and is commonly used as a measure of platelet size (4). The morphology of platelets remains fairly constant during their lifespan. In other words, the regulation of platelet function and aging is primarily aimed at maintaining platelet mass (platelet count multiplied by MPV) and persistent haemostatic potential. It has been put forward that the inverse relationship between platelet count and MPV in physiological and some pathological conditions reflect the tendency to maintain haemostasis by preserving a constant platelet mass (23).

In a recent review about MPV and its clinical use for diagnostic/prognostic purposes of non-haematological diseases, it was emphasized that there is inter individual

Table I. Differences between the patients with ARF during the active stage and in remission compared with the control group

	ARF in active stage (n=70)	ARF in remission (n=70)	Control (n=70)	p-value		
				p*	p**	p***
Haemoglobin (g/dL)	12.1±1.4	12.8±0.9	13.3±0.8	NS	NS	NS
WBC (mm³)	9,319±3,201	7,668±2,197	7,334±1,660	<0.001	NS	<0.001
Platelet count (mm³)	385,900±105021	337,914±87434	286,600±57,300	<0.001	0,001	<0.05
MPV (fL)	8.1±1.4	8.2±1.5	7.8±0.8	NS	NS	NS
MPV/platelet count	0.022±0.008	0.026±0.01	0.028±0.006	<0.001	NS	<0.05

p*: Between ARF patients during the active stage and the control group.
p**: Between ARF patients in remission and the control group.
p***: Between patients during the active stage of ARF and ARF patients in remission.
ARF: Acute rheumatic fever, WBC: White blood cell, MPV: Mean platelet volume, NS: Not significance

Table II. WBC, CRP, ESR, MPV, platelet count and MPV/platelet ratio in ARF patients with or without carditis

	ARF arthritis (n=29)	ARF carditis (n=41)
WBC (K/uL)	9,255±4,029	9,365±2,512
CRP (mg/dL)	4.5±5.9	5.1±5.3
ESR (mm/h)	56.6±26.0	63.3±28.8
MPV (fL)	7.8±1.4	8.3±1.4
Platelet count (x10⁹/L)	372,241±106,369	395,560±104,282
MPV/platelet ratio (fL/(10⁹/L)	0.023±0.008	0.02±0.008

p>0.05
WBC: White blood cell, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, MPV: Mean platelet volume, ARF: Acute rheumatic fever

variability in healthy subjects and its inverse correlation with platelet count (24). However, in some studies, it was found that there is a relationship between MPV increase and some cardiovascular events and mortality rate (5,6). This is also true for rheumatologic diseases like familial Mediterranean fever (7), Behçet's disease (8,9), inflammatory bowel disease (10) and rheumatic arthritis (11). It was found that there is converse relationship between MPV and disease activity. Although there is an inverse relationship between MPV and rheumatological disease activity in some studies, in rheumatoid arthritis patients, MPV was found to be increased in comparison with a control group (12). In Sert et al.'s (12) study which included 40 ARF patients, (8 had carditis, 32 arthritis) it was reported that MPV was decreased during the acute phase and it increased after treatment in patients with ARF. The authors claimed that in ARF, inflammatory cytokines such as IL-1 and IL-6 affect megakaryopoiesis and this causes the increase in platelet number and decrease in MPV values (12). However, Ozdemir et al. (13) reported that they did not find any relationship between MPV and rheumatic carditis in a study including 53 patients with rheumatic carditis. Karpuz et al. (15) investigated whole blood parameters of children with rheumatic valvular heart disease and they found elevated MPV levels compared with healthy controls. Also, in Çelik and Çelik (16) study, they found elevation of MPV in patients with valvular regurgitation. Contrary to these studies, our study includes patients with ARF both during the active stages and remission periods, and we did not find any relationship between MPV and ARF either in arthritis or in carditis.

Even though there are contradictory results for MPV and inflammatory diseases, it was found that the MPV/platelet count ratio was more sensitive than MPV alone in hepatocellular carcinoma (17), deep vein thrombosis (18) and non-ST elevation acute myocardial infarction (19). Also, we found a statistically significant relationship between the MPV/platelet ratio and the acute phase of ARF. This could be explained by increased megakaryopoiesis in inflammatory diseases but, both platelet count and MPV value must be considered.

The strength of our study is the evaluation of MPV, MPV/platelet ratio, CRP and ESR both during the acute phase and the remission phase of ARF.

Study Limitations

In our study, there were some limitations. Firstly, our sample size was small for the analysis of MPV and the MPV/platelet count ratio with the subgroups of the major signs of ARF. Secondly, due to the retrospective design of our study, we did not evaluate the long-term prognosis of our patients.

In the literature, this is the first study investigating the MPV/platelet ratio and ARF during the acute phase and after remission.

Conclusion

Rather than MPV, the MPV/platelet ratio can be used as inflammatory marker in ARF. However, prospective studies with a larger number of patients are needed to evaluate the efficacy of the MPV/platelet ratio.

Ethics

Ethics Committee Approval: The Ethical Committee of our institution approved the study (with approval number: 60116787/020/28852 on 16/08/2013).

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Design: D.G., Data Collection or Processing: F.A., Analysis or Interpretation: M.D., Writing: F.A.

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

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Original Article

Uncovering the Barriers to Exclusive Breast Feeding for Mothers in a Rural Setting in Southern India

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ABSTRACT

Aim: Exclusive breastfeeding (EBF) is very important for the growth and development of the baby. The purpose of this study was to identify the prevalence of EBF practices, to assess the factors associated with those practices, and to find the barriers to EBF practices in the rural community.

Materials and Methods: A cross-sectional study based on systematic questioning was carried out involving 252 mothers with children between the ages of 6 and 9 months in the southern Chennai community and the nearby districts of Chengalpattu and Kanchipuram from October 2018 to October 2019. Multiple logistic regression was used to determine factors associated with EBF.

Results: Seventy percent (184/252) of mothers following EBF habits had a child older than six months. About 58% of mothers started breastfeeding within one hour of birth, and 32.53% reported colostrum feeding. An apparent shortage of milk (58.82%) was a common problem reported by the mothers leading to EBF discontinuation. Children of working mothers [Odds ratio (OR) 3.32; 95% confidence interval (CI) 1.13, 9.70], urban dwellers (OR 6.67; 95% CI 1.12, 39.66) and children in urban areas (OR 12.47; 95% CI 2.05, 75.90) were less likely to be breastfed exclusively as indicated in the multivariate regression analysis. No relationships were found between the child's gender, method of childbirth, medical advice, or nutritional management before meals and EBF.

Conclusion: Working mothers and those living in urban areas were at greater risk of non-compliance with EBF. The national impact of urban sprawl and the impact on EBF activities should be studied in depth.

Keywords: Breastfeeding, exclusive, habits, community

Introduction

Exclusive breastfeeding (EBF) is defined as exclusively breastfeeding for the initial six months of life excluding oral rehydration solution (ORS), drops of medicine, or syrups (vitamins, minerals) (1). EBF promotes physical and mental health during childhood and beyond (2-6). In addition to EBF for 6 months as a universal recommendation, with its proven links in improving child survival and reducing illnesses (7-9); only 37-39% of babies in low- and middle-income countries have breastfeeding money (10,11).

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In India, breastfeeding is the norm and meets EBF's global targets (12). A recent national study estimated that the country's EBF levels are about 55% (13). However, a survey from various urban slums of India found very low estimated rates of 8% to 37% (14-19).

Various publications are available regarding EBF practices from a variety of contexts. The mother's experience of childbearing and rearing is strongly influenced by social norms and ideas, cultures, and personal thoughts. Therefore, it is difficult to integrate EBF practice decisions, but research has shown that EBF levels are linked to maternal age, education, employment; infant sex, age, access to health care, accommodation and multi media exposure or counselling (20-24). Other factors contributing to EBF are the history of maternity visits, prenatal counselling, influence from health care providers, early nutrition, early onset, and type of delivery (25-27). The direction and magnitude of these organizations, however, were not universal, and this points to the strong influence of contextual approaches to EBF actions. One of the most frequently identified barriers to EBF in various studies and cases is inadequate breast milk (17,28).

However, evidence surrounding feeding practices is limited to the Tamil Nadu region, which is why this study investigated the status of EBF practices, examined the factors associated with these practices, and identified barriers to EBF practices in the community.

Materials and Methods

Study Design and Sample Selection

A community-based study was conducted in the southern state of Chennai and in the combined districts of Chengalpattu and Kanchipuram from October 2018 to October 2019. All 6-to 9-month-old infants were enrolled and a simple evaluation application was made to recruit study participants. The study approval was obtained from the Ethics Committee, Chettinad Hospital and Research Institute (No. 92/IHEC/12-16) and written approval was obtained from all participants.

Variable Options

Independent variables were selected based on extensive literature reviews. The maternal characteristics recorded were age, education, occupation, type of family, place of residence, number of maternity visits, delivery method, lactation counselling, breastfeeding initiation, frequency of feeding, feeding habits before childbirth, gender, use of a neonatal intensive care unit and birth order. In the case of working mothers, years of current work, working hours, type of leave available, kindergarten availability, whether the child is allowed at the work place, nursing leave, night shifts, and the presence of a non-mother caregiver were also assessed.

Data Collection

Face-to-face questions were asked to the mothers about their breastfeeding habits. They are abbreviated as EBF (breast milk excluding ORS, drops of medicine, syrups up to six months in total) and non-breastfeeding (1). Some of the information collected includes the variables as described above.

Statistical Analysis

Data were analyzed with SPSS Version 20.0 software. Descriptive analysis was performed to reveal the required number of people to research the characteristics of the infants and mothers. A simple adjustment of the settings was used to determine the relationship between each variable and the state of the different effects -EBF- and l. Results are shown as inconsistencies in p-values. The variables considered to be included in most analyses were selected with respect to the previous literature (3,15,23,27). All variables with a p-value of less than 0.20 in bivariate analysis were taken, after adjusting for other variables. Age and gender were considered static and adjusted to the final model. Corresponding factors of $p<0.05$ were considered statistically significant.

Results

A total of 252 mothers with infants under 9 months of age were interviewed in this study. Descriptive sample analysis, divided into feeding performed only for six months, is presented in Table I. The prevalence of EBF was found to be 73.01%. More than half of the mothers (51.58%) reported being working mothers, and 42.9% had received basic education. Before giving birth, 89.68% of mothers had received medical advice and planned to breastfeed their babies for at least six months. About 66% of mothers received 4 or more check-ups before delivery and 51.5% received maternity care. They were all brought to the clinic. About half (52.38%) of the mothers underwent surgical resection.

This study found that 58.72% of mothers started breastfeeding within one hour of birth, but only 32.53% reported colostrum. In addition, 19.80% of mothers reported pre-lacteal treatment, providing anything other than breast milk for babies in the first three days of life.

Honey (33.42%), sugar water (19.73%), plain water (15.81%), and infant formula (8.92%) were infant formula-fed. Of the participants, 26.98% reported stopping breastfeeding before the age of six months. Of them, 8.82% stopped EBF at 1 month, 11.76% at both 2 and 3 months, 29.41% at four months and 38.23% at five months. The mother's response to questions about the problems that led to breastfeeding discontinuation was not revealed (23.52%), due to sore nipples (14.70%), due to breast implants (2.94%) or due to poor milk supply (58.82%).

In the final model of relapse after rehabilitation ($p<0.2$) - we found that children of working mothers [Odds ratio (OR) 3.32; 95% confidence interval (CI) 1.13, 9.70], urban dwellers (OR 6.67; 95% CI 1.12, 39.66) and those in urban areas (OR 12.47; 95% CI 2.05, 75.90), and those brought to a public hospital (OR 5.45; 95% CI 1.44, 20.60) had little chance of EBF. There were absolutely no relationships found between the child's sex, type of delivery, breastfeeding counselling, or first feeding and EBF (Table II).

