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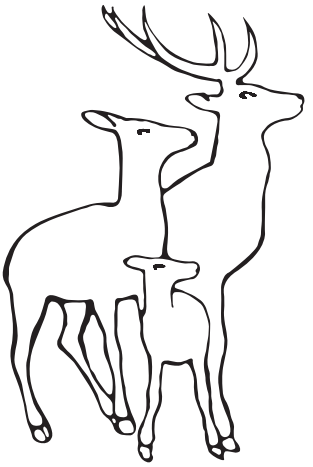

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YAYIN SAHİBİ
Yayınlayan Kurumlar Adına
Murat YURDAKÖK

SORUMLU YAZI İŞLERİ MÜDÜRÜ
Enver HASANOĞLU

DÖKÜMANTASYON
Ali DÜZOVA

YAYINLAYAN
Türkiye Milli Pediatri Derneği,
Hacettepe Üniversitesi Çocuk Sağlığı Enstitüsü
Uluslararası Çocuk Merkezi

EDİTÖR ADRESİ
Çocuk Sağlığı ve Hastalıkları Dergisi
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Faks: (312) 305 22 64

YAYIN İDARE MERKEZİ
The Turkish Journal of Pediatrics Editör Ofisi
Hacettepe Üniversitesi
İhsan Doğramacı Çocuk Hastanesi
06100 Ankara
Tel : (312) 305 26 76
Faks: (312) 305 22 64

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BASIM YERİ
Meteksan Matbaacılık ve Teknik Sanayi A.Ş.
Beytepe No: 3, 06530 Bilkent, Ankara, Türkiye
Tel: (312) 266 44 10 (Pbx)

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Original Article

Children with chronic-refractory autoimmune cytopenias: a single center experience

Tuba Hilkay Karapınar[1], Ersin Durgun[1], Yeşim Oymak[1], Nesrin Gülez[2], Yılmaz Ay[1], Ferah Genel[2], Salih Gözmen[1], Erkin Serdaroğlu[3], Sultan Aydın Köker[1], Ersin Töret[1], Canan Vergin[1]

Departments of [1]Pediatric Hematology-Oncology, [2]Pediatric Immunology and [3]Pediatric Nephrology, Dr. Behçet Uz Children Research and Training Hospital, İzmir, Turkey.

ABSTRACT

Background and objectives. Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoietic lineage cells. The differential diagnosis of children with autoimmune cytopenias requires much time and laboratory investigations. The aim of the present study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children at a single children's hospital.

Method. Between February 1997 and September 2015, chronic/refractory autoimmune cytopenias patient data were evaluated retrospectively. Twenty-three patients were assessed in this study.

Results. The median duration of following was 2.6 years (4 months-18.5 years). The median age of diagnosis was 3.1 years (6 months-16 years). A total of 13 patients (56.5%) had single-lineage and 10 (46.5%) had multi-lineage cytopenias. The most frequent single-lineage cytopenia was thrombocytopenia, followed by anemia. In 22 of the patients, cytopenias was detected before the primary diseases. All of the patients were treated with corticosteroids or intravenous immune globulin as first-line treatment. Ten patients (43.5%) needed second or further-line immunosuppressive therapies that patients diagnosed as systemic lupus erythematosus, hypogammaglobulinemia, or common variable immunodeficiency. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primer disease. Cytopenias were continued in 14 patients.

Conclusion. Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed in the clinical course. Taking the new targeted treatment options into consideration; early diagnosis is likely to become more important in the near-future in order to begin the treatment for the underlying disease as early as possible.

Key words: immune cytopenia, childhood, immune deficiency, autoimmunity.

Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoietic lineage cells. In some instances, they are idiopathic or occur as a manifestation of other underlying disorders, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome (ALPS), tumors, medications (penicillin, cephalosporins, sulphonamides, nonsteroidal anti-inflammatory drugs, etc) or infections.[1-3] Most children with autoimmune cytopenias have idiopathic disease. However, sometimes, the hematological manifestations precede the clinical onset of underlying immune disorders. So, the differential diagnosis of children with autoimmune cytopenias requires much time and laboratory investigations.[4-6]

The management of patients with chronic-refractory autoimmune cytopenias is

✉ Tuba Hilkay Karapınar
thkarapinar@yahoo.com

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heterogeneous and complex. Typically, these patients are treated with nonspecific immune suppression. Some of those patients respond to first-line immunosuppressive therapy, although many of them require second or further-line therapies. The primary goal of the therapies is directed at the underlying cause of the autoimmunity.[4,7] However, therapies of autoimmune cytopenias may be insufficient still more, because auto-immunity has been increasingly recognized as an important component of immune disorders.[2,4,8]

There have been a few reports of chronic/ refractory autoimmune cytopenias in childhood.[2,4,9-11] Therefore, the aim of this study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children followed up at our hospital.

Material and Methods

Study design, selection of patients and definitions

A total of 337 files of information belonging to patients with chronic or refractory autoimmune cytopenias except tumors were evaluated retrospectively at our hematology department between February 1997 and September 2015. Cytopenias due to medications, infections or idiopathic were excluded. Ultimately, patients with immune deficiency or autoimmune diseases (23 patients) were included in this study.

Cytopenias were the reduction of the blood cell types in the peripheral blood. Thrombocytopenia was defined as a platelet count <100x10^9/L according to an International Working Group criteria.[12] Autoimmune hemolytic anemia (AIHA) was defined as anemia (hemoglobin<10 g/dl) with evidence of hemolysis (reticulocytosis and positive direct antiglobulin test). The lower limit of the absolute number of neutrophils was accepted as 1000/μL in infants and 1500/μl in children over 12 months of age.[13] The diagnosis of autoimmune neutropenia (AIN) was accepted a diagnosis of exclusion. When the cytopenias had persisted more than 3 months, they were accepted as chronic or refractory cytopenias.[12-14]

The diagnosis of Common variable immunodeficiency (CVID) was based on 4 criteria: Age above 4 years, low serum IgG (Immunoglobulin G; reduced by more than two standard deviations below the mean for age), with or without low serum IgA (Immunoglobulin A) diminished antibody response to immunizations, and exclusion of other potential immune diseases.[15] Combined immune deficiency was accepted as evidence of a profound T cell defect along with evidence of a humoral defect.[16] Patients fulfilling the revised diagnostic criteria for ALPS were classified as ALPS patients: Chronic non-malignant lymphoproliferation, elevated double negative T cells (CD4-/CD8-, CD3+, TCR $\alpha\beta$+) in peripheral blood circulation and defective *in vitro* Fas-mediated associated.[17] Systemic lupus erythematosus (SLE) was diagnosed according to Systemic Lupus International Collaborating Clinics classification criteria.[18]

Statistical Methods and Ethical Permission

Data were analyzed using SPSS 15.0. The results are presented as the mean, standard deviation, median, absolute number, or percentile. Comparison of median values between independent groups was carried out with the Mann Whitney test. This study was approved by local ethics committee of the Dr. Behçet Uz Children's Research and Training Hospital (number: 2018/233).

Results

Our protocol included evaluation of the following variables for chronic- refractory cytopenias: Children were evaluated using a standard diagnostic approach, obtained thorough history, physical examination, laboratory screening tests (complete blood count, direct antiglobulin test, liver and renal function tests, measurements of immunoglobulin (IgA, IgM, IgG, and IgE), C3 and C4 complements

and antinuclear antibody levels, standard peripheral blood flow cytometry analysis ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+56^+$ and if necessary double negative T cells) , serology of several viral pathogens (TORCH, EBV, hepatitis, and HIV) and bone marrow aspiration evaluation (morphologic, cytogenetic).

The patients with immune thrombocytopenia (ITP) were treated with corticosteroids (30 mg/kg/d, 3 days, followed 3 days 20 mg/kg/d, then 2 days 10 mg/kg/d, pulse methylprednisolone) or a single dose of intravenous immune globulin (IVIG) at 1 g/kg as first-line treatment. Further use of IVIG was dependent on clinical response to the initial dose. The patients with AIHA were treated much like ITP as first-line treatment. The treatment of AIN was oral or intravenous antibiotics depending on the severity of bacterial infections as first line treatment. For refractory cases of ITP, AIHA, or both, second or further-line immunosuppressive therapies including rituximab, mycophenolate mofetil (MMF), cyclosporine A, azathioprine, and chloroquine have been used.

All of the patients were followed up for a maximum 18.5 years or until they recovered from cytopenia. The median duration of following was 32 months (between 4 months-18.5 years). Twenty-three of the patients with chronic or refractory autoimmune cytopenias (6.8%) had an immune deficiency or an autoimmune disease. All of the patients were younger than 18 years of age at initial application. The median age of diagnosis was 3.1 years (between 6 months-16 years) and the ratio of male/female was 1.3. A total of 13 patients (56.5%) had single-lineage cytopenias and 10 patients (46.5%) had multi-lineage cytopenias (Table I).

Table I. Cytopenia detected peripheral blood cell lines.

Cell lines	Number of patients (n=23)
Platelets	9
Neutrophils	1
Erythrocytes	3
Erythrocytes + Neutrophils	2
Erythrocytes + Platelets	5
Platelets+ Neutrophils	2
Erythrocytes + Neutrophils+ Platelets	1

A total of 22 patients had cytopenia at initial application. During follow-up, it took 0-77 months to identify the underlying diseases beneath the cytopenias. Two of them were diagnosed with SLE at initial application. So, excluding these 2 patients, primary diseases were diagnosed a median 5 months after cytopenias had occurred. Only one patient was initially diagnosed as CVID, in this case cytopenia developed during the following years.

Last diagnoses of the patients are given in Table II. Two of the patients were diagnosed as ALPS, 3 of the patients were diagnosed as CVID, 8 of the patients were diagnosed as hypogammaglobulinemia, 5 of the patients were diagnosed as SLE, 2 of the patients were diagnosed as Wiskott–Aldrich syndrome (WAS), one was diagnosed as combined immunodeficiency, one was diagnosed as DiGeorge syndrome, and one was diagnosed as celiac disease. DiGeorge syndrome and Wiskott-Aldrich syndrome were diagnosed by genetic mutation analysis.

All of the patients were treated with corticosteroids or IVIG as first-line treatment. Ten patients (43.5%) needed second or further-line immunosuppressive therapies. These patients were diagnosed as SLE, hypogammaglobulinemia, or CVID. Rituximab was used on 6, MMF was used on 5, cyclosporine A was used on 3, and azathioprine was used on 3 patients. Two patients had undergone splenectomy. Only one patient with neutropenia needed granulocyte-colony stimulating factor treatment. He was diagnosed with combine immune deficiency (Table II).

A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primer disease. Of these 8

Table II. The clinical features of patients.

Patients	Age at Application (Year)	Sex	Cytopenia(s) at Application	Hb (g/dl)	ANC (μL)	PLT (x10^9/L)	Immune Disorder	Time* (mounth)	Treatment	Current Status of Cytopenia(s)
1	4.3	F	AIHA+ITP	9.1	1815	54	DiGeorge syndrome	77	First-line	CwR
2	7	M	AIHA+ITP	11.4	3320	13	SLE	0	Chloroquine	Recovered
3	2	M	ITP	11.5	2200	55	CVID	66	First-line	Continue
4	8	M	AIHA+ITP	8.6	2910	4	HG	6	First-line	CwR
5	13	F	AIHA+ITP	9.9	3250	7	SLE	0	Azathioprine	Recovered
6	7	F	AIHA	7.4	6140	340	ALPS	27	Rituximab, MMF	CwR
7	2.3	M	AIHA+ITP	8.3	2680	67	HG	4	MMF, Rituximab, cSA, splenectomy	CwR
8	0.7	M	ITP	11.5	3210	34	HG	12	MMF, Rituximab, cSA Azathioprine, splenectomy	CwR
9	11	F	AIHA+AIN	9.6	240	158	ALPS	23	First-line	CwR
10	3	M	ITP	11.6	7930	10	Celiac disease	9	First-line	Recovered
11	1.5	F	ITP	11.2	16000	11	HG	5	First-line	Recovered
12	3	M	AIHA	7.9	2000	569	CVID	32	Rituximab, MMF, cSA	Exitus
13	11	F	ITP	11.8	4390	17	SLE	2	Chloroquine, Azathioprine	Recovered
14	1.3	F	AIHA+AIN	8.4	10	432	HG	6	First-line	Recovered
15	2	M	AIN	11.1	690	714	CID	2	First-line, G-CSF	CwR
16	0.5	F	ITP+AIN	11.7	830	26	HG	5	First-line	CwR
17	1.5	M	ITP	12.9	1940	3	HG	2	First-line	Recovered
18	1	F	AIHA	3.7	5650	465	HG	1	Rituximab	CwR
19	8.5	F	ITP	14.4	3580	13	SLE	3	First-line	CwR
20	13.5	F	ITP+AIN	13.0	1070	98	CVID	36**	Rituximab	CwR
21	16	M	ITP	13.9	6790	130	SLE	4	MMF	Recovered
22	7	M	Pancytopenia	9.0	1190	8	WAS	3	First-line	Continue
23	1	M	ITP	12.4	13070	34	WAS	8	First-line	Continue

*: Diagnosis time of between cytopenias and underlying diseases, **: Cytopenia was occurred after diagnosis of CVID
Hb: hemoglobin, ANC: absolute number of neutrophils, PLT: platelet, M: male, F: female, AIHA: autoimmune hemolytic anemia, ITP: immune thrombocytopenia, AIN: autoimmune neutropenia, SLE: systemic lupus erythematosus, HG: hypogammaglobulinemia, CVID: common variable immunodeficiency, ALPS: autoimmune lymphoproliferative syndrome, CID: combined immune deficiency, WAS: Wiskott–Aldrich syndrome, G-CSF: granulocyte-colony stimulating factor, CwR: Continue with recurrens, MMF: mycophenolate mofetil, cSA: cyclosporine A.

patients; 4 were diagnosed with SLE, 3 with hypogammaglobulinemia and 1 with celiac disease. The patient with celiac disease recovered from autoimmune cytopenia after starting gluten-free diet. Cytopenias had continued in 14 of the patients, 11 of them had a least one recurrence. The Clinical Features of Patients are given in Table II. None of these patients had received bone marrow transplantation. No malignancy was detected. One patient with CVID died.

Discussion

Cytopenia is one of the most common autoimmune manifestations in immune disorders. The clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children were evaluated in current study. Twenty-three of the patients with chronic or refractory autoimmune cytopenia (6.8%) had an immunodeficiency or an autoimmune disease. Currently, there are a few reports examining the autoimmune cytopenias in childhood. In their large series with 326 CVID patients, Wang et al.[10] reported that 11% of patients had a history of autoimmune hematologic disease and 19 of patients (5.8%) had the first episode of thrombocytopenia or AIHA prior to the diagnosis of immunodeficiency. A slight male predominance was observed. The ratio of male/female (M/F) was found 1.3. While this ratio has reported as <1 in adults, it has reported as variable in childhood (M/F=0.7-2.7) in previously studies. Also a tendency for male predominance in younger children was mentioned, similar to our finding.[11,19]

The median age of detection of cytopenia was 3.1 years (between 6 months-15.9 years). The median age at initial cytopenia was reported approximately median 5 years in children with chronic or refractory autoimmune cytopenia in previous studies (5-5.7 years).[7,9,11] We found that the diagnosis of primary disease was delayed up to 77 months (median 5 months) after cytopenias had occurred. There are currently a few reports examining the delay between the occurrence of cytopenia and diagnosis of immune disorders. Savaşan et al.[20] reported that the delay of diagnosis in patients with CVID was approximately 8 years. Wang et al.[10] reported that 5.8% of patients had the autoimmune cytopenia prior to the diagnosis of immunodeficiency and they reported that ITP occurs a median of 2 years before a diagnosis of CVID can be made. In a French Cohort, onset of Evans syndrome (ES) was concurrent with diagnosis of SLE in 3/13 patients, but was later within a 1- to 10 year period in 10/13 patients.[11]

A total of 13 patients (56.5%) had single-lineage cytopenia and 10 patients (46.5%) had multi-lineage cytopenia in our study. The most frequent single-lineage cytopenia was thrombocytopenia, followed by anemia, in accordance with reports in the literature.[5,21-24] The percentage of multi-lineage cytopenia has been given different ratios in previous studies: Some of studies have given its ratio as 52-60.9% in their series.[5,22-25] Also, Bride et al.[7] reported that 24/30 patients had multi-lineage cytopenia in patients with relapsed/refractory autoimmune cytopenia. However, Resnick et al.[23] have reported that 4.2% percentage of CVID patients have multi-lineage cytopenia.

Different definitions for ES have been made in the previous studies. Some of them defined it as a multi-lineage cytopenia due to autoimmune destruction of the cells, others defined it as AIHA and ITP with or without AIN.[4,5,11,26-29] Recently, in a French National cohort study, they observed that ES is associated with immune manifestations including hypogammaglobulinemia.[11] As a result, it is suggested that patients with ES (defined as an autoimmune multi-lineage cytopenias) should be screened for ALPS, CVID, and SLE.[4]

Three of the patients were diagnosed with CVID and 2 of the patients were diagnosed with ALPS. Approximately 20% percentage of patients with CVID can be diagnosed at less than 20 years of age.[23] Also, it was reported that, ALPS did not have a high incidence in children

with Evans syndrome.[4] So, the scantiness of patients with CVID and ALPS may be due to the study being made with a pediatric population. Eight of the patients (34.7%) were diagnosed with hypogammaglobulinemia (deficiency of IgA and/or IgG). Immune thrombocytopenia has been reported to occur in approximately 1/200 of patients with selective IgA deficiency. Also, ITP and AIHA were seen in selective IgA deficiency which has been reported in previous studies.[21,30] It is reported that various associated immune clinical or laboratory manifestations (lymphoproliferation, other autoimmune disease, and hypogammaglobulinemia) were observed in 93 children (60%) with ES.[11] As a possibility, maybe some of our patients with hypogammaglobulinemia will fulfill the criteria for ALPS, CVID or SLE in the future.

A total of 5 patients had beem diagnosed as SLE. None of them had neutropenia. Hematologic manifestations have been reported as a percentage of 34-38% in childhood SLE.[26] Also, it was reported that, autoimmune cytopenias were significantly more frequent in the childhood SLE group.[31] Hematologic abnormalities associated with SLE can include lymphopenia, hemolytic anemia and thrombocytopenia. However, neutropenia is uncommon in SLE.[32-34] Our results are compatible with previous studies for SLE patients. Moreover, it is reported that the absence of neutropenia in children with SLE suggests that AIN is not a relevant mechanism in this disease.[26]

Two of the patients were with diagnosis of WAS. Thrombocytopenia is one of the major component of WAS. Pathophysiology of thrombocytopenia in patients with WAS is not clear but generally anomaly in actin cytoskeleton has been accused.[35] Beside this, immune mediated clearance of platelets may have a role in thrombocytopenia of patients with WAS.[36] Recently, it was shown that WAS is linked to defective regulatory T (Treg) cells and impaired T cell development.[8] Therefore we included these patients in our study.

Five of the patients with multi-lineage cytopenias required second-line treatments. In a large series, Aladjidi et al.[11] reported that 69% of their patients had needed several second-line treatments in children with ES. It is known that the management of patients with chronic-refractory autoimmune cytopenias is complex. Corticosteroids and IVIG are recommended as first-line therapy in a newly diagnosed patient. Second-line therapies are varied depending on the cell lineages affected and underlying disease. For example, patients with autoimmune cytopenias due to SLE are typically treated with medications for SLE, patients with ALPS are often treated with corticosteroids or MMF, whereas patients with CVID are often treated with IVIG per month.[3,4,7,12]

In the current study, none of the patient treated with rituximab had recovered from cytopenia. A number of studies have shown remarkable efficacy with rituximab in autoimmune cytopenias because it targets B cells.[3,4,22] It is suggested that when a patient does not respond to rituximab, a calcineurin inhibitor may be used because the underlying autoimmune disease can be caused by a T-cell disorder. Also, it is reported that a patient with partial response to rituximab may benefit from a drug that suppresses both B and T cells, such as MMF.[4] However, therapies for autoimmune cytopenias may still be insufficient since we still do not know much about the biology of autoimmune cytopenias. As a result, often times these patients are treated similarly to patients with primary disease.[3,4,22] As we improve our understanding about the underlying biological mechanisms, new treatment options including bortezomib, belimumab, tocilizumab and epratuzumab will be used for autoimmune cytopenias.[11] Recently, sirolimus has been recommended as a first or second line agent for patients with chronic-refractory autoimmune cytopenias 2016.[7] During our study, none of our patients were treated with those agents.

Two patients who failed to respond to medical treatment had to undergo splenectomy. Both of

them had hypogammaglobulinemia. If a patient has an immune deficiency, splenectomy should have been avoided. However, sometimes splenectomy may be opted for patients who have persistent diseases.[4,12,27]

In the current study, a total of 8 patients (34.7%) recovered from cytopenia. The rate of recovery from autoimmune cytopenia in this study is similar to that in previous childhood studies.[6,26] Cytopenias had continued in 14 patients and 11 of them had a least one recurrence. Recurrent episodes were treated in a similar manner. In a long-term outcome of a French study, 74% of those patients (67/90) had experienced a relapse.[11]

No malignancy was detected in our patients. Various malignancies (including lymphoma, Hodgkin's disease, or breast cancer) have been reported in patients with immunodeficiency or autoimmune disease.[11] However, it has been reported that none were diagnosed with malignancy.[11]

One patient with CVID died. No patient death has been reported for autoimmune cytopenias due to SLE, whereas 7-36% mortality rates have been reported for autoimmune cytopenias due to other immune disorders.[11,26]

Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed during the clinical course. Taking the new targeted treatment options into consideration; early diagnosis is likely to become more important in the near-future in order to begin treatment for the underlying disease as early as possible.

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Original Article

Orbital complications of pediatric rhinosinusitis: A single institution report

Göksel Turhal[1], Sercan Göde[1], Baha Sezgin[1], İsa Kaya[1], Aykut Bozan[2], Raşit Midilli[1], Bülent Karcı[1]

[1]Department of Otorhinolaryngology, Ege University Faculty of Medicine, İzmir; [2]Department of Otorhinolaryngology, Medical Park Tarsus, Mersin, Turkey.

ABSTRACT

Background and objectives. Rhinosinusitis is one of the most common infections of childhood. Rhinosinusitis usually limits itself in the pediatric age group, however orbital and intracranial complications may arise in some of the patients. The purpose of the study was to retrospectively analyse the previous treatments and outcomes in pediatric rhinosinusitis patients with orbital complications.

Methods. The effect and prognosis of medical treatment and endoscopic sinus surgery are reported in this study. Twenty-five pediatric patients diagnosed with complicated rhinosinusitis between January 2008 - February 2016 were included in the study. Orbital complications, examination findings, radiological imaging and blood counts were retrospectively collected from patient files. The duration of hospitalization, source of the infection, complications, previous medical and surgical treatments were also retrospectively assessed.

Results. Average age of the patients were 8.84 ± 4.02 years (range: 1-16 years). The mean length of stay in hospital was 6.72 ± 3.28 days. Hospitalization in surgically treated group was higher than primarily medically treated group. However this difference could not reach to a statistically significant level ($p > 0.05$). Mean hospitalization time was found 5.21 ± 2.51 and 8.43 ± 2.87 days in patients diagnosed with preseptal cellulitis and subperiosteal abscess respectively. Hospitalization in patients with subperiosteal abscess was higher than preseptal cellulitis and a statistically significant difference was detected ($p < 0.05$).

Conclusion. Morbidity and mortality of orbital complications which are the most common complications of pediatric rhinosinusitis, could significantly be reduced by using appropriate treatment methods and an early diagnosis. Conservative therapy is an effective method for patients with preseptal cellulitis and most cases of orbital cellulitis in children.

Key words: pediatric rhinosinusitis, orbital complications, endoscopic sinus surgery, complicated rhinosinusitis, preseptal cellulitis.

Rhinosinusitis is the inflammation of the mucosal lining of paranasal sinuses and the nasal cavity.[1] Rhinosinusitis usually limits itself in the pediatric age group, however orbital and intracranial complications may arise in some of the patients.[2] Orbital complications are classified into five groups according to the Chandler classification: preseptal cellulitis (preseptal edema or inflammatory edema), orbital cellulitis, subperiosteal abscess, orbital abscess and cavernous sinus thrombosis.[3] Allergies, immunodeficiency, anatomic anomalies and ciliary dysfunction are usually associated in complicated cases.[4] Early diagnosis of these complications are important in terms of reducing morbidity and mortality rate. Warning symptoms and signs of orbital complications are: inflammatory edema of the eyelids, orbital edema, exophthalmus, ophthalmoplegia,

✉ Göksel Turhal
gokselturhal@gmail.com

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diplopia and decrease in the visual acuity.[5] History and physical examination are adequate in most of the patients for the diagnosis of acute rhinosinusitis, however radiologic imaging is required in most of the complicated rhinosinusitis patients.[6]

Response to medical treatment is quite good and most of the patients can be managed with medical treatment alone.[5,7] In complicated cases refractory to medical treatment, the existence of an abscess has to be considered and sought radiologically. Opthalmoplegia and deterioration of visual acuity are indications for promt surgical intervention.[8,9] Timing of surgery is controversial in orbital cellulitis unresponsive to medical treatment.

The aim of this study was to retrospectively analyze previous treatments and outcomes of pediatric rhinosinusitis patients with orbital complications. Since most of the reports rely on single-institution case series, results of this study which focuses on orbital complications would guide the clinicians in treating these specific group of patients.

Material and Methods

Twenty-five pediatric rhinosinusitis patients with orbital complications between January 2008 - February 2016 were included in the study. This study was carried out at a tertiary academic center in concordance with international ethical standards and the World Health Organisation Helsinki Declaration. It was approved by the institutional review board (Approval no: 14-1.1/10 – 18.02.2016- Ege University School of Medicine). Informed consent was obtained from the legal guardian in all patients.

Demographic data, orbital complications, physical examination findings, radiological imaging and laboratory results were retrospectively investigated. Diagnosis was made with clinical and laboratory findings. Duration of hospitalization, source of the infection, complications, previous medical and surgical treatments were also retrospectively assessed.

Primary purpose was obtaining clinical demographic data and treatment results, wheras the secondary purpose was the evaluation of treatment failures and assessing special concerns.

The anatomical structure of the paranasal sinuses change in the pediatric population due to the relatively rapid growth. Therefore, the patients were divided into three groups and the results were stratified based on different age groups which are given in Table I.

Patients diagnosed with preseptal or orbital cellulitis were treated with broad-spectrum antibiotics. The antibiotherapy was initiated according to the suggestion of the pediatric infectious diseases consultant. If the patients didn't show clinical and/or laboratory (decrease in leucocytosis and proinflammatory markers)

Table I. Results stratified according to different age groups.

Groups	Number of patients	Preseptal cellulitis	Periorbital cellulitis	Subperiosteal abscess	Diplopia	Mean length of stay in hospital (days)	Treatment		Outcome recurrence
							Medical	Medical + Surgical	
<6 years	8	6	1	1	-	4.37	6	2	1
Between the age of 6-12	11	7	1	3	-	10.27	7	4	1
>12 years	6	2	-	3	1	4.00	2	4	-
Total	25	15	2	7	1	7.50	15	10	2

improvement despite a broad-spectrum antibiotics of 48-72 hours and if there was radiologically proven orbital or subperiosteal abscess, patients were treated with surgery. A computed tomography (CT) scan was performed to all patients during the first admission. The surgical procedure included endoscopic sinus surgery and drainage of the abscess.

Statistical analysis was made using computer software (SPSS version 22.0, SPSS Inc. Chicago, IL, USA). Chi-square (X^2) exact tests were used for the comparison of categorical data while Wilcoxon and Mann-Whitney U tests were used for the analysis of non-parametric variables based on the distribution pattern of the data. Data were expressed as "mean (standard deviation; SD)", percent (%), minimum-maximum, Odds Ratio (OR); 95% confidence interval (CI) and "median (Interquartile range; IQR)" where appropriate. $p < 0.05$ was considered statistically significant.

Results

Average age was 8.84 ± 4.02 years (range: 1-16 years). Twelve patients (48%) were female and 13 patients (52%) were male. The mean follow-up time was 33.84 ± 3.48 months. The mean length of hospital stay was 6.72 ± 3.28 days. Fifteen patients had preseptal cellulitis (60%), seven (28%) had subperiosteal abscess, one (4%) patient had diplopia related to isolated sphenoid sinusitis and two (8%) patients had orbital cellulitis with involvement of the infraorbital and buccal area.

Fifteen patients were treated with medical therapy only, ten patients were treated with both surgery and medical therapy. One patient with preseptal cellulitis didn't improve with medical therapy for 48 hours and endonasal endoscopic surgery was performed.

Mean length of hospital stay was 7.50 ± 3.83 and 6.20 ± 2.88 days for surgically treated and only medically treated patients respectively. Hospitalization duration was higher in patients treated with surgery compared to patients with only medical treatment ($p > 0.05$). Mean hospitalization time was 5.21 ± 2.51 and 8.43 ± 2.87 days in patients diagnosed with preseptal cellulitis and subperiosteal abscess respectively ($p < 0.05$).

Thirteen (68%) of the patients had signs of proptosis, periorbital edema and hyperemia, seven (28%) patients had decreased visual acuity, four (16%) patients had eyelid edema and pain and one (4%) patient had diplopia.

Ethmoid mucocele was found in two patients who were treated with surgery. No recurrences or further complications were observed in 24 patients during the follow-up. One patient (4%)

Table II. Findings of the patients.

	Mean Age	Gender (Female/Male)(%)	Medical Therapy	Medical + Surgical Therapy	Length of stay in hospital	Recurrence (number of patient)
Preseptal cellulitis(60%)	8.06	7(28%)/8(32%)	15	-	5.21	1
Periorbital cellulitis(8%)	12.5	0/2(8%)	-	2 (endoscopic sphenoethmoidectomy)	5.00	-
Subperiosteal abscess(28%)	8.42	4(16%)/3(12%)	-	7 (endoscopic sphenoethmoidectomy + orbital decompression)	8.43	1
Diplopia with isolated sphenoid sinusitis (4%)	16	1(4%)/0	-	1 (optic decompression)	3.00	-
Total (100%)	8.84	12(48%)/13(52%)	15(60%)	10(40%)	6.72	2

had recurrence and required additional surgery two years after the initial treatment. The patients with mucoceles didn't describe any history of trauma or prior surgery. Comparison of the patients' findings regarding orbital complications are shown in Table II.

Nine (36%) of the patients were treated with ceftriaxone, 11 patients (44%) were treated with ceftriaxone and metronidazole, 3 patients (12%) with ampicillin and sulbactam, 1 patient (4%) with vancomisin and ceftriaxone and 1 patient (4%) with meropenem. The administration of antibiotherapy was started on the first day and mean length of medical therapy was 7.50 days. All patients were discharged with an oral antibiotic therapy after at least 7 days.

All patients who were treated with surgery underwent functional endoscopic endonasal sinus surgery. Patients who had orbital or subperiosteal abscess underwent orbital decompression and abscess drainage, whereas patients with deterioration of the vision underwent both orbital and optic decompressions. One patient required external abscess drainage in addition to the endonasal treatment (Fig. 1). Surgical treatment was comprised of one optic nerve decompression, two endoscopic sphenoethmoidectomies and seven endoscopic sphenoethmoidectomies+orbital decompressions. None of the patient's paranasal sinus bacterial cultures revealed any microbiological agents.

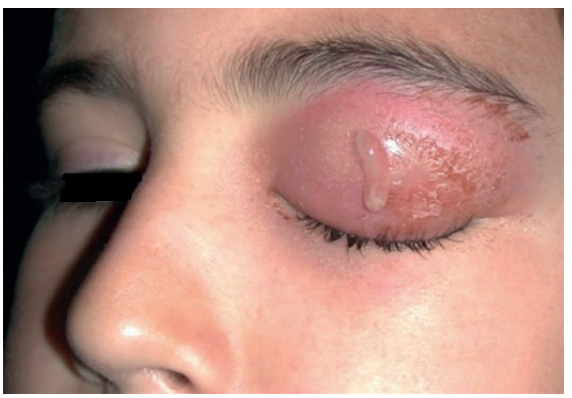

Fig. 1. Preoperitve photo of a 6-year-old girl with orbital abscess requiring external drainage.

Dicussion

Acute rhinosinusitis is a relatively frequent disease of the childhood. The reported incidence of orbital complications is 6% in a retrospective report.[10] The most frequent orbital complication in this study was preseptal cellulitis (60%) followed by subperiosteal abscess (28%) which is similar with previous studies.[11,12] Because most orbital complications are managed successfully with medical therapy alone, it has been suggested that patients with 1st and 2nd stage orbital complications can be treated with broad-spectrum antibiotics alone. However it is generally accepted that surgical intervention is necessary in patients unresponsive to medical therapy within 48 hours despite broad-spectrum antibiotics, documented subperiosteal or intraorbital abcess and reduced visual acuity or color vision.[13,14] Some authors suggest waiting 48-72 hours with medical therapy alone if the vision is normal in case of a subperiosteal abscess.[8]

According to Oxford et al.[15], the most common isolated organism was *Streptococcus milleri* in 7 of the 23 patients with subperiosteal orbital abscess. Hwang et al.[16] reported that streptococcus viridans was the most common isolated agent in complicated pediatric rhinosinusitis. Devrim et al.[17] reported that most common isolated organism was *Staphylococcus auerus* (41.9%) followed by coagulase-negative staphylococcus (25.8%) and *Haemophilus influenza type b* (6%) in orbital and preseptal sellulitis patients. Culture of the offending organism should be made whenever possible because it will guide the antibioterapy. However all of the patients had negative bacterial culture results. All patents were on oral antibiotics before admission, thus negative culture was an expected outcome. Stokken et al.[18] reported no growth (30.8%), Streptococcus milleri (30.8%), and normal flora (19.2%) in complicated pediatric acute bacterial rhinosinusitis patients. In general etiological agents could be identified in only 20-30% of the cases so treatment is usually initiated empirically.

In this study, 15 patients with preseptal cellulitis and two patients with orbital cellulitis were treated with medical treatment alone, however two of these patients with orbital cellulitis didn't respond to medical therapy and were treated with surgery. One of these two patients developed ophthalmoplegia and decrease in visual acuity preoperatively. Patients treated with medical therapy and patients treated with surgery after failed medical therapy healed well without any morbidity.

Patients with stage 1 and 2 orbital complications must be closely monitored for voluntary eye movements and visual acuity before proceeding with surgery. Orbital decompression is suggested in patients with proven abscess in CT scans.[15] Drainage and orbital decompression using the endonasal endoscopic approach was applied on 7 patients with CT proven subperiosteal abscess (Fig. 2). Abscess drainge via an external approach was applied in one of these patients because the patient was not improving following postoperative second week (Fig. 1). In the case of a proven abscess in contrast enhanced CT, the preferred treatment method is abscess drainage via an endonasal endoscopic approach. External approach or combined endonasal and external approaches could be used in necessary cases. The recent advances in endoscopic visualization has significantly decreased the need for external approaches. In the case of frontal sinusitis, external approaches may be necessary. Indications for these procedure includes disease related to a type 4 frontal cell. Infections that reach subcutaneous tissue could also be treated with an external approach. Many authors suggest removing the anterior ethmoid cells and lamina papyracea for the drainage of subperiosteal abscess is adequate.[16] Hospitalization time was related with the type of treatment and complication. Patients who were treated both surgically and medically with subperiosteal and orbital abscess were hospitalized significantly longer than patients with preseptal cellulitis. Caglar et al.[19] analysed treatment costs of pediatric preseptal cellulitis cases in 54 patients and reported that sinusitis was associated with longer length of stay and higer hospital costs.

Rhinosinusitis with orbital complications accompanied with ethmoid mucoceles constitute a specific group that needs to be handled separately. One of the patient in this study developed recurrent orbital cellulitis and this patient had an ethmoid mucocele which was not apparent at the first admission, but was clearly visible during the second admission. The patient was diagnosed with left preseptal cellulitis at the first admission and full recovery was achieved with intravenous 100 mg/kg/day ceftriaxone treatment. After two years, the patient was again diagnosed with a right etmoid mucocele with subperiosteal abscess

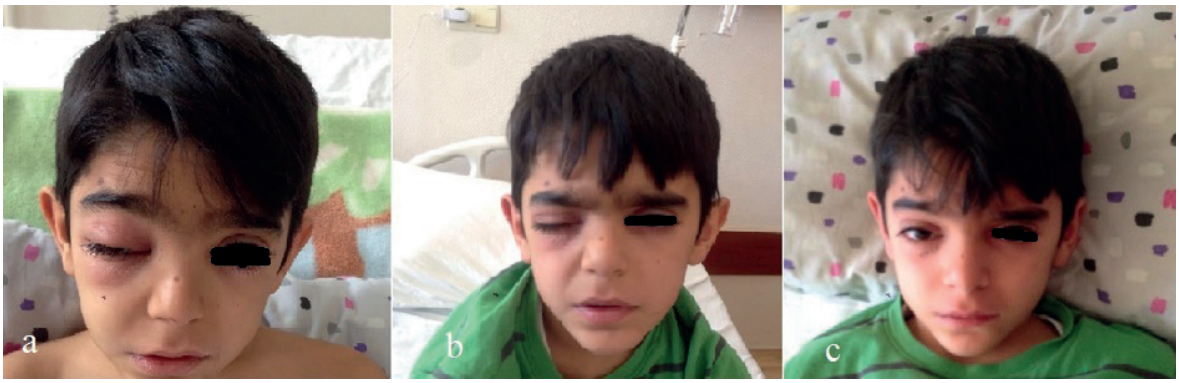

Fig. 2. Preoperative and postoperative photos of a 7-year-old boy with subperiosteal abscess after endoscopic endonasal orbital decompression and broad-spectrum antibiotics (a: preoperative, b: postoperative 2nd day, c: postoperative 4th day).

and required surgery. Endonasal endoscopic surgery (orbital decompression and drainage of the abscess) was performed. Because of recurrent complicated sinusitis, the patient was consulted to pediatric immunology clinic. Addition to the complicated sinusitis, the patient also had recurrent episodes of pneumonia and gastroinstesinal infections. The patient than was diagnosed with IgG deficency and is regularly treated with intravenous immunoglobulin (Figs. 3 and 4). No predisposing underlying medical issues were present in the other patients. Immunological work up could be performed in recurrent complicated pediatric rhinosinusitis cases. One has to be careful about ethmