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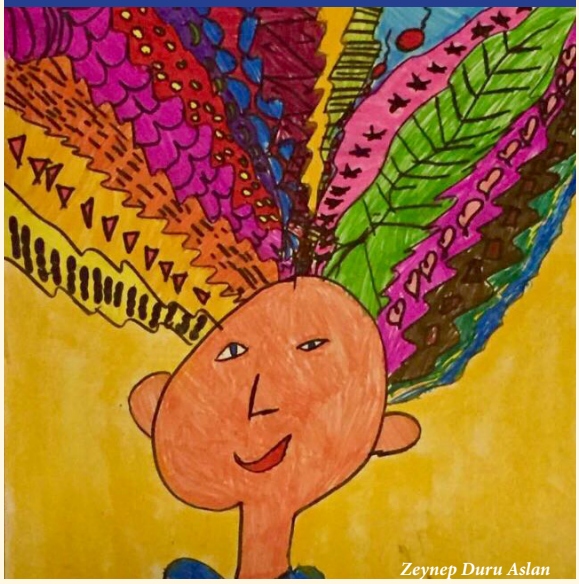

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PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med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

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

STROBE statement, a checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/);

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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Cover letter: The cover letter should include statements about manuscript type, single-journal submission affirmation, conflict of interest statement, sources of outside funding, equipment (if applicable), approval of language for articles in English and approval of statistical analysis for original research articles.

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The "p" value defined as the limit of significance along with appropriate indicators of measurement error and uncertainty (confidence interval, etc.) should be specified. Statistical terms, abbreviations and symbols used in the article should be described and the software used should be defined. Statistical terminology (random, significant, correlation, etc.) should not be used in non-statistical contexts.

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

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Unique research papers cover clinical research, observational studies, novel techniques, and experimental and laboratory studies. Unique research papers shall consist of a heading, abstract, keywords related to the main topic of the paper, introduction, materials and methods, results, discussion, acknowledgements, bibliography, tables and figures. The text shall not exceed 2500 words. Case reports must contain rare cases or those that are unique in diagnosis and treatment, those which contribute to current knowledge and provide educational information, along with the introduction, case reporting and discussion sections. The whole text must not exceed 1500 words. Reviews are texts in which a current subject is examined independently, with reference to scientific literature. The whole text must not exceed 18 A4 paper sheets. Letters to the Editor must be manuscripts, which do not exceed 1000 words, with reference to scientific literature, and those written in response to issued literature

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Abstract: A summary of the manuscript should be written in English. References should not be cited in the abstract. Use of abbreviations should be avoided as much as possible; if any abbreviations are used, they must be taken into consideration independently of the abbreviations used in the text.

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

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Study Limitations: Limitations of the study should be discussed. In addition, an evaluation of the implications of the obtained findings/results for future research should be outlined.

Conclusion: The conclusion of the study should be highlighted.

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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Case reports should present cases which are rarely seen, feature novelty in diagnosis and treatment, and contribute to our current knowledge. The first page should include the title in English, an unstructured summary not exceeding 50 words, and key words. The main text should consist of introduction, case report, discussion and references. The entire text should not exceed 1500 words (A4, formatted as specified above). A maximum of 10 references shall be used in case reports.

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Review articles can address any aspect of clinical or laboratory pediatry. Review articles must provide critical analyses of contemporary evidence and provide directions for future research. **The journal only accepts and publishes invited reviews.** Before sending a review, discussion with the editor is recommended.

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Editorial

Dear Journal of Pediatric Research Readers,

We are so proud and happy to welcome you to the third issue of The Journal of Pediatric Research in 2018.

In the third issue of 2018, we present to you 13 articles including 7 research articles and 6 case reports from different disciplines. Four articles contain endocrinological studies. The first research we present evaluates the effect of obesity on the choroid tissue of the eye and the second article is an investigation into the effects of exercise and osteoporosis in Down syndrome children. Osteoporosis is a problem in Down syndrome children and research investigating the effect of exercise on bone mineral density in Down syndrome children contributes to the literature in this respect. The next piece of research is about oxidative stress and antioxidative enzymes in Type I diabetes mellitus. A novel HNF4A mutation in a case with MODY also contributes to the literature. We present in this issue a piece of research about tick bites and tick species which is also responsible from Crimean Congo hemorrhagic fever and three pediatric surgery articles covering peptic ulcer perforation, neck mass and diaphragmatic evantrations. In addition, two articles focusing on Familial Mediterranean Fever and two rare conditions, peripheral facial palsy and Arthrogryposis-Renal dysfunction-Cholestasis syndrome, also aim to help the reader improve their clinical knowledge.

We would like to acknowledge the members of our editorial board reviewers, authors and Galenos Publishing House for preparing the third issue of 2018. We look forward to your scientific contributions in our future issues.

We hope you benefit from these articles.

Best wishes

Aslı Aslan MD,
Associate Professor of Pediatrics,
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Original Article

Does Obesity Affect the Ocular Choroid Tissue in Children and Adolescents?

Bediz Özen[1], Hakan Öztürk[1], Gönül Çatlı[2], Bumin Dündar[2]

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ABSTRACT

Aim: Obesity may cause microangiopathic changes associated with the inflammatory process. The choroid tissue of the eye is one of the most highly vascularized tissues of body and supplies the outer 1/3 of the retina. Thinning in choroid tissue is an indicator of damage. Few studies have investigated obesity-induced choroid tissue damage in children, and their findings are inconsistent. The purpose of this study was to investigate changes in choroid tissue thickness in non-diabetic children and adolescents using optic coherence tomography (OCT) and the association with metabolic risk factors.
Materials and Methods: One hundred fifty-six eyes of 38 obese and 40 healthy children and adolescents aged 10-18 were included in the study. The bilateral choroidal thicknesses were measured. We then investigated correlations between choroidal thickness and age, body measurements, pubertal stages, systolic and diastolic blood pressures, homeostasis model assessment insulin resistance and lipid values.
Results: Mean choroidal thicknesses measured using OCT were 284.4±34.9 µm in the obese group and 316.3±39.7 µm in the control group (p=0.018). Choroidal thickness in the obese group decreased as body mass index (BMI) standard deviation scores (SDS) increased (r=-0.390, p=0.000).
Conclusion: Mean choroidal thickness was lower in obese children and adolescents in this study compared to the healthy controls and thinning in the choroid tissue was more pronounced as BMI-SDS values increased. Increased adipose tissue may result in a susceptibility to damage by thinning choroid tissue.
Keywords: Choroidal thickness, optical coherence tomography, pediatric obesity

Introduction

The prevalence of childhood obesity is growing. Obesity may cause microangiopathic changes associated with the inflammatory process (1,2). Microvascular changes caused by obesity may result in damage to the optic nerve, retinal nerve fiber layer (RNFL) and choroidal regions, and damage can be revealed in the early period with optic coherence tomography (OCT). The layers of the eye can be visualized in a painless, rapid and non-invasive way using OCT (3). The choroid is one of the most highly vascularized tissues of body and it supplies the outer 1/3 of the retina. The choroid also plays important anatomical and physiological roles, including ocular thermoregulation, the regulation of intraocular pressure and growth factor secretion. Thinning of choroid tissue is a damage indicator (4,5). Previous studies have investigated choroidal thickness in healthy children (6-9). However, few studies have investigated the effect on choroidal and retinal structure in obese children, and their results are inconsistent. The purpose of this study was to investigate changes in choroid tissue thickness in non-diabetic children and adolescents using OCT and the association with metabolic risk factors and pubertal stages.

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Materials and Methods

Consent form was filled out by all participants. The study was approved by the İzmir Tepecik Training and Research Hospital Local Ethics Committee (approval number: 29.12.2014/20). All procedures were conducted in line with the ethical principles of the Declaration of Helsinki.

Inclusion criteria for study and control subjects:

- Age 10-18 years
- No neurological diseases
- No history of ocular disease or surgery
- Children and parents being compliant with examinations
- Subjects with spherical values between -0.50 D and +0.50 D were enrolled.

Exclusion criteria for study and control subjects:

- Presence of diabetes mellitus or any systemic disease
- Use of systemic corticosteroids
- Non-compliance with OCT measurement
- History of ocular trauma and dense media opacities

Seventy-six eyes of 38 obese children and adolescents aged 10.1-17.2 years presenting to the İzmir Tepecik Training and Research Hospital Pediatric Endocrinology Clinic, Turkey, between January 2015 and May 2016, and 80 eyes of 40 healthy children and adolescents aged 10.2-18.0 years were included in the study. The demographic characteristics of the obese and control groups were recorded from their medical files. Body measurements, blood pressure values and pubertal stages were assessed by an experienced pediatric endocrinologist. Pubertal stages were classified based on Tanner's system (10). Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured to the nearest 0.1 kg using a calibrated balance scale with the subject unclothed. Body mass index (BMI) was determined using the formula weight (kg)/height squared (m^2). Established reference values for Turkish children were employed to calculate the standard deviation scores (SDS) for weight, height and BMI (11). Obesity was diagnosed on the basis of World Health Organization definitions (12). Blood pressure was measured in all cases following a period of rest. Measurements were taken at least three times at 10-minute intervals. Individuals with systolic and/or diastolic blood pressure values greater than the 95th percentile were considered hypertensive (13). Blood glucose, insulin and serum lipids in the case of obese subjects were measured using an automatic analyzer from fasting venous specimens collected on that day. Insulin resistance using the homeostasis model assessment insulin resistance (HOMA-IR) was calculated using the formula fasting insulin (μIU/mL) × fasting glucose (mg/dL)/405 (14). All cases underwent detailed eye examinations performed by the same ophthalmologist. Best corrected visual acuities were measured, detailed anterior segment examination was performed with a slit-lamp biomicroscope, intraocular pressure measurement using Goldman applanation tonometry, ocular motility evaluation and optic nerve and retinal examination with a 90 dioptric lens. For pupil dilation, 1% cyclopentolate hydrochloride (Sikloplejin R; Abdi İbrahim İlaç Sanayi, İstanbul) eye drops were applied twice at 5 min intervals, and the mean of three measurements performed 30 min after the final application using an autorefractometer (Canon RK-F1) was taken. Ocular biometry was measured by the LenStar biometer (Haag-Streit, Switzerland)

Choroidal thickness was measured manually using OCT (Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany) in increased imaging depth mode in order to optimize choroidal resolution. Automatic real time eye tracking was performed. Choroidal thickness was measured between the outer border of the hyper-reflective retinal pigment epithelium and the inner border of the choroidal-scleral junction. Measurements were performed bilaterally by two independent masked observers. Choroidal thicknesses were measured using OCT, 500 μm nasal (N500) and 500 μm temporal (T500) from the foveal center (C). Mean choroidal thickness values were recorded. All OCT imaging was performed between 09.00 and 11.00 in order to avoid diurnal variation. Statistical analysis was performed using the choroidal thickness values measured and the mean thereof. Choroidal thicknesses were compared between the control and obese groups. We then investigated correlations between choroidal thickness and age, pubertal stages, body measurements, systolic and diastolic blood pressures, fasting insulin, HOMA-IR and lipid values.

Statistical Analysis

Statistical Package for Social Sciences (SPSS 20.0; IBM, USA) software was employed for statistical analyses. The Kolmogorov-Smirnov test was used to evaluate the normality of the sample distribution. Mean and standard deviation values are provided for all parameters. Pearson correlation analysis was used to assess relations for normally distributed variables. Spearman correlation analysis was applied to non-normally distributed variables. A value of $p<0.05$ was considered statistically significant.

Results

Mean ages were 12.8±2.1 years in the obese group (n=38) and 12.9±2.4 in the control group (n=40). The difference between the two groups was not statistically significant (p=0.99). Also, no significant difference was determined between the two groups in terms of sex distributions, pubertal stages or mean systolic and diastolic blood pressures. BMI-SDS was 3.0±0.4 in the obese group compared to 0.5±0.4 in the control group (p<0.0001). Fasting blood glucose values were within normal limits in both groups (control group: 82.1±8.8 mg/dL, obese group: 85.3±9.9 mg/dL, p=0.65). As anticipated, morning fasting insulin and HOMA-IR values were statistically significantly higher in the obese

individuals compared to the controls (obese group fasting insulin: 19.6±9.8, control group fasting insulin: 8.3±3.1 mIU/mL, p=0.02, obese group HOMA-IR: 4.7±2.7, control group HOMA-IR: 1.9±0.7, p=0.01). There was no difference between the two groups in terms of serum lipid levels. Clinical and laboratory characteristics of the obese and control groups are shown in Table I. Between the axial length measurement in study (22.7±0.6 mm) and control (22.8±0.5) groups, there was no statistically significant difference (p=0.211). No statistically significant difference was determined between sex and both eyes in terms of choroidal thickness values measured using OCT (p=0.81). When the central, nasal and temporal quadrants were assessed individually in terms of choroidal thicknesses, choroidal thinning was observed in all quadrants in the obese group compared to the controls, but the difference was not statistically significant. However, mean choroidal thickness values were 284.4±34.9 µm (range, 230-378 µm) in the obese group and 316.3±39.7 µm (range 293-348 µm) in the control group. This difference was statistically significant (p=0.018). Choroidal thickness values in the study groups are shown in Table II. The relations between clinical and laboratory variables and choroidal thickness were analysed. Age and pubertal stage were positively correlated with choroidal thickness, although no statistical significance was determined (p>0.05). No correlation was determined between choroidal thickness and blood pressure, serum fasting glucose, HOMA-IR or lipid levels. In the obese group, choroidal thickness decreased as BMI-SDS values increased (r=-0.390, p<0.0001). In the control group, although negative correlation was observed between increasing BMI and choroidal thickness, this correlation was not statistically significant (r=-0.112, p=0.079). Pearson correlation analysis results between choroidal thickness and clinical and laboratory data are shown in Table III.

Table I. Clinical and laboratory characteristics of the study groups

Clinical or laboratory characteristics	Control (n=40)	Obese (n=38)	p value[a]
Gender (male/female)	21/19	18/20	0.81[b]
Age (years)	12.9±2.4	12.8±2.1	0.99
Puberty stage (pre-pubertal/pubertal)	11/29	10/28	0.98[b]
BMI-SDS	0.5±0.4	3.0±0.4	0.000
Systolic BP (mmHg)	106.1±9.1	111.8± 9.4	0.19
Diastolic BP (mmHg)	66.3±6.7	69.2±9.3	0.28
Fasting glucose (mg/dL)	82.1±8.8	85.3±9.9	0.65
Fasting insuline (mIU/mL)	8.3±3.1	19.6±9.8	0.02
HOMA-IR	1.9±0.7	4.7±2.7	0.01
Triglycerides (mg/dL)	125.3±62.0	138.5±72.9	0.07
LDL-cholesterol (mg/dL)	89.3±18.6	96.9±25.7	0.06
HDL-cholesterol (mg/dL)	46.1±11.3	43.2±10.3	0.72

[a]Student's T test, [b]Chi-square test
BMI-SDS: Body mass index-standard deviation score, BP: Blood pressure, HDL: High density lipoprotein, HOMA-IR: Homeostasis model assessment of insulin resistance, LDL: Low density lipoprotein

Discussion

The choroid, one of the most highly vascularized tissues in the body, is particularly susceptible to diseases leading to microvascular complications. Like other ocular structures, choroidal thickness may vary throughout childhood. Examination of choroidal thickness provides important information in the diagnosis and management of various ocular and systemic diseases leading to chorioretinal inflammatory changes (4,15). Previous studies have shown choroidal thinning independent of stage of retinopathy in Type II diabetes (16). Lower choroidal thickness has also been observed compared to healthy controls in several diseases, such as hypertension, rheumatoid arthritis, systemic lupus

Table II. Choroidal thickness in control and obese children

Choroidal thickness	Control (n=40)	Obese (n=38)	p value
Central (C) (µm)	320.5±40.0	288.5±35.0	0.112
Nasal (N500) (µm)	303.5±39.1	271.4±34.6	0.068
Temporal (T500) (µm)	325.1±40.0	293.4±35.1	0.082
Avarage (µm)	316.3±39.7	284.4±34.9	0.018

Table III. Correlation analysis of choroidal thickness with the clinical and laboratory parameters of the study groups

	Control		Obese	
Clinical or laboratory characteristics	r	p value	r	p value
Age	0.161	0.157	0.197	0.099
Puberty stage	0.142	0.214	0.156	0.116
BMI-SDS	-0.112	0.079	-0.390	0.000
Systolic BP	-0.159	0.164	-0.165	0.570
Diastolic BP	-0.145	0.209	-0.166	0.124
Fasting glucose	0.029	0.227	0.155	0.308
Fasting insulin	0.077	0.566	0.172	0.093
HOMA-IR	0.211	0.099	0.290	0.059
Triglycerides	0.014	0.731	0.189	0.231
LDL-cholesterol	0.073	0.632	-0.174	0.210
HDL-cholesterol	0.056	0.755	0.094	0.178

BMI-SDS: Body mass index-standard deviation score, BP: Blood pressure, HDL: High density lipoprotein, HOMA-IR: Homeostasis model assessment of insulin resistance, LDL: Low density lipoprotein

erythematosus, and obstructive sleep apnoea (17-20). Chronic microvascular systemic inflammation is implicated in the development of all these diseases. Obesity and severe obesity have become an increasingly severe public health problem in children in recent years (21,22). Obesity can lead to systemic and ocular complications. The RNFL and the thickness of choroid tissue can be affected by obesity. Previous studies have shown that obesity causes a thinning in RNFL thickness in children (23,24). Low level systemic inflammation is known to occur in obesity (25-27). For may also have the potential to affect the choroid layer. Previous studies have reported normative data concerning choroidal thicknesses in healthy children and adolescents. Read et al. (6) reported a mean subfoveal choroidal thickness of 330±65 mm (range, 189-538 mm) in 194 healthy children aged 4-12. In addition, they determined normal choroidal thicknesses of 312±62 mm at age 4-6, 337±65 mm at age 7-9, and 341±61 mm at age 10-12. Based on these findings, they reported that choroidal thickness increases from early childhood. We also determined a positive correlation, although not at a statistically significant level, between age and pubertal stage and choroidal thickness. In The Copenhagen Child Cohort 2000 Eye Study of 1323 children aged 11-12, Li et al. (7) determined a mean subfoveal choroidal thickness of 369±81 mm, but determined no relation between choroidal thickness and sex. We also observed no significant difference between the sexes in terms of choroidal thickness values (p>0.05). Bidaut-Garnier et al. (8) measured a mean subfoveal choroidal thickness of 341.96±74.7 mm and reported that the choroid was thinner in the nasal region than in the temporal region. In their study of healthy children under 18, Lee et al. (9) determined greater choroidal thicknesses in the macular region in all quadrants investigated compared to adults. They also emphasized that pediatric subfoveal choroidal thickness is disposed to thinning with age and refractive error. Subjects with refractive error were excluded from our study. The mean choroidal thickness measured with OCT in the healthy children and adolescents we enrolled as the control group was 316.3±39.7 µm. These values are in agreement with previous studies. Few studies have investigated choroidal thickness in obese children, and their findings are inconsistent. In their study of obese children aged 5-15, Erşan et al. (28) determined a mean choroidal thickness of 301.95±56.72 mm in the control group and of 270.20±56.13 mm in the obese group (p=0.014). They reported that this thinning might be due to microvascular complication. In contrast to that study, Bulus et al. (29) reported a mean choroidal thickness of 348.43±73.21 mm in the control group and of 385.77±6.09 mm in obese children. Choroidal thickness increased in the obese group (p=0.017). The authors suggested that choroidal thickening might be attributed to obesity-related vascular changes and increased adipocyte tissue. We observed thinning of choroidal thickness in all the measured quadrants in obese children compared to the healthy controls. Mean choroidal thicknesses were 316.3±39.7 µm (range 293-378 µm) in the control group and 284.4±34.9 (range, 230-360 µm) in the obese group. This difference in choroidal thicknesses was statistically significant (p=0.018). Thinning in choroidal thickness in obese cases may be associated with chronic systemic inflammation and microvascular disturbance (25-27). Oxidative stress and hypoxia may occur in obesity. In addition, changes in leptin and adipokines levels, adipose tissue dysfunction and insulin resistance may also occur. The production of inflammatory cytokines and reactive oxygen species increases due to the oxidative stress. Apoptosis and tissue necrosis are then triggered as a result. Studies have shown that oxidative stress may be a significant factor in cell death (30-33). Vascular endothelial damage, oxidative stress and chronic inflammation may impair the permeability and nutrition of microvascular structures. This may then give rise to thinning of choroid tissue. Choroidal thickness measurement may be affected by diurnal variation. We performed our measurements at the same time interval, between 09.00 and 11.00, in order to avoid diurnal fluctuation. The border of the chorioscleral junction was measured manually. Choroid OCT images were taken by two independent masked observers. Studies concerning the reliability and repeatability of this manual measurement method have reported powerful correlation between measurements and the individuals performing them (34-36). There are a number of limitations to this study. Plasma levels of inflammatory mediators such as adiponectin, leptin and interleukin-6 could not be measured. However, the metabolic and vascular effects of these mediators were evaluated indirectly by measuring insulin, lipid and glucose levels. No studies have shown whether changes in choroidal thickness values will occur through weight loss in obese individuals. Prospective observational studies involving weight control are needed in order to reveal more clearly the effect of obesity, and therefore the chronic inflammatory process, on choroidal tissue.

Conclusions

In conclusion, this study shows a lower mean choroidal tissue thickness in obese children and adolescents compared to healthy controls. In addition, the decrease in choroidal tissue thickness becomes more marked as BMI-SDS values increase. An increase in adipose tissue may result in a susceptibility to retinal damage. Long-term observational studies are now needed in order to confirm the findings of this cross-sectional study.

Ethics

Ethics Committee Approval: The study was approved by the İzmir Tepecik Training and Research Hospital Local Ethics Committee (approval number: 29.12.2014/20).

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ö., H.Ö, Concept: B.Ö., H.Ö, G.Ç., Design: B.Ö., H.Ö, G.Ç., Data Collection or Processing: B.Ö., H.Ö, G.Ç., Analysis or Interpretation: B.Ö., H.Ö, G.Ç., B.D., Literature Search: B.Ö., H.Ö, G.Ç., B.D., Writing: B.Ö., H.Ö, G.Ç.

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Original Article

The Effect of Exercise on Bone Mineral Density in Patients with Down Syndrome

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ABSTRACT

Aim: Down syndrome (DS) is a predisposing factor for osteoporosis. The aim of this study is to investigate the effect of resistance and jumping exercises on the bone mineral density (BMD) and bone mineral content (BMC) of patients with DS.

Materials and Methods: DS and normal individuals aged between 10 and 30 years old were randomized into an exercise group and a control group: a DS without-exercise group (n=15), DS exercise group (n=17), control without-exercise group (n=18), and control exercise group (n=20) were designated. A supervised 45-minute period of exercise was given to the DS exercise group and control exercise group three times per week for six months. The lumbar total and femoral neck BMD and BMC were measured both before and after six months of exercise using dual-energy X-ray absorptiometry.

Results: The change in BMD and BMC over six months did not differ significantly between the exercise and control groups. The BMD and BMC of the DS exercise group decreased in the vertebral and femur areas over six months. The study on resistance and jumping exercises over six months did not lead to a significant change in the total BMD and BMC in the vertebral and femoral neck areas of the patients with DS.

Conclusion: The decrease in BMD and BMC over six months in the DS exercise group is not a predictable result.

Keywords: Down syndrome, exercise on bone mineral density, childhood, adult

Introduction

Physical activity is important for bone formation in order to maintain bone mass. Exercise training has positive effects on peak bone mass during childhood. An active life style and high-intensity physical activity are recommended to reduce the risk of osteoporosis in adulthood (1). Down syndrome (DS) is one of the most common types of genetic abnormalities related to disability. Hypotonia and joint hyper-flexibility are skeletal problems that cause morbidity in people with DS (2). Osteoporosis risk increases in people with DS as they age (3). However, the life expectancy of people with DS has increased recently due to improvements in health care. Mechanotransduction is bone formation due to mechanical stress. Mechanical stimuli cause the stimulation of osteocyte proliferation and the differentiation of osteoblasts, collagen synthesis and bone mineralization (4). For these reasons, we preferred resistance training and intensive physical activities as the exercise type for our study. Resistance training is effective in maintaining and increasing bone mineral density (BMD) (5). Intensive physical activities, such as basketball, jumping, and gymnastics, are effective in increasing vertebral

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and hip BMD while physical activity is important for gaining and providing bone mass (6,7). Children with DS have low BMD. Hypotonia and decreasing muscle strength in DS may lead to decreased bone mass. The purpose of this study is to assess the effect of loading exercise treatment on the BMD of patients with DS.

Materials and Methods

Design

The initial study population consisted of 40 male and 40 female subjects (10-30 years old); 40 with DS; and 40 healthy controls. The mildly mental retarded DS participants, were recruited from two special schools by advertisement, and the healthy group attended high schools and universities in İzmir. The treatment group was divided into an exercise group and a control group. The control group consisted of 20 DS (mean age 19.80±4.1 years) and 20 healthy people (mean age 20.0±4.9). The exercise group consisted of 20 DS with a mean age of 22.01±2 years and 20 healthy people with a mean age of 20.0±4.2 years. The study was completed with 70 subjects in six months [two subjects in the healthy control group and five subjects in the DS control group did not accept the second dual-energy X-ray absorptiometry (DXA) measurement; three subjects in the DS exercise group could not complete the exercise study]. DXA measurements were performed to evaluate the benefit of the exercise treatment after six months of training. The third DXA measurement could not be performed in seven subjects in the healthy control group and eight subjects in the healthy exercise group due to not being available at the given time. The study was completed with 55 subjects within 12 months. Patients in the DS group were living with their families under good dietary and environmental conditions. The individuals in all groups were euthyroid. The DS, healthy men and women neither had systemic diseases nor were on any medication, vitamin or mineral affecting bone mineralization. The blood calcium and 25-hydroxyvitamin D (25 OHD) levels of participants were not evaluated before the study. Musculoskeletal deformities were not ascertained. All participants were non-smokers and they did not use alcohol during the study period. Informed consent was obtained from all subjects and their parents. The study was approved by the Ege University Local Ethics Committee (approval number: 08-12.1/14).

Participants, Therapists

The loading and resistive exercise training program consisted of three 45-minute exercise sessions per week for six months. The training included the following exercises: back extension exercise, squatting, resistance hip abduction, trunk flexion, bridge, side bridge, abdominal curl-up, jumping and upper extremity resistive exercise. The lifting weight was determined as 50-70% of their one repetition maximum and was not increased during the study. Jumping exercises were repeated 100 times per session. The resistive training was repeated 10 times for each exercise type. The exercise treatment was given to the healthy training group as a home program that was followed up on via phone call from the supervisor physician. The exercise group with DS was trained by the supervisor physician during the first three months and was trained by a physical education teacher while simultaneously being monitored by the supervisor physician during the second three months. The exercise treatment for subjects with DS was given in their schools.

Outcome Measures

The bone mineral content (BMC) g, BMD gr/cm^2 and bone area cm^2 were determined using DXA (HOLOGIC 4500 A). The BMD, BMC and bone area were measured at the beginning of the study, at six and twelve months after the start. BMD, BMC and bone area of the lumbar spine (L1-4, anteroposterior), femoral neck, and femur total were derived using spine software and femur software. In our laboratory, the intra-class correlations for repeated measurements, including the subregions, were 0.933 for femoral neck BMD, 0.975 for femoral neck BMC, 0.880 for femur total BMD, 0.985 for femur total BMC, 0.990 for lumbar total BMD and 0.996 for lumbar total BMC.

Statistical Analysis

The comparisons between the patients and controls were made using the t-test or the Mann-Whitney U test in the case of significant deviation from the normal distribution. A one-way analysis of variance (ANOVA) was used to test the hypotheses regarding the equality means between the groups for the following subject characteristics: age, body weight, height and body mass index (BMI). The Bonferroni post-hoc test was used to determine the differences between the groups. Intention to treat analysis was used for missing data at the first year of the study, with the sixth month's data of the missing subjects used for the analysis.

Results

The characteristics of the subjects, such as weight, age, and BMI, were similar at the baseline and at the completion of the study. The height of the DS control group was significantly lower than the two healthy groups (Table I). The baseline lumbar spine BMD and BMC as well as the femoral neck and total BMD and BMC of the DS group were significantly lower than those of the healthy groups (Table II). During the study period, BMD and BMC values of the lumbar spine and femoral neck showed no significant difference between patient and control groups, and no significant difference was observed in any of these three locations between the initial and post-training values (Table II). The BMD values in the lumbar spine, femoral neck and total femur decreased in the DS exercise

Table I. Demographic characteristics of the study groups

	Normal control	Normal exercise	DS control	DS exercise
	(n=18)	(n=20)	(n=15)	(n=17)
Age	20.11±5.21	19.65±4.49	19.60±5.93	22.41±2.69
Initial weight	63±14.49	59.5±18.94	58.74±18.64	64.2±13.4
Weight at 6th month	64.4±14.5	60.6±17.5	58.6±16.5	64.54±13.5
Weight at first year	65.4±14.2	58.9±15.3	59±17	64.7±12
Initial height	165.8±2.7	165.6±2.0	151.2±2.1*	158.4±1.4
Height at 6th month	166.4±2.6	165.9±1.9	151.5±2.0*	159±1.2
Height at first year	168.7±2	164.4±2	151.1±1*	158.5±2
Initial BMI	22.6±0.74	21.4±1.1	25.1±1.7	25.4±1.0
BMI at 6th months	23.0±0.80	21.7± 0.9	25.3±1.8	25.4±1.0
BMI at first year	23.4±1	21.4±0.6	25.6±1.4	25.5±0.9

BMD: Bone mineral density, BMC: Bone mineral content, BMI: Body mass index, DS: Down syndrome
*p<0.05

group at the sixth month of the study; despite this, these values were increased non-significantly at the sixth month in the other three groups. The lumbar spine and femoral neck BMC values of the DS exercise group decreased at the sixth month of the study, while the BMC values of the other three groups increased (Table II). The changes in the BMC values were not significant. The BMD and BMC values of the DS exercise group, which decreased at the sixth month, showed a non-significant increase at the end of the first year of the study.

Discussion

Several studies have determined that individuals with DS have reduced BMD in comparison to healthy controls (8,9). Several investigators determined that adynamic bone formation takes place in people with DS (10). The present study shows that people with DS have lower BMD and BMC in the spine and hip than to normal controls, which is in accord with the results of previous studies. In the study of Angelopoulou et al. (11), the lumbar BMD in men and women with DS were 25% lower than those of normal controls; Baptista et al. (12) reported that subjects with DS had 20% lower lumbar BMC than the healthy subjects. In our study, the lumbar BMC, femoral neck and femur total were lower in patients with DS compared with normal controls. Angelopoulou et al. (11) observed that the muscle strength of mentally retarded people with DS was lower than that of healthy people and mentally retarded people without DS. Matute-Llorente et al. (13) determined that adolescents with DS who perform longer periods of physical activity have higher BMD Z-scores than those who perform less physical activity. Experimental studies indicate that high-impact activities should lead to high mechanic stimuli and thus be over the osteogenic index (14). It is reported that the jumping exercises are good for femur and vertebra BMD (14). In the study of Fuchs et al. (15), the femoral and vertebral BMD of healthy subjects were higher than those of the control group, who jumped over the 61 cm sized box; the ground reaction force was determined to be 8.8 more than their body weights. Witzke et al. (16) observed that the femoral neck and trochanter BMD were higher in the exercise group than in the controls after a plyometrics jumping exercise study that took place over nine months, with exercises being completed three times a week and 360 times per exercise session. The present study involved jumping exercises 100 times per exercise session, three times a week. The ground reaction force was determined to be 3-4 times more than the subjects' body weights with the jumping style used in our present study as in previous studies. Experimental studies indicate that the load on an L4-5 vertebra is about 2200 N during the two-leg-raising and trunk flexion positions. At the same time, the load on the femur and lumbar vertebra is six times more than the body weight during the squat exercise (17). Ferry et al. (18) determined a significant increase at the lumbar spine, total hip BMC and lumbar spine BMD after a one-year physical training program study. Nichols et al. (19) observed an increase in BMD at the femoral neck but not with the lumbar total of adolescent girls during a 15 month resistance exercise program. In the study of González-Agüero et al. (20), which consisted of 25 minute sessions per week

Table II. Comparison of bone mineral density between groups

		Normal control	Normal exercise	DS control	DS exercise
		n=18	n=20	n=15	n=17
Lumbar total BMD	Initial	0.936±0.165	0.916±0.14	0.795±0.149	0.871±0.136
	6th month	0.964±0.156	0.942±0.129	0.815±0.136	0.868±0.149
	First year	0.960±0.150	0.950±0.150	0.830±0.120	0.870±0.130
Femur neck BMD	Initial	0.827±0.142	0.825±0.114	0.794±0.124	0.827±0.129
	6th month	0.844±0.152	0.838±0.135	0.799±0.15	0.814±0.129
	First year	0.850±0.130	0.840±0.120	0.810±0.100	0.820±0.120
Femur total BMD	Initial	0.942±-0.182	0.921±0.121	0.844±0.133	0.877±0.129
	6th month	0.956±0.156	0.935±0.133	0.862±0.169	0.876±0.136
	First year	0.968±0.156	0.930±0.120	0.870±0.169	0.880±0.136
Lumbar total BMC	Initial	57.96±19.38	54.38±15.00	42.66±12.52	50.2±12.93
	6th month	59.84±18.07	56.21±15.27	44.77±12.27	49.89±11.99
	First year	60.20±17.04	57.33±15.33	46.15±15.27	50.80±12.03
Femur neck BMC	Initial	4.33±1.05	4.21±13.27	3.54±0.66	3.78±0.89
	6th month	4.49±1.10	4.46±0.98	3.71±0.77	3.68±0.92
	First year	4.49±1.10	4.49±0.94	3.79±0.72	3.76±0.72
Femur total BMC	Initial	3.36±11.72	32.12±9.06	25.44±8.01	28.14±7.46
	6th month	33.72±11.43	33.57±9.88	26.26±7.86	28.58±7.83
	First year	27.33±17.03	31.69±10.08	26.78±7.00	28.48±7.48
Mean ± SD	-	-	-	-	-

BMD: Bone mineral density, BMC: Bone mineral content, SD: Standard deviation, DS: Down syndrome

of a conditioning and plyometric jump training program for 21 weeks, increases in the total and hip-region BMC in the DS exercise group were found compared with the DS non-exercise group in the training program. In this study, we considered the previous exercise studies on bone formation and decided to perform the jumping and resistance exercise experimentation for patients with DS in order to increase their BMD and BMC. However, the results of the present study did not comply with the previous studies. We did not find a significant increase in the lumbar spine and femoral BMD and BMC in the exercise group. Chilibeck et al. (21) did not observe an increase in BMD at the lumbar spine and in the femur of young adults during a 20-week resistance exercise study. It was reported that the osteogenic effect on bone is greater when implementing the jumping and resistance exercises together (14). We performed both jumping and resistance training for two groups. Soomro et al. (22) did not observed any significant difference on young females who were given osteoporosis preventing exercises versus walking. They have postulated that the short duration of the study and small sample size resulted in a non-significant outcome. In the present study, we observed a non-significant increase in the lumbar total, femoral neck and total BMD and BMC in the healthy study group, healthy control group and control group with DS, but we observed a non-significant decrease in the lumbar total and femoral neck BMD and BMC in the study group with DS at the sixth month of the study. Matute-Lorente et al. (23) observed non-significant increase in BMC and BMD values with DS while healthy controls showed more significant changes after undergoing whole body vibration training. They suggested that exercise training might have higher bone resorption and lower bone formation. Puustjärvi et al. (24) demonstrated that a reorganization in bone collagen with mechanic loading increases bone strength; however, they reported that a BMD decrease does not affect bone strength after mechanic loading (25). No study has researched the collagen structure of DS patients and the relationship between collagen structure and BMD. McKelvey et al. (26) determined that adults with DS had lower bone

formation markers than healthy adults. They claimed that these markers are significantly associated with low BMD in people with DS. It was supposed that the insufficient trabecular connection in bone in people with DS is due to a decreased message transmission between bone cells, which causes a mineralization defect (25). In the present study, the non-dynamic bone structure and lower bone formation of the subjects with DS might have caused an insufficient response of the bone to mechanic loading. The femoral neck, which contains less trabecular bone than a vertebra, might be insufficient with respect to the micro damage during the jumping exercise sessions. The exercise protocol was taught to the healthy exercise group as a home program. The healthy exercise group was controlled by the researchers via phone call on a weekly basis which decreased the study value. Another limitation of our study is the patient number. We could not reach the patient number that was needed. In our study, we observed a non-significant increase in all the parameters of the healthy groups and DS control group, while there was a non-significant decrease in the BMD and BMC of the DS exercise group. In the present study, we could not analyze the bone formation markers, blood calcium and 25 OHD.

Conclusion

Individuals with DS have an increased risk of osteoporosis. Weight bearing exercises might be designed and applied considering their special musculoskeletal structure in order to prevent osteoporosis. Despite the limitations of this study, the present study can be useful in the making of specific exercise protocols for patients with DS. More detailed and longer duration studies that contain more subjects are needed to express the frequency and intensity of exercise required for people with DS who are at risk for osteoporosis and low bone density.

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Ethics

Ethics Committee Approval: The study was approved by the Ege University Local Ethics Committee (approval number: 08-12.1/14).

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.D., C.Ö., Concept: G.D., C.Ö., Design: G.D., C.Ö., F.Ö., Data Collection or Processing: G.D., S.H., Analysis or Interpretation: G.D., M.D., C.Ö., S.H., Literature Search: G.D., M.D., Writing: G.D., M.D., C.Ö., S.H.

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Original Article

Evaluation of Child Cases Admitted for Tick Bite and Tick Species in İstanbul

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ABSTRACT

Aim: The Crimean-Congo Haemorrhagic Fever (CCHF) is a tick-borne infection that has a high mortality. In Turkey, the total number of cases reported between 2002-2014 was 9.069 and 440 of them died. The aim of this study is to evaluate the demographic characteristics of the children with the complaint of tick bite and to determine the species of the ticks seen in İstanbul.

Materials and Methods: A hundred sixty two tick bite cases were analyzed with respect to demographic, clinical and laboratory findings between January and December 2014. The blood samples for whole blood count, prothrombin time, activated partial thromboplastin time, alanine aminotransferase, aspartate aminotransferase and creatinine were obtained from all cases and they were followed up for 10 days. The ticks removed from patients were classified by the Department of Parasitology in the Veterinary Faculty of İstanbul University.

Results: The mean age of the patients was 6.1±3.7 years and 76% of them were male. Eighty four (52%) of the cases had additional complaints other than tick bite. The most frequently complaints were nausea/vomiting (26%), fever (19%) and cough (14%). We found that 34 (20%) of them had abnormal laboratory results such as elevated liver enzymes, leukocytosis, leukopenia, thrombocytopenia and prolonged prothrombin time. August was the month in which most patients applied (56 cases, 34%). The regions of body that were most bitten by the ticks were the extremities (35.8%). The total number of larvae, nymphs and adult ticks were found as 4, 88 and 14 respectively.

Conclusion: This study showed that the tick species in the İstanbul province were largely different from the species causing CCHF. Although the incidence of CCHF in İstanbul is lower than in other regions of Turkey, children and their families who live in or travel to rural areas in Istanbul should be informed about this disease.

Keywords: Crimean-Congo Haemorrhagic Fever, child, tick bite

Introduction

The Crimean-Congo Haemorrhagic Fever (CCHF) is a tick-borne infection which is characterized by fever, haemorrhage, liver dysfunction and it has a high mortality. CCHF is caused by CCHF virus (CCHFV) which is a member of the Nairovirus genus from the Bunyaviridae family and it is transmitted to humans by infected ticks (1). In 1944, a haemorrhagic fever disease was first described in the Crimea. In 1956, it was named as CCHF after the virus was isolated from a feverish patient in the Congo (2). CCHF has been reported over a wide area including Asia, Africa and Europa similar to the distribution of the ticks. However, most of the cases are seen around the axis extending from South Russia and Black Sea region to Africa (3). The first case in Turkey was identified in Tokat province in 2002. The majority of the cases were reported from the Central Anatolia and Eastern Black Sea regions (4,5). In Turkey, the total number of cases reported

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between 2002 and 2014 was 9.069 and 440 of them died. While the mortality rate is about 5% in Turkey, worldwide the mortality rate has been reported between 20-50% (2,6). The aim of this study is to evaluate the demographic characteristics of the children with the complaint of tick bite and to determine the species of the ticks seen in İstanbul.

Materials and Methods

This study was performed in the Child Emergency Department of Kanuni Sultan Süleyman Training and Research Hospital between January and December 2014. A hundred sixty two cases of tick bites were retrospectively analyzed in terms of demographic, clinical and laboratory findings. The cases were evaluated using the algorithm of the Ministry of Health's "approach to people with tick bites". Firstly, the tics were removed with pincers from the patients and the wound was cleaned with antiseptic solution. The patients were evaluated for clinical signs and symptoms. Afterwards, whole blood count, prothrombin time (PT) activated partial thromboplastin time, international normalized ratio, alanine aminotransferase, aspartate aminotransferase and creatinine were obtained from all cases. The patients were followed up for 10 days and the findings were recorded. The ticks removed from patients were classified according to morphological characteristics by the Department of Parasitology in the Veterinary Faculty of İstanbul University.

Results

A hundred sixty two patients who were admitted to our hospital were retrospectively evaluated. The mean age of the patients was 6.1±3.7 years (range 0.5-16 years), 76% of them were male. When the patients were analyzed according to age groups, 58 of them (35%) were between 0-4, 62 (38%) were between 4-8, 30 (19%) were between 8-12 and 12 (7%) were between 12-16 years of age. The mean weight of the patients was 22.2±11.8 kg (range 8-70). Seventy eight (48%) of the patients had no complaint except for the tick bite. However, 84 (52%) of them had additional complaints; nausea and vomiting (22; 26%), fever (16; 19%), fever and cough (12; 14%), erythema (8; 10%), fatigue (6; 7%), abdominal pain (6; 7%), headache and sore throat (6; 7%), diarrhea (4; 5%), arthralgia (3; 4%) or skin eruption (1; 1%). Although the mean values of the laboratory parameters are within the normal range, we found that 34 (20%) of them had abnormal results; 12 of them had slightly elevated liver enzymes, 7 of them had leucocytosis, 7 of them had slightly prolonged PT, 4 of them had leucocytosis and elevated liver enzymes, 3 of them had thrombocytopenia and 1 of them had leukopenia. In follow up, all of these laboratory abnormalities improved (Table I). When we evaluated the diagnosis of the patients, we found that 53 (33%) of them had additional diseases; 35 of them had upper respiratory tract infection, 13 of them had acute gastroenteritis, 3 of them had superficial skin infection, 1 of them had urticaria and 1 of them had rubella. None of the patients developed the clinical and laboratory findings related with CCHF in follow up.

When the admission time was evaluated, it was found that there were no patients who applied to hospital in December or January. Most patients applied in August (56

Table I. Demographics and laboratory results of the patients

	n (%)
Age (y) (Mean ± SD)	6.1±3.7
0-4	58 (35%)
≥4-8	62 (38%)
≥8-12	30 (19%)
≥12	12 (7%)
Gender n (%)	
Male	123 (76%)
Female	39 (24%)
Weight (kg) (Mean ± SD)	22.2±11.8
Body regions bitten by ticks	
Extremities	58 (35.8%)
Head-neck	52 (32%)
Trunk	45 (27.7%)
Genital-groin	7 (4.3%)
Complaint	
Tick bite only	78 (48%)
Additional complaint	84 (52%)
Leukocyte (mm^3)	10.297±2.845
Platelet (mm^3)	287.425±68.419
ALT (UI/L)	16.5±4.2
AST (UI/L)	29.8±8.6
Creatinine (mg/dL)	0.54±0.1
PT (sec)	12.6±0.9
aPTT (sec)	29.2±3.1
INR	1.0±0.1

INR: International normalized ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, aPTT: Activated partial thromboplastin time, SD: Standard deviation, PT: Prothrombin time

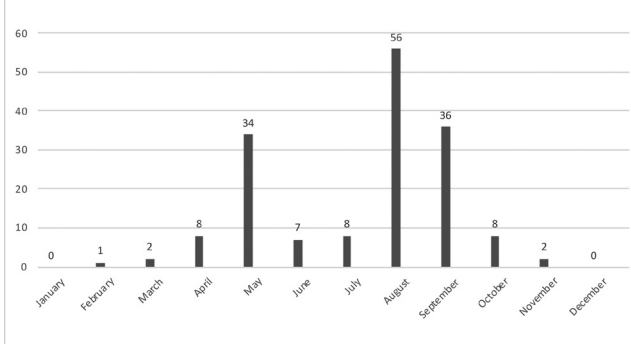

Figure 1. Number of case per month

cases, 34%), September and May were second (36 cases, 22%) and third (34 cases, 20.9%) respectively (Figure 1). All of the cases except one had contacted with ticks in rural areas in the İstanbul region. A hundred thirty one of the ticks (81%) were removed in hospital and 31 (19%) of them were removed at home. When the body regions which were bitten by ticks were evaluated, it was found that 52 of them (32%) were in the head-neck region, 45 of them (27.7%) were in trunk, 58 of them (35.8%) were in the extremities and 7 of them (4.3%) were in genital-groin region (Table I). A hundred six ticks that were removed from the cases were able to be classified but 56 samples were excluded from classification due to fragmentation. The classified ticks included 14 (13%) adults, 88 (83%) nymphs and 4 (4%) larvae. The total number of larvae and nymphs were 92; 43 (47%) of them were *Hyalomma* spp., 38 (41%) were *Ixodes* spp., 10 (11%) were *Rhipicephalus* spp. and 1 (1%) was *Haemaphysalis* spp. (Table II). There were 14 adult ticks including 1 *Hyalomma marginatum*, 1 *Rhipicephalus sanguineus*, 4 *Ixodes ricinus*, 6 *Rhipicephalus turanicus* (Table III).

Discussion

Although, epidemics of CCHF have been reported from different countries in Europa, Africa and Asia in previous years, the first case in Turkey was not reported until 2002. In the following years, the number of reported cases from Turkey increased. The total number of cases between 2002 and 2014 was 9.069, 440 of these have died (1,2,4,6). CCHF can be transmitted to humans via exposure to blood or body fluids of patients or infected animals or rarely by nosocomial transmission as well as by infected tick bite. For this reason, health workers and patients' close relatives are considered as an at risk group as well as farmers and rural residents (2). Because the ticks are inactive in low temperatures, CCHF is more common in warm weather months, especially between April and October (6-8). The admission time of our cases was consistent with this, although the number of cases declined in June and July. We think that this decline in the number of cases is related to the month of Ramadan. This is because most people in İstanbul go to their hometown or holiday places for the Ramadan holiday every year. The course of the disease includes incubation, pre-haemorrhagic, haemorrhagic and convalescent periods. The duration of the incubation period ranges from 2 to 12 days. Clinical symptoms include fever, headache, chills, myalgia, vomiting, diarrhea, conjunctival haemorrhage, rash, petechiae and purpura. In the following periods, unconsciousness and coagulopathy may develop and result in coma and death (2,6). The basis of treatment is supportive care. Although, ribavirin is the only drug used in the treatment of this disease, its efficacy is controversial (9-12). In our study, 84 of the patients had additional signs and symptoms and 34 of them had abnormal laboratory results but none of the clinical and laboratory findings were severe. Although these patients have at least one or two risk factors, all of these resolved in follow up. Also, additional diseases that were detected in 53 of the patients were treated and followed closely. The previous studies reported that the majority of adult cases with tick bite lived in rural areas and engaged in agriculture and animal husbandry (4,6,9). Similarly, the majority of children with tick bite belonged to families who lived in rural area (8,13,14). All of the cases in our study lived in rural areas or participated in weekend trips to these places and 73% were under 8 years of age. The preventive measures include staying away from the regions where the ticks live and using closed shoes and clothes (2,15). In accordance with these recommendations, when the areas of attachment of the ticks were examined, we found that unprotected body parts such as the head, neck and extremities are mostly affected. *Hyalomma genus* of Ixodes ticks, especially *Hyalomma marginatum* (H.m.) *marginatum*, *H.m. rufipes*, *H. anatolicum anatolicum* have been reported as the main vector and reservoir for CCHF (2,4,6). In the previous studies on tick fauna, 46 tick species were found in Turkey and 38 of them belong to the Ixodidae family. *Ixodes* spp, *Hyalomma* spp., *Haemaphysalis* spp., *Dermacentor* spp. and *Rhipicephalus* spp. were the most common species of ticks. However, *H.m. marginatum* is the main vector for CCHF in Turkey (14,16-21). In a study in İstanbul, it was found that 27% of the tics were *Ixodes ricinus* and 50% were *Hyalomma aegyptium* (22). Similarly, in a study in the Thrace region, it was found that the main vector responsible for bites was *H. aegyptium* (23). Also, in another study conducted on the

Table II. The classification of larvae and nymphs

Genus	Larvae	Nymph	Total
Hyalomma spp.	1	42	43 (47%)
Ixodes spp.	3	35	38 (41%)
Rhipicephalus spp.	0	10	10 (11%)
Haemaphsalis spp.	0	1	1 (1%)
Total	4	88	92 (100%)

Table III. The classification of adult ticks

Species	Adult male	Adult female	Total
Hyalomma marginatum	1	0	1 (7%)
Ixodes ricinus	0	4	4 (29%)
Rhipicephalus turanicus	6	0	6 (43%)
Rhipicephalus sanguineus	1	0	1 (7%)
Dermacentor niveus	1	0	1 (7%)
Haemapyhsalis otophila	0	1	1 (7%)
Total	9	5	14 (100%)

tortoises in the Thrace region, 81% of the ticks were found to be *H. aegyptium* (24). Similar to the other studies in Turkey, *Hyalomma* spp. and *Ixodes* spp. were the most common genus in our study. When the adult ticks were evaluated, *Rhipicephalus turanicus* was the most common species and *H. marginatum* was detected in only one case.

Study Limitation

Our study was carried out in the European region of İstanbul. Therefore, this study may be insufficient to evaluate all of İstanbul.

Conclusion

This study showed that tick species in the İstanbul province were largely different from those species causing CCHF. It is necessary to know the seasonal and regional characteristics of this disease and the geographical distribution of these tick species. Thus, unnecessary investigations and hospitalization can be avoided. Although the incidence of CCHF in İstanbul is lower than in other regions of Turkey, children and their families who live in or travel to rural areas in İstanbul should be informed about this disease and its prevention methods.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article

Serum Antioxidative Enzymes Levels and Oxidative Stress Products in Children and Adolescents with Type I Diabetes Mellitus

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ABSTRACT

Aim: Type I diabetes mellitus (TIDM) is an oxidative stress condition in addition to being a chronic metabolic disease. In this study, our aim is to investigate the activity of antioxidative enzymes and the products of oxidative stress in children and adolescents with TIDM and compare the findings with those in healthy control subjects.

Materials and Methods: The study enrolled 41 children and adolescents with TIDM (mean age 11.4±3.3 years; 21 female, 20 male) and 25 healthy subjects (mean age 11.3±3.1 years; 8 female, 17 male) with a similar age and gender distribution. Serum samples were obtained to detect the antioxidative enzymes of paraoxonase (PON), arylesterase (ARE), oxidation degradation products of malondialdehyde (MDA) and also zinc which acts as an antioxidant.

Results: We found a significant decrease in PON activity and zinc levels in diabetics compared to the healthy controls (p=0.021; p<0.001, respectively). Zinc was negatively correlated to hemoglobin A1c (r=-0.317, p=0.049). MDA and ARE did not show a significant difference in the TIDM patients compared to the healthy subjects.

Conclusion: Zinc level and PON activity were lower in diabetic children and adolescents. Further studies with larger samples are required to confirm their roles in the following and prognosis of TIDM.

Keywords: Antioxidant, oxidative stress, paraoxonase, Type I diabetes mellitus, zinc

Introduction

Type I diabetes mellitus (TIDM) is the most common metabolic disorder resulting in the destruction of insulin producing pancreatic β-cells by lymphocytic infiltration (1). Genetic predisposition is important in the development of TIDM (2). In addition, some environmental factors such as viral infection, vaccines, low levels of vitamin D and dietary factors during infancy may trigger the development of TIDM in those individuals with genetic susceptibility (2). Some metabolic and physiologic processes lead to reactive oxygen species (ROS) in the body. ROS are highly reactive molecules derived from the reduction of oxygen and can be harmful to some cell structures such as carbohydrates, nucleic acids, lipids and proteins (3). Their elimination is provided by the antioxidant defence system (4). Oxidative stress is the loss of balance between prooxidant and antioxidant systems (5). Oxidative stress may play a role in the pathogenesis of human diseases. Many studies have investigated the relationship between oxidative stress parameters and various

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diseases such as some cancers, cardiovascular disease, Type II diabetes, cataracts and aging (5-9). Oxidative stress is believed to play a role both in the initial pathology of diabetes and in the development of vascular complications during the course of the disease (10-12). It can cause irreversible damage to the β-cells of the pancreatic islets (13). As a result, diabetic patients are susceptible to developing atherosclerotic cardiovascular diseases at early ages compared to healthy subjects (12). Many antioxidants are produced in the body to prevent the harmful effects of these oxidants (1). This study measured paraoxonase (PON) and arylesterase (ARE) activities as antioxidants, and the level of malondialdehyde (MDA), an end product of lipid peroxidation and the level of zinc which is a trace element acting as antioxidant in children with TIDM and also healthy control subjects. We aimed to compare them between groups and to investigate whether these parameters are associated with metabolic control, gender and diabetes duration.

Materials and Methods

Study Groups

The patient group consisted of 41 children and adolescents with TIDM (mean age 11.4±3.3 years, 20 males and 21 females). The patients were diagnosed according to criteria provided by American Diabetes Association (14) and the presence of positive autoimmune antibodies. Exclusion criteria were as follows: other systemic diseases, abnormal renal/hepatic biochemical values or macrovascular complications. The control group consisted of 25 healthy subjects (mean age 11.3±3.1 years; 8 female, 17 male). The study was approved by the University of Health Sciences, İstanbul Haseki Training and Research Hospital Local Ethics Committee (approval number: 48-11/10/2013). Written informed consent was obtained from each child included in the study or their parents before enrolling in the study. All patients had been treated with fast- and longacting insulin therapy from the onset of the disease. Data about the duration of illness and onset of the disease in those children with TIDM were obtained from the parents. Biochemical parameters such as glycosylated hemoglobin A1c (HbA1c) levels were determined in each child. The patients were also divided into subgroups according to their gender, glycaemic control (optimal and suboptimal glycaemic control: <9%; poor glycaemic control: ≥9%) (12) and duration of disease (≤1 year; >1 year).

Biochemical Analysis

Venous blood samples were collected after overnight fasting and were centrifuged at 2.000 rpm for 20 minutes; serum samples were stored at -70 °C until assayed. PON and ARE activities were measured by an enzyme-linked immunosorbent assay (ELISA) technique using an ELISA kit (Eastbiopharm, USA). The PON activity was determined using paraoxon as the substrate and measured by increases in the absorbance at 412 nm due to the formation of 4-nitrophenol. ARE activity was determined by measuring the rates of phenyl acetate and paraoxon hydrolysis at 548 nm. MDA was analyzed by a spectrophotometric method. MDA was measured using thiobarbituric acid (TBA) reaction substance production in the following manner. 50 µL of sample was added to 750 µL of acetic acid (20%), 100 µL SDS (8.1%), 750 µL TBA and 350 µL distilled water. The mixture was heated at 100 °C for 45 min. Then, 0.5 mL of distilled water and 2.5 mL of butanol-pyridine 15:1 were added to the mixture and incubated. Then, the absorbance at 532 nm was determined. Zinc was detected by a spectrophotometric method. HbA1c was analyzed using ion-exchange high performance liquid chromatography (Adams A1c, Arkray).

Statistical Analysis

SPSS (Statistical Package for the Social Science) 15.0 for Windows was used for the statistical analysis. Qualitative data are presented as counts and percentages. The association between qualitative variables was assessed using a chi-square test. Quantitative data are presented as mean ± standard deviation for normally distributed data or otherwise as median and interquartile range. Student's t-test for independent samples was used to check for differences between two independent groups of normally distributed data and also by the Mann-Whitney U test. Spearman correlation coefficient was used to assess the relations between quantitative variables not following a normal distribution. $P<0.05$ was considered as statistically significant.

Results

The study group comprised of 41 children and adolescents that were diagnosed with TIDM. Some markers related to oxidative stress were compared with a known control group of 25 healthy children and adolescents.

The mean age in the group was 11.4±3.3 years (range 3.5-18 years) and was similar to the control group 11.3±3.1 years (range 6-16 years) ($p=0.912$). There were 21 female/20 male (51.2% female/48.8% male) in the TIDM group and 8 female/17 male (32.0% female/68.0% male) in the control group. The gender distribution was similar in both groups ($p=0.127$). The main characteristics of the groups in the study are summarized in Table I. The average time from diagnosis of TIDM to participating in this study was 34.0±49.2 months (range 0-192 months). The average HbA1c value in the patients with TIDM was 11.0±2.5% (range 6.8-15.3%). While 28 of the children with TIDM had poor metabolic control, 13 patients had good metabolic control (Table I).

Regarding PON activities and zinc levels, we found statistically significant lower values for the diabetics compared to the controls ($p<0.001$, $p=0.021$, respectively). Also, ARE activity was lower in the diabetics vs. the controls,

Table I. Demographic features and biochemical data of the diabetic and control groups

Parameters	Type I diabetics	Control group	p value
Age (years)	11.4±3.3 (3.5-18)	11.3±3.1 (6-16)	0.912
Gender (females, %)	21 (51.2)	8 (32.0)	0.127
Duration of diabetes (month)	34.0±49.2 (0-192)	-	-
Long-acting insulin (U/day)	15.1±8.7 (4-36)	-	-
Fast-acting insulin (U/day)	20.2±11.7 (6-45)	-	-
HbA1c (%)	11.0±2.5 (6.8-15)	-	-
Zinc (mcg/dL)	103.9±51.9 (0.98-278)	163.8±40.6 (88-244.6)	**<0.001****
Malondialdehyde (nmol/L)	108.1±166.5 (18-502)	123.7±181.3 (18-502)	0.402
Paraoxonase (ng/mL)	10.60±12.05 (3.6-37.65)	13.82±13.87 (3.3-37.65)	**0.021***
Arylesterase (ng/mL)	0.19±0.32 (0.06-1.18)	0.28±0.47 (0.06-1.98)	0.177

*p<0.05, **p<0.001, Data are mean ± standard deviation, HbA1c: Hemoglobin A1c

Table II. Biochemical parameters comparison between diabetic children Type I diabetes mellitus and controls according to gender

		Type I diabetics		Control group	
		Mean ± SD	p value	Mean ± SD	p value
Zinc (mcg/dL)	Female	88.73±32.33	0.095	158.28±41.40	0.651
	Male	119.90±63.62		166.40±41.28	
Malondialdehyde (nmol/L)	Female	101.11±171.32	0.151	159.93±213.49	0.380
	Male	115.43±165.53		106.69±168.63	
Paroxonase (ng/mL)	Female	10.55±12.28	0.314	19.11±16.27	0.539
	Male	10.65±12.12		11.33±12.34	
Arylesterase (ng/mL)	Female	0.22±0.37	0.449	0.39±0.67	0.793
	Male	0.17±0.25		0.22±0.36	

Data are mean ± SD, SD: Standard deviation

but not statistically significant (p=0.177). MDA levels of the diabetic patients were not statistically significant different from those of the controls (p=0.402) (Table I). We compared the parameters measured in the diabetic patients and the control group according to gender (Table II). We did not observe any significant difference between female and male children for PON, ARE activities, MDA and zinc levels (Table II).

We stratified the patients according to disease duration above and below one year. We did not observe any significant difference between children having a diabetes duration above one year and those below one year for PON, ARE, MDA and zinc (Table III). We compared the parameters measured in the diabetic group according to metabolic control. There was not a statically significant difference between children with poor and good metabolic control for PON, ARE, MDA and zinc (Table IV). A negative correlation was observed between zinc and HbA1c in children with TIDM (Rho=-0.317, p=0.049).

Table III. Biochemical parameters in Type I diabetes mellitus patients according to diabetes duration ≤1 year and >1 year

	Duration of diabetes		
	≤1 year	>1 year	
	Mean ± SD	Mean ± SD	p value
Zinc (mcg/dL)	107.45±51.03	101.37±55.03	0.655
Malondialdehyde (nmol/L)	95.64±161.72	124.71±177.30	0.613
Paraoxonase (ng/mL)	9.83±12.23	11.54±12.40	0.714
Arylesterase (ng/mL)	0.17±0.29	0.22±0.35	0.924

Data are mean ± SD, SD: Standard deviation

Table IV. Biochemical parameters in Type I diabetes mellitus patients according to hemoglobin A1c levels <9% and ≥9%

	HbA1c <9%	HbA1c ≥9%	
	Mean ± SD	Mean ± SD	p value
Zinc (mcg/dL)	89.24±38.51	111.11±57.20	0.206
Malondialdehyde (nmol/L)	101.20±157.64	116.82±176.84	0.813
Paraoxonase (ng/mL)	9.16±11.11	11.54±12.86	0.309
Arylesterase (ng/mL)	0.08±0.04	0.25±0.37	0.472

Data are mean ± SD, SD: Standard deviation, HbA1c: Hemoglobin A1c

Table V. Correlations of zinc, malondialdehyde, paraoxonase and arylesterase with age, duration of diabetes and hemoglobin A1c in children with Type I diabetes mellitus

		Age	Duration of TIDM	HbA1c	Zinc
Zinc (mcg/dL)	rho	-0.193	-0.087	**-0.317***	-
	p	**0.126**	0.591	**0.049***	-
Malondialdehyde (nmol/L)	rho	**0.299***	0.211	0.051	-0.108
	p	0.016*	0.192	0.758	0.390
Paraoxonase (ng/mL)	rho	0.201	-0.003	0.057	0.231
	p	0.112	0.987	0.732	0.062
Arylesterase (ng/mL)	rho	**0.335***	0.264	0.185	0.170
	p	**0.007***	0.100	0.259	0.172

*p<0.05, HbA1c: Hemoglobin A1c, TIDM: Type I diabetes mellitus

Age was positively correlated with ARE and MDA in the diabetic subjects (Rho=0.335, p=0.007; rho=0.299, p=0.016 respectively) (Table V).

Discussion

Free oxygen radicals interact with cellular components such as proteins, lipids and nucleic acids and start lipid peroxidation (15). In an organism, production of free oxygen radicals and antioxidant defence mechanisms are in balance, and as long as this oxidative balance is kept, oxidative stress cannot damage the organism (1,15). Diabetes mellitus is associated with an endogenous inflammatory process and oxidative stress (1,10,13,16,17). The destruction of insulin producing β cells in TIDM patients elevates the plasma sugar level (18). It is a thought that high glucose levels trigger oxidative stress and increase ROS in diabetics (18). In addition, the balance between oxidative and antioxidant processes is sensitive to the plasma glucose level (19). Therefore, an increase in ROS is generally accompanied by a decrease in antioxidant defence in TIDM patients (18,19). Prolonged oxidative stress may be associated with chronic complications of diabetes. As a result, diabetic patients are predisposed to atherosclerosis beginning at an early age (20). Most of the studies addressing these mechanisms were performed with diabetic adult patients (17,20-22). Therefore, the present study aimed to evaluate the biochemical markers of oxidative stress in children with TIDM. The end product of lipid peroxidation, MDA is an important marker of oxidative stress (23). High MDA levels were showed in diabetics (21-24). In some studies, statistically significantly higher levels were reported for patients with poor metabolic control than in patients with suboptimal and optimal metabolic control (17,21,23,25). These studies suggested that high glucose levels lead to lipid peroxidation and consequently to increased MDA (25). Erciyas et al. (23) proposed that the elevated MDA levels in children with TIDM with poor metabolic control may lead to vascular complications. Also, they recommended that MDA should be added to the routine laboratory evaluations in the follow-up of these patients (23). In contrast to these studies, Reis et al. (20) reported low MDA levels in patients with TIDM. In our study, MDA was similar between children with TIDM and the healthy controls. Also, MDA was not different between the groups in terms of disease duration, glycaemic control and gender. The reason for the different results in the studies is probably that many different enzymes and proteins play a role in oxidative stress. Enzymes with important functions in the fight against free radicals are known as antioxidants. PON and ARE have antioxidant and antiatherogenic effects. They are encoded by the same gene (26). Although PON shows polymorphic change, ARE does not show a genetic polymorphic change (26). There are studies indicating that PON polymorphism is a genetic predisposition to the complications of diabetes (27,28). Also, although the two enzymes have different natural substrates, the PON has the ability to hydrolyse phenylacetate, the natural substrate of ARE. PON prevents lipid oxidation which plays an important role in the development of micro- and macro-vascular disease (12,27). Studies showed that PON activity was statistically significantly lower in patients with Type I diabetes compared to control groups (12,28,29). Craciun et al. (12) did not observe a correlation between PON activity and HbA1c in children with TIDM. In our study as in that of Craciun et al., (12) PON activity in the patient group was statistically significantly lower than in the controls but its correlation with HbA1c was not observed. Although ARE activity was lower than the control group; this difference

was not statistically significant. This result can be attributed to the fact that ARE activity is weaker than PON activity. However, the reason for decreased PON activity in patients with TIDM observed in our study as with studies of other investigators is still not fully understood (29). A possible explanation could be a modification of the enzyme's active centre affected by the glycation process. Even though we did not observe a negative correlation between the HbA1c value and PON activity, we speculated that lower PON activity in Type I diabetic patients could be the result of chronic hyperglycemia. Răchişan et al. (30) showed lower activities of PON and ARE in girls with TIDM than boys with ARE. There was no difference in PON and ARE activities in terms of gender in our study. In a study using a diabetic rat model, MDA and blood glucose were reduced in rats with TIDM treated with curcumin but superoxide dismutase and insulin increased (13). Curcumin is a kind of spice extensively used in Asian countries. It has antioxidant and anti-inflammatory effects (13). The antioxidant treatment is thought to improve beta-cell dysfunction, but the results are uncertain (31). Zinc is an essential element for the storage, secretion and action of insulin (31). In addition, it is a key co-factor of many antioxidant enzymes and also helps decrease the effects of inflammatory substances and oxidative stress (31). Zinc stimulates the synthesis of metallothionein, which cleanses hydroxyl radicals (31). Zinc transportation to insulin vesicles is facilitated by ZnT8 which is a transmembranic protein (31,32). Antibodies against ZnT8 are produced in patients with TIDM (33). A study from Sweden showed that low zinc in drinking water is associated with the risk of developing Type I diabetes during childhood (33). Lin et al. (34) did not observed a significant difference in zinc levels between diabetics and controls. As opposed to this study, serum zinc levels in our study were significantly lower in the diabetic patient group than in the control group. The reason for decreased zinc levels in our study is not clear. But a negative correlation with HbA1c was observed in this study. This result suggests that the decrease in zinc may be due to hyperglycemia. The small sample size was the main limitation of this study.

Conclusion

PON, ARE activities and zinc levels were lower in children and adolescents with TIDM, but the decrease in the ARE activity was not statistically significant. In addition, a negative correlation was observed between zinc and HbA1c. Our results showed that the antioxidant defence systems decreased in children with TIDM. We conclude that antioxidant enzymes should be at normal levels to prevent or delay the complications of Type I diabetes in children, so we suggest that children with TIDM should adopt more physical activity, a healthier diet and less stressful lifestyle.

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Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Haseki Training and Research Hospital Local Ethics Committee (approval number: 48-11/10/2013).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article

Evaluation of Dynamic Postural Balance in Pediatric Familial Mediterranean Fever Patients

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ABSTRACT

Aim: Familial Mediterranean Fever (FMF) is an autoinflammatory and chronic disorder. Colchicine has been prescribed to treat FMF since 1972. Balance is a complex function of the neuromuscular system. The aim of this study is to determine 1) if there is a connection between FMF and dynamic balance, 2) if colchicine use affects balance, and 3) if the disease severity score is related to a disruption in balance.
Materials and Methods: The study examined 50 pediatric patients with FMF and 130 healthy age- and sex-matched children as control subjects. Dynamic postural stability was measured using the Biodex Stability System (BSS).
Results: The stability indices were significantly higher in the FMF group than in the controls. There was no relationship between the FMF disease severity score and the three stability indices, while the colchicine dose was related to all three stability indices.
Conclusion: By detecting any change in balance status early using a simple, safe, objective measurement of balance via the BSS in FMF patients, neuromyopathy could be identified earlier and unwanted outcomes prevented.
Keywords: Dynamic balance, Biodex Stability System, colchicine, Familial Mediterranean Fever, children

Introduction

Familial Mediterranean Fever (FMF) is an autoinflammatory disorder with autosomal recessive inheritance that is characterized by recurrent episodes of fever, arthritis, and serosal inflammation (1,2). Although its attacks are self-limited, AA type amyloidosis is the most prominent and life-threatening consequence of FMF. FMF is common in Turks, Jews, Arabs, Armenians and other Mediterranean basin and Middle East populations (3).

Balance is a complex function of the neuromuscular system (4,5). It is controlled by sensory input, central processing and neuromuscular responses. Vestibular, proprioceptive and visual inputs are the major sensory inputs (6). Controlling static and dynamic balance is essential for daily living and participating in physical exercise and sports (7). Although healthy subjects can maintain a stable posture and balance automatically, it can be challenging if the sensory inputs are disrupted or brain functions are damaged (8,9). The Biodex Stability System (BSS) is reliable for evaluating dynamic postural balance in healthy (10-12) and blind (13) individuals and has been used to evaluate postural balance in recent years (10-12,14). Pain processing can cause balance disorders (15). A possible explanation for this is that in central nervous system pain processing, the balance control circuit and pain-induced inhibition of muscles share the same pathways and

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pain has a negative influence on proprioceptive feedback from painful structures (8,9). Colchicine has been prescribed to treat FMF since 1972. It reduces the frequency of attacks and prevents the development of amyloidosis (16,17). Colchicine is also used to treat gout and Behçet's disease. Its most common and reversible side effects are nausea, abdominal cramps and diarrhea (18,19). High doses of colchicine can cause severe side effects, such as disseminated intravascular coagulation, bone marrow suppression and renal damage (20). However, toxicity is also observed with standard doses (21). Neuromyopathy related to colchicine presents as distal areflexia, mild sensory changes and weakness of the proximal muscles and increased creatine kinase levels and low motor unit potentials on electromyography. To our knowledge, no study has investigated the influence of FMF on the balance system. Therefore, this study aims to see 1) if there is a connection between FMF and dynamic balance, 2) if colchicine use affects balance, and 3) if the disease severity score is related to a disruption in balance.

Materials and Methods

The study examined 50 child patients with FMF (19 males, 31 females) and 130 healthy age- and sex-matched (65 males, 65 females) children as control subjects. All subjects were recruited from the Gaziosmanpaşa Health Research and Practice Center, Pediatric outpatient clinics between June and September 2013. The study was approved by the Gaziosmanpaşa University Clinical Research Ethics Committee (approval number: 12-BADK-046). All procedures were conducted after written informed consent was obtained from patients or guardians. The diagnosis of FMF was established using the Tel-Hashomer criteria (22). All patients were in remission and on colchicine treatment. None of the patients or controls were taking any drugs that could affect balance, other than colchicine for those in the patient group. Individuals who had psychiatric, neurological or orthopedic problems, muscle disease, limited mobility, motor deficits, inflammatory arthritis, previous spinal surgery, vertigo, diabetes, or visual or auditory problems were excluded from the study. The oral dose of colchicine is 0.5, 1 or 1.5 mg per day in children <5, 5-10, or >10 years old, respectively (23). A recent study in Turkey proposed that children with FMF should be prescribed colchicine according to body weight and surface area; however, the mean colchicine dose was calculated to exceed the age-based dosage (24). The FMF disease severity scores were calculated based on the age at disease onset, number of attacks per month, amyloidosis status, arthritis status, colchicine dose and the presence or absence of erysipelas-like erythema at the time of admission. Scores of 3-5, 6-8, and ≥9 were considered to reflect mild, moderate and severe disease respectively (25). Demographic data and information on disease duration and current colchicine dose were retrieved from our hospital database or collected during patient interviews. Dynamic postural stability was measured using the BSS. There is a movable balance platform that provides up to 20° of surface tilt in a 360° range of motion. The platform was combined with computer software (3.1 Biodex), which allows the device to serve as an objective assessment of balance. The measure of postural stability includes the overall (OASI), anterior-posterior (APSI) and medial-lateral (MLSI) stability index scores. The scores range between 0° and 20° for all stability indexes. Poor balance is identified with a high stability index score (26). A dynamic postural balance score was obtained from measurements of the BSS at level 8. The difficulty levels of the system range from 1 (most difficult) to 8 (easiest). It was decided to set the platform at level 8 because this meant that it could be used in all subjects, allowing testing of all patients at the same level. Results were calculated as the mean of three measurements made at 20-second intervals. The BSS parameters were compared between the FMF patients and control group. All data were analyzed using the Statistical Package for the Social Sciences ver. 18.0 (SPSS, Chicago, IL, USA). Analysis of variance was performed using Dunnett's post hoc test to analyze between-group differences. Differences between FMF patients and the control group were assessed using the Mann-Whitney U test when the parameters were not normally distributed and the Student's t-test for normally distributed continuous variables. $P<0.05$ was considered significant.

Results

The demographic and clinical features of the FMF patients and matched controls are summarized in Table I. There were no significant differences between the groups with regard to age, sex and body mass index (BMI). The dynamic postural balance results for the groups are also shown in Table I. The results were based on the average of the three tests. The OASI, APSI and MLSI were significantly higher in the FMF group than in the controls. There was no relationship between the FMF disease severity score and the three stability indices, while the colchicine dose was related to all three stability indices (Table II).

Discussion

This study demonstrated that FMF can influence postural balance. In addition, we found a relationship between the stability indices and the colchicine (the main treatment for FMF) dose, but no correlation between the stability indices and arthritis or the FMF disease severity score. Achieving proper postural balance is a complex process. Visual, vestibular and proprioceptive sensory inputs need to be integrated centrally. Visual and peripheral sensory inputs and muscle strength

Table I. The demographic characteristics and stability indices of Familial Mediterranean Fever patients and healthy controls

	Familial Mediterranean Fever patients		Healthy controls		p value
Sex	31 girls (52%)		65 girls (54.2%)		0.348
	Mean	**Standard deviation**	**Mean**	**Standard deviation**	
Age	11.31	2.82	10.66	1.78	0.140
Body mass index	17.88	3.85	18.66	3.46	0.311
Overall stability index	1.14	0.77	0.85	0.52	**0.025**
Anteroposterior stability index	0.79	0.47	0.59	0.38	**0.039**
Mediolateral stability index	0.68	0.47	0.51	0.35	**0.034**

Table II. Correlation analysis of Familial Mediterranean Fever patients ("r" values)

	Stability indices		
	Overall	**Anteroposterior**	**Mediolateral**
Colchicine dose	**-0.400***	**-0.388***	**-0.439***
Disease severity score	0.064	0.030	0.093

*p<0.01

are important determinants of balance (5,27-30). Treede et al. (15) and Hassan et al. (8) explained balance disorder as a function of common or close pathways of pain processing, the balance control system and pain-induced muscle inhibition (27). FMF is a chronic disease characterized by abdominal pain, arthritis, chest pain and synovitis. Based on the discussion above, pain processing might lead to decreased balance control in FMF patients, and several potential mechanisms might be responsible for the balance impairment observed in FMF patients. Pain associated with synovitis in FMF patients might be related to balance impairment, although all of our patients were free from attacks during the study and had no pain. Therefore, pain played no role in balance impairment in our patient group. Higher FMF disease severity scores reflect severe disease activity (25). We hypothesized that higher severity scores were related to impaired balance, but there was no relationship between the FMF disease severity scores and the stability indices in our study. Aydoğ et al. (31) and Ekdahl et al. (32) evaluated the impact of disease activity on balance control in rheumatoid arthritis (RA) patients. While Aydoğ et al. (31) found no relationship for balance with disease duration, the inflammatory marker Disease Activity Score 28 or C-reactive protein (CRP) levels, Ekdahl et al. (32) determined that CRP levels affected balance control (5). The Health Assessment Questionnaire (HAQ), which has been used widely to evaluate disability in daily activities, can reflect the severity of disease (33). Luoto et al. (34) and Aydoğ et al. (31) found a significant association between high HAQ scores and a poor performance on the balance test in RA patients. Aydoğ et al. (31) concluded that balance was affected by functional status rather than RA disease activity. In our study, we did not detect inflammatory activity, measure the CRP or assess HAQ scores and thus cannot compare these parameters in our patients. Neuromuscular complications associated with colchicine use are one of the less recognized causes of neuromyopathy (35). Colchicine-dependent neuromyopathy is reversible, in that the laboratory and clinical abnormalities resolve after colchicine withdrawal (20). Given the availability of colchicine over the counter and its wide use in FMF patients, neuromyopathy might be more common than expected. In our study, there was a positive correlation between colchicine doses and stability indices.

Study Limitations

Our study is not without limitations. We measured balance during attack-free periods, and thus could not compare balance between attack and attack-free periods and assess the effects of pain. The second limitation was the lack of an evaluation of the effects of vestibular, visual and proprioceptive sensory inputs. Future, prospective studies on the effects of these sensory inputs on balance might provide more accurate results in FMF patients. A third limitation of the study was its cross-sectional design, which prevented us from determining a cause-effect relationship. Another limitation was the disease severity index used in our study is no longer up to date. The new activity index known as the Autoinflammatory Disease Activity index should be used in the future in prospective studies.

Conclusion

Due to the complex control of balance, impairment of any control component, such as sensory inputs, central processing or neuromuscular responses, could cause a balance disorder. Peritonitis, pleuritis and arthritis, which cause pain in FMF, could affect the central center of balance. The main medication used in FMF patients, colchicine, can cause neuromyopathy and may lead to balance disorders. By

detecting any change in balance status early using a simple, safe, objective measurement of balance with the BSS in FMF patients, neuromyopathy could be identified earlier and unwanted outcomes prevented.

Ethics

Ethics Committee Approval: The study was approved by the Gaziosmanpaşa University Clinical Research Ethics Committee (approval number: 12-BADK-046).

Informed Consent: All procedures were conducted after written informed consent was obtained from patients or guardians.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Medical Practices: R.Y., A.İ., N.Ö.K., N.Ç., Concept: R.Y., A.İ., N.Ö.K., N.Ç., Design: R.Y., A.İ., Data Collection or Processing: R.Y., A.İ., N.Ö.K., N.Ç., A.G., Analysis or Interpretation: R.Y., A.İ., N.Ö.K., N.Ç., A.G., Literature Search: R.Y., A.İ., N.Ö.K., N.Ç., A.G., Writing: R.Y.

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

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Original Article

Retrospective Comparison of Moderate and Severe Diaphragmatic Eventration in Children: Efficiency of Radiological Classification

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ABSTRACT

Aim: Diaphragmatic eventration (DE) is a congenital or acquired elevation of the hemi-diaphragm. The indications for surgery may be challenging because clinical symptoms do not always correlate with radiological severity. We aim to identify the factors for the necessity and the efficiency of thoracoscopic diaphragmatic plication (TDP) in children with DE.

Materials and Methods: A retrospective cross-sectional analysis of patients treated for DE (April 2006-August 2017) was performed. Demographics, type of DE, laterality, associated malformations and clinical symptoms were evaluated. Patients were grouped in two groups (moderate and severe) according to their diaphragmatic elevation levels on X-ray at admission. The severe DE group (SDE, n=14) had a DE of more than 2 vertebral bodies whereas the DE was 2 vertebral bodies or less in the moderate DE group (MDE, n=16). The groups were then compared regarding the necessity of TDP. The efficiency of TDP was analyzed by comparison of the outcome of patients who underwent TDP with that of conservative management.

Results: There were 30 DE cases with a median age of 13.75 months. DE was acquired in 5 patients. The right side was the dominant side (21/30). The most common clinical symptoms were pneumonia (21) and respiratory distress (7) while 6 cases were asymptomatic. Acquired DE and respiratory distress were significantly higher in the SDE group. Four patients (25%) in the MDE group and 13 patients (92.9%) in the SDE group required TDP (p=0.000). The total number of cases of pneumonia was significantly higher in the conservatively treated patients in the follow-up (p=0.023).

Conclusion: Two vertebral bodies may be an efficient cut-off level to discriminate between MDE and SDE. Absolute indications for TDP are SDE, acquired DE and respiratory distress at admission. Patients that are conservatively treated are more prone to pneumonia.

Keywords: Child, diaphragm, risk factors, thoracoscopy

Introduction

Diaphragmatic eventration (DE) is defined as the elevation of the hemidiaphragm without defects of continuity. The generally accepted concept of its management includes conservative and surgical treatment options for asymptomatic and symptomatic cases respectively. Respiratory symptoms such as pneumonia and dyspnea constitute the common indications for surgical intervention; however, these symptoms do not always correlate with the severity of the pathology and hence the decision for surgery may sometimes be challenging.

Thoracoscopic diaphragmatic plication (TDP) has been performed on children for decades and has been shown to be effective and safe previously (1-3). However, no classification or a surgical approach algorithm has been proposed for this anomaly to date. The purpose of this report is to identify the factors for the necessity and the efficiency of TDP in children with DE.

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Materials and Methods

Patients

The study was approved by the Ege University Local Ethics Committee (approval number: 17/7.2), the medical records of children who were diagnosed as DE in a tertiary medical center between April 2006 and August 2017 (n=30) were reviewed. Written consent was obtained for all patients. Records of demographic data, clinical, radiological and operative findings and outcomes were collected.

Radiological Classification and the Comparison of Moderate and Severe Diaphragmatic Eventration

All patients were retrospectively evaluated and grouped according to the elevation level of the affected hemidiaphragm via X-rays taken at admission. The diaphragm dome height was determined on the postero-anterior radiograph by drawing a horizontal line tangent to the diaphragm dome and extending this to the vertebral column (Figure 1). The expected level of the hemidiaphragm was one vertebral body higher for the right side. Patients with a diaphragmatic elevation of more than the height of 2 vertebral bodies at admission were classified as severe DE group (SDE, n=14). The moderate DE group (MDE, n=16) included those patients admitted with a diaphragmatic elevation of 2 vertebral bodies or less. The groups were then compared regarding their demographics, preoperative findings, indications and outcomes.

Management of Diaphragmatic Eventration

The eventration of the diaphragm was detected by X-ray in all patients, and paradoxical movement of the diaphragm was confirmed with either fluoroscopy or ultrasonography when needed. All procedures were performed by four board-certified surgeons via three trocars (3-5 mm) and plications were performed with non-absorbable interrupted sutures (silk 2/0) on the posterolateral-anteromedial axis of the hemidiaphragm in a reefing fashion.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or as median and range where appropriate. Categorical variables are expressed as numbers and percentages and analyzed for comparisons using the Pearson chi-square test. Comparison of groups was performed by univariate analysis using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). Positive predictive values (PPV) and negative predictive values (NPV) for TDP were calculated on crosstabulation of groups (SDE and MDE) with treatment groups (conservative and surgical). The Mann-Whitney U test was used to compare differences in median age at admission, mean follow-up period and number of cases of pneumonia on follow-up.

Results

Overall Study Group

There were 30 DE cases (16 boys, 14 girls) with a median age of 13.75 months (2-180 months). DE was acquired due to prior thoracic surgery in 5 patients. The most common associated malformations were cardiac pathologies (4) and pectus carinatum (2) whereas there was also one from each subsequent pathology; Chilaiditi syndrome, thymoma, hydrocephaly, gastroesophageal reflux and corrected hiatal hernia. The right side was the dominant side (21/30). The most common clinical manifestation was pneumonia (n=21). Respiratory distress was evident in 7 cases. Six cases were asymptomatic. At initial admission, there were 16 patients in the MDE group and fourteen patients in the SDE group. These two groups were similar in terms of median age, gender distribution, laterality and associated anomalies. Acquired DE was significantly higher in the SDE group (p=0.009). Clinical symptoms were similar between the groups except for respiratory distress which was slightly higher in the SDE group (p=0.044). Radiologically, mean diaphragmatic elevation at admission was 1.53 and 3.20 for MDE and SDE groups respectively (p=0.000). Atelectasis on computed tomography (CT)-scan showed no significant difference between the groups. Table I depicts the demographics, clinical and radiological characteristics of both groups at admission. The type of DE was the only significant variable; hence a multivariate analysis was not performed. PPV and NPV were calculated for assessment of the effect of patients' characteristics on the type (surgical or conservative) of the management (Table II). For the necessity

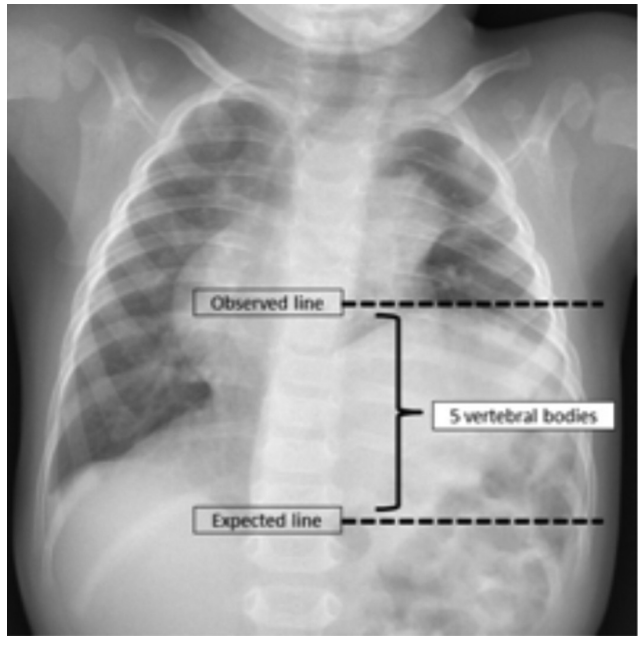

Figure 1. Postero-anterior chest radiograph with measurement of the elevated hemidiaphragm dome-corresponding vertebral body (T9)

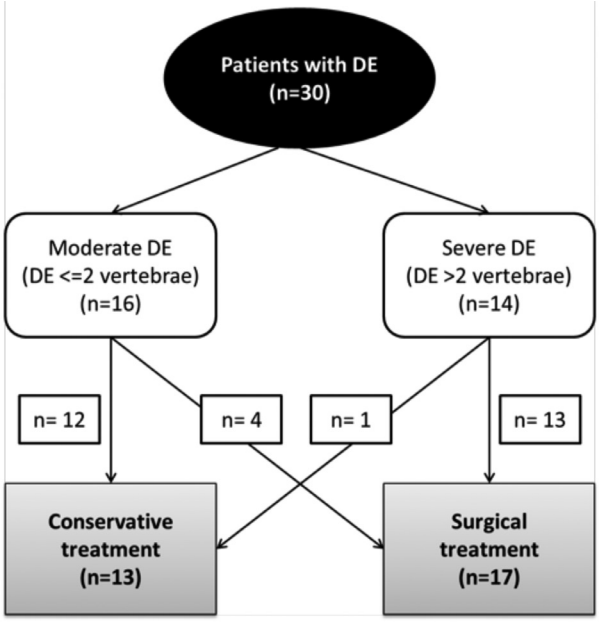

Figure 2. Flowchart of patient groups and outcome
DE: Diaphragmatic eventration

of TDP; acquired DE had a PPV of 80% (p>0.05), respiratory distress had a PPV of 85.7% (p=0.017) and the presence of atelectasis on CT-scan had a PPV of 90.9% (p=0.050). Four patients (25%) in the MDE and thirteen patients (92.9%) in the SDE groups required TDP (p=0.000). Indications for TDP were pneumonia in 11 and respiratory distress in 6 patients. Three asymptomatic patients with associated malformations (history of thymectomy, pectus carinatum and history of cardiac operation) required TDP later in the course due to developing respiratory distress in 1 case, and severe elevation of the diaphragm (more than 4 vertebral bodies) in 2 cases.

Switching Patients

Four patients with pneumonia symptoms required TDP in the MDE group. Indications were newly developed respiratory distress and ipsilateral lower lobe atelectasis in 3 cases and a recurrence of pneumonia in 1 case.

In the SDE group, one patient with a previous history of cardiac operation was followed up and did not receive surgical treatment due to the resolution of the eventration in the third postoperative month (Figure 2).

Surgically Treated Patients

At the end of a median follow-up period of 1 month before surgery, TDP was performed on 17 children (median operation age of 14 months). Major indications were pneumonia in 11 and respiratory distress in 6 patients. Three patients with very SDE (4-5 vertebral bodies) underwent TDP although they were asymptomatic.

All procedures were completed via three trocars (3-5 mm) with non-absorbable interrupted sutures to create one plication line in the posterolateral-anteromedial axis. Transient pleural effusion in 2 patients and pneumothorax in 1 patient developed as minor post-operative complications which resolved within a few days. Colonic perforation occurred in one patient with Chilaiditi syndrome (second patient of the series) and this was repaired laparoscopically. The median postoperative hospital stay was four days (3-30 days) for this group.

Table I. Comparison of groups' characteristics at admission

		MDE (n=16)	SDE (n=14)	p value
Median age (months)		16.25 (2-180)	11.5 (4-156)	>0.05
Gender				
Male		8	8	>0.05
Female		8	6	
Type				
Congenital		16	9	0.009
Acquired		0	5	
Laterality				
Right		11	10	>0.05
Left		5	4	
Associated malformation		3	7	>0.05
Symptoms	Asymptomatic	3	3	>0.05
	Pneumonia	13	8	>0.05
	Respiratory distress	1	6	0.044
	Vomiting	1	1	>0.05
Mean diaphragmatic elevation (VB)		1.53	3.20	0.000
Atelectasis on CT scan		4/8	7/10	>0.05

MDE: Moderate diaphragmatic eventration, SDE: Severe diaphragmatic eventration, VB: Vertebral body, CT: Computerized tomography

All patients benefited from surgical intervention clinically and radiologically. There was no recurrence, 4 hospital admissions and 1 episode of postoperative pneumonia in the median postoperative follow-up period of 11 months (6-17 months).

Conservatively Treated Patients

Thirteen patients were managed conservatively for a median follow-up period of 16 months (2-32 months). Of these, DE persisted at the same level (1 vertebral body above the expected level) in only 2 patients whereas DE was resolved radiologically in the remaining ones. There were 7 hospital admissions and 6 episodes of pneumonia within the follow-up period (Table II).

Discussion

Thoracoscopic diaphragmatic plication was first reported in 1995 (4). The first pediatric case was performed on a new born in 1998 (5). With the advancement of this minimally

Table II. Comparison of conservatively treated and surgically treated patients' characteristics and positive predictive value and negative predictive value for the necessity of thoracoscopic diaphragmatic plication

Patient characteristics		Conservatively treated (n=13)	Surgically treated (n=17)	PPV for TDP	NPV for TDP	p value
Admission	Gender Male Female	 7 6	 9 8	 56.3% 57.1%	 42.9% 43.7%	>0.05
	Type of DE Congenital Acquired	 12 1	 13 4	 52% 80%	 20% 48%	>0.05
	Laterality Right Left	 8 5	 13 4	 61.9% 44.4%	 55.6% 38.1%	>0.05
	Associated malformation	4	6	60%	56.3%	>0.05
	Asymptomatic	3	3	50%	40%	>0.05
	Pneumonia	10	11	52.4%	33.3%	>0.05
	Respiratory distress	1	6	85.7%	50%	0.017
	Vomiting	1	1	50%	42.9%	>0.05
	Atelectasis on CT scan	1/5	10/13	90.9%	57.1%	0.050
Follow-up 6th	Total number of hospital admissions	7	4	N/A	N/A	>0.05
	Total number of pneumonia	6	1	N/A	N/A	0.023
	Median follow-up period (months)	16 (2-32)	11 (6-17)	N/A	N/A	>0.05

DE: Diaphragmatic eventration, PPV: Positive predictive value, NPV: Negative predictive value, TDP: Thoracoscopic diaphragmatic plication, CT: Computerized tomography, N/A: Not applicable

invasive technique, TDP has become a good alternative for DE (6). However, the number of studies that have investigated minimally invasive surgery for DE is limited. Becmeur et al. (7) presented 18 thoracoscopic pediatric cases in 2005. To date, less than 100 cases have been presented in English literature (Table III). This is the first study to classify DE according to the elevation level of the hemidiaphragm on chest radiography and to evaluate the efficiency of thoracoscopic plication by comparing surgically treated patients with conservatively followed-up cases.

There has been controversy regarding conservative versus surgical treatment for DE. Absolute indications include recurrent life-threatening pneumonia and respiratory distress. A functional deficit of the ipsilateral lung and SDE have also been reported to be an indication for surgical repair (1). However, a functional deficit of the lung and SDE remain controversial due to their subjective nature. What is more, the severity of DE is not always in parallel with clinical symptoms. Some patients are asymptomatic even though the radiological grade of DE is high, and some patients with MDE may develop recurrent pneumonia or respiratory distress, as was seen in our series. Therefore, an algorithm for the management of DE is necessary and is considered in this report.

Minimally invasive diaphragm plication techniques have emerged as equally effective and less morbid alternatives to open plication. When it is considered that the lungs continue to grow until the age of nearly ten years, surgical therapy seems reasonable to provide space for future pulmonary development (2). We have preferred surgical repair in patients with SDE who present with pneumonia and respiratory distress. In grey zone patients with MDE, the decision for surgery has been made according to the presence of atelectasis in the ipsilateral lower lobe on CT-scan. Our results indicate that radiological classification with a cut-off level of two vertebral bodies is efficient. What is more, the surgically treated group benefited from the treatment in terms of their postoperative outcomes whereas the conservatively treated patients seemed to be more prone to pneumonia although they had less SDE. Hence, we think that patients with SDE should undergo TDP without delay. Acquired DE is the only exception to performing early TDP. Although it is frequently necessary (PPV for TDP was as high as 80% in acquired DE in our series), phrenic nerve injury may spontaneously recover within 6 weeks as has been previously reported (6,8,9). We prefer to observe these patients for at least three months following surgical trauma. There were five acquired DE cases in our study group out of which 4 (3 cardiac surgery, one thymectomy) required TDP and one had resolved without plication by the 3rd month of follow-up. DE symptoms may range from wheezing to life-threatening respiratory distress requiring mechanical ventilator

Table III. The summary of pediatric thoracoscopic diaphragmatic plication series in English literature

Series	Patients (n)	Gender (M/F)	Mean age	Laterality (R/L/B)	Acquired/ congenital	Symptoms/indications	X-ray level	Assoc. malf.	Comment for CT-scan	Technique	Complication	Follow-up
Becmeur et al. (7)	10	6/4	17 months	N/A	1/9	Rec. pneumonia: 7 Dyspnea: 2 Rib deformity: 1	N/A	5 (50%)	N/A	Thoracoscopy	Conversion: 2 Subcutaneous emphysema: 1 Recurrence: 0	16 months
Borruto et al. (1)	8	5/3	1.6 years	6/2/0	0/8	Rec. pneumonia: 5 Persistent cough: 1 Thor. deformity: 1 Asymptomatic: 1	N/A	N/A	N/A	Thoracoscopy	Recurrence: 2	12 months
Hu et al. (10)	27	21/6	12.7 months	21/6/0	9/18	Asymptomatic: 11 Resp. distress: 6 Lung collapse: 3 Vent. dependence: 2 Rec. pneumonia: 5	Postop. descending level: mean 2.6 vertebrae (1-4.5)	N/A	N/A	Thoracoscopy for right DE Laparoscopy for left DE (4/6)	Reelevation: 6 Pneumothorax: 1	10.5 months (1-35)
Fujishiro et al. (6)	13	N/A	7 months	3/10/0	N/A	N/A	N/A	N/A	N/A	Thoracoscopy: 10 Laparoscopy:3	Conversion: 0 Recurrence: 1	N/A
Miyano et al. (16)	20	15/5	20.7 months	5/13/2	12/8	Resp. distress: 20 Vent. dependence: 9 O2 dependence: 13	N/A	N/A	N/A	Laparoscopy: 13 Thoracoscopy: 7	Conversion: 1 (thoracoscopy) Recurrence: 6 (laparoscopy) Atelectasis: 2 (thoracoscopy: 1 Laparoscopy: 1)	2.6 years
Present series	17	9/8	14.7 months	13/4/0	4/13	Rec. pneumonia: 11 Resp. distress: 6 Severe DE: 12 Atelectasis on CT: 10	Severe DE cut-off level: 2 VB	6 (35.3%)	Atelectasis as a relative indication	Thoracoscopy: 17	Conversion: 0 Recurrence: 0 Pleural eff.: 2 Pneumothorax: 1 Colonic perf.: 1	11 months (6-17)

M: Male, F: Female, R: Right, L: Left, B: Bilateral, Assoc.: Associated, Malf.: Malformations, CT: Computerized tomography, Rec.: Recurrent, Thor: Thoracic, Resp.: Respiratory, Vent.: Ventilator, Postop.: Postoperative, DE: Diaphragmatic eventration, Eff.: Effusion, N/A: Not applicable

support in small children or frequent respiratory infections and exercise intolerance in older children (2). Some investigators suggest that very severe eventration that occupies much of the hemi-thorax should be repaired on that basis alone for fear that they might interfere with postnatal lung development (3). Indeed, symptoms do not correlate with the severity of DE as in some of our cases. There were six asymptomatic patients with an elevation of the affected hemidiaphragm up to 5 vertebral bodies. In contrast, lower lobe atelectasis was detected in 4 MDE patients with an elevation of the diaphragm of less than two vertebral bodies in our series.

Acquired DE and respiratory distress were the only two variables to be found to significantly affect the outcome in our series. All thoracoscopic procedures were performed successfully. Postoperative minor complications included two pleural effusions and one pneumothorax. There was a colonic perforation in a patient with right DE and Chilaiditi syndrome. After the plication procedure, immediate remission of symptoms was noted in our patients. Minimal access surgery may offer a more rapid improvement with a shorter recovery period (1,10-12). The principle of the operation is to decrease the surface of the redundant diaphragm by plicating it to an acceptable level. In this way, the repair improves the movement of the diaphragm during respiration and achieves physiologic pulmonary function. The global muscular force of the diaphragm increases after unilateral plication with a gain of 30% in trans-diaphragmatic pressure (13,14). Literature reports several experiences about the efficiency and safety of thoracoscopy in the treatment of DE in terms of less ventilation impact and better outcomes (7,10-12,14-16). The duration of the procedure was not longer than open surgery. In our department, thoracoscopic plication is preferred as the gold standard operation for the treatment of DE.

Conclusion

We consider that TDP is efficient in cases of DE. It offers all the benefits of minimal-invasive surgery with significantly better respiratory outcomes compared to conservative management. Early thoracoscopic plication should be considered for children with absolute indications including SDE, acquired DE (following observation for at least 3 months) and respiratory distress. Relative indications are MDE with a history of pneumonia and the presence of atelectasis on CT-scan.

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Ethics

Ethics Committee Approval: The study was approved by the Ege University Local Ethics Committee (approval number: 17/7.2).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.D., E.D., C.Ö., A.E., Concept: Z.D., Design: Z.D., Data Collection or Processing: Ü.Ç., E.D., Analysis or Interpretation: Z.D., Ü.Ç., E.D., Literature Search: Z.D., E.D., Writing: Z.D.

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Original Article

Lymphadenopathies: An Annoyance or Not?

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ABSTRACT

Aim: The aim of this study was to evaluate the cases hospitalized with lymphadenopathy in terms of demographic and clinical characteristics, lymph node involvement regions, infection markers and their diagnoses.

Materials and Methods: The medical records of 56 children with lymphadenopathy between 2014 and 2017 were reviewed retrospectively at Ege University, Children's Hospital, General Pediatrics Unit. Demographic characteristics, clinical findings and accompanying complaints of the cases were examined. Laboratory tests including complete blood count, sedimentation rates and other biochemical parameters were measured. Lymph nodes were assessed via ultrasonographic examination in terms of distribution, number, size and structure. Lymph node regions were described as anterior or posterior cervical, supraclavicular, submandibular, axillary, epitrochlear, inguinal or popliteal. Laboratory results, microbiological studies and histopathological examination results of the patients were evaluated.

Results: Among the 56 patients enrolled in the study, 31 (55.4%) were male, 25 (44.6%) were female and the median age was 3.7±7.1 years. The most frequent involvement location of the enlarged lymph nodes was the cervical area. Others occurred in the axillary, inguinal or supraclavicular regions. The median results of the white blood cell, C-reactive protein and erythrocyte sedimentation rates were 13.670±9760/mm^3, 1.9±5.4 mg/dL and 42±51 mm/h respectively. Ultrasonographic evaluation showed that lymph nodes were diagnosed with reactive hyperplasia in 69.6%, suppurative lymphadenopathy in 23.2% and suspected malignancy in 7.1%. Most of the cases with lymphadenopathy resulted from a benign condition.

Conclusion: Lymphadenopathy is a common complaint of childhood, mostly benign. The etiology should be elucidated using full history, careful physical examination, follow-up, laboratory and imaging methods. A good physical examination and follow-up of the clinical features of the lymph node are more important than the laboratory and imaging methods. If there is no change in lymph node size in the follow-up, further studies should be performed.

Keywords: Lymphadenopathy, childhood, benign conditions

Introduction

Lymph nodes that are located in various parts of the body interconnected through lymphatic channels are the most important part of the immune system. The lymphatic system consists of approximately 600 lymph nodes. The lymphatic fluid, an ultra-filtered form of blood that is rich with lymphocytes, is free to circulate through ducts and is transported to the right lymphatic duct or thoracic duct. The lymphatic fluid is connected to the system via the right and left subclavian venules via these ducts. Knowing the location of the lymph node and the location of its drainage helps to clarify lymph node pathologies. The appropriate and timely inflammatory response when antigens enter the body mostly through the gastrointestinal and respiratory system leads to the production of antibodies and cytokines and T cell proliferation in lymph nodes. This inflammatory response enlarges the lymph nodes (1). Lymphadenopathy (LAP) usually describes all pathological conditions of the lymph nodes while lymphadenomegaly explains the swollen lymph node. Detected lymph nodes in two or more non-adjacent regions are described as "generalized LAP". Localized LAP refers to the involvement of only one lymph node region. LAP is a common clinical problem which can occur at any age and one

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of the leading causes of general outpatient admissions (2,3). Due to the fact that LAP can be the first finding of malignant diseases especially in childhood, it is a cause of serious anxiety in families. Palpable supraclavicular, iliac, popliteal, epitrochlear nodes greater than 0.5 cm, inguinal nodes larger than 1.5 cm, and cervical/axiller nodes larger than 1 cm are considered abnormal (4). The most common causes of lymphadenopathies are infections (5). Other causes including autoimmune diseases, histiocytosis, malignant diseases, lipid storage diseases, drug reactions and granulomatous diseases might be related to the enlargement of the lymph nodes (6). Since possible malignant diseases may be a finding; detailed physical examination of the patient, careful examination of the lymph nodes regarding changes in size, consistency and settlement are absolutely necessary. In the current study, we aimed to evaluate retrospectively the demographic and clinical data, lymph node involvement, infection markers and diagnoses of the patients hospitalized with LAP.

Materials and Methods

The medical records of 56 children with lymphadenopathy between 2014 and 2017 were reviewed retrospectively at Ege University, Children's Hospital, General Pediatrics Unit. Demographic characteristics, clinical findings and accompanying complaints, and also the laboratory markers such as white blood cells (WBC), sedimentation rate and several biochemical investigations of the cases were examined on admission. Ultrasonographic examination of the lymph nodes assessed their distribution, number, size and structure. Lymph node regions were described as anterior or posterior cervical, supraclavicular, submandibular, axillary, epitrochlear, inguinal or popliteal. Palpable supraclavicular, iliac, popliteal, epitrochlear nodes greater than 0.5 cm, inguinal nodes larger than 1.5 cm and cervical/axiller nodes larger than 1 cm are considered abnormal. The laboratory results, viral serologic studies and histopathological examination results of the patients were evaluated. For the patients who, as a result of their physical examination and laboratory tests, were thought to be infected, appropriate antibiotics were administered and they were recalled for check-up. Histopathologic examination was performed on patients whose lymph node size did not regress by the end of four or more weeks, and in whom there was no indication of an infectious disease.

Statistical Analysis

The data were analyzed using the statistical package for social sciences (SPSS) (version 17). All data were described as means and standard deviations or medians and ranges. Categorical variables were expressed by percentages. Pearson correlation analysis was used for the parameters. A p value <0.05 was statistically significant.

Results

Among the 56 patients enrolled in the study, 31 (55.4%) were male, 25 (44.6%) were female and the median age was 3.7±7.1 years. Table I shows the demographic and other clinical features of our cases. Overall, 23 (41.1%) cases with swelling on the neck, 3 cases (5.4%) on inguinal and 2 (3.6%) cases in the axilla region were admitted to the hospital. Associated symptoms including sore throat, fever, cough and pain in the lymph node were observed. Ten patients (17.9%) had hepatomegaly, 10 (17.9%) splenomegaly and 5 (8.9%) hepatosplenomegaly on physical examination. When the lymph nodes were classified according to size as <1, 1-3, and >3 cm, 15 (26.8%) patients had lymph nodes of 1-3 cm and 38 (67.9%) had lymph nodes of >3 cm. There was no history of any drugs that may have caused the enlargement of the lymph node in the patients. In the acute LAP group, 42 (89.3%) patients of the 47 had benign etiologies and 5 (10.6%) had been diagnosed with malignancies. Of the 9 patients presenting with chronic LAP, only 2 (22.2%) had malign etiologies (one had Hodgkin lymphoma and the other non-Hodgkin lymphoma), whereas 6 had benign lesions (4 of them had non-specific reactive hyperplasia and two patients were diagnosed with *Mycobacterium tuberculosis*). Inflammatory markers were examined in all the patients. The median of the WBC, C-reactive protein and erythrocyte sedimentation rate (ESR) were 13.670±9760/mm^3, 1.9±5.4 mg/dL and 42±51 mm/h respectively. The laboratory features are summarized in Table II. Positive Epstein-Barr virus immunoglobulin M (IgM) was detected in 6 (10.7%) of the patients, and in 1 (1.8%) cytomegalovirus IgM was seen. Viral pathogens were not found in the other patients. The median of lactate dehydrogenase was 244±109 U/L (normal range: 142-297). Ultrasonographic examination was performed on 50 (89.3%) of the patients and it was found that 39 (69.6%) of the patients had reactive features and that 4 (7.1%) had possible malignant lymph nodes. Neck tomography in 22 (39.3%) of our patients showed abscess formation in the lymph node in 12 (54.6%) of these. Lymphadenitis accompanied by abscess was surgically drained and necessary microbiological samples were sent to the microbiology laboratory. The most common microorganism was *Staphylococcus aureus*. Mycobacteriological examination of three patients revealed *M. tuberculosis*, and mycological examination revealed fungal infection in two. Two other patients had a parasitic infection. Histopathologic examination was performed on 26 patients. Sixteen of all the patients were diagnosed with reactive hyperplasia, 4 with Hodgkin lymphoma, 3 with non-Hodgkin lymphoma, one with langerhans cell histiocytosis, one with dermatopathic lymphadenitis and one patient was diagnosed with Kikuchi Fujimato disease (Table II).

Table I. The demographic and clinical characteristics of patients hospitalized with lymphadenopathy	
Demographic features	
Age [median (IQR)]/years	3.7 (7.1)
Gender, n (%)	
Male	31 (55.4)
Female	25 (44.6)
Causes for admission, n (%)	
Neck swelling	25 (44.6)
Neck swelling + fever	18 (32.1)
Throat pain	1 (1.8)
Axillary swelling	3 (5.4)
Inguinal swelling	2 (3.6)
Supraclavicular swelling	-
Supraclavicular swelling + fever	2 (3.6)
Neck + inguinal swelling	-
Neck + axillary swelling	4 (7.1)
Neck + supraclavicular swelling	-
Axillary + inguinal swelling	1 (1.8)
Duration [median (IQR)]/days	7 (11)
Duration, n (%)	
Acute	47 (83.9)
Chronic	9 (16.1)
Infection history before admission, n (%)	
Yes	35 (62.5)
No	21 (37.5)
Antibiotic use before admission, n (%)	
Yes	29 (51.8)
No	27 (48.2)
Associated symptoms, n (%)	
Fever	19 (33.9)
Sore throat	1 (1.8)
Sore throat and fever	1 (1.8)
Rash	2 (3.6)
Weight loss	2 (3.6)
Night sweating	1 (1.8)
Fever, weight loss and night sweating	1 (1.8)
Cough and fever	2 (3.6)
Rash and fever	2 (3.6)
Arthralgia	1 (1.8)
Arthralgia and fever	1 (1.8)
Thrombocytopenia	1 (1.8)
Earache	1 (1.8)
Cough	1 (1.8)
Not associated symptoms	20 (35.7)
Extension, n (%)	
Generalized	12 (21.4)
Local	44 (78.6)
Site distribution of lymph nodes, n (%)	
Cervical	23 (41.1)
Submandibular	7 (12.5)
Cervical + submandibular	7 (12.5)
Cervical + axillary	5 (8.9)
Cervical + inguinal	3 (5.4)
Inguinal	3 (5.4)
Axillary	2 (3.6)
Cervical + inguinal + submandibular	1 (1.8)
Cervical + supraklavikular	1 (1.8)
Axillary + inguinal + submandibular	1 (1.8)
Cervical + axillary + inguinal preauriküler	1 (1.8)
Size, n (%)	
<1 cm	2 (5.4)
1-3 cm	15 (26.8)
>3 cm	38 (67.9)
Structure of the lymph node, n (%)	
Soft	32 (57.1)
Fixed and stiffed	23 (41.1)
Inflamed appearance	1 (1.8)
Other organ involvement on physical examination, n (%)	10 (17.9)
Hepatomegaly	10 (17.9)
Splenomegaly	5 (8.9)
Hepatosplenomegaly	

IQR: Interquartile range

Table II. Laboratory, radiologic and histopathological characteristics of the patients (n=56)	
Biochemical analyses	
Peripheral blood smear, n (%)	
Normal	49 (87.5)
Atypical lymphocytes	7 (12.5)
Blasts	-
WBC [median (IQR)]/mm^3	13.670 (9760)
CRP [median (IQR)]/mg/dL	1.9 (5.4)
ESR [median (IQR)]/mm/h	42 (51)
LDH [median (IQR)]/U/L	244±109
UA (mean ± SD)/mg/dL	3.6±1.2
Chest X-ray, n (%)	
Normal	53 (94.6)
Mediastinal LAP	3 (5.4)
Radiology	
Lymph node USG, n (%)	
Reactive lymphadenitis	39 (69.6)
Suspected malignancy	4 (7.1)
Suppurative	13 (23.2)
Abdominal USG, n (%)	
Normal	44 (78.6)
Organomegaly	4 (7.1)
Abdominal lymph node	5 (8.9)
Organomegaly and abdominal lymph node	3 (5.4)
***Neck tomography, n (%)**	
Abscess	12 (54.6)
Benign findings	5 (22.7)
Malign findings	5 (22.7)
Microbiological investigations, n (%)	
EBV	6 (10.7)
CMV	1 (1.8)
Staphylococcus aureus	8 (14.3)
Streptococcus pneumoniae	3 (5.4)
Mycobacterium tuberculosis	3 (5.4)
Parasitic infection	2 (3.6)
Fungal infection	2 (3.6)
****Histopathological diagnosis, n (%)**	
Reactive hyperplasia	4 (15.4)
Hodgkin's lymphoma	3 (11.6)
Non-Hodgkin's lymphoma	16 (61.6)
Dermatopathic lymphadenopathy	1 (3.8)
Kikuchi-Fujimoto disease	1 (3.8)
Langerhans cell histiocytosis	1 (3.8)

CMV: Cytomegalovirus, EBV: Epstein-Barr virus, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, IQR: Interquartile range, LDH: Lactate dehydrogenase, SD: Standard deviation, UA: Uric acid, WBC: White blood cell
*(n=22), **(n=26)

Discussion

LAP is a common clinical scenario in childhood and most of the cases result from a benign condition. Although it can be the manifestation of a serious systemic disease or malignancy, its percentage is quite low. Detailed history, appropriate and timely evaluation of physical examination and laboratory findings provide a guide for the differential diagnosis in the evaluation of the LAP. Qualified-close clinical follow-up and the determination of high/low risk factors for malignancy can prevent unnecessary investigation in the diagnosis of enlarged lymph nodes. Growth in lymph nodes usually occurs during the first two weeks of antigenic stimulation and is expected to decrease within 4-6 weeks after the end of the stimulation (3). In the absence of regression, the patients should be re-evaluated via histopathological examination. In our study, similar to the studies in the literature, most of the cases seemed to be the result of benign causes (87.5%), and only 7 patients were diagnosed with a malignant disease. Totally, a specific etiology was found in 35 (62.5%) of the patients. The overall percentage of malignant disorders was 12.5% in our study. A study from our country conducted by Oguz et al. (7) reported that a specific etiology was found in 58% of the patients, and malignant disease was seen in 24.3%. In the study of Moore et al. (8) a malignant cause was found in 11.6% of the children who had undergone lymph node biopsy. In another study in which 185 patients were evaluated, it was stated that benign pathologies were seen in 33.5% of the patients, and 64.5% of them had a swollen lymph node that was characterized by reactive features (9). The duration of lymphadenopathies is significant in determining their cause. If the complaints go on for less than four weeks, it is considered an acute lymph node enlargement; complaints continuing longer than 4 weeks are described as chronic LAP (10). It is reported that the duration of LAP is significantly longer for malignant disorders and usually occurs as chronic LAP (11). In this study, with the exception of 5 patients diagnosed with malignant disorders, benign causes were found in most of the cases of hospitalized LAP to be characterized with a duration less than 4 weeks. Oguz et al. (7) evaluated 457 patients, 218 of whom had acute LAP and 98.2% of them were diagnosed with benign diseases. Two hundred thirty nine patients presented as chronic LAP, and 132 (55.2%) of these 239 patients had benign etiologies, whereas 107 had malignancies (44.8%) (8). It is important to be careful about malignancy and to perform histopathologic examination of those lymph nodes that persist for more than four weeks and do not regress in size. Localized lymph node enlargement usually occurs in acute diseases such as localized infections or tooth decay. Firstly, patients with localized LAP should be examined thoroughly and screened for infections (12). Cervical lymph nodes are often enlarged due to a variety of infections of the head and neck or due to some systemic infections such as Epstein-Barr virus, cytomegalovirus infection or toxoplasmosis (13). It is emphasized that none of the lymph nodes detected at the supraclavicular region were benign and immediate biopsy was recommended (7). The supraclavicular area is generally related to malignancy in all ages. Another study reported that 4.8% of 185 patients had a swollen lymph node at the supraclavicular region and these were mostly diagnosed with non-Hodgkin lymphoma (9). In our study, a swollen lymph node was detected at the supraclavicular region in 2 (3.6%) patients and one of them was classic Hodgkin lymphoma and the other was Kikuchi-Fujimoto disease. Generalized LAP is usually a sign of an underlying systemic disease or Epstein-Barr virus, HIV, lymphoma or autoimmune disorders. The most common malignant disease in childhood is non-Hodgkin's lymphoma, causing extensive lymph node enlargement (14-16). In our study, out of a total of 12 patients with generalized lymph node, one of them was diagnosed with B-cell lymphoma and the others were diagnosed with benign conditions. Local or generalized LAP is not an indication of malignancy. The size of the lymph node is an important factor in order to distinguish the etiology. In general, a size of more than 2 cm is the upper limit for a malignancy or a granulomatous disease (17). In a study by Soldes et al., (18) predictive parameters for malignancy were reported as lymph nodes bigger than 1 cm in size. Oguz et al. (7) found that 85.6% of the patients with lymph nodes larger than 3 cm had malign diseases. In the same study, it was said that small lymph nodes that were under 1 cm were benign and that those between 1-3 cm could not indicate a clear evaluation concerning the disease. Although lymph node size might give an idea about diseases, there is no clear limiting value in the malignant-benign distinction (12). There was no correlation between malignancy and size of the lymph node in our study ($p>0.05$). To rule out malignancy, excisional biopsy was applied to 26 (46.4%) of our cases. Histopathologically, the most frequent malignancies were Hodgkin and non-Hodgkin lymphoma. Despite biopsy, a specific etiology could not be found in some cases hospitalized with LAP (61.6%). Similar to our study, Oguz et al. (7) could not find any specific cause with biopsy in 46.2% patients and no specific disease was detected in them.

Conclusion

Lymph node enlargements are a common condition in childhood which can obsess the families and physicians in some cases. They are usually associated with infections. A detailed history, complete physical examination and also the size and duration of the complaints are very important in determining the cause. Lymphadenopathies that are at the supraclavicular region, larger than 2 cm diameters, going on longer than 4 weeks and not responding to non-specific antibiotic treatment should be suspected in terms of malignancy.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: Ş.G., Z.K., G.K., Design: Ş.G., Data Collection: Ş.G., Analysis and Interpretation: Z.K., Literature Search: Z.K., G.A., Writing: Ş.G.

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

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Case Report

A Rare Cause of Acute Abdominal Pain in Childhood: Peptic Ulcer Perforation

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ABSTRACT

Four children with a mean age of 12 years were referred to our emergency department with a history of abdominal pain. Examination revealed tenderness in the lower abdomen, in particular the left iliac fossa. The youngest child, who was 3 years old, also presented with shock. Abdominal X-rays revealed free air under both hemidiaphragms. Subsequent surgery was administered as the primary treatment of three patients and a partial resection was performed in the remaining one. The youngest child died and the others were discharged. These cases emphasize that although uncommon, alternate diagnoses must be kept in mind in children presenting with lower abdominal pain.
Keywords: Child, intestinal perforation, abdominal pain

Introduction

Peptic ulcer disease (PUD), which may be complicated by severe hemorrhage or perforation, is a rare cause of life-threatening abdominal pain in children. Perforation is the second most common complication of PUD following acute gastrointestinal hemorrhage (1,2). The disease is characterized by a loss of tissue penetrating gastrointestinal mucosa. Although the exact etiology of peptic ulcer perforation still remains unclear, some factors such as stress, an underlying disease or corticosteroid/non-steroidal anti-inflammatory drugs are proven to play a role (2,3). Peptic ulcers are often localized in the anterior wall of the duodenum. The gold standard of diagnosis is endoscopy. In case of perforation, there is a free air under the diaphragm diagnostic and another radiologic examination is not required (3,4). However, an absence of free air under the diaphragm does not rule out the diagnosis of perforation (4). The aim of this case series is to emphasize that gastrointestinal perforation should be considered in the differential diagnosis in children (especially adolescents) presenting with acute abdominal pain even in the absence of dyspeptic symptoms. Four children (2 girls, 2 boys) with a mean age of 12 years (3-16 years) who presented with peptic ulcer perforation (PUP) were included in this study. Three had perforated duodenales and one had a perforated gastric ulcer. The characteristics of the patients are presented in Table I. Informed consent was given by all parties involved.

Case Reports

Case 1

A 16 years-old boy presented to the emergency department with a history of intermittent pre-prandial epigastric pain over a 3-month period. Cholelithiasis was detected in his prior examination at another healthcare facility. Upon admission,

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Table I. Characteristics of patients

Age	**16**	**3**	**13**	**16**
Gender	**Male**	**Female**	**Female**	**Male**
Presentig symptom	Abdominal pain	Abdominal pain, fever, vomiting	Abdominal pain, abdominal distension and vomiting	Abdominal pain
Duration of symptoms	3 months/1 hour	10 days	4 days	8 days
Family history	Negative	Negative	Father-duodenal ulcer perforation	Negative
Physical examination	Epigastric voluntary defense	Ill appearance, abdominal distension and peritoneal signs	Diffuse tenderness and rigidity	Diffuse tenderness and rigidity
WBC	23800/mm^3	3470/mm^3	19500/mm^3	19500/mm^3
CRP	Negative	Negative	16.44 mg/dL	2.5 mg/dL
X-ray	Bilateral subdiaphragmatic free air	Not performed	Multiple air-fluid levels	Not performed
CT scan	Not performed	Not performed	Not performed	Free air in the abdominal cavity
Localization of ulcer	Duo denum anterior wall	Greater curvature of stomach	Duodenum	Duodenum anterior wall
Treatment	Primary suture, omental patch	Partialre section of stomach	Primary closure	Primary suture and omental patch
Histology	Acute benign ulcer	Acute gastric ulcer and perforation	Chronic gastritis	Ulcer repair tissue
Oral feeding	3rd day	-	7th day	4th day
Hospital stay	6 days	-	13 days	7 days

WBC: White blood cell, CT: C-reactive protein, CRP: Computed tomography

his physical examination revealed that there was rigidity in his epigastrium. Laboratory tests revealed an elevated white blood count (23.800/mm^3) and neutrophil predominance (21.900/mm^3). Bilateral sub-diaphragmatic free air was seen on X-ray (Figure 1). Abdominal ultrasonography was normal except for cholelithiasis. Urgent surgical exploration revealed an area of perforation on the duodenum anterior wall (3.4 mm) and a surrounding indurated, edematous and fragile area. Primary repair was performed with an omental patch. Oral feeding was commenced on the third day postoperative. The patient was given antiulcer therapy with anti-biotherapy and subsequently discharged.

Case 2

A 3-years-old girl, who had been on antibiotics for a urinary tract infection for 10 days, presented with abdominal pain, fever and vomiting. On physical examination, she had an ill appearance and her clinical picture was suggestive of hypovolemic shock. She had abdominal distension and peritoneal signs. Routine blood tests were normal except for leukopenia (3470/mm^3) and moderately high levels of amylase (178 IU/L), hyponatremia (123 mEq/L) and hypocalcemia (6.6 mg/dL). She was operated on urgently after fluid resuscitation.

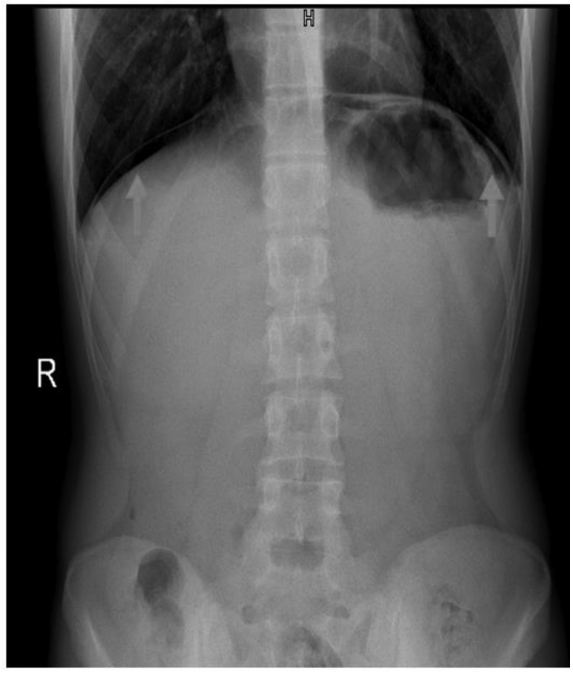

Figure 1. Arrows: bilateral sub-diaphragmatic free air on X-ray

The area of perforation on the greater curvature was resected and primarily repaired. On day 5 postoperative, she died in intensive care unit due to brain death with severe brain edema on computed tomography (CT) scan and no sign of cranial perfusion on scintigraphy. A histological evaluation confirmed an acute gastric ulcer and perforation.

Case 3

A 13-year- old girl had suffered from abdominal pain, abdominal distension and bilious vomiting for 4 days. A CT scan was obtained that showed left lower lobe pneumonia and ileus in another center. On physical examination, there were diffuse tenderness and rigidity on the abdomen. Multiple air-fluid levels and dilated bowel loops were seen on X-ray scanning (Figure 2). Laboratory tests revealed leukocytosis, elevated C-reactive protein (CRP) and aspartate aminotransferase (AST) levels whereas other biochemical values were within normal limits. The patient underwent emergency surgery. Bile and purulent fluid was aspirated, adhesiolysis of the bowel loops and primary repair of the duodenal perforation were performed. Antibiotherapy and gastroprotective medication were used during postoperative follow-up.

Case 4

A-16-years old boy who had previously taken anti-constipation therapy elsewhere presented with abdominal pain for 8 days. His past medical history revealed that he had had an appendectomy. Diffuse tenderness and rigidity were noticed on abdominal examination and leukocytosis and an elevated CRP level necessitated a CT scan which showed free air in the abdominal cavity (Figure 3). Urgent exploration revealed an area of perforation on the duodenum anterior wall that was repaired with primary suture and an omental patch. Antibiotherapy and gastroprotective medication were used during the postoperative follow-up.

Discussion

PUD is rare in children. Therefore, it may be missed or only diagnosed upon the presentation of complications such as hemorrhage or perforation. Modern effective medical therapy has reduced the incidence of PUD and the need for surgical treatment (4). Nevertheless, due to complications of the disease, there is still a significant morbidity and mortality rate. Especially in adolescents, as a rare cause of abdominal pain, PUP should be considered in the differential diagnosis. PUD usually occurs in adulthood. However, it has also been reported in children (3-5). Most peptic ulcers in children occur between the ages of 8 and 17 years (mean 12 years) (3). Also, PUP is more common in adolescents than in other age groups and also is more common in males. Hua et al. (3) previously reported a significant predominance of adolescents (90.4%) and males (80.7%). However, in our study, gender distribution was equal in our limited number of patients. If we exclude the youngest patients from our series we can also conclude that there is a male predominance in adolescents. Patients are usually referred to the emergency department with acute abdominal pain and peritoneal signs are seen on physical examination. Perforation of the anterior surface of the stomach leads to the first sign which is often sudden, intense abdominal pain. Posterior wall perforation leads to tenderness and guarding, which often radiates pain to the back. Schwartz at al. (6) reported a patient with PUP, who presented to an emergency department with acute abdominal and shoulder pain. In our study, acute abdominal pain (mean 5.75 days) and peritoneal signs were observed as the most common findings. We noted that 50% of patients had vomiting and one patient had fever. There were three patients with diffuse peritoneal signs and one of our patients had localized defense. A study by Lee et al. (7) reported differently on a 30-month-old case who presented with acute massive hematochezia. A clinical study in 73 children showed that younger patients especially presented with vomiting, older patients mostly presented with pain which was similar to our study (2). Case 2 had vomiting and cases 1 and 4 had pain while case 2 had both of these symptoms. In research from Shangai, it shows that a family history of peptic ulcer is a very strong risk factor in PUD [odds ratio

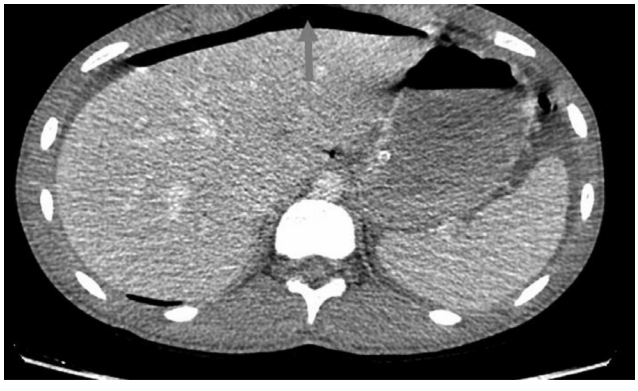

Figure 2. Computed tomography scan showed free air before operation

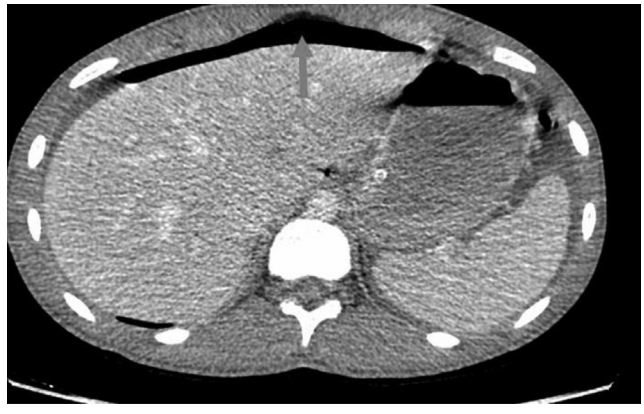

Figure 3. Computed tomography scan showed free air before operation

(OR)=4.94, 95% confidence interval (CI)=3.69-6.61] (8). In the development of duodenal ulcers, García-González et al. (9) made a report that the carriage of the IL-1B-511*C/IL-1B-31*T/IL-1B + 3954*C/IL-1RN*2 allele combination in addition to bacterial and environmental factors play a key role in the development of duodenal ulcers. One of our patients has a positive family history. In a study from Philadelphia, leukocytosis was evaluated in patients who applied after 24 hours in five of seven cases and in one of four patients applied within 24 hours (10). In our study 3 patients who presented in the acute period had leukocytosis, but case 2 who presented at day 10 had leukopenia. Even though extra luminal free air is the most common and consistent finding of gastroduodenal perforation, it may be absent at the onset of symptoms as was shown in 30%-50% of patients (11). Perforation sites can be often categorized by the CT findings, for instance: ulceration or a focal defect of the gastroduodenal wall, air bubbles in contact with the stomach or the duodenum, abrupt wall thickening associated with adjacent "dirty fat" density and local fluid between the duodenum and the pancreatic head (12). One of our patients had extraluminal free air on X-ray, one on CT, which confirms that absence of free air in imaging studies does not exclude gastrointestinal perforation. Duodenal ulcers were approximately 20-30 times more prevalent than gastric ulcers (2,3). In a recent study from Poland, duodenal ulcer perforations were more common (13). We noted 3 cases of duodenal ulcer and one of gastric ulcer. Surgical options range from localized ones (ie, bleeding, perforation or obstruction) to definitive ulcer operations. Definitive ulcer surgeries (eg, highly-selective vagotomy, truncal vagotomy with gastric drainage or partial gastrectomy) aim to reduce acid secretion and hence decrease the ulcer recurrence rate (2-4). Since these procedures include operative time and can be associated with increased perioperative morbidity and long-term adverse physiologic sequelae, in our cases, localized therapy was performed. Dakubo et al. (14) conducted a retrospective and prospective hospital-based study in Ghana. In their study, simple closure with an omental patch at a rate of 94.3%, truncal vagotomy and drainage with a rate of 3.2% and Billroth II partial gastrectomy with a rate of 2.2% were performed. Laparoscopic repairs were also reported (15) but open surgery still remains the approach of choice in the majority of centers for these patients. Omental patch is a preferred method to minimize the risk of leakage. PUP should be suspected in adolescents who suddenly develop severe, diffuse abdominal pain. We also conclude that although PUP is rare during childhood, it is a life-threatening condition.

Ethics

Informed Consent: The verbal consent was taken from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.Y., Z.D., E.U.S., Concept: A.Y., M.K., E.U.S., Design: A.Y., C.T., E.U.S., Data Collection or Processing: M.K., C.T., Analysis or Interpretation: A.Y., Z.D., E.U.S., Literature Search: A.Y., C.T., M.K., Writing: A.Y., Z.D., E.U.S.

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

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Case Report

Familial Mediterranean Fever Mimicking Wilson's Disease: A Case Report

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ABSTRACT

Wilson's disease (hepatolenticular degeneration) is an autosomal recessive defect in cellular copper transport. Impaired biliary copper excretion leads to an accumulation of copper mostly in the liver, brain and cornea. Familial Mediterranean Fever (FMF) is an autosomal recessive autoimmune disease as a result of a mutation in the *MEFV* gene encoding pyrin protein characterized by recurring fever and polyserositis attacks. In this report, we describe a Turkish female child with cholestatic hepatitis of unknown etiology who was later diagnosed with typical FMF.
Keywords: Familial Mediterranean Fever, Wilson's disease, liver, cryptogenic cirrhosis, cholestasis

Introduction

Wilson's disease (hepatolenticular degeneration) is an autosomal recessive defect in cellular copper transport. Impaired biliary copper excretion leads to an accumulation of copper mostly in the liver, brain and cornea. Different clinical manifestations of hepatic copper accumulation (acute hepatitis, chronic hepatitis, acute liver failure, cirrhosis) have been seen in Wilson's disease (1,2). The autosomal recessive disease Familial Mediterranean Fever (FMF), as the name suggests, is predominantly found in the Mediterranean populations; this includes Turks, Arabs, Armenians and non-Ashkenazi Jews. The disease is characterized by febrile, recurrent inflammatory attacks of the serosal membranes, causing a prolonged self-limitation (3). FMF has been linked to liver failure if left untreated over an extended period; this connection, however, has rarely been reported. In this report, we describe a patient admitted with acute cholestatic hepatitis who was later diagnosed with typical FMF.

Case Report

A 7-year-old female presented with jaundice, 2-3 reported instances of yellow-coloured stools and pruritus over a 5-day period. She had had abdominal pain, vomiting and poor feeding during the previous two days. The patient was seen regularly for health maintenance visits and she was generally a healthy child. She was up to date with her immunizations at that time. Verbal consent was taken from the patient's parents. Physical examination indicated poor overall health. However, her growth and development were considered normal. Her spleen and liver were enlarged with inferior margins of 4 cm and 5 cm respectively under the costal line. Blood analysis results revealed haemoglobin of 11.3 g/dL, white blood cell count of

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14.100/mm^3, with 72% neutrophils and a platelet count of 703.000/mm^3. The erythrocyte sedimentation rate (ESR) was elevated at 32 mm/h. C-reactive protein (CRP) was also elevated at 3.3 mg/dL. Prothrombin time, partial thromboplastin time and fibrinogen values were markedly abnormal. Liver function tests revealed elevated levels of aspartate aminotransferase (1.835 U/L), alanine aminotransferase (1.114 U/L), lactate dehydrogenase level (698 U/L), γ-glutamyl transferase (128 U/L), total bilirubin (20.45 mg/dL) and conjugated bilirubin (10.8 mg/dL). Her condition was evaluated as acute hepatitis with hyperbilirubinemia, coagulopathy and not accompanied encephalopathy (fulminant hepatitis). Antibody screening for hepatitis A, hepatitis B and hepatitis C viruses and for Epstein-Barr virus were negative. Toxoplasmosis was negative. Cytomegalovirus (CMV) antigen and CMV DNA were also negative. Tests for anti-liver-kidney microsomal anti-bodies Type I, anti-soluble liver antigen, anti-liver-pancreas antigen, anti-smooth muscle antibodies and anti-nuclear antibodies were all negative. Alpha-1 antitrypsin and sweat test were normal. The serum level of ceruloplasmin was 24 mg/dL (normal >20 mg/dL) and non-ceruloplasmin-bound copper level was elevated at 38 mcg/dL (normal <15 mcg/dL). The urinary copper level was 387 mcg/24 h and 128 mcg/24 h (normal ≤30 to 40 mcg/24 h). Her neurological examination was normal. The patient was administered a combination treatment of N-acetylcysteine (Asist 10%, Hüsnü Arsan Medical, İstanbul, Turkey) (5 mg/kg/h), ursodeoxycholic acid (Ursofalk, Aris Medical, İstanbul, Turkey) (15 mg/kg/d), zinc (Zinco-220, Berko Medical, İstanbul, Turkey) (2 mg/kg/d) and ampicillin (Ampisina, Mustafa Nevzat Medical, İstanbul, Turkey) due to the indication of liver insufficiency with unknown etiology. A liver biopsy was also performed. At the one-week follow-up, regarding the examination of the liver biopsy, all coagulation markers and transaminase levels had returned to normal levels quickly with the exception of cholestatic hepatitis. This quick decline of transaminase levels was attributed to the zinc supplementation. In spite of her normal ceruloplasmin levels, Wilson's disease was originally considered due to elevated urinary copper levels. D-penicillamin (Metalcaptase TBL, Actavis Medical, Czech Republic) treatment (250 mg) was initiated; increased to 500 mg after 1 week. A histological examination of the liver biopsy specimens showed moderate fibrosis with portal inflammations (Figure 1-3). Copper and orcein staining were negative and the dry liver copper weight was 21.8 mcg/g. The abnormal liver function improved and the patient was discharged. During the follow-up period, liver function tests were normal, however, an enlarged liver and spleen with elevated ESR and CRP levels were still being noted. A very detailed history of the patient revealed that she had been having attacks of arthralgia with fever three to four times per year, which now made FMF a strong possibility. At that time, a molecular analysis for Wilson disease's proved negative and informed consent was obtained in order to perform a

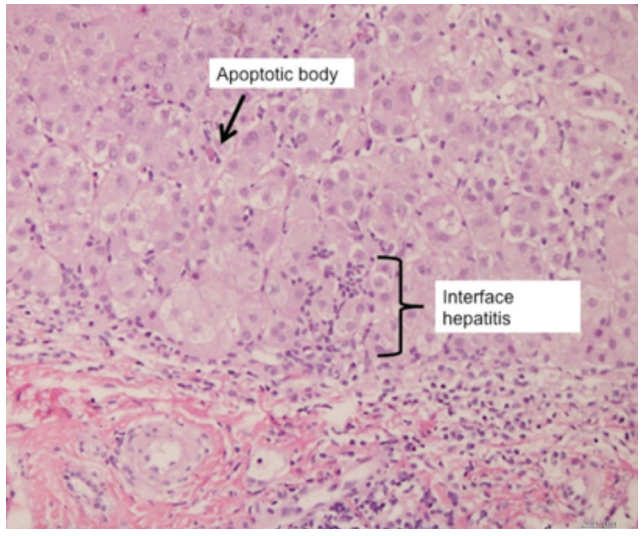

Figure 1. Interface hepatitis and apoptotic body (hematoxylin and eosin, x20)

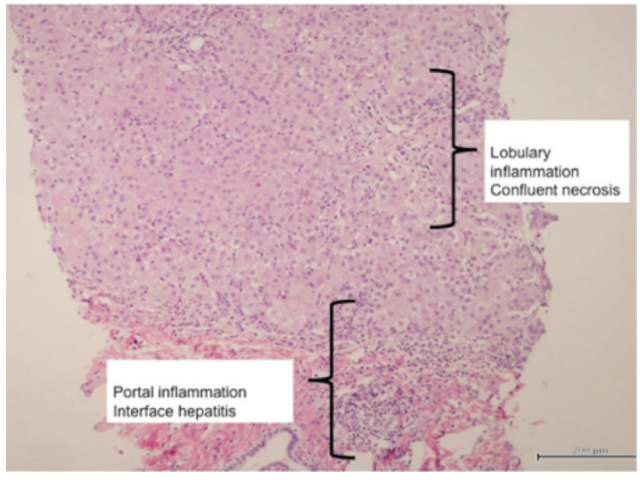

Figure 2. Lobulary inflammation, portal inflammation and confluent necrosis (hematoxylin and eosin, x10)

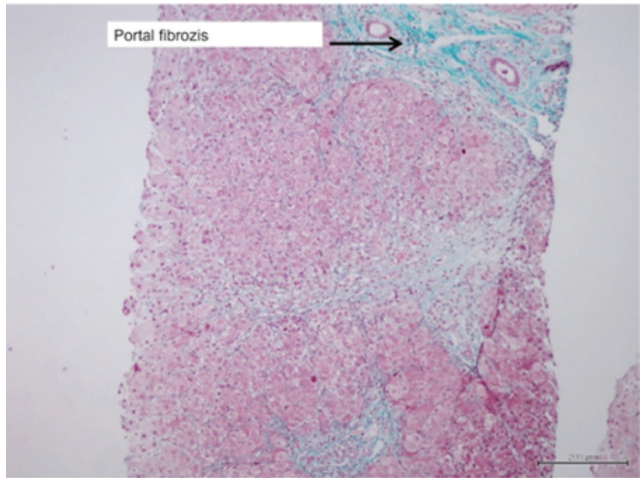

Figure 3. Portal fibrosis (hematoxylin and eosin, x10)

genotype analysis. After informed consent was obtained, genomic DNA was then extracted from the peripheral blood cells. Results showed a homozygous M694V mutation. Colchicine therapy at an average daily dose of 1.0 mg/day was initiated immediately, with d-penicillamin treatment being decreased. The patient responded to colchicine treatment and d-penicillamin treatment was completely discontinued.

The diagnosis of FMF was given after the history of arthralgia and fever attacks (≥3 attacks per year) became known, the homozygous M694V mutation was detected and there was a response to the colchicine therapy. Therefore, she presented two major and one minor criteria of FMF and the genetic mutation of FMF.

A follow-up examination one year later showed the patient had no clinical signs and all laboratory tests were within the normal ranges.

Discussion

The most prominent characteristics of FMF are short, acute attacks of abdominal pain and fever accompanied by serosal, synovial and cutaneous inflammation. While the liver is one of the most affected organs seen in FMF disease, Nonamyloid liver disease concomitant with FMF has rarely been reported. Korkmaz and Kaşifoğlu (4) reported mild hyperbilirubinemia in 11 of 41 adult patients and elevated transaminases levels in 9.7% percent of patients during acute FMF attacks. Migita et al. (5) described one female patient with hepatitis who was later diagnosed as typical FMF. In another study, Rimar et al. (6) reported an association between FMF and non-alcoholic steatohepatitis. Seventy-four percent of their FMF patients had originally been referred for assessment due to chronic liver disease. In the literature, however, only limited case reports involving recurrent acute cryptogenic hepatitis are available (7). Sari et al., (8) reported two cases with Budd-Chiari syndrome associated with FMF. They were characterized by a hypercoagulable state or ongoing inflammation that activated coagulation through endothelial damage, possibly leading to thrombosis. Unal et al. (9) found liver involvement in 11 of 58 pediatric FMF cases. One of their patients, originally admitted with acute cholestatic hepatitis and later diagnosed with FMF, was phenotypically similar to our patient in this case report. The molecular analysis of our patient for FMF revealed a homozygous M694V mutation. Tweezer-Zaks et al. (10) had previously suggested the possible association between FMF and homozygous M694V MEFV mutation with cryptogenic cirrhosis. They speculated that mutated MEFV may play a modifier role in cryptogenic cirrhosis. Unal et al. (9) determined that, while M694V allele mutations were a strong indicator of FMF, mutations without the underlying clinical manifestations had been observed. Future studies analyzing *MEFV* gene sequences in a large cohort of patients with liver involvement are needed to truly verify this possibility. This case study emphasizes the fact that FMF may present with cryptogenic hepatitis during the early years of life. Clinicians in Mediterranean countries (such as Turkey) where the prevalence of FMF is high must consider this when making their differential diagnosis.

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Ethics

Informed Consent: The verbal consent was taken from the patient's parents.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.T., M.K., Concept: C.T., M.K., S.A., Design: C.T., M.K., Ç.Ö.E., Data Collection and Processing: C.T., Ç.Ö.E., F.Y., Analysis and Interpretation: C.T., Ç.Ö.E., S.A., Literature Search: C.T., Ç.Ö.E., Writing: C.T., Ç.Ö.E., S.A.

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Case Report

A Novel *De Novo* Missense Mutation in *HNF4A* Resulting in Sulfonylurea-Responsive Maturity-onset Diabetes of the Young

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ABSTRACT

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes, with autosomal-dominant inheritance, which usually develops before 25 years of age. MODY is classically caused by a heterozygous mutation of genes known to affect insulin production or secretion. Heterozygous inactivating hepatocyte nuclear factor 4A (***HNF4A***) mutations, one of the rare subtypes of MODY, cause impaired insulin secretion and subsequent glucose intolerance especially in adolescence. Conversely, *HNF4A* mutations are also known to be associated with macrosomia and hyperinsulinemic hypoglycemia in newborns. Herein, we report a rare cause of diabetes resulting from a novel heterozygous mutation in the ***HNF4A*** gene. In conclusion, genetic testing should be considered in order to establish an accurate diagnosis and provide an opinion in determining the appropriate type of treatment.

Keywords: Maturity-onset diabetes of the young Type I, macrosomia, HNF4A, monogenic diabetes, child

Introduction

Maturity-onset diabetes of the young (MODY) is a monogenic subgroup of diabetes mellitus characterized by autosomal dominant inheritance, non-insulin diabetes onset usually before 25 years of age and decreased insulin production or secretion response to glucose. At least 13 different genes have been reported to be associated with MODY to date (1). Approximately 1-2% of patients with diabetes have a monogenic type (2). The inactivating mutations in the nuclear transcription factor 1 homeobox A (*HNF1A*), the hepatocyte nuclear factor 4 homeobox *(HNF4A)* and the glucokinase *(GCK)* are the most common causes of MODY (3). Other genes associated with MODY are infrequently detected: *HNF1B, IPF, NEUROD, PDX1, KLF11, CEL, PAX4, BLK, ABCC8* and *KCNJ11* (3). While the heterozygous inactivating mutations in the *GCK* gene lead to asymptomatic mild fasting hyperglycemia, mutations in the genes of *HNF1A* and *HNF4A* lead to progressive failure in insulin secretion and worsening of glucose tolerance with age (4). *HNF4A* is a member of the steroid/thyroid hormone receptor superfamily and plays a major role in glucose stimulated insulin secretion. Homozygous HNF4A mutation is lethal at the early embryonic stage (5). However,

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heterozygous *HNF4A* mutations have a Janus effect on glucose metabolism, which leads to either macrosomia and hyperinsulinemic hypoglycemia during infancy or MODY Type I in adulthood (6). The mutations of the genes involved in MODY are typically inherited from affected parents. However, a few *de novo* mutations have also been reported to date (4,7). In this study, we report on an interesting patient with MODY Type I that resulted from a novel and *de novo* mutation in the *HNF4A* gene.

Case Report

A 14-year-old girl was referred to our outpatient clinic due to fatigue and polyuria; and hyperglycemia was detected afterwards. She was born full term after an uneventful pregnancy with a birth weight of 5.500 gr [4.9 standard deviation (SD) score]. Her parents were healthy and there was no consanguinity between them. The family history revealed no diabetes. Physical examination of the case revealed a height of 163 cm (SD score 0.29), weight of 64.7 kg (SD score 1.2) and body mass index (BMI) of 24 kg/m^2 (SD score 1.2). Acanthosis nigricans or stria was not found. A puberty examination according to the Tanner scale was stage 5 and she had a regular pattern of menstrual periods. On admission, laboratory analyses showed hyperglycemia, a relatively low level of C-peptide, elevated glycated hemoglobin (HbA1c), a low level of triglycerides and negative autoantibodies regarding diabetes (Table I). Urine analysis revealed 2+ glycosuria and no ketosis. The parents had normal fasting blood glucose and HbA1c levels. These findings indicated a most probable diagnosis of MODY. We initiated an insulin glargine only treatment (0.2 unit/kg/day). Postprandial hyperglycemia was rarely observed and no significant hypoglycemia was seen with this treatment. HbA1c decreased to 6.3%.

Table I. The laboratory values of the patient at the diagnosis

Parameters	Patient value	Normal range
Glucose (mg/dL)	137	60-100
Total cholesterol (mg/dL)	137	<170
Triglyceride (mg/dL)	38	<150
LDL-cholesterol (mg/dL)	87	<130
HDL-cholesterol(mg/dL)	42	>45
C-peptide (ng/mL)	1.66	0.9-7.1
Hemoglobin (gr/dL)	12.8	12-15.6
Glycated hemoglobin (HbA1c) (%)	8.8	4-6.0
Anti-thyroid peroxidase (IU/mL)	0.9	0-9
Anti-thyroglobulin (IU/mL)	1.1	0-4
Anti-tissue transglutaminase (U/mL)	1.8	0-20
Anti-insulin antibody (%)	6.2	<8.2
Glutamic acid decarboxylase (IU/mL)	0.26	0-10
Islet cell antibody	Negative	Negative

HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Molecular Analysis

Genomic DNA was extracted from peripheral blood leukocytes of the patient and her parents by using MagNA Pure LC DNA Isolation Kit I (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. All coding exons and exon-intron boundaries of the *HNF4A* gene were amplified by polymerase chain reaction (PCR). After purification of PCR products, mutational analysis was performed by direct sequencing of the coding exons and flanking introns of the *HNF4A* gene in an ABI PRISM 3500 genetic analyzer (Applied Biosystems, Foster City, California, USA). As a reference sequence, NM_175914 (obtained from GenBank accession number) for HNF4A was used. While the father and mother had no mutation, analysis of the patient revealed a p.C93Y (c.278G>A) heterozygous novel change in the third exon of *HNF4A* (Figure 1). This missense mutation was not found in the Ensembl and Human Gene Mutation Database (HGMD). It was interpreted to be "disease causing" by the Mutation Taster Software (test score: 0.999999999999997) (http://www.mutationtaster.org). The cysteine residue in position 93 is highly conserved across different species (Figure 2). Insulin treatment was stopped and low-dose sulfonylurea (5.0 mg/day in two doses) initiated as soon as the diagnosis of MODY 1 was made. After five months of the administering, glucose monitoring was within

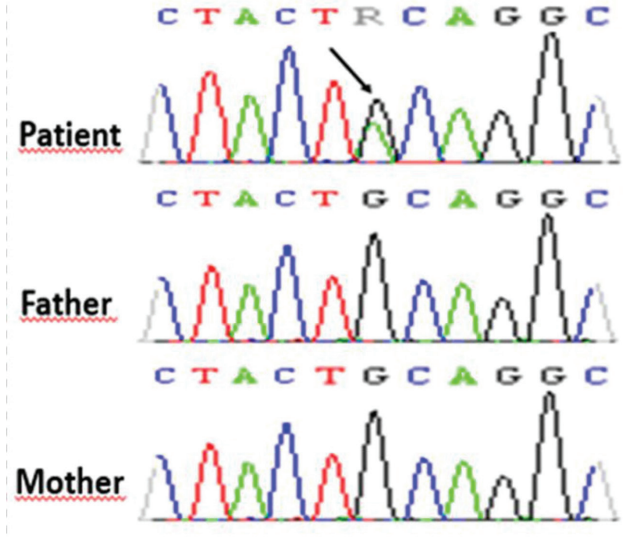

Figure 1. Partial sequence traces for the *HNF4A* gene of the father, mother and patient. Analysis of the patient revealed heterozygous and ***de novo*** a G-to-A (R=A) substitution (c.278G>A) that changes cysteine to tyrosine amino acid (p.C93Y) in exon 3

Transcript IDs	Species	Amino acid alignments around position 93
ENST00000316673	Human (Homo sapiens)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSPTRG00000013519	Chimpanzee (Pan troglodytes)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSMMUG00000006464	Monkey (Macaca mulatta)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSFCAG00000008178	Cat (Felis catus)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSMUSG00000017950	House Mouse (Mus musculus)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSGALG00000004285	Chicken (Gallus gallus)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSTRUG00000009982	Pufferfish (Takifugu rubripes)	...VDKDKRNQCRY C RLKKCFRAGMK...
ENSDARG00000021494	Zebrafish (Danio rerio)	...VDKDKRNQCRY C RLKKCFRAGMK...
T23H4.2	Nematode (Caenorhabditis elegans)	...VTKNKRNACRA C RLQKCVKAGMK...
ENSXETG00000001775	Frog (Xenopous tropicalis)	...VDKDKRNQCRY C RLKKCFRAGMK...

Figure 2. Partial protein alignment of *HNF4A* gene from different species around position 93. The cysteine residue in position 93 is highly conserved. Cystein is a polar neutral amino acid whereas tyrosine is a polar hydrophilic amino acid. C93Y mutation may change the secondary or tertiary structure of HNF4A protein and impair its function

the normal range during sulfonylurea treatment and no hypoglycemia was observed. Laboratory evaluation revealed fasting glucose at 111 mg/dL, insulin at 11 IU/mL, C-peptide at 2.2 ng/mL and HbA1c at 5.8%.

Discussion

GCK and *HNF1A* mutations are responsible for the majority of MODY cases. Of all MODY cases, 20-50% are caused by *GCK* and *HNF1A*, approximately 10% are from a mutation of *HNF4A* or *HNF1B* (3). Studies from our country reported that GCK is the most common subtype (9-11). In the present study, we have identified a novel heterozygous G-to-A substitution at 278 position (c.278G>A) that changes cysteine to tyrosine amino acid (p.C93Y) in exon 3 in HNF4A, which leads to MODY Type I. Flanagan et al. (8) have reported a different *de novo* HNF4A mutation at the same position (p.C93S, c.278 G>C) leading to a diazoxide responsive hyperinsulinemic hypoglycemia that was diagnosed within the first week of life in a patient born with macrosomia (4.100 gr). Our group (9) did not detect HNF4A mutations in 42 children diagnosed with MODY, but Ağladıoğlu et al. (10) analyzed 43 patients with MODY and identified two cases with the same heterozygous HNF4A mutations. One of the cases had a missense mutation (c.416C>T), which is associated with Type II diabetes mellitus in the HGMD. The other patient was carrying both heterozygous HNF4A (c.416C>T) and HNF1A mutations. As distinct from those two cases, our case had a *de novo* mutation. Findings of the multicenter study by Stanik et al. (4) underlined that *de novo* mutations of cases with MODY are more frequent than previously assumed. As a result, the authors emphasize the importance of genetic testing for MODY in patients without a family history (4). Thanabalasingham et al. (12) reported that measurable serum C-peptide is valuable in the diagnosis of MODY in individuals diagnosed with diabetes before 30 years of age. Moreover, they speculated that a family history of diabetes, presence or absence of autoantibodies regarding diabetes and metabolic disturbances (e.g. insulin resistance) were less important than previously thought (13). In the present case, the age at onset of diabetes, the negative family history, normal BMI, an absence of autoantibodies to pancreatic cell fragments and a measurable C-peptide level indicated MODY and a novel mutation in *HNF4A* was detected subsequently. Consistent with the literature, this patient was highly responsive to even low doses of sulfonylurea with no hypoglycemia. The majority of MODY Type I individuals are born with macrosomia (>4.000 gr) similar to the offspring of women with diabetes (13). Macrosomia is related to considerable fetal and maternal morbidity (13). In case of maternal diabetes, incremental glucose exposure to the fetus via the placenta results in incremental fetal insulin secretion and macrosomia develops subsequently due to insulin-mediated growth. However, the mother is normoglycemic in cases of *de novo* heterozygous *HNF4A* mutations and

associated fetal macrosomia is thought to be related to a different, yet unknown mechanism. *HNF4A* mutations are thought to have dual opposite roles in insulin secretion from beta cells (13). While these mutations usually lead to increased insulin secretion and subsequent hypoglycemia in newborns (not seen in our case), this effect is switched to impaired insulin secretion in adulthood resulting in glucose intolerance. Pearson et al. (5) asserted the underlying etiology of macrosomia in cases with a *HNF4A* mutation is associated with incremental endogenous insulin production. Since they found that 56% of the newborns with a heterozygous *HNF4A* mutation were macrosomic, birth weight was considered to be related with individual genetic characteristics as well as the maternal intra-uterine environment (6). All in all, *HNF4A* gene mutation should be considered in differential diagnosis of macrosomic newborns in spite of a negative family history for diabetes or hypoglycemia. Unlike MODY 2 cases, progressive hyperglycemia becomes evident in individuals of MODY 1 and MODY 3. Therefore, besides dietary treatment, they frequently require pharmacotherapy such as sulfonylureas, which usually allows for better glycemic control especially in children and young adults (4,7,14). It is well known that MODY 1 and MODY 3 cases are likely to develop microvascular complications at a similar rate compared with those of Type I or II diabetes (14). We switched the treatment of our case from insulin to sulfonylurea (glibenclamide 5 mg/day, b.i.d) when the diagnosis of MODY 1 was genetically proven. In follow-up, glycemic control was better and no hypoglycemia was observed. The transcription factor of HNF4A is an activator of genes involved in the control of lipid homeostasis as well as glucose metabolism (15). It has been demonstrated that *HNF4A* mutation carriers have low circulating triglycerides and apolipoprotein concentrations (16). In line with this, in another study, it was shown that a *HNF4A* knockout mouse had reduced fasting serum levels of total cholesterol, high-density lipoprotein, triglycerides and apolipoprotein (16). Our patient, similarly, had low serum level of triglycerides, which suggests an essential role of *HNF4A* in the complex transcription factor network that controls lipid regulation. In conclusion, herein, we described a rare cause of diabetes resulting from a novel and *de novo* heterozygous mutation in the *HNF4A* gene. We emphasize that genetic testing is crucial for both establishing an accurate diagnosis and providing an option to determine whether patients are sensitive to sulfonylurea or not. In addition, we underline that genetic testing of *HNF4A* might be considered for carefully selected patients born with macrosomia without hypoglycemia or a family history of diabetes.

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Ethics

Informed Consent: Consent form was obtained from the patient and her parents.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A., A.A., E.B., Concept: S.A., K.D., T.R.Ö., Design: S.A., B.Ö., A.A., Data Collection or Processing: S.A., K.D., T.R.Ö., Analysis or Interpretation: S.A., A.A., E.B., Literature Search: K.D., B.Ö., Writing: S.A., A.A., K.D.

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

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Case Report

A Rare Case of Cholestasis: Arthrogryposis, Renal Tubular Disorder and Cholestasis Syndrome

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ABSTRACT

Arthrogryposis, renal tubular dysfunction and cholestasis (ARC) syndrome is a rare, autosomal recessive multisystem disorder. Severe growth retardation, ichthyosis, recurrent febrile disease, platelet abnormalities, sensorineural hearing loss, hypotonia and corpus callosum dysgenesis were later included as further features of this syndrome. We present a case of ARC syndrome diagnosed by genetic analysis.
Keywords: Arthrogryposis, ichthyosis, cholestasis, renal tubular disorder

Introduction

Arthrogryposis, renal tubular dysfunction, cholestasis (ARC) syndrome was originally described in 1973 by Lutz-Richner and Landolt (1) severe growth retardation, ichthyosis, recurrent febrile disease, platelet abnormalities, sensorineural hearing loss, hypotonia and corpus callosum dysgenesis were later included as further features of the syndrome (1,2). This multisystemic disease due to an autosomal recessive hereditary transmission occurs as a result of *VPS33B* or *VIPAR* gene mutations (3,4). Most of the patients are lost during the first year of their lives due to dehydration, acidosis, sepsis and particularly profuse bleeding due to diagnostic invasive interventions (1,2,4). We present here a case of ARC syndrome diagnosed by genetic analysis.

Case Report

A thirty-five-day old female infant was referred to our hospital with jaundice and lack of weight gain. The parents of the infant, born at term weighing 2.620 g, were second degree relatives. At the referral time, her weight was 2.800 g (<3 p%), height 51 cm (3-10 p%) and head circumference 33 cm (<3 p%). Physical examination revealed jaundice of the skin and sclera, generalized hypotonia, dry skin, bilateral club feet and hepatomegaly. There was no history of acholic stool and it was not observed during follow up either. Laboratory results were as follows: white blood cell count 15.240/mm^3, hemoglobin 7.9 g/dL, platelet count 397.800/mm^3, C-reactive protein 1.45 mg/L, glucose 77 mg/dL, aspartate aminotransferase (AST) 133 U/L, alanine aminotransferase

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(ALT) 169 U/L, total bilirubin 9.72 mg/dL, direct bilirubin 4.51 mg/dL, alkaline phosphatase (ALP) 1142 U/L, gamma glutamyl transpeptidase (GGT) 31 U/L, prothrombin time 11.81 secs, international normalized ratio 1.03 and activated partial thromboplastin time 20.39 secs. Peripheral smear findings showed leucocytosis and normal thrombocytes. Thyroid function tests, viral hepatitis markers, alfa feto-protein levels were normal. Tandem mass spectrometry and urinary organic acid analysis were normal but urine amino acid chromatography showed generalized aminoaciduria. Blood pH was 7.21, bicarbonate 17 mmol/L and base deficit -8.3. urinary pH was 8.5 with glucosuria and proteinuria and fractional phosphate absorption was 0.4. These findings led to the diagnosis of proximal renal tubular acidosis (RTA). The liver and biliary system were normal in abdominal ultrasonography (USG), but pelvic ectasia and dysplasia were found at the left kidney. Cranial USG and tomography were normal. Hip ultrasound revealed a grade 3 dislocation on the right side and a grade II dislocation on the left. Bilateral sensorineural hearing loss was observed under brainsteam evoked response audiometry testing. The dermatological examination was consistent with ichthyosis. Based on these clinical and laboratory findings, ARC syndrome was considered. Due to expected severe bleeding disorder and lack of family consent, organ biopsies were not performed and genetic analysis revealed a homozygotic p.Gly514 Ser mutation in the *VPS33B* gene (Figure 1).

During follow up, growth retardation and ichthyosis progressively increased, growth parameters were below the third percentile at the age of three years. Cranial magnetic resonance imaging (MRI) showed corpus callosum dysgenesis and cerebellar displacement called Arnold Chiari malformation Type I. There were several oral bleeding episodes during teething. These bleedings could be stopped only by applying local anticoagulant. The patient had multiple febrile episodes and recurrent suppurative otitis media with *Pseudomonas aeruginosa* growth in ear culture due to hearing aid apparatus. The patient was lost in her last febrile episode because of severe intractable septicaemia.

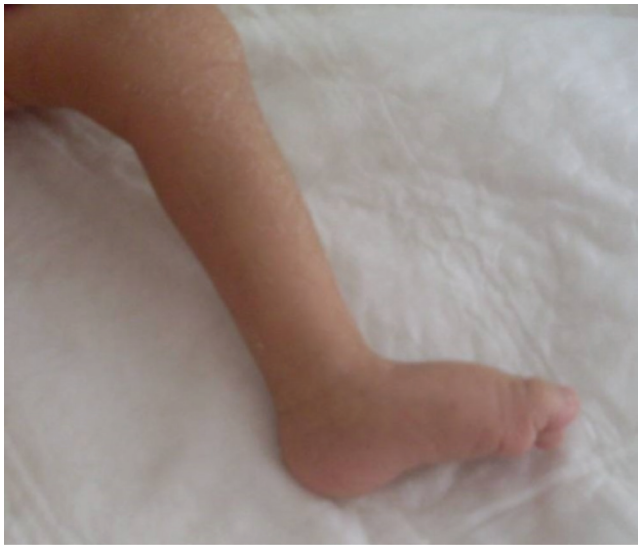

Figure 1. Club foot, icterus and dry-scaly skin of patient

Discussion

ARC syndrome is a rare disease and its incidence is not known. Most cases of ARC syndrome have been reported in Pakistani, Saudi Arabia, Oman, North Africa, Asia, Italy and Portugal. In Turkey, the disease was described for the first time in 2005 in two siblings and only a few cases have been added to them since then (5,6). However, in a study including a series of 90 infants with cholestasis in Korea, 46% were diagnosed as extrahepatic biliary atresia, 30% as neonatal hepatitis and 7% were diagnosed as having ARC syndrome due to a demonstrated *VPS33B* mutation (7). It was believed that some patients die without being diagnosed properly.

Arthrogryposis, which is thought to occur as a result of the degeneration of frontal motor neurons, is one of the distinguishing features of this syndrome. Characteristics of arthrogryposis include contractures, clubfeet, fractures and hip dislocations (4,6). Hip dislocation and clubfeet in our patient were considered as characteristics of arthrogryposis. Renal tubular dysfunction is observed in all patients and it is usually Fanconi type RTA associated with glucosuria, phosphaturia and proteinuria and less frequently as renal diabetes insipidus. Renal USG reveals nephrocalcinosis and dysplastic kidneys and renal biopsy shows glomerulosclerosis, tubular degeneration, calcification and glomerulocystic appearance (4,6,7). In our patient, there were dysplastic kidneys and Fanconi type RTA. Cholestasis and hepatomegaly exist in all patients, a mild elevation of ALT, AST and ALP levels in association with a normal GGT level are particularly seen in this syndrome (1-4). Normal GGT levels are also seen in progressive familial intrahepatic cholestasis syndromes but without arthrogryposis and ichthyosis. Liver histology is described as a decrease in biliary ductus and proliferation, giant cell formation, pigment deposition, extramedullary hematopoesis and portal fibrosis which are non-specific features (4,7). Recently, because of a high risk of profuse bleeding, genetic analysis is recommended instead of biopsy (4). In our patient, ALT, AST and ALP levels were elevated and GGT was normal. A liver biopsy was not performed because of severe bleeding risk and family consent was lacking; thus, the diagnosis was confirmed by genetic analysis. Severe growth retardation is seen in all patients and severe acidosis, diarrhea, recurrent febrile diseases and renal losses account for this growth retardation (2,4,7). Our patient also had severe growth retardation despite all the supportive therapies. Ichthyosis may not be present at birth in many cases and it usually occurs after the first month of life

(4). Our patient had ichthyosis unresponsive to treatment, worsening gradually over time. The central nervous system symptoms of this syndrome are hypotonia, sensorineural hearing loss and corpus callosum dysgenesis or agenesis (1,4,7). Our patient was using a hearing aid apparatus due to sensorineural hearing loss and cranial MRI had shown corpus callosum dysgenesis and Arnold Chiari Type I malformation. It is not known if Arnold Chiari malformation is a component of ARC syndrome or a coincidence because there was no published data to be found in the medical literature. Tendency to spontaneous bleeding is reported in these patients and it is considered that Grey Platelet syndrome and a dysfunction of alpha granules are the causes of these bleeding episodes (4,8). In a case report, spontaneous nasal bleeding could be stopped only by the delivery of a platelet suspension (8). Our patient had several prolonged oral mucosal bleeding episodes despite normal coagulation tests. These bleedings were stopped by use of local anticoagulants and platelet suspensions were not given. Genetic mapping of ARC syndrome was described as a mutation of chromosome 15q26.1 in *VPS33B* gene. This gene encodes the *VPS33B* protein that is involved in the vesicular trafficking pathway (4). This protein, existing in various organs, is involved in intercellular synaptic transmission, vesicular exocytosis and general secretion. Akbar et al. (9) have shown that the *VPS33B* gene is involved in phagosome and endosome maturation and is responsible for the recognition of microorganisms. *Staphylococcus species*, *Escherichia coli* and *Pseudomonas aeruginosa* were shown as causative agents (8,9). Episodes of suppurative otitis media caused by *Pseudomonas aeruginosa* were observed in our patient. Treatment in ARC is usually symptomatic. However, recently, there has been a reported case of a child with ARC syndrome from Iran undergoing a liver transplantation and still surviving after five years (10). Further prognostic improvements are expected for this condition. In conclusion, ARC syndrome is a cause of cholestasis and it should be particularly kept in mind in certain geographical locations including Turkey. In an infant presenting with cholestasis that is associated with ichthyosis and renal tubular dysfunction, ARC syndrome should be considered.

Ethics

Informed Consent: The verbal consent was taken from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.T., A.B., F.C.Ö., Concept: Y.T., Y.A., Design: Y.T., S.E.A., Data Collection and Processing: Y.T., A.B., R.S., Analysis and Interpretation: Y.T., S.E.A., Literature Search: Y.T., R.S., F.C.Ö., Writing: Y.T., Y.A., R.S., S.E.A.

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

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Case Report

Post-traumatic Delayed Peripheral Facial Palsy

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ABSTRACT

Peripheral facial palsy in children is very rare in comparison to adults. The most common cause is idiopathic. Another rare cause of peripheral facial palsy in children is trauma. It occurs after head trauma, mostly due to temporal bone fracture as an early onset paralysis after trauma. Early onset facial palsy is usually due to direct damage to the facial nerve whereas there is some controversy about the etiology of late onset facial palsy. In this article, a child patient whose peripheral facial palsy developed six days after a head injury is presented, and the etiopathogenesis and the treatment of delayed traumatic facial palsy after temporal bone fracture is discussed.
Keywords: Head trauma, facial palsy, child, etiopathogenesis, treatment

Introduction

Facial paralysis (FP) in children is an uncommon condition, although it is common in adults. Its estimated annual incidence is thought to be approximately 2.7/100.000 in children younger than 10 years (1). In spite of this, there are several described causes of facial nerve paralysis in children, in approximately 40-75% of cases, the cause of unilateral FP is still unknown and it remains idiopathic (2). The most common known causes of FP are Herpes Simplex Type I, varicella zoster virus, Ebstein-Barr virus, hemophilus influenza, tuberculosis, Lyme disease, adenovirus, rhinovirus, acute and/or chronic otitis media, ear infection with cholesteatoma, mastoiditis, vasculitis, inflammatory disease such as Henoch-Schönlein purpura, Kawasaki syndrome and neoplastic tumors (1,2). FP due to fracture of the temporal bone after head trauma is not common. It accounts for 1.5-5% of the causes of FP in children, and is mostly seen immediately following a head injury (3-5). The incidence of delayed facial palsy after head injury was found to be approximately 0.6-2.2% (6). In this article, a child patient whose peripheral facial palsy developed six days after head injury is presented, and the etiopathogenesis and the treatment of delayed traumatic facial palsy following a temporal bone fracture is discussed.

Case Report

A-8-year-old girl with right peripheral FP was sent to our ear, nose and throat (ENT) clinic from the emergency service. In her medical history taken from her family, she had fallen from a bicycle 6 days earlier. On the day of the accident, she arrived at the emergency service, a computed brain tomography was taken and it appeared normal. Her physical and neurological examinations revealed no abnormalities and she was discharged. Five days after her head trauma her family noticed immobilization of the right side of her mouth and the inability to close her right eye, and they applied to the emergency department. There was no complaint such as ear pain, bleeding from the ear, hearing loss or vertigo. On otological examination, the right ear drum was intact, but there was hemotympanum in the anterior zone of her right ear drum. Her neurologic examination revealed the development

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of a House-Brackmann Grade III-IV peripheral FP on the right side (Figure 1). The other examinations were normal. In the patient's hearing test, an average 28 dB conductive hearing loss was found in the right ear (Figure 2). High resolution temporal bone computed tomography (HRTBCT) of the patient was taken, which revealed hemorrhage and edema in the right middle ear cavity and a mastoid cellular and longitudinal temporal bone fracture without bone chain damage (Figure 3a, 3b). It was seen that the fracture line crossed at the geniculate ganglion of the facial nerve (Figure 4). Corticosteroids were administered to the patient at a dosage of 1 mg/kg/d and it was decreased progressively and stopped after 18 days. By the end of one week, FP had begun to improve. She was examined after one month; her FP and hearing deficit were resolved completely. Written informed consent was obtained from the patient's parents.

Discussion

The trauma patient constitutes most of the patients who apply to the emergency department (7). Traumatic head injury is a smaller group of these patients. Basal skull fractures account for 21% of all skull fractures. In approximately 7-8% of these individuals, temporal bone fractures occur. In the pediatric population, the incidence of temporal bone fracture

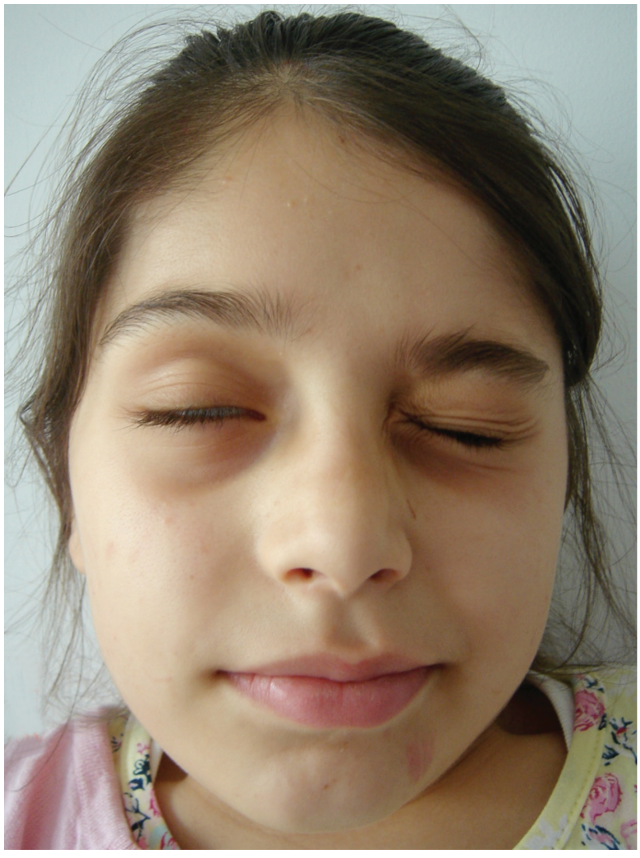

Figure 1. The patient with right peripheral facial palsy

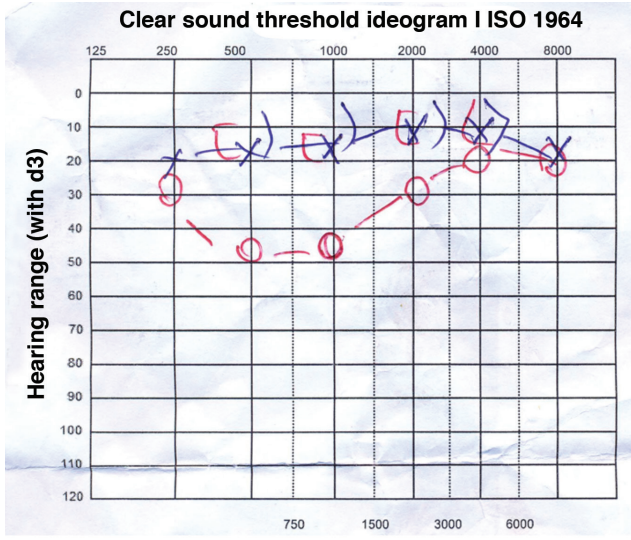

Figure 2. The hearing test of the patient after trauma

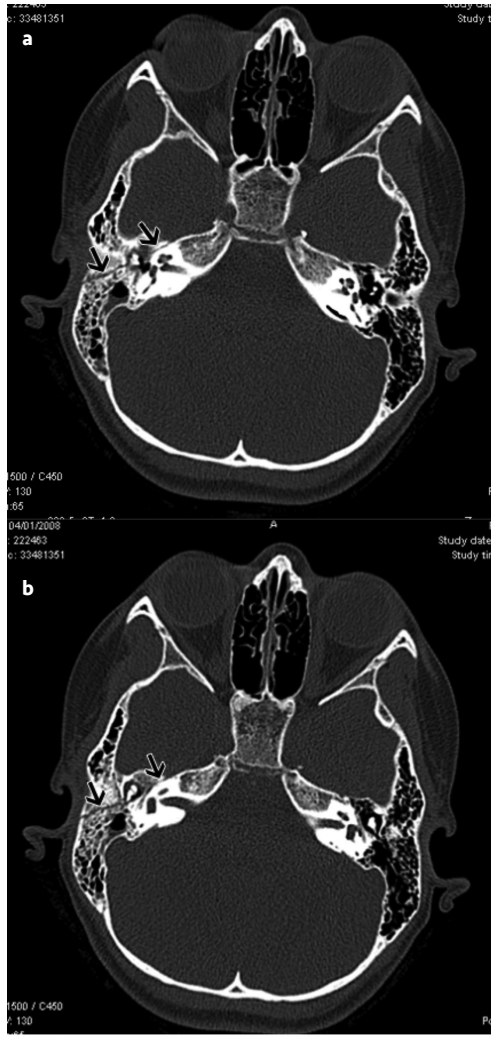

Figure 3a, 3b. The image of coronal section high resolution computed tomography of the patient

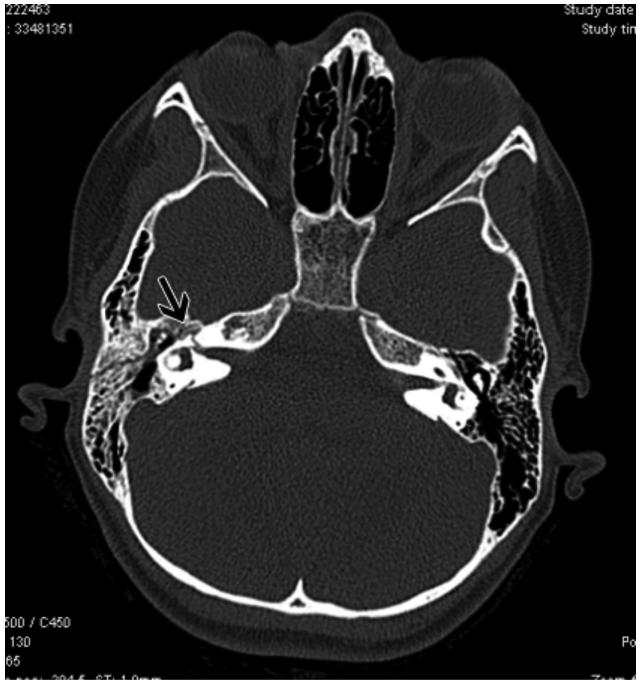

Figure 4. The fracture line which crossed the geniculate ganglion section of facial nerve (arrow)

is not common (7). Temporal bone fractures are classified as longitudinal, transverse and mixed with respect to the long axis of the petrous bone: in longitudinal fractures; the fracture line is parallel to the long axis of the petrous bone, in transverse fractures; the fracture line crosses the long axis of the petrous bone, in mixed fractures; there are both fracture lines (8). Up to 80% of all temporal bone fractures are longitudinal as was seen in our patient. Longitudinal fractures very often pass through the external auditory canal, and FP occurs in 10-20% of these cases (6,9). Transverse fractures are uncommon and account for only 15% of temporal bone fractures. In these patients, trauma usually occurs in the occipital and frontal region. Facial nerve paralysis occurs in 50% of transvers fractures, and the paralysis is likely to be immediate in onset. Mixed type fractures are very rare, they account for 5% of all temporal bone fractures (9). The severity of head trauma affects both the severity of temporal bone fracture and the development of FP. Presently, the use of seat belts and more recently airbags has dramatically decreased the incidence of temporal bone fractures (10).

In the immediate onset of facial nerve palsy after trauma, the nerve is either completely lacerated or contused at the fracture site (4,6). The delayed presentation of FP is seen typically 1-10 days after injury. In our patient, FP developed over 6 days. There are different ratios about post-traumatic delayed facial palsy in different studies. Turner found a 2.2% ratio in their study while Puvanendran et al. (6) found the ratio to be 0.3%. To our knowledge, there is no study in the literature about this issue in children. The pathophysiology of post-traumatic delayed facial palsy is not clearly known. Some theories have been suggested about this issue: in the facial canal, the area occupied by the facial nerve is only 30-50% of the cross-sectional area of the canal. The remainder of the facial canal is occupied by blood vessels with connective tissue loosely arranged around the nerve. Delayed facial palsy is possibly the result of bleeding into the facial canal. An increasing size of a hematoma in the limited non-expanding bony tube could press on the facial nerve. If the pressure were of a mild degree, there would only be a neuropraxia, or conductive block due to segmental demyelination. If the damage were more severe, there could be axonal damage with denervation (6). The other theory is that ultimately the blood supply of facial nerve is cut off due to the trauma and this causes ischemic damage to the nerve. Some authors postulate a similar mechanism for this facial weakness as in Bell's palsy with a possible inflammatory reaction in and around the nerve, or a swelling of the nerve in the canal which could lead to ischemia. The vascular damage such as delayed arterial spasm, arterial or venous thrombosis, external compression from bony fragment or soft tissue edema are other etiological causes (4,5,9). The symptoms and clinical findings in temporal bone fractures change depending on whether there is a longitudinal or transverse fracture line. Longitudinal fractures very often pass through the external auditory canal, and usually tear the tympanic membrane producing bleeding from the external auditory area and leading to conductive hearing loss. Transverse fractures cause vertigo, hemotympanum and sensorineural hearing loss (6). In this condition, otological examination should be carried out and the patient should be referred to an ENT specialist. HRTBCT imaging should be taken to assess facial nerve damage. In the treatment of post-traumatic FP, it is important to identify patients with immediate or delayed onset facial palsy. The treatment protocol should as below (10):

- In cases of immediate FP after trauma and electrophysiologically severe with a clear cut fracture line on the fallopian canal on high-resolution CT (HRCT), surgery is performed as soon as possible; depending on the patient's neurologic status. Facial nerve repair is achieved by reanastomosis of the severed ends or, in cases with significant loss of nerve tissue, cable grafts using the great auricular nerve, the sural nerve or the cervical plexus as donor sites (4).

- In cases of immediate FP in the absence of a visible fracture line on HRCT, a medical treatment with steroids is given. The patient is followed with electrophysiological tests over a period of 3-6 months. Crushing or stretching injuries without cutting the nerve cause interruptions in nerve electric conduction. Stretching injuries heal more slowly than crushing injuries and they differ in range and degree. Surgery may be performed if there is no recovery in terms of both

electrophysiological tests and clinical tests 6 months after trauma (9,10).

- In cases of FP a few days after trauma, even if a visible fracture line is present on HRCT, the patient is followed with medical treatment. Steroids are given to reduce inflammation and edema in the nerve. Corticosteroid treatment initiated at a dosage of 1 mg/kg/d is given for 3 weeks (1,7).

The time of surgery time is controversial. If surgery is performed as early as possible, the functional and aesthetic results are more likely to be better than if the surgery is delayed. If the nerve was cut, the repair of it should be completed within 72 hours from the onset of the trauma (2). Prognosis of post-traumatic FP depends on the time of onset of the paralysis, the degree of paralysis and the site of the injury. Delayed post-traumatic FP usually has a good prognosis. Most patients of post-traumatic facial injuries recover with conservative treatment. Surgery and reanimation are rarely required (9). As a consequence; although the incidence of post-traumatic FP in children is very low, as soon as this issue is recognised, it must be immediately treated. Emergency service doctors should be alert to all issues concerning children with head-injuries. When a child with post-traumatic FP comes to an emergency department, the emergency department physician should take a detailed history and perform a detailed physical examination, arrange a cranial CT scan and refer the patient to a brain surgeon and ENT specialist to establish the most appropriate treatment as quickly as possible.

Ethics

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

Peer-review: Externally peer-reviewed.

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

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Case Report

A Rare Cause of Neck Mass: Pilomatrixoma

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ABSTRACT

Pilomatrixoma (pilomatricoma) (Malherbe's calcifying epithelioma), although rare, is the second most frequent benign skin tumour in childhood. It originates from the hair follicles. It is most commonly seen on the head and in the neck region, it also may be located on the upper extremities. Diagnosis may be confused with other skin tumours and malignancies. In this case, we report on a patient who presented with a mass on the neck diagnosed as a pilomatrixoma.
Keywords: Pilomatricoma, pilomatrixoma, skin tumour, child

Introduction

Pilomatrixoma is a benign tumour of the skin which originates from the cortex of hair follicles and is named as "calcifying epithelioma". Although rare, it is the second most common benign tumour of the head and neck, which are its most common locations. The majority of cases occur in the first two decades of life. It is a dermal tumour which is generally a well-demarcated, hard, mobile superficial nodule that grows slowly and it rarely shows malign transformation. In this case, we report on a patient who presented with a mass on the neck diagnosed as a pilomatrixoma.

Case Report

A 7-year old boy was admitted to the pediatric emergency department because of a swelling on the left side of the neck. It was first noticed four months previously, grew increasingly and did not respond to antibiotic treatment. He did not have fever, anorexia, sweating or weight loss in his history. On physical examination, a palpable pea-sized mass, approximately 20x10 mm in size, was noted in the left posterior cervical region. The mass was mobile, hard and well-demarcated (Figure 1, 2). Lymphadenopathy (LAP) was not present. Further physical examination was normal. According to laboratory findings, hemogram, peripheral smear, erythrocyte sedimentation rate and other biochemical tests were normal, C-reactive protein was negative, serologic analyses were normal, toxocara immunoglobulin (Ig) M and toxoplasma IgM-IgG were negative. Ultrasound examination showed a solid mass, 14x9 mm in size, on the left posterior cervical region and no organomegaly in the abdomen. The purified protein derivative (PPD) of tuberculin test was normal (3 mm). A local excision of the mass was performed. Gross examination of the specimen showed a 20x50x10 mm firm white nodule with a calcified cut surface and capsule. Pilomatrixoma was diagnosed with the appearance of basaloid hair matrix cells and eosinophilic anucleated shadow cells under histologic examination. The patient's recovery was uneventful. No malign transformation was observed and no recurrence was seen after a follow-up period of 8 months. Verbal consent was given by the patient's parents.

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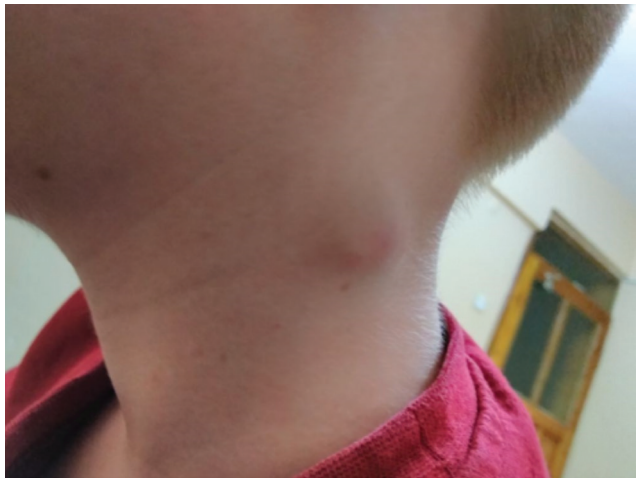

Figure 1. A palpable pea-sized mass, approximately 20x10 mm in size, in the left posterior cervical region

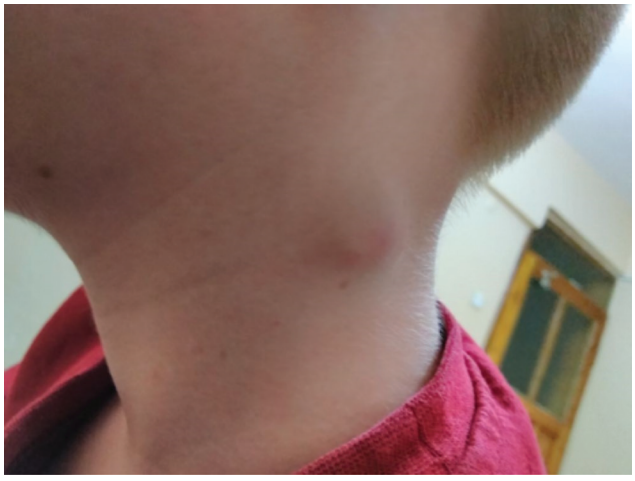

Figure 2. A palpable pea-sized mass, approximately 20x10 mm in size, in the left posterior cervical region

Result

Pilomatrixoma is a rare benign skin tumour. Despite being well known by dermatologists and pathologists, it causes diagnostic difficulties for pediatricians since it is rarely encountered in pediatrics. Rather than requiring a large number of laboratory tests in patients with chronic-subacute LAP, excision after clinical examination will facilitate diagnosis and avoid unnecessary further testing.

Discussion

LAP is usually benign in childhood with a common prevalence. Aggressive management or biopsy in due time is the most important factor to avoid serious or specific diseases. In children, the most common causes are infections of viruses, bacteria or mycobacteria. Acute localized lymphadenitis may have unilateral or bilateral LAP. In our case, fever, tenderness and fluctuance were not detected. Staphylococcus or group A beta hemolytic streptococci are frequently seen in etiologies (1,2). Also, tooth decay or anaerobic microorganisms in periodontal diseases cause LAPs (1). No tooth decay or history were noted in our patient. Subacute-chronic lymphadenitis is a lymph node that grows in days and weeks, painless or mildly painful, with no fever, sometimes fluctuating and usually without prodromal findings. Mycobacterial infections, cat scratch diseases, cytomegalovirus (CMV), toxoplasmosis-toxin infections, human immunodeficiency virus (HIV), sarcoidosis are considered during the differential diagnosis, but less frequently neoplasms should also be considered. Tuberculous lymphadenitis has unilateral, painless LAP and fistulisation is seen very commonly. Positive X-ray findings have been found in 30-70% of cases and the existence of a PPD over 15 mm is consistent with tuberculosis (1,2). In our case, since the fistula was absent, the chest X-ray was normal and PPD was 3 mm; tuberculosis was not considered. In addition, CMV, HIV toxoplasma and toxocara tests were found to be negative in the serological analyses. Cat scratch disease is seen as a self-limited localized LAP caused by Bartonella henselae after contact with cat litter or scratching. In this case, the LAP grows at the bite/scratch site 1-8 weeks after infection and clinical findings of anonymous fever, encephalitis, neuroretinitis, granulomatous conjunctivitis, hepatosplenic involvement, atypical pneumonia and thrombocytopenic purpura may be seen (3,4). In our case, cat scratch disease was not considered because there was no incident of cat contact. Childhood neoplastic (malignant) diseases are also among the causes of LAP. In particular, leukaemia, Hodgkin's disease, non-Hodgkin's lymphoma, solid tumour metastases (neuroblastoma, nasopharyngeal carcinoma, rhabdomyosarcoma and thyroid cancers), histiocytosis (langerhans cell histiocytosis, Haemophagocytic syndromes) should be kept in mind for differential diagnosis (5). In our case, the absence of symptoms B, no malignancy findings in the physical examination and the findings of the laboratory examination results being normal, it was decided to perform a lymph node biopsy for a definitive diagnosis. Neoplastic diseases were not considered. Pilomatrixoma is a rare benign tumour of childhood which frequently develops from the hair follicles of subepidermal tissue (6). They are usually asymptomatic and patients often present with only a solitary, firm mass that grows slowly under the skin (7). While a typical single lesion of 0.5-3 cm in size is typically seen, large size lesions and a number of familial lesions have also been reported (6,8). Gardner's syndrome may be seen with familial adenomatous polyposis, myotonic muscular dystrophy and Turner's syndrome (9,10). In our case, a unilateral, solitary 2x1 cm mass was seen similar to cases reported in the literature and also no accompanying syndrome or anomaly were detected. The patient's history and physical findings

gave rise to thoughts of pilomatrixoma; however, according to the literature, less than 50% of cases are thought to be pilomatrixoma before a pathological diagnosis is made (11). In our case, a lymph node biopsy was performed to eliminate the possibility of the existence of malignancy due to the history and physical examination findings and the diagnose was set after the histopathologic evaluation. A well-defined solid mass that is localized in the dermis and subcutaneous fat tissue, as seen with ultrasonography (USG), which causes focal scarring in the dermis is typical for pilomatrixoma. A target lesion can be seen in the centre. An acoustic shadow is observed due to calcification in the central nidus (6). However, in our case, the mass was interpreted via USG as only a smoothly bounded solid mass in the subcutaneous area. The reason for not considering pilomatrixoma during USG is thought to be because the differential diagnosis report in which pilomatrixoma was considered was not presented by the patient to the radiologist. Treatment is usually set as surgical excision and rare recurrence may be seen in incomplete excision (11). Malign pilomatrixomas are very rare and mostly seen in adults (12). Total excision was performed in our case and no recurrence was detected in the follow-up period of 8 months.

Ethics

Informed Consent: Verbal consent was given by the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.T., A.Y., Concept: C.T., E.U.S., Design: C.T., E.U.S., Data Collection and Processing: C.T., A.Y., Analysis and Interpretation: C.T., E.U.S., Literature Search: C.T., E.U.S., Writing: C.T., E.U.S.

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