Table I. Descriptive statistics of the sample stratified by exclusively breastfed for 6 months

Parameters		**Total (n=252)**	**No EBF (n=68)**	**EBF (n=184)**	**p-value**
Mother's Age	<20	5.55	5.88	5.43	0.54
	20-30	69.84	76.47	67.39	
	>30	24.60	17.64	27.17	
Family type	Nuclear	52.38	47.05	54.34	0.29
	Joint	47.61	52.94	45.65	
Working mother		48.41	67.64	41.30	0.01*
Employed in	Urban	47.61	47.05	47.82	0.02*
	Semi-Urban	29.36	44.11	23.91	
	Rural	23.01	8.82	28.26	
Sex of baby	Female	51.58	47.05	53.26	0.55
	Male	48.41	52.94	46.73	
Birth order	1	64.28	17.46	64.13	0.74
	2	33.59	32.35	34.78	
	3	1.58	2.94	1.08	
Place of delivery	Govt Hospital	17.46	32.35	11.95	0.01*†
	Private Hospital	82.53	67.64	88.04	
NICU admission		20.63	17.64	21.73	0.80†
Lactation counseling		89.68	88.23	90.21	0.74
Initiation of breastfeeding	Immediately	32.53	32.35	32.60	0.81
	Less than 1 hour	26.19	26.47	26.08	
	1-2 hours	22.22	17.64	23.91	
	>2 hours	19.05	23.52	17.39	
Frequency of feeds (times)	<8	14.28	32.35	7.60	0.000*
	8-14	84.92	64.70	92.39	
	≥15	0.79	2.94	0.00	
Prelacteal feeds		19.80	35.29	14.13	0.01*†
Gripe water		34.12	55.88	26.08	0.003*

*p<0.05, †- Fischer exact test
EBF: Exclusive breastfeeding, NICU: Neonatal intensive care unit, Gout: Government

In further analysis to determine the reasons behind high non-EBF, significant differences were found between both groups in terms of breaks (p=0.03) and night shifts (p=0.02). However, it has also been noted that working years, working hours, or type of leave did not affect the discontinuation of breastfeeding (Table III).

Table II. Multivariate Logistic Regression between maternal parameter sand lack of exclusive breast feeding

Parameters OR[a]		**Univariate Analysis**		**Multivariate Analysis**	
		p-value[a]	**aOR[b]**	**p-value[b]**	
Working mother	Yes	2.91 (1.29-6.81)	0.01*	3.32 (1.13-9.70)	0.02*
	No (Ref)				
Employed in	Urban	3.15 (0.83-11.85)	0.09	6.67 (1.12-39.66)	0.03*
	Semi-urban	5.90 (1.51-23.10)	0.01*	12.47 (2.05-75.90)	0.006*
	Rural (Ref)				
Place of birth (Hospital)	Govt	3.52 (1.35-9.15)	0.01*	5.45 (1.44-20.60)	0.01*
	Private (Ref)				
Prelacteal feed	Yes	3.31 (1.32-8.28)	0.01*	1.07 (0.30-3.82)	0.90
	No (Ref)				

[a]-Bivariate logistic regression analysis
[b]- Multivariate logistic regression analysis adjusted for factors with p<0.20
[a]OR: Adjusted odds ratio; Govt: Government

Table III. Job statistics of working mothers stratified by exclusively breastfeeding for 6 months

Parameters		**Total (n=122)**	**Non EBF (n=46)**	**EBF (n=76)**	**p-value**
Present job years	<1	9.83	8.69	10.52	0.61
	1-2	19.67	26.08	15.78	
	>2	70.49	65.21	73.68	
Working hours	<5	8.19	8.69	7.89	0.99
	5-8	60.65	60.86	60.52	
	>8	31.14	30.43	31.57	
Leave	Maternity	65.57	65.21	65.78	0.54
	Earned	4.91	0.00	7.89	
	Commuted	1.63	0.00	2.63	
	Partial pay	6.55	8.69	5.26	
	Unpaid leave	2.13	26.08	2.63	
Baby allowed in workplace		34.42	21.73	42.10	0.10
Presence of Creche		29.50	21.73	34.21	0.30
Nursing breaks		47.54	30.43	57.89	0.03*
Night shifts		29.50	9.67	39.47	0.02*
Baby caretaker	Spouse	13.11	6.45	15.78	0.69
	In-Laws	59.01	4.51	57.89	
	Appointed nanny	16.39	16.12	13.15	
	Play home	11.47	6.45	13.15	

*p<0.05
EBF: Exclusive breastfeeding

Discussion

This study not only investigated the status of EBF practices and their related factors, but also identified post-traumatic stress disorder among mothers with 6- to 9-month-old infants in the southern Chennai community and the combined districts of Chengalpattu and Kanchipuram. This study found that almost half of mothers were working and this affected EBF three times, which also led to further (29,30). Also, urban and urban workplaces pose a significant risk to breastfeeding by a factor of 6 and 12 times respectively, which may be the result of outpouring, while those in rural areas tend to work at home / stay at home with the child. This was based on the discovery of Chen et al. (30).

The rates EBF was found to be higher in our study (73.01%) compared to the national average from the National Family and Health Survey-4 (55%) (13). A survey by the Department of Women and Children Development, Government of India, in 2013-2014 found an EBF rate of 65.1% in rural areas (31). Interestingly, studies conducted in countries such as Jordan (32), Qatar (33), and Saudi Arabia (34) reported extreme low EBF rates of 2.1%, 24.3%, 37% and a better rate of 50.8% in Sri Lanka (35).

About 58.72% started breastfeeding within one hour. Of those who could not, 78.21% failed to do so due to the surgical phase. These findings show that only 47.7% of mothers at the health care facility start within 1 hour, which indicates an effect of the type of delivery (36). Implementation of breastfeeding was also affected by the surgical interventions received by women among other subjects (37-39). A mother's inability to breastfeed her baby immediately after surgery delayed early initiation. In addition, the initiation of breastfeeding within 1 hour of delivery has also been found as a prediction of continued breastfeeding (40,41). However, this was not the result of our research findings.

Unlike other studies, 32.53% of children in this study were fed colostrum. Colostrum nutrition was supported by pre-lacteal habits, as found in previous studies. Honey (33.42%) and sugar water (19.73%) were the first reported feeds given to infants in this study. In India, it is common practice to use this as a precautionary measure (42,43). We found that babies who received early breastfeeding were three times more likely to breastfeed especially in bivariate analysis. Prelacteal administration has also been reported as a risk factor for EBF failure in previous studies (44).

Our study did not find that the mother's age was a predictor of EBF. A review of Emmanuel's books has shown the association of maternal age with higher EBF levels (45). Similar studies in rural Uttar Pradesh (46) and southern India (36) found a strong correlation between EBF and maternal age. However, the combined results of systematic reviews obtained, where 7 out of 12 studies did not reveal any relationship between the mother's age and EBF, while the remaining 5 found significant relationships between the two (47).

EBF counselling for mothers, various breastfeeding educational materials, and breastfeeding support videos have been found to be strongly associated with EBF practices in studies conducted in India by Chudasama et al. (48) and Patil et al. (49). All mothers included had at least one visit before delivery, and were all referred to facilities; about 90% of them received EBF counselling. However, it was not found to have a positive EBF effect in our study. Those who delivered in public hospitals had a fivefold risk of EBF compared to those mothers who delivered in antenatal hospitals. This can be seen in the absence of appropriate advice and funding from the government, although research on this is limited.

Study Limitations

A few limitations within this study need to be noted. The definition of EBF used here according to 24 hour - recall period is subjected to bias and misreporting. It was difficult for the women to recall the use of some supplements from memory. A different approach such as long-term follow-up with women to see if they are using EBF can produce better evidence of smaller ingredients. In addition, the mother's perception of milk deficiency was present in 58% of cases, so water or other water management may have been higher than normal. However, we have tried to reduce inaccuracy by including mothers of infants who are 6 to 9 months old in the study. On the other hand, this study was conducted publicly with the required sample size and full response, so there is a good external performance that allows us to reproduce what we have obtained from other people in the country.

Conclusion

EBF practices among mothers in the community of southern Chennai and the adjoining districts of Chengalpattu and Kanchipuram was found to be higher than the universal levels recommended by the World Health Organization and also was above the national average. Moreover, this study found that working mothers and those residing in urban areas were at higher risk of not adhering to EBF. These finding should be studied in greater depth to

understand the national impact of increasing urbanization and the impact on EBF practices as well as maternal and infant immediate and long-term health.

Ethics

Ethics Committee Approval: The study approval was obtained from the Ethics Committee, Chettinad Hospital and Research Institute (No. 92/IHEC/12-16).

Informed Consent: Written approval was obtained from all participants.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: S.V., A.M., Design: S.V., A.M., K.S., Data Collection or Processing: S.V., A.M., K.S., Analysis or Interpretation: S.V., A.M., K.S., J.V., U.L., Literature Search: S.V., J.V., U.L., Writing: S.V., A.M., Manuscript Review: K.S., J.V., U.L.

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

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Original Article

Childhood Asthma and Vitamin D-case Control Study in an Academic Tertiary Care Hospital

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ABSTRACT

Aim: Vitamin D is suggested to be involved in the pathogenesis of childhood asthma. It is one of the most researched hypotheses but previous reports are inconclusive. The objective of this study was to investigate the association between vitamin D and asthma; and its association with level of control of asthma in children.

Materials and Methods: This case control study was conducted in a tertiary care teaching hospital among children of 6-12 years of age. Children with bronchial asthma who were under follow-up in a respiratory clinic were enrolled as the cases. Healthy children with no history of bronchial asthma were enrolled as the controls. Serum 25 hydroxyvitamin D was measured via a chemiluminescence method.

Results: There was high prevalence of vitamin D deficiency (<20 ng/mL) among children with asthma compared to control and was associated with occurrence of asthma p=0.000; Univariate analysis of the relationship between asthma and vitamin D showed that decreased vitamin D levels were associated with significantly increased odds of asthmatic state (p<0.001). In multivariate analysis after adjustment for age, body mass index and sex, the relationship between vitamin D and asthma increased; but it was not associated with level of control of asthma.

Conclusion: There is high prevalence of vitamin D deficiency among children with asthma. Furthermore, the results did not consistently support that vitamin D levels associate with level of control of asthma.

Keywords: Asthma, child, prevalence, vitamin D

Introduction

Bronchial asthma is a heterogenous respiratory disease usually associated with hyper responsiveness and airway inflammation. Childhood asthma prevalence is increasing in developing countries despite improved knowledge of its pathophysiology and better management strategies (1). The development of asthma is associated with many immunological markers. There are upcoming reports on the potential link between vitamin D and asthma. Increased asthma prevalence parallels the vitamin D deficiency pandemic and adds to significant morbidity. The increased prevalence of asthma can be due to various genetic factors, environmental factors like urbanization or nutritional factors. Among the hypotheses related to nutritional factors, some current research focuses on the association of asthma with vitamin D deficiency. Vitamin D is an immunomodulator involved in innate and acquired immunity (2,3).

The association between vitamin D and bronchial asthma is not yet clear. Previous studies show a high prevalence of

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vitamin D deficiency in childhood asthma (4-6) but some studies reported no difference in vitamin D levels (7,8). Some studies even show that high levels of vitamin D increase the risk of atopy (9). Recent metanalysis show mixed results for the association of vitamin D with asthma (3). Prevalence of vitamin D deficiency varies according to geographical location, latitude, or population studied. High prevalence has been reported even in sun replete areas. Factors like urbanization, genetic factors, decreased outdoor activities, decreased sun exposure, low physical activity, obesity, or deficient diet can lead to an increased prevalence of vitamin D deficiency. Additionally, the reported prevalence varies according to varying cut-off levels for defining deficiency, different methods for vitamin D estimation and the season of vitamin D estimation (10). Country wide reports show conflicting results on the prevalence of vitamin D deficiency in asthma; especially those from Asian countries (11). Racial/ethnic-specific associations between vitamin D insufficiency and asthma in children is also reported (12). Vitamin D receptor gene polymorphism has been associated with asthma phenotypes (2). There are studies which reported the effects of prenatal vitamin D exposure (13-18). Hollams (19) in a cohort study reported low vitamin D at 6 years is a predictor of asthma at 14 years of age. Van Oeffelen et al. (20) reported serum vitamin D measured at age 4 is inversely associated with asthma at 4-8 years whereas vitamin D measured at age 8 is positively associated with asthma. Randomized controlled trials give inconclusive reports on vitamin D supplementation (21). Rapid correction of very low vitamin levels shows short-term benefits but no long-term effects (22). The threshold level of vitamin D level to be maintained in asthma is unknown. Available evidence does not confirm or rule out the benefits of vitamin D supplementation and, if found useful, would be a cheaper alternative with less side effects.

With childhood asthma being highly prevalent and with its significant morbidity, it is important to know its association with vitamin D. There is a scarcity of literature from Asian countries, and these have conflicting reports. This may be due to variations in geographical location and populations studied. Therefore, it is important to know the situation in our area, which is a tropical region. The objective of the present study was to investigate the association of vitamin D in children with asthma compared to normal children in our region and to determine whether vitamin D status is related to the level of control of asthma.

Materials and Methods

This case control study was conducted in an academic tertiary care hospital. Cases were recruited from the pediatric respiratory clinic of our institution. All consecutive cases of respiratory physician diagnosed asthma in the age group of 6-12 years with good compliance when they came for follow-up in the respiratory clinic were recruited as cases. Children with acute illness, chronic diseases like heart disease, neurological disease, diseases which affect vitamin D metabolism such as liver disease, renal disease, a history of vitamin D supplementation in the previous 6 months, or on drugs that affect vitamin D supplementation such as anticonvulsants were excluded. The controls were recruited from 6-12-year-old healthy children with no history of bronchial asthma with exclusion criteria similar to that of the cases, coming to immunization clinic/follow-up in out-patient clinic. Written informed parental consent or assent from all participants were obtained wherever necessary. Approval of the institutional ethics committee was obtained.

Parents and children were interviewed to collect data regarding age, sex, weight, height, body mass index (BMI), average sun exposure duration between 10 a.m.-3 p.m., average outdoor physical activity in the previous month and asthma control status in those with history of asthma. The Indian Academy of Pediatrics growth chart was used for height, weight and BMI cut-off levels. Those with sun exposure during 10 a.m. to 3 p.m. of at least 6 hours per week were considered as having adequate sun exposure. Physical activity of at least 1 hour daily was considered as adequate. Venous blood was collected and Serum 25 hydroxyvitamin D [25(OH)D] estimation was done in all cases and controls using the chemiluminescence method. A vitamin D level <20 ng/mL was considered deficient (11). According to Global Initiative for Asthma (GINA) guidelines, children with asthma were grouped into 3 groups according to their level of control of asthma (1).

Statistical Analysis

Statistical analysis was carried out using R statistical software version 3.0. Univariate analysis was performed to assess the distribution of baseline variables. In a multiple logistic regression model, analyses were adjusted for potential confounding by age, sex, BMI, adequate physical activity and sun exposure. The results are presented as frequencies (number) and percentages for categorical variables (non-parametric data) and mean ± standard deviation (SD) for continuous variables (parametric data) and median and interquartile range (IQR) for non-parametric variables. The statistical tests, chi-square test (χ^2) for categorical variables, independent sample t-test, Mann-Whitney U test, One-Way ANOVA and Kruskal-Wallis tests for continuous

variables were used for analysis. The odds ratio (OR) with a 95% confidence interval was also calculated to assess the association of variables with vitamin D deficiency. P-values of the likelihood ratio tests were used to test for statistical significance in logistic regression analyses. All p-values are two-tailed, and statistical significance was defined as $p<0.05$.

Results

Out of 193 participants, there were 101 cases of asthma and 92 controls. The median (IQR) vitamin D level was 20.76 (15,31) ng/mL. The median (IQR) age was 8.5 (7,11) years. There were 113 (58.5%) male children. Mean (SD) height was 129.6 cm (14) with 184 (95.3%) children in the normal range. Mean (SD) weight was 27 kg (9.8) among which 10 (5.2%) were underweight, 181 (93.8%) were normal and 2 (1%) were overweight. Mean (SD) BMI was 15.6 (3.6), out of which 32 (16.6%) were thin, 112 (58%) were normal, 31 (16%) were overweight and 18 (9.3%) were obese. There was adequate sun exposure for only 66 (34%) children. There was adequate physical activity for 119 (61%) children with mean physically active hours per week of 8.41 (4.63) hour. The measured vitamin D levels stratified according to month of examination did not show much variation as shown in Figure 1.

Vitamin D deficiency was seen in 88 (45.6%) children. Median (IQR/age was 8 (7,10) in those with vitamin D sufficiency and 9 (7,11) in those with deficiency. Among children with vitamin D sufficiency 65 (61.9%) were males and among those with deficiency 48 (54.5%) were males.

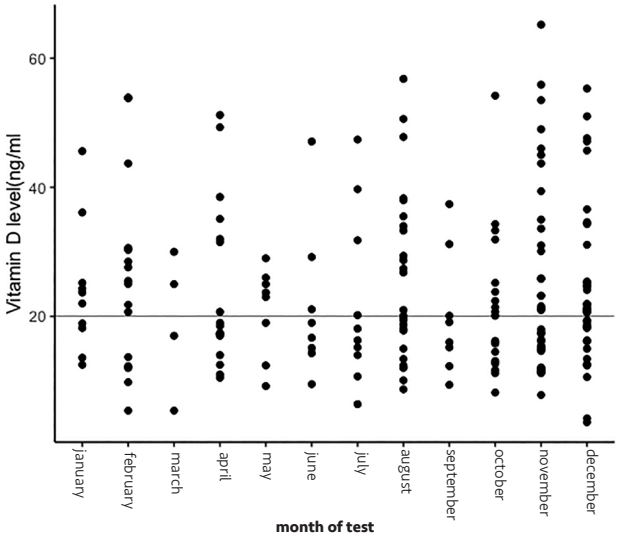

Figure 1. Scatter diagram showing vitamin D levels (ng/mL) and month of test

Mean (SD) BMI was 15.4 (3) kg/m^2 among children with vitamin D deficiency and 15.8 (3.9) kg/m^2 among those with sufficiency. Adequate sun exposure was seen in 33 (31.4%) and 33 (37.5%) in the vitamin D sufficient and deficient groups respectively. Physical activity was adequate in 68 (64.8%) and 51 (58%) in the sufficient and deficient groups with mean (SD) active hours per week 8.49 (4.4) and 8.31 (4.9) respectively. Mean (SD) height was 130 (14.5) cm in the vitamin D deficient and 129 (13.6) cm in sufficient groups. Mean (SD) weight was 26.8 (9) kg in the deficient and 27 (10.26) kg in sufficient groups. Height was normal in 101 (96.2%) sufficient and 83 (94.3%) deficient groups and weight was normal in 98 (93.3%) and 83 (94.3%).

Characteristics of both asthma cases and controls and the differences between the two groups are shown in Table I. Median vitamin D levels in the cases and controls were 17.30 ng/mL and 30.45 ng/mL respectively; this difference was statistically significant ($p<0.001$). Also, vitamin deficiency was significantly associated with asthma cases ($p<0.001$). Adequate physical activity was significantly high, and BMI was significantly lower in the asthma cases.

Among the 101 children with asthma, 65 (64.3%) children had vitamin D deficiency. However, among the controls only 23 (25%) had deficiency. Vitamin D deficiency was associated with the occurrence of asthma ($p=0.000$) (Figure 2). However, the level of control of asthma and vitamin D deficiency did not show any significant association (Table II).

Univariate analysis of the relationship between asthma showed that decreased vitamin D levels were associated with significantly increased odds of asthmatic state ($p<0.001$). Multivariate analyses of the relationship between asthma and vitamin D levels with adjustment for age, sex, BMI, adequate physical activity and sun exposure were conducted. As shown in Table III, a significant relationship between vitamin D levels and asthma can be seen in multivariate

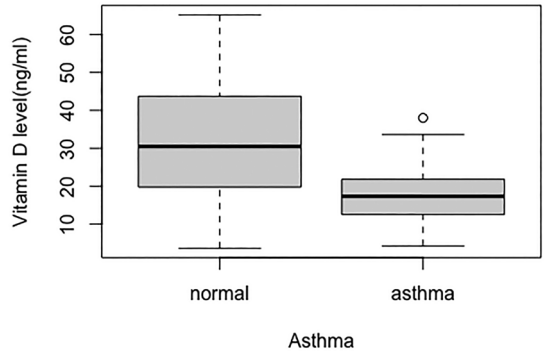

Figure 2. Boxplot showing association between vitamin D levels and asthma

Table I. Characteristics of participants

	Asthma (n=101) (%)	Controls (n=92) (%)	p-value
Age in year [median (IQR)]	8 (6,11)	9.00 (7,10)	0.288*
Gender-male	62 (61.4)	51 (55.4)	0.489**
Weight [median (IQR)] - Underweight - Normal - Overweight	24 (18.31) 9 (8.9) 92 (91.1) 9 (8.9)	27.5 (20.35) 1 (1.1) 89 (96.7) 2 (2.2)	 0.032* 0.018**
Height [median (IQR)] - Normal	128 (114.141) 94 (93.1)	130.5 (121.138) 90 (97.8)	0.231* 0.221**
BMI [median (IQR)] - Thinness - Normal - Overweight - Obese	14.3 (13,16) 17 (16.8) 70 (69.3) 9 (8.9) 5 (5)	14.6 (13.19) 15 (16.3) 42 (45.7) 22 (23.9) 13 (14.1)	 0.057* 0.001**
Adequate sun exposure	39 (38.6)	27 (29.3)	0.229**
Physical activity - Total hr/week-mean (SD) - Adequate	 9.69 (5.14) 72 (71.3)	 6.99 (3.5) 47 (51.1)	 <0.001*** 0.006**
Vitamin D - Level (ng/mL) [median (IQR)] - Deficiency	 17.3 (12.22) 65 (64.4)	 30 (19,43) 23 (25)	 <0.001* <0.001**

*Mann-Whitney U test, **Chi-square test,*** t-test
SD: Standard deviation, IQR: Interquartile range

Table II. Characteristics of asthma cases according to level of control of asthma

	Well controlled (%)	Partly controlled (%)	Uncontrolled (%)	p-value
Age in year [median (IQR)]	8 (6,10.5)	8.5 (6,10.8)	9 (6,11.5)	0.809**
Gender-male	25 (62.5)	23 (60.5)	14 (60.9)	0.982***
Weight [median (IQR)] - Underweight - Normal	20 (17.29) 4 (10) 39 (90)	25 (20.32) 2 (5) 36 (90)	25 (17.30) 3 (13) 20 (87)	 0.237**
Height [median (IQR)] Normal	121 (114.136) 38 (95)	128 (118.139) 36 (94)	131 (113,141) 20 (87)	0.699** 0.422***
BMI [median (IQR)] - Thinness - Normal - Overweight - Obese	13.8 (13.15) 5 (12.5) 33 (82.5) 2 (5) 0 (0)	15.5 (14.17) 6 (16) 23 (60) 5 (13) 4 (10)	14.5 (13.16) 6 (26) 14 (60) 2 (8.7) 1 (4.3)	 0.076** 0.166***
Adequate sun exposure	16 (40)	17 (44)	6 (26)	0.340***
Physical activity - Total hour per week-mean (SD) - Adequate	 10 (4.7) 29 (72)	 9.5 (5.9) 26 (68)	 8.6 (4.3) 17 (73.9)	0.399* 0.879***
Vitamin D deficiency	25 (62.5)	29 (76.3)	11 (47.8)	0.075***

*One-Way ANOVA, **Kruskal-Wallis test, ***Chi-square
SD: Standard deviation, IQR: Interquartile range

Table III. Univariate and multivariate analysis of relationship of asthma with variables

Variable	Univariate OR (95%CI)	p-value	Multivariate OR (95%CI)	p-value
Age	0.94 (0.82-1.07)	0.333	0.99 (0.83-1.19)	0.939
Gender	1.28 (0.72-2.27)	0.402	1.31 (0.62-2.77)	0.481
BMI	0.88 (0.81-0.96)	0.005	0.86 (0.76-0.97)	0.017
Vitamin D	0.89 (0.85-0.92)	<0.001	0.87 (0.83-0.91)	<0.001
Sun exposure	1.51 (0.83-2.78)	0.176	1.09 (0.49-2.45)	0.825
Physical activity	1.15 (1.07-1.23)	<0.001	1.19 (1.09-1.31)	<0.001
Weight	0.97 (0.94-1.00)	0.057	-	-
Height	0.99 (0.97-1.01)	0.327	-	-

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index

analysis. Increased vitamin D levels were associated with a greater decrease in the probability (p<0.001) of asthma compared with univariate analysis (OR 0.89 and 0.87).

Discussion

Vitamin D deficiency is common and was seen in half of our study participants similar to other hospital-based studies (6,23). However, another study showed higher prevalence but their population area constituted urban and semirural areas (22). One quarter of normal children had vitamin D deficiency in spite of our region being a tropical region receiving substantial amounts of sunlight. The median serum vitamin D level was 20.76 ng/mL. There was adequate average daily sun exposure for only one third of the children, but it did not differ significantly between the sufficient and deficient groups. The month of test of vitamin D did not show much variation in vitamin D levels which in turn shows that seasonal variation is not significant. Other factors like skin color, clothing, genetic or dietary factors can affect vitamin D levels. In order to determine the exact prevalence of vitamin D deficiency, population-based studies are required. As our study was a hospital-based study, from our data, we cannot suggest screening of normal children for subclinical vitamin D deficiency.

The vitamin D deficient and sufficient groups did not differ much by age, sex, height, weight, BMI, adequate sun exposure or physical activity. Even though dietary factors contribute to only 10-15% of vitamin D level, supplementation or food fortification may be considered for subclinical vitamin D deficiency because of its crucial role during the growing age.

An increased prevalence of vitamin D deficiency (<20 ng/mL) is seen in children with asthma and it is statistically significant. Two thirds of asthma cases had vitamin D deficiency compared to only one fourth of the controls. Similar results are reported by other Indian studies. Awasthi and Vikram (6) reported vitamin D insufficiency (<30 ng/mL) in 84% of asthma cases and 28% of controls. Median vitamin D level was low in the asthma group compared to the controls. Two of the proposed reasons for low vitamin D levels in asthma patients are decreased sun exposure and restricted physical activity. However, in our study, adequate sun exposure and physical activity were significantly more in the asthma group. We conducted univariate analysis and found a strong inverse association between serum vitamin D levels and asthma. In the multivariate analysis, after adjusting for age, sex, BMI, sun exposure and physical activity, this relationship increased compared to the univariate analysis (OR=0.89-0.87). It confirms the association between lower vitamin D levels and asthma.

An explanation for the potential association between vitamin D and the development of asthma is that adequate vitamin D levels during pregnancy and early childhood prevent respiratory infections, help lung development, and thus decrease asthma risk (18). Vitamin D deficiency affects the development of immune tolerance and the epithelial barrier function in early childhood and thus relates to the development of asthma (18,19). Vitamin D has a major role in immune homeostasis. The vitamin D receptor is associated with the transcription of genes related to inflammation and immunomodulation of respiratory epithelium which is a part of the innate immune system and contains a high level of the enzyme which converts 25(OH)D3 to its active form. This active form of vitamin D has a local effect on infection responses and is effective in alleviating inflammation due to infections. Also, through direct induction of antimicrobial peptide gene expression, it is involved in innate immunity. Vitamin D also affects the adaptive immune system through

Th1, Th2 and regulatory T-cells. Vitamin D activates anti-inflammatory cytokines and inhibits proinflammatory cytokines. Vitamin D shifts T-lymphocyte response between Th1 phenotype and Th2 (3). The reason for low vitamin D levels in asthma may be due to increased demand due to excessive consumption in asthma with recurrent inflammation and so higher amounts may be required for immune functions. However, there is no consensus for the optimal serum levels to be maintained in asthma.

Asthma guidelines focus on the attainment of asthma control. However, there is no single measure to assess asthma control. Our study has not found any association between vitamin D deficiency and the level of control of asthma. Previous studies report an inconsistent association between the level of control of asthma and vitamin D levels; different studies used different methods to assess the level of control and different cut-off points for vitamin D deficiency. Kavitha et al. (24) did not identify any significant relationship between vitamin D levels and asthma control according to GINA in children aged 5-15 years but a study conducted by Kaaviya et al. (25) identified a significant relationship between vitamin D levels and asthma control according to GINA in children aged 5-15 years. A case control study conducted by Awasthi and Vikram (6) showed poorer asthma control associated with decreasing vitamin D levels in children aged 1-15 years. A study carried out in Thailand by Krobtrakulchai et al. (26) in 125 asthmatic children showed no association between vitamin D levels. Gupta et al. (27) conducted a study that assessed the control of asthma with the Asthma Control Test and found that lower vitamin D levels were associated with poor asthma control. Recent trials in children and adults with asthma revealed an inconsistent effect of vitamin D supplementation on symptom control (28-31).

Study Limitations

High serum parathyroid hormone level is the true indicator of vitamin D deficiency. It was not measured in this study. Longitudinal studies are required to prove the cause-effect relationship of vitamin D levels and asthma and interventional studies are needed to investigate the effect of vitamin D supplementation on asthma. We assessed the level of control of asthma using GINA symptom control history for the period of the previous 4 weeks only. A study which takes into consideration different methods to assess the level of control of asthma with a sufficient sample in all groups of the levels of control is required to assess this association. Studies focusing on vitamin D level variations and asthma parameters, which may depend on the genotype or phenotype of children with asthma, are needed to clarify the effect of vitamin D status on asthma.

Conclusion

Vitamin D deficiency is prevalent in children with asthma in the present setting. There was no significant association between serum vitamin D levels and asthma control status.

Ethics

Ethics Committee Approval: Approval of the institutional ethics committee was obtained (IEC no: 08/26/2017/MCT, date: 28/07/2017).

Informed Consent: Written informed parental consent or assent from all participants were obtained wherever necessary.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: V.A., I.Y., B.S., Design: V.A., I.Y., B.S., Data Collection or Processing: V.A., I.Y., B.S., Analysis or Interpretation: V.A., I.Y., B.S., Literature Search: V.A., I.Y., B.S., Writing: V.A., I.Y., B.S.

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Original Article

Evaluation of Cardiac Findings in Mucopolysaccharidosis Type III Patients

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ABSTRACT

Aim: To investigate cardiac involvement in patients diagnosed with mucopolysaccharidosis type III (MPS III) in a university hospital in Turkey.

Materials and Methods: This descriptive cross-sectional study was performed in a university hospital by examining the files of 49 MPS III patients who were admitted between January 1998 and December 2019.

Results: The mean age of the participants was 12.24±5.21 years (range: 1-26). The mean age at which the patients underwent echocardiography was 6.90±4.82 years. MPS IIIA, IIIB, IIIC, and IIID subtypes were present in 24 (49.0%), 19 (38.8%), 5 (10.2%), and 1 (2.0%) patient, respectively. Among the MPS III patients who had echocardiographic evaluation (n=44), 32 patients (72.7%) had pathological cardiac findings, while 12 patients (27.3%) had normal cardiac findings on echocardiographic examination. The most common cardiac pathologies were those related to mitral valve [valve insufficiency 52.3% (n=27), valve thickening 43.2% (n=25), and prolapse 38.6% (n=23)]. Tricuspid insufficiency (34.8%, n=8) was seen only in MPS IIIA. Mitral insufficiency and aortic valve thickening were significantly more common among females (p=0.014, p=0.025, respectively).

Conclusion: Patients with MPS III should be closely monitored for cardiac pathologies and especially mitral valve insufficiency, which are more prevalent among females.

Keywords: Mucopolysaccharidosis III, cardiac, mitral valve, tricuspid valve, sex

Introduction

Mucopolysaccharidoses (MPS) are hereditary lysosomal storage diseases in which specific enzymes that ensure the destruction of glycosaminoglycans are deficient due to genetic defects (1). In this group of diseases, a total of 11 enzyme deficiencies have been identified and examined under seven types (Type I, II, III, IVA, VI, VII, IX) (2).

Sanfilippo syndrome [MPS type III (MPS III)] is the most common type among MPS (3). In MPS III, there are four subtypes (A, B, C, D), all of which are caused by a disruption in the heparan sulfate catabolism. The enzymes involved are heparan N-sulfatase (encoded by the *SGSH* gene), α-N-acetyl-glucosaminidase (encoded by the *NAGLU* gene), acetyl α-glucosaminidase N-acetyltransferase (encoded by the *HGSNAT* gene), and N-acetylglucosamine-6-sulphatase

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(encoded by the *GNS* gene), respectively (4). Although very rare (1 in 70,000), each of these four subtypes has devastating effects on children (5). Additionally, MPS III shows autosomal recessive transition (6).

A clinical picture emerges where the central nervous system is affected and somatic findings are less common. The clinical course of MPS III can be discussed in three phases (7). In the first period of the disease (usually starting between 1 to 4 years of age), there is a developmental delay, especially in speech. In the second period, a marked behavioral disorder characterized by hyperactivity and sleep disturbance accompanies the picture. Usually, loss of skills acquired after ten years and a slow progression to a vegetative state are monitored eventually. Cardiovascular system disorders in MPS III disease have also been reported in the literature (8,9). However, as in other MPS patients, significant clinical differences can be observed between patients.

Data in the literature regarding cardiac involvement in MPS III patients is scarce (6), the most common cardiac findings in patients with MPS were reported as thickening of the mitral valve with accompanying prolapse, insufficiency, and less frequently, stenosis (10).

Materials and Methods

We aimed to examine the cardiac involvement in MPS III disease in a University Hospital in Ankara, Turkey.

This descriptive cross-sectional study was carried out by examining the files and records in the hospital automation system of patients diagnosed with MPS III who were admitted between the 1st January 1998 and the 31st December 2019, to Hacettepe University, Department of Pediatric Metabolism. Data were obtained from the hospital's electronic medical records (Nucleus automation system, MONAD software and counseling, Ankara, Turkey) and the patients' files. Ethical approval was obtained from the Hacettepe University Clinical Research Ethics Committee, and this study was performed in accordance with the ethical standards of the Declaration of Helsinki (GO 18/901-09).

During the study period, the files of 144 patients diagnosed with MPS were reviewed. Forty-nine patients with a diagnosis of MPS III were included in the analysis (Figure 1). MPS III was diagnosed based on the patients' specific enzyme levels and/or genetic analysis.

Echocardiographic findings were evaluated according to the subgroups of MPS III (IIIA, IIIB, IIIC, and IIID). The primary outcome variable of the study was "the thickening of the mitral valve leaflet". Secondary outcome variables

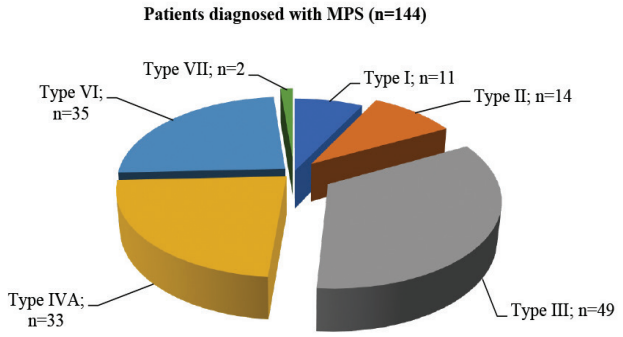

Figure 1. Diagnostic distribution of MPS patients (n; number of patients)
MPS: Mucopolysaccharidosis

were mitral valve prolapse, mitral valve insufficiency, tricuspid valve thickening, tricuspid valve prolapse, tricuspid insufficiency, atrial valve thickening, bicuspid aorta, atrial valve prolapse, aortic insufficiency, left ventricular ejection fraction and fractional shortening, and prognosis. The independent study variables were age and sex.

Transthoracic echocardiography was performed with Vivid E9 with an XD clear echocardiography device (GE Healthcare, General Electric Company, Wauwatosa, WI, USA). All echocardiographic studies were performed by an experienced cardiologist with a comprehensive knowledge of echocardiographic examination. Cardiac chamber quantifications were made according to pediatric guidelines (11) and the severity of valvar (aortic, mitral and tricuspid) regurgitations were defined according to the 2003 guidelines as absent, mild, moderate or severe (12).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) (SPSS for Windows, Version 25.0, Chicago, IC, USA) program was used for statistical analysis. The results are presented as mean and standard deviation for numerical variables, and frequency and percentage for categorical data. The suitability of numerical variables to normal distribution was evaluated using skewness and kurtosis. Parametric variables were compared with the independent samples t-test for two groups and One-Way ANOVA for more than two groups. The chi-square (or Fisher's exact) test was used for comparing categorical variables. A p-value of <0.05 was considered sufficient for statistical significance.

Results

Out of the 144 patients diagnosed with MPS, 49 patients with a diagnosis of MPS III were included in this analysis. Twenty-six of the patients (53.1%) were female, and twenty-

three (46.9%) were male. The mean age was 12.24±5.21 years (range: 1-26) (Table I and Table II). The youngest patient with cardiac involvement (mitral insufficiency) was 1.5 years old. When the relationship between cardiac involvement and age was examined, it was found that the patients' cardiac involvement increased with age (p=0.031). Nineteen (43.2%) patients had mitral valve thickening, 32 patients (72.7%) had pathological cardiac findings, while 12 patients (27.3%) had normal cardiac findings on echocardiographic examination. During echocardiographic evaluation, none of the patients with cardiac involvement had severe pulmonary involvement, mechanical ventilator support, or any non-invasive ventilation support. In addition, no cardiomyopathy findings were found in any of the patients.

While making comparisons between the subtypes, there was only one patient in the D subtype. Hence, this patient was not included in the comparisons. However, there was a statistically significant difference between the subtypes with respect to tricuspid insufficiency (Table III). Left ventricular ejection fraction and fractional shortening were not significantly different between the subtypes (F=0.363, p=0.698 and F=0.879, p=0.423, respectively).

Concerning the comparisons made according to the prognosis, there was no significant difference in any categorical or numerical variables.

When cardiac findings were compared by sex, there was a substantial difference in some numerical and categorical variables (Table IV). Although no significant difference was found between ejection fraction values, a considerable variation was found between fractional shortening values (t=1.718, p=0.087 and t=2.278, p=0.033, respectively).

Discussion

Our study demonstrated that the most common MPS III was type IIIA (n=24, 49.0%). Thirty-two patients (72.7%) had pathological cardiac findings, while 12 patients (27.3%) had normal cardiac findings on echocardiographic

Table I. Description of categorical variables and dichotomous cardiac findings

Variable		n	%
Sex	Female	26	53.1
	Male	23	46.9
Type	3A	24	49
	3B	19	38.8
	3C	5	10.2
	3D	1	2
Echocardiography	Absent	5	10.2
	Present	44	89.8
Thickened mitral valve leaflet*	Absent	25	56.8
	Present	19	43.2
Mitral valve prolapse*	Absent	27	61.4
	Present	17	38.6
Mitral insufficiency*	Absent	21	47.7
	Mild	22	50
	Moderate	1	2.3
Thickened tricuspid valve*	Absent	42	95.5
	Present	2	4.5
Tricuspid valve prolapse*	Absent	43	97.7
	Present	1	2.3
Tricuspid insufficiency*	Absent	36	81.8
	Mild	8	18.2
Bicuspid aortic valve*	Absent	43	97.7
	Present	1	2.3
Aortic valve thickening*	Absent	38	86.4
	Present	6	13.6
Aorta valve prolapse*	Absent	43	97.7
	Present	1	2.3
Aortic insufficiency*	Absent	37	84.1
	Mild	6	13.6
	Moderate	1	2.3
Prognosis	Exitus	6	12.2
	Follow-up	38	77.6
	Unfollowed	5	10.2

*: Only 44 of the 49 patients had echocardiographic evaluation

Table II. Description of numeric variables and numerical cardiac findings

Variable	n	Mean	SD	Min.	Max.
Age	49	12.24	5.21	1	26
Age at diagnosis	49	5.25	3.00	0.83	14
Follow-up time	39	6.43	5.19	0.17	19.81
Age at echocardiography*	44	6.90	4.82	0.83	25
Ejection fraction*	44	71.20	4.94	61	81
Fractional shortening*	44	41.91	4.41	33	49

*: Only 44 of the 49 patients had echocardiographic evaluation
SD: Standard deviation, min.: Minimum, max.: Maximum

Table III. Comparison of cardiac findings by subtypes

Variable	Subtype	Absent	Present	$\chi 2$	p-value*
Thickened mitral valve leaflet	3A	12	11	1,491	0.520
	3B	11	7		
	3C	2	0		
Mitral valve prolapse	3A	12	11	2,536	0.309
	3B	13	5		
	3C	2	0		
Mitral insufficiency	3A	9	13	2,154	0.370
	3B	11	7		
	3C	1	1		
Thickened tricuspid valve	3A	21	2	2,142	0.542
	3B	18	0		
	3C	2	0		
Tricuspid valve prolapse	3A	22	1	2,187	1.000
	3B	18	0		
	3C	2	0		
Tricuspid insufficiency	3A	15	8	8,521	0.010
	3B	18	0		
	3C	2	0		
Bicuspid aortic valve	3A	22	1	2,187	1.000
	3B	18	0		
	3C	2	0		
Aortic valve thickening	3A	18	5	2,224	0.414
	3B	17	1		
	3C	2	0		
Aorta valve prolapse	3A	22	1	2,187	1.000
	3B	18	0		
	3C	2	0		
Aortic insufficiency	3A	18	5	3,914	0.102
	3B	17	1		
	3C	1	1		

$\chi 2$: Chi-square test value, *Fisher's exact test.

examination. The most common cardiac pathologies were those related to mitral valve [valve insufficiency 52.3% (n=27), valve thickening 43.2% (n=25), and prolapse 38.6% (n=23)]. Tricuspid insufficiency (34.8%, n=8) was seen only in patients with MPS IIIA. Mitral insufficiency and aortic valve thickening were significantly more common among females.

The deposition of heparan sulfate in the tissues leads to various anomalies. In addition to the symptoms of the nervous system, in particular, they can cause respiratory, ear, nose and throat, musculoskeletal, gastroenterological, ocular, and cardiac symptoms (13,14).

Followed by pneumonia, cardiorespiratory insufficiency has been reported to be the most common cause of death in MPS III syndrome (13). However, it is not clear whether the cause of cardiorespiratory insufficiency is due to respiratory problems or cardiac origin. Indeed, in our study, we could not find any evidence that cardiac pathologies increase the mortality rate.

Table IV. Comparison of cardiac findings regarding sex

Cardiac findings	Sex	Absent	Present	χ2	p-value
Thickened mitral valve leaflet	Female	11	13	2,597	0.135
	Male	14	6		
Mitral valve prolapse	Female	13	11	1,154	0.359
	Male	14	6		
Mitral insufficiency	Female	7	17	7,291	**0.014***
	Male	14	6		
Thickened tricuspid valve	Female	22	2	1,746	0.493
	Male	20	0		
Tricuspid valve prolapse	Female	23	1	0.853	1.000
	Male	20	0		
Tricuspid insufficiency	Female	17	7	4,809	0.054
	Male	19	1		
Bicuspid aortic valve	Female	24	0	1,228	0.455
	Male	19	1		
Aortic valve thickening	Female	18	6	5,789	0.025
	Male	20	0		
Aorta valve prolapse	Female	23	1	0.853	1.000
	Male	20	0		
Aortic insufficiency	Female	19	5	0.957	0.428
	Male	18	2		

χ2: Chi-square test value, *:

It has been noted that the symptoms of MPS IIIA start earlier and progress faster than MPS IIIB and IIIC (15,16). Furthermore, it has been reported that the IIIA subtype is more common in Sanfilippo patients than the other subtypes (17). Additionally, MPS IIID has been reported to be very rare and heterogeneous (18,19). Similarly, in our study, subtype A was more common with a rate of 49% (n=24), and only one case of subtype D (2%) was detected. Similar to other studies, the subtype with the most common pathological cardiac findings was type IIIA. However, only tricuspid insufficiency was statistically significant. This finding may be due to difficulties in the analysis of rare diseases. Although it was stated that subtype A was diagnosed earlier, our study did not support this data.

It has been reported that the cardiovascular system is also affected in MPS III disease. In addition, many studies have noted that cardiovascular involvement in MPS I, II, and VI patients are higher than in MPS III and IV (9, 20-22). MPS VII has not been reported on yet as it is very rare (10).

In a study conducted in Spain with 55 MPS III patients, it was reported that only one of the cases with subtype A had cardiac valve involvement and four of them had mild cardiomyopathy (17). Also, in a recent study in Taiwan evaluating the echocardiographic findings of 26 patients with MPS III, the incidence of heart valve involvement was reported as 38% (8). In that study, it was reported that the most common cardiac pathology was mitral regurgitation, followed by aortic regurgitation (8).

In another recent study performed with 45 MPS III patients, the age at which the first echocardiography was performed was similar to ours. They reported a slightly lower incidence (60%) of abnormal cardiac findings than our result (6). Similarly, in this study, the incidence of mitral valve problems were more common than other valves.

Compatible with previous studies, in our study, the most common cardiac pathology was associated with the mitral valve. In addition, tricuspid valve involvement in MPS III patients observed in our study is remarkable and had not been reported in previous studies. Furthermore, another important finding of our research is that features of cardiac involvement may differ by sex. To the best of our knowledge, our study is the first to mention this significant difference.

Study Limitations

This is a retrospective study based on hospital records. Thus, the reliability of the recorded data might be a concern in this type of research. However, we consider that the recorded information is reliable since the cardiac evaluations were performed by an experienced cardiologist with a comprehensive knowledge concerning echocardiographic examination. Despite repeated follow-ups of the patients, the evaluation of all patients was based on their first-recorded echocardiographic findings. Since MPS is a progressive disease, it would be of value to observe the cardiac involvement changes over time.

Conclusion

According to our results, the majority of patients with MPS III have cardiac involvement and it is most commonly related to the mitral valve, therefore these patients should be closely monitored by echocardiography. Tricuspid insufficiency was seen only in those patients with MPS IIIA. Mitral insufficiency and aortic valve thickening were significantly more common among females.

MPS IIIA subtype and female sex may increase the risk of cardiac pathologies. Although verification by future studies is warranted, considering the relatively large number of patients in our study, our results are important.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Hacettepe University Clinical Research Ethics Committee, and this study was performed in accordance with the ethical standards of the Declaration of Helsinki (GO 18/901-09).

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Medical Practice: B.B.G., E.A., H.S.S., Concept: A.T., T.C., Design: B.B.G., H.S.S., Data Collection or Processing: E.A., D.A., Analysis or Interpretation: E.A., D.A., A.T., T.C., H.S.S., Literature Search: T.C., A.D., H.S.S., Writing: B.B.G.

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

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Case Report

A Rare Case of Burkitt Lymphoma in a 13-year-girl

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ABSTRACT

Burkitt lymphoma is rare Non-Hodgkin's lymphoma type in childhood. The differential diagnosis includes dental infection and osteomyelitis. Therefore, patients often refer to the dentist first. Early diagnosis has great importance on prognosis. We aimed to broaden the perspective of dentists about this disease and confirm the diagnosis radiographically and clinically.

Keywords: Burkitt lymphoma, dental abscess, jaw, paediatric

Introduction

Burkitt lymphoma (BL) first described by Dr. Dennis Burkitt in 1958 (1). It is a sub-type of Non-Hodgkin's lymphoma, and is also an aggressive neoplasm with three variants that are endemic (African), sporadic (American), and immunodeficiency associated [human immunodeficiency virus (HIV) associated] (2).

Although the histopathological features of these three forms are similar, their clinical features are different in terms of age distribution and prevalence. The endemic form that is frequently found in Africa is widely reported to occur in early childhood at a peak age of 6 years; in contrast, the sporadic form does not appear in a particular geographic area and occurs mainly in children and adolescents. The endemic form commonly involves the jaw bone, especially the maxilla, and typically presents as tooth mobility, jaw expansion, and also systemic signs such as an abdominal mass (2). However, jaw involvement in the sporadic form is not common (3).

The HIV associated subtype represents 35-40% of Non-Hodgkin's lymphomas appearing in the HIV positive population. BL is silent in the early stages, but it grows and expands rapidly. Signs and symptoms vary depending on the primary location and extent of the tumour (4).

Intraoral findings are rarely observed at the beginning of the disease. To emphasize the role of the dentist in the early detection of BL due to the importance of early diagnosis of this rare disease, we present a case of BL in a 13-year-old girl with hypermobile teeth, pain, and numbness on her jaw.

Case Report

This case report was approved by Başkent University Institutional Review Board (D-KA 20/24) on the 12th February 2020. Informed consent was obtained from the parent of the participant. The authors certify that they have obtained the patient consent form. In the form, the patient has given her consent for her radiological images and other clinical information to be reported in the journal. The patient

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(parent) understands that her name or initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

A 13-year-old girl applied to the Dental Clinic of Başkent University Adana Dr. Turgut Noyan Research and Practice Center with severe dental infections presenting with mandibular and maxillary hypermobile teeth, pain, and numbness of right mandibular region. The patient had been treated for 2 months in another clinic with the diagnosis of dental abscess, receiving intensive antibiotic treatment, and abscess drainage was performed several times by a dentist. These treatments provided temporary relief but symptoms reappeared in a short time.

The patient, whose past medical history was nonspecific, had no history of progressive systemic symptoms such as fatigue, weakness, or fever. Extraoral examination revealed facial asymmetry caused by a soft, painless swelling of the right cheek. Some enlarged lymph nodes were observed. Intraoral examination showed symptoms such as juvenile periodontitis, enlarged gingival mucosa, and several teeth caries. The molar and premolar teeth in both right jaws were mobile and swelling was also observed in the gingiva surrounding these teeth. Purulent pus was not detected. There was no oral sign of disease in the buccal and palate mucosa, upper and lower lips.

In radiological examination, several root resorptions, and chronic periapical infections were determined. On panoramic radiograph, the patient was found to have alveolar bone resorption adjacent to the maxillary and mandibular canines, premolars, and molars with the appearance of floating teeth. Periapical X-rays also attracted attention to the radiological appearance such as a punch hole on the maxillary alveolar bone (Figure 1).

An incisional biopsy was performed on the mandibular molar gingiva. Histopathological findings of the gingiva showed infiltration consisting of small to medium round hyperchromatic vesicular nucleus. Narrow eosinophilic cytoplasm atypical cells were observed under the epidermis in the prepared sections (Figure 2).

Immunohistochemical staining was positive for CD10, CD20, and MUM1, which are specific to B-cell lymphocytes and late stages of B-cell differentiation. It was negative for CD2, CD3, Tdt, MPO, Cyclin D1, CD34, CMYC. Ki-67 staining was positive in more than 85-90% of the cells, revealing high proliferative activity. Overall, the histopathology results supported the diagnosis of BL. Also, there was no Epstein-Barr virus (EBV) genome (EBV-).

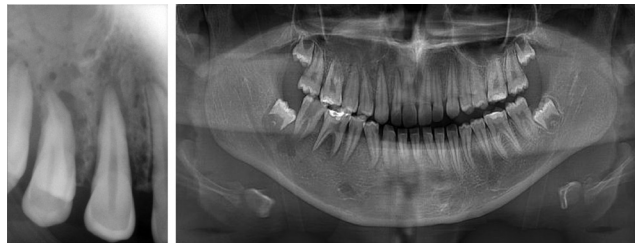

Figure 1. Periapical radiographs have punch holes on the maxillary alveolar bone; panoramic radiograph has alveolar bone resorption and floating tooth appearance from right maxillary and mandibular canines to molars

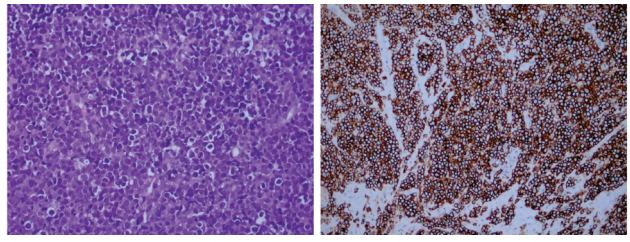

Figure 2. Histopathological evaluation of the patients mandibular molar gingiva

On abdominal examination, a mass in the midline of the pelvis extending to the umbilicus was revealed by the paediatrician. Additionally, nodular masses and accompanying fluid with multiple diffusion restriction in the proximal part of the pancreas corpus, behind the uterus in the pelvic region in the pericecal area and the largest behind the uterus in both ovarian loges were detected via lower abdomen magnetic resonance imaging.

After our prompt biopsy, the extent of the disease was identified, and the patient was immediately referred to the Haematology-Oncology Department for definitive care and chemotherapy was initiated.

Discussion

Most often affecting the paediatric population, BL is a potentially curable aggressive neoplasm (5).

Dental radiography can play an important role in the diagnosis of BL. Radiographic changes in the jaw are usually found before the clinical dental signs of the disease appear and can be noticed primarily by dentists (5). In this present case, floating teeth, chronic gingival abscesses, root resorptions of the mandibular posterior teeth, loss of lamina dura, pulpal infiltration with tumour cells, and osteolytic lesions were the problems detected before other symptoms appeared, in accordance with the literature (6,7). Thus, the presence of floating teeth on plain radiography reflecting the destructive process (punch hole appearance)

in the maxilla and mandible might suggest sporadic BL as well as infectious, hematologic, or metabolic causes including Langerhans cell histiocytosis (7). Moreover, the unexplained hypermobility of teeth and the displacement of developing teeth with cortical weakening in a child were signs that created suspicion regarding malignant lesions (2).

BL should be diagnosed as quickly as possible and requires prompt intervention because it is a rapidly growing high-grade malignant neoplasm (2). However, the clinical features of oral lymphoma are not specific enough for dentists to diagnose, and there is an attempt to solve the misdiagnosed problem by dental treatment. For example, dentists often evaluate hypermobile teeth with a history of jaw pain or floating teeth with resorption of the adjacent alveolar bone as a dental abscess or Langerhans cell histiocytosis, and the prognosis may worsen because the correct diagnosis is delayed. Therefore, dentists should be more attentive in the presence of alveolar bone resorption and/or floating tooth symptoms.

BL is an aggressive neoplasm with poor prognosis. However, its treatment success depends on its stage. A study on oral BL reported that even stage III patients had a 97% survival rate. In contrast, the prognosis of stage IV BL is extremely poor: the survival rate of stage IV patients is substantially lower at 27%, suggesting that early diagnosis of BL is crucial (8). Luckily, since the disease was diagnosed early in our case, most organs were not yet involved and the disease did not progress clinically.

Oral signs of BL include the loosening and extrusion of molar teeth, the premature shedding of primary molars and the premature eruption of permanent molars, gingival enlargement and swelling of the alveolar regions and jaw (5). In our case, almost all of these symptoms were observed in accordance with the literature. However, the sporadic type of BL can involve one quadrant of the jaw, while the endemic type can involve all four quadrants. In this case, the patient had involvement of two quadrants; the right lower and upper jaws. This clinical finding suggests that the BL type in the patient may be an endemic form but it had not progressed to other regions of the jaw as it was diagnosed early.

Since the differential diagnosis of BL is acute dental infection and osteomyelitis (5), when these are not supported radiologically, these findings may lead the dentist to make a wrong diagnosis, as in our case. In such a situation, the patient's disease may have progressed beyond treatment and be fatal. In our case, the patient had been diagnosed with dental abscess and was given intensive antibiotic treatment and abscess drainage was carried out twice over a period of two months, and the real diagnosis of the disease was delayed because no detailed radiographic examination was performed.

According to the literature, the disease does not show systemic symptoms such as weakness, fever, hepatosplenomegaly, and is diagnosed in children only with intraoral and extraoral findings. It has been reported that the loss of lamina dura is noticeable radiologically. In our case, there were no systemic findings and the disease was diagnosed completely via oral findings. Notably, the presence of punch hole in the alveolar bone on periapical radiographs distinguishes this case from others (7,9).

Furthermore, in a case of BL in 15-year-old girl, attention was drawn to misdiagnosis of this disease. In that case report, the dentist tried to treat the swelling of the patient in the maxillary area with root canal treatment, initially considering it to be endodontic lesion as in our case (9).

In addition to the facial mass in the sporadic form, toothache and paresthesia on the lip are frequently seen (5). Similar to the literature, our patient had had toothache and paraesthesia in the right lower lip for 3 months.

The sporadic form of BL is more common in boys than in girls and is most commonly seen between the ages of 3-8. The endemic form occurs in both genders and the average age is 11. In our case, the age of the patient was 13 and the gender was female, making us think that the diagnosis was more suitable for the endemic form (2). EBV appears in 97% of endemic LB, but only in 20-30% of sporadic cases (10). The EBV genome was not present in our case.

Abdominal mass is frequently seen in older children and adults in the sporadic form of BL (11). The patient we present in this paper has a mass in the midline of the pelvis extending to the umbilicus.

Conclusion

This case suggests the possibility of dental complaints as an initial clinical manifestation of BL and emphasizes the role of dentists in the early detection of this disease to improve prognosis, as early diagnosis and immediate chemotherapy treatment are important in increasing the survival rate.

Ethics

Informed Consent: Informed consent was obtained from the parent of the participant.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.Y., E.S., B.H., Design: B.Y., E.S., B.H., Data Collection or Processing: B.Y., E.S., B.H., Analysis or Interpretation: B.Y., E.S., B.H., Literature Search: B.Y., E.S., B.H., Writing: B.Y., E.S., B.H.

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

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Case Report

Mucopolysaccharidosis Type-II with Pathognomonic Skin Appearance: A Case with Pebbling Sign

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ABSTRACT

Mucopolysaccharidosis type-II (MPS-II) is an X-linked lysosomal storage disorder. Here, we report an 8-year-old boy with pebbling sign in the scapular region, coarse facies, claw hand, diastolic murmur, and hepatomegaly. With decreased iduronate-2-sulfatase activity and hemizygous mutation in the *IDS* gene, the diagnosis was MPS-II. Pebbling sign is a rare but pathognomonic sign of MPS-II.

Keywords: Mucopolysaccharidosis, Hunter disease, pebbling sign

Introduction

Mucopolysaccharidosis (MPS) is a group of progressive lysosomal storage disorders caused by mutations of the genes encoding lysosomal enzymes that have a role in the degradation of glycosaminoglycans (GAG). MPS type-II is characterized by dermatan and heparan sulfate storage in all tissues due to iduronate sulfatase (IDS) enzyme deficiency caused by *IDS* gene mutations (1). It was first described in two brothers by Hunter (2) in 1917. Hunter syndrome (MPS type-II) is an X-linked inherited disease, whereas all the other types of MPS are autosomal recessively inherited. Although it is almost exclusively seen in males, there are a few rare female cases reported. Its incidence is estimated to be between 1:100,000 and 1:170,000 male births (3).

The disease is known to have severe, intermediate and mild types. The clinical findings of the severe form resemble Hurler syndrome (MPS type I-H), but corneal opacities are not seen. Life expectancy is 10-20 years. Central nervous system involvement, progressive airway disease or cardiac disease may lead to death. The mild type of the disease may be clinically confused with Scheie syndrome (MPS type I-S). Generally neurological manifestations are not seen.

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Diagnosis is made by determining a deficiency of IDS activity and mutation analysis. Enzyme replacement therapy (ERT) is the current treatment option (3).

In all types of MPS, generally the skin has a thickened appearance and it loses its elasticity. Unlike the other types of MPS, in Hunter syndrome, a pathognomonic cutaneous lesion which is known as the pebbling sign may be seen in the scapular region in particular, but may also be seen on the arms and on the thighs. These lesions are generally 2-10 mm in diameter, ivory white in color, papular or nodular in structure and placed in a reticular pattern (4-6). The aim of this case report is to emphasize the importance of recognizing the pebbling sign in the differential diagnosis of skin MPS-II from other types and to initiate ERT without a delay.

Case Report

An 8-year-old boy was admitted to our hospital with the complaint of abnormal skin appearance in the scapular region which was first recognized at the age of 6 years. He had symmetric, shiny, ivory-white colored, and rough surfaced lesions on the scapulae. Although the lesions persisted, diagnosis could not be made. There was nothing remarkable in the prenatal and natal history. He had not had any previously known systemic diseases. At the age of 3 months, the patient had undergone a surgery for inguinal hernia. His parents were not consanguineous and there was no similar family history. On physical examination; his weight was 20.5 kg (50th percentile), and height was 111 cm (25th percentile). There were mild coarse facial features (Figure 1), a pebbling sign on the back (Figure 2), joint stiffness, mild claw hands (Figure 1), a 2/6 diastolic murmur, hepatomegaly (3 cm palpable in the midclavicular line), and an umbilical hernia.

Routine hematological and biochemical examinations revealed no abnormalities. Echocardiography detected 2nd degree aortic valve insufficiency. His ophthalmological examination and hearing test were completely normal. Multisystemic involvement together with the pebbling sign led us to the diagnosis of MPS-II. Elevated amounts of urinary total GAG [243.5 mg/mol creatinine (N:<80)], more specifically increased urinary dermatan and heparan sulfate, with a low plasma iduronate-2-sulfatase level [0,0 nmol/mL/4 h (N:494-1113)] and hemizygous p.N63D (c.187A>G) mutation in the *IDS* gene confirmed the diagnosis of MPS type-II. The intelligence quotient of the patient was found to be within normal limits for his age. ERT was initiated at a dosage of 0.5 mg/kg/week. The patient has now been on ERT for 2 years. The pebbling sign has not disappeared, but improvements in joint and liver involvement have been observed. Informed consent, including visual use, was obtained from the patient's parents.

Discussion

Hunter syndrome is a rare lysosomal storage disease characterized with variability both in the age of disease onset and severity of clinical findings (1). The severe form of the disease begins at the age of 2-4 years with prominent systemic symptoms and signs which are accompanied by neurological involvement and developmental delay. These patients are generally lost in the 1st or 2nd decade of life because of heart failure, obstructive airway disease or neurological complications. In the milder form of the disease, clinical signs and symptoms are not prominent

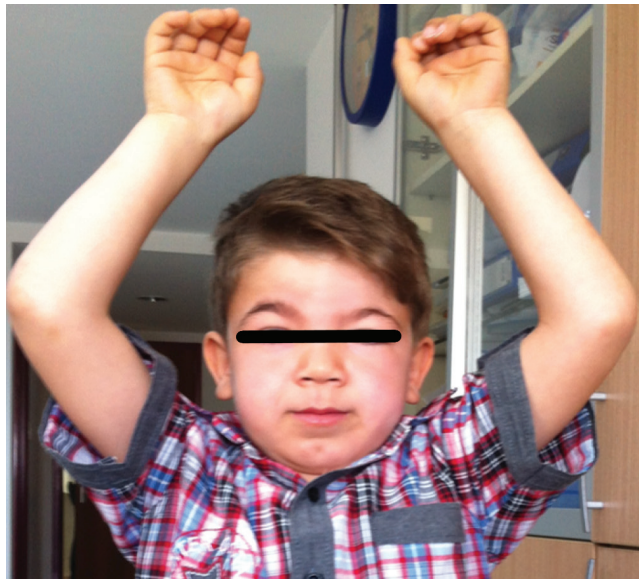

Figure 1. Coarse facial features, claw hands and joint stiffness

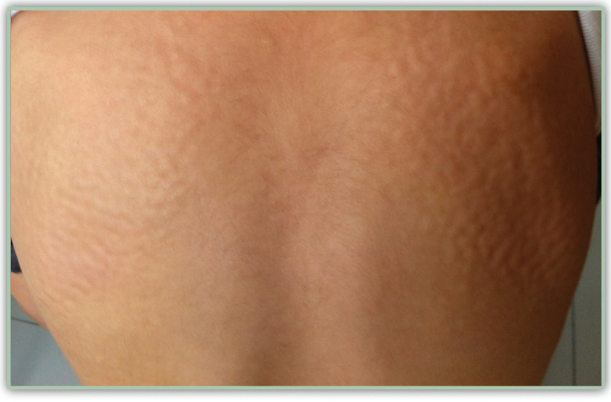

Figure 2. The pebbling sign: Papular lesions seen in a reticular pattern in the bilateral scapular region

enough to put forward a specific diagnosis. Patients usually have normal mentality and survive into their forties or fifties. Coarse facial features with thick lips and macroglossia, macrocephaly, hypertrophic adenoids and tonsils, hearing loss, hepatosplenomegaly, umbilical and inguinal hernias, heart valve insufficiency, joint stiffness, and contractures are the common and frequently seen features of all subgroups of MPS II patients (3). However, very little is known about the definitive incidence and the impact on the prognosis of cutaneous lesions known as pebbling sign (4-7). From a report of 11 MPS type-II patients aged between 18 months and 31 years, typical skin lesions were only seen in 2 of them (5). These skin lesions often create a reticular appearance, are colored ivory-white, and sometimes consist of papules and nodules. They may be seen symmetrically in the scapula, neck, and the posterior axillary, and pectoral regions. They may also be on the upper extremities and the sides of the thighs (3). The pebbling sign is reported to appear before the age of 10 years and spontaneously recover (4-8). While other systemic findings of our patient ameliorated during the 2-year follow-up period up to the age of 10, the pebbling sign had not disappeared to date. He is the only patient with pebbling sign out of 18 MPS type-II patients followed up in our clinic.

We report this case as the pebbling sign is a rare but an alarming feature for the diagnosis of Hunter disease that requires the early initiation of ERT before the development of other undesirable systemic manifestations.

Ethics

Informed Consent: Informed consent, including visual use, was obtained from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.H.İ., B.Ş.Y., F.D.B., Design: S.K., D.K., M.K., H.N.Ö.M., Data Collection or Processing: A.H.İ., B.Ş.Y., F.D.B., S.K., D.K., Literature Review: S.K., D.K., Editing Assistance: M.K., H.N.Ö.M., Writing: A.H.İ., B.Ş.Y., F.D.B.

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

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Case Report

Late Diagnosis of Progressive Pseudorheumatoid Dysplasia in an Adolescent Patient: A Case Report and Review of the Literature

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ABSTRACT

We present an adolescent girl with severe kyphoscoliosis and inability of walking due to progressive pseudorheumatoid dysplasia (PPD) who was initially misdiagnosed as juvenile idiopathic arthritis for 9 years. The lack of inflammatory joint involvement and inflammatory laboratory parameters, along with the characteristic radiological findings should raise suspicion for PPD.

Keywords: Progressive pseudorheumatoid dysplasia, childhood, late diagnosis, juvenile idiopathic arthritis, intensive rehabilitation treatment

Introduction

Progressive pseudorheumatoid dysplasia (PPD), known as spondyloepiphyseal dysplasia tarda with progressive arthropathy, is a rare genetic bone disorder characterized by the progressive degeneration of articular cartilage affecting both the axial skeleton and peripheral joints (1). PPD occurs due to loss-of-function mutations in the *WNT1-inducible signaling protein 3* gene, currently known as Connective tissue growth factor, Cysteine-rich 61, Nephroblastoma over-expressed *(CCN6)* gene (2). The prevalence of PPD is estimated at one per million children with a slight predominance among females and consanguineous populations (1).

The mean age ranges between 3 and 8 years (3). PPD is characterized by polyarticular involvement, gait abnormalities and fatigability. Symptoms may clinically mimic those of juvenile idiopathic arthritis (JIA) resulting in a late diagnosis. The absence of inflammatory joint involvement and raised inflammatory laboratory parameters, characteristic radiological findings (enlarged epiphyses, platyspondyly), and poor response to anti-inflammatory/immunosuppressive treatment should raise suspicion for PPD (4). Due to the rarity of the disease, to-date literature is limited (approximately 64 studies) among which 28 are case reports and 27 are case series with less than 10 patients (1,5-7); even fewer are the large series counting more than 30 PPD patients (1,8).

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This is the first report of a PPD patient in Greece, an adolescent girl who was misdiagnosed and treated as JIA for 9 years.

Case presentation

A 13.5-year-old girl presented to our department due to severe pain in both hips and gradual difficulty of walking. Familiar history was uneventful for rheumatic and autoimmune diseases. Parents were not consanguineous and the child had normal facial appearance and intelligence. Since the age of 4 years, the girl presented pain and progressive stiffness and swelling of small joints of hands and both ankles. She was then diagnosed as suffering from seronegative polyarticular JIA. Treatment with corticosteroids (5 mg prednisolone/day), methotrexate (10 mg/week) and folic acid (5 mg/week) was initiated. Due to poor response, treatment was scaled up including subcutaneous etanercept (25 mg/week) one year later; however, etanercept had also minimal clinical impact. Despite treatment, the patient gradually presented back pain. Radiology of the spine showed kyphoscoliosis and platyspondyly, which was not taken into account at that time. Consequently, during 9 years the girl remained under disease-modifying antirheumatic drugs (DMARD) and anti-TNF treatment with no improvement, instead worsening of her symptoms resulting in progressive emergence of difficulty of walking.

Upon examination at our department, the girl presented on wheelchair, unable to walk on her own. Her weight and height were short for age (<3rd percentile). She had severe kyphoscoliosis of thoracic spine and restricted mobility of cervical spine and coxofemoral joints. There was enlargement and contractures of almost all peripheral joints, i.e. elbows, wrists, metacarpophalangeal, distal and proximal interphalangeal joints of both hands and ankles, resulting in severe restriction of their movement (Figures 1a, b, c). Enlargement in peripheral joints was of bony consistence resulting in camptodactyly, while no active arthritis was detected.

Laboratory investigation showed normal white blood cell count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factors, antinuclear and anti-cyclic citrullinated peptide antibodies. Ophthalmologic examination was normal. Review of the child's medical history showed no signs of raised CRP/ESR since the beginning of the disease. Radiograph of the hands revealed diffuse osteopenia, widening of the metacarpal and phalangeal epiphyses and loss of joint space without erosive lesions (Figure 1d). Radiology of the hips and spine showed degenerative changes with almost complete destruction of hip joints and platyspondyly (Figures 1e, f, g). No soft tissue swelling or erosions could be seen. Peripheral quantitative computed tomography (pQCT; slices 4, 14 and 38% of tibia

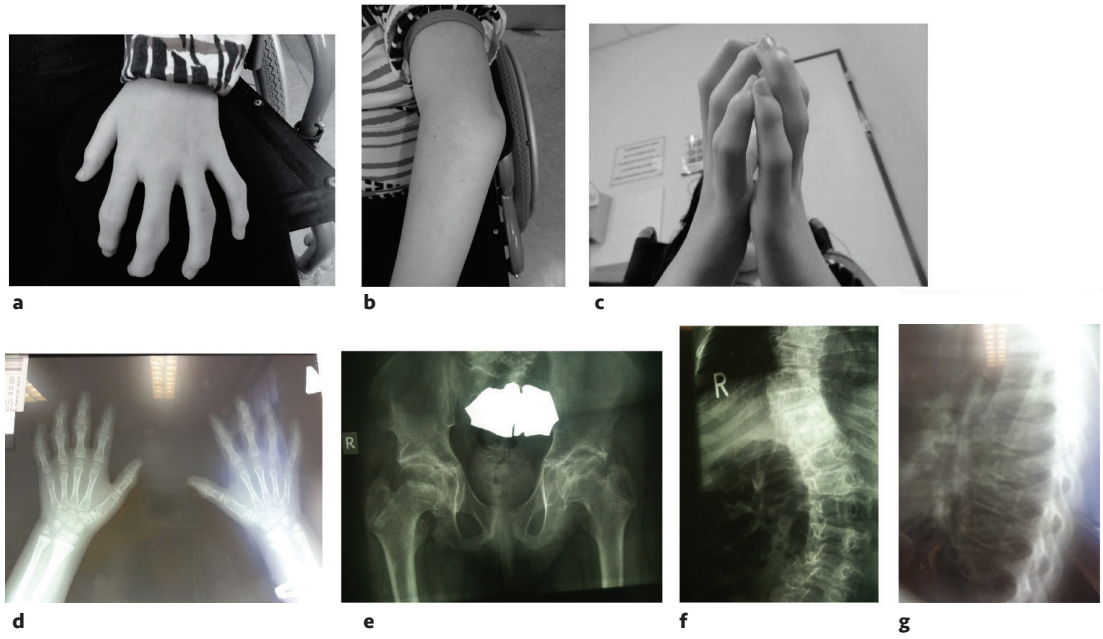

Figure 1. Patient 13 years old: Enlargement and contractures of metacarpophalangeal and interphalangeal joints (a) with limitation of extension of the fingers (b); and enlargement and contractures of the elbow (c). Hand X-rays showing osteopenia, enlargement of the metaphyses and epiphyses at the metacarpals and phalanges, with loss of joint space, without erosions (d). Coxofemoral X-rays showing severe degenerative changes (e) and spine X-rays showing severe scoliosis (f) and flattened thoracic and lumbar vertebral bodies with irregularities of their surfaces (platyspondyly) (g)

length) showed extremely reduced trabecular volumetric density (86 mg/cm^3), cortical volumetric density and derived bone strength (slice 38% - 344 mm^3).

The aforementioned findings raised suspicion for PPD. Direct sequencing of all coding genes and their flanking regions of the *CCN6* gene revealed the c.156C>A (p.Cys52*), and the c.248G>A (p.Gly83Glu) mutations both located in exon 2, confirming the diagnosis of PPD (Figure 2).

Intensive rehabilitation treatment was initiated. One year later, the patient started walking with crutches and the mobility of almost all joints and the parameters of pQCT were significantly improved. Currently, the girl is able to walk on her own. Given that the range of motion of her hips remains minimal (inability to climb stairs and dress alone) she has been referred to orthopedic surgeons and a total-hip replacement arthroplasty is being planned.

The proxies of the patient have signed a consent to publication form and the signed form is held by the treating institution.

Discussion

PPD is a progressive autosomal recessive disorder which caused decreased joint mobility, osseous swelling of the interphalangeal and other joints, and platyspondyly (1).

The disease is due to biallelic mutations in the *CCN6* gene located on chromosome 6q22 (2). This gene encodes a 354-amino acid protein, a member of the connective tissue growth factors *CCN* gene family. The CCN6 protein is expressed in skeletal-derived cells and participates in angiogenesis, maintenance of cartilage integrity and bone growth. Seventy-six *CCN6* gene mutations have been reported so far, the majority of which located in exon 2 (1,2). In our case, we identified two mutations, the most frequent pathogenic mutation c.156C>A (p.Cys52*), and the c.248G>A (p.Gly83Glu) both located in exon 2; these mutations have been described before in children with PPD (9).

Clinically, patients appear normal at birth, have subtle symptoms by the age of 3 years and develop later a progressive degenerative joint disease characterized by enlarged metacarpophalangeal and interphalangeal joints, kyphoscoliosis, short stature and severe reduction in almost all joints' mobility with multiple contractures (Table I) (6). The characteristic radiologic findings include platyspondyly, large femoral and tibial epiphyses, enlarged epi-metaphyseal portions of the metacarpals and phalangs, and narrow joint spaces (1,4). Cervical spine involvement seems to be a significant prognostic factor due to severe neurological manifestations that may be caused by spinal canal stenosis (1).

The diagnosis of PPD is challenging with many children initially misdiagnosed as JIA (4,10). The differential diagnosis also includes myopathy, camptodactyly-arthropathy-coxa-vara-pericarditis syndrome and some types of mucopolysaccharidosis. Despite the onset of first symptoms

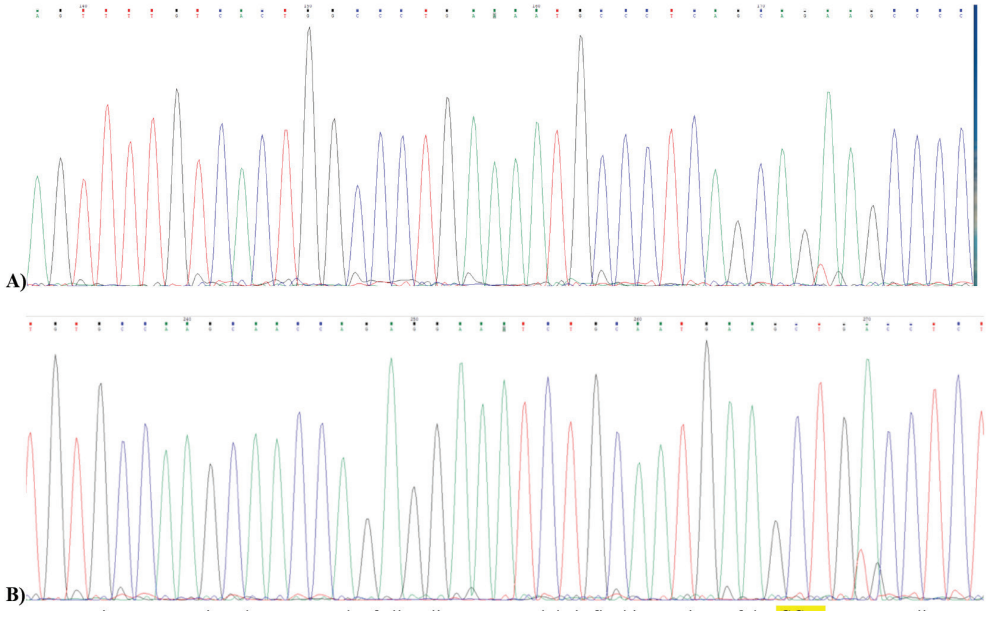

Figure 2. Direct sequencing chromatograph of all coding genes and their flanking regions of the *CCN6* gene revealing (A) the c.156C>A (p.Cys52*) and (B) the c.248G>A (p.Gly83Glu) mutations in the patient

Table I. Main clinical, laboratory and radiologic features of progressive pseudorheumatoid dysplasia (PPD) compared to juvenile idiopathic arthritis

Clinical, laboratory and radiologic features	**PPD**	**JIA**	**Patient's features**
Mean age at onset (years)	3-8	1-3 and 8-10	4
Familiar history (relatives with similar symptoms, consanguineous marriage)	+	uncommon	-
Joint contractures	+	+	+
Inflammatory joint involvement	-	+	-
Extra-skeletal clinical manifestations	-	+	-
Inflammatory laboratory markers	-	+	-
Radiological findings			
Soft tissue swelling	-	+	-
Bony erosions	-	+	-
Narrow articular space	+	+	+
Ankylosis	+	+	+
Narrowed joint space, epiphyseal dysplasia, enlarged epiphyses and irregular acetabulum	+	-	+
Platyspondyly	+	-	+
Kyphoscoliosis	+	+/-	+
Anterior ossification defects in vertebrae	+	-	-
Spondyloepiphyseal dysplasia	+	-	+
Response to anti-rheumatic/anti-inflammatory treatment	poor	+	no

PPD: Progressive pseudorheumatoid, JIA: Juvenile idiopathic arthritis

in early childhood, the diagnosis of PPD is usually delayed up to the second decade of life, and patients often receive unnecessary anti-inflammatory and immunosuppressive treatments (6). Although no diagnostic criteria have been established, "typical PPD" is defined as the presence of early-onset stiffness and pain in multiple joints, enlarged interphalangeal joints, normal inflammatory parameters, and the absence of extra-skeletal manifestations (Table I) (1). It is thus critical to reevaluate the clinical clues in cases without recovery or good response to treatment, as the patient described herein, and discuss such cases in multidisciplinary councils. Moreover, a lateral radiograph of the spine or a full skeletal survey could direct towards the diagnosis, which is thereafter usually confirmed by molecular genetic testing (9,10). However, genetic testing of genomic DNA extracted from blood leucocytes may be negative given that intronic mutations in *CCN6* can only be detected by analyzing mRNA from cultured skin fibroblasts (11). Thus, a skin biopsy could be a useful diagnostic tool in cases with suspicion of PPD and negative mutation screening of genomic DNA (1). Genetic testing is recommended for family counselling (3).

The treatment of PPD remains supportive. The poor response to DMARD is typical of PPD, whereas anti-inflammatory treatment has also minimal clinical impact (6). Early-onset rehabilitation therapy is of utmost importance to preserve joint mobility. Surgical interventions include hip and knee joint replacement, realignment of the lower limbs and spinal surgery (4). The best timing for joint replacement depends on the patient's condition, but it is not recommended before epiphyseal closure to avoid leg length discrepancy, namely before the ages 12-18 for girls (usually 15-16 years) and 14-19 for boys (usually 18-19 years) (1).

PPD is a non-inflammatory skeletal disorder clinically simulating JIA. Increased awareness and timely recognition of this disease could spare children of unnecessary treatments and offer access to family genetic counselling. Larger studies could refine our understanding of the pathogenesis of PPD allowing hopefully the development of novel etiological treatments.

Ethics

Informed Consent: The proxies of the patient have signed a consent to publication form and the signed form is held by the treating institution.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.A., Design: E.A., Analysis or Interpretation: A.P., M.M., Data Collection or Processing: A.P., Critical Review: M.A.K., A.P., O.P., M.M., G.S., V.P., E.A.

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

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

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Letter to the Editor

The Neurodevelopmental Outcome of Severe Neonatal Haemolytic and Non-hemolytic Hyperbilirubinemia

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Keywords: Neonatal, haemolytic, hyperbilirubinemia

Sir,

I have read an interesting study by Çolak et al. (1) published in the June 2020 issue of the Journal of Pediatric Research. The authors studied the neurodevelopmental outcome of severe neonatal haemolytic and nonhemolytic hyperbilirubinemia. The study design, the protocol for conducting the study, was not mentioned in the article. The pivotal aspects of planning a clinical research are the calculations of the sample size and this was not highlighted.

In Table I (1), the Bilirubin/Albumin ratio is given as number (percentage). Although it is statistically insignificant, the value which is given in the haemolytic and non-haemolytic column could not be understood i.e. [3 (21.4%) vs 9 (40.9%)].

The author found dehydration in 85.7% of the non-haemolytic group as compared to 90.9% in the haemolytic group of neonates. However, only 7.1% received IV fluid in the non-haemolytic group as compared to 68.1% of the haemolytic group. Is there any specific reason for this?

The authors mentioned in the results that 2 neonates develop cerebral palsy (CP) and one neonate had deafness in the haemolytic group. Additionally, they found that 4 neonates received exchange transfusion in the same group. Is there any correlation between the exchange transfusion and the development of CP or deafness in the infants?

To my knowledge, this is the best study of the neurodevelopmental outcomes of severe neonatal hyperbilirubinemia carried out by the author.

Ethics

Peer-review: External and internal peer-reviewed.

Financial Disclosure: The author has no sources of support for this work.

Reference

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Response to the Letter

Dear Editor,

We would like to thank you for the opportunity to respond to the issues raised in Dr. Amar Taksande's letter. First of all, the study design was a retrospective observational cohort study. The study started in May 2015 with a file system scan of 36 patients hospitalized due to indirect bilirubinemia over 25 mg/dL in our Neonatal Intensive Care Unit between June 2013 and January 2015. All patients were contacted via their registered phone numbers whilst in the age range 18-24 months to invite them to our hospital to perform "Bayley Scales of Infant Developmental Assessment scale II" (BSID). We did not perform power analysis since we received all patients who met the inclusion criteria within the specified time interval.

Secondly, the bilirubin/albumin ratio mentioned in Table I shows the number of patients whose bilirubin/albumin ratio should be planned according to the risk category of the baby (1). As stated in the table, there was no statistical difference between the two groups in terms of the number of patients.

Thirdly, we indicate the presence of dehydration if there is a weight loss exceeding physiological limits on that specific postnatal age. However, we do not perform IV fluid replacement for all dehydrated infants in our Clinic of Neonatal Intensive Care Unit if they are vigorous and active, have sufficient oral intake, have normal blood pressure and sufficient urine output. We usually prefer physiological rehydration in such infants by supporting breastfeeding or offering infant formulas. Therefore this difference did not surprise us.

And finally, as seen in Table II, in the hemolysis group, one patient with cerebral palsy and hearing loss who had undergone exchange transfusion, and one patient with only hearing loss who hadn't received exchange transfusion were identified.

Although it is not the best study on this subject and have some limitations, it may be a study with good results in terms of the neurodevelopmental outcomes of severe hyperbilirubinemia.

Thanks you very much for your kind and affirmative comments.

Best regards.

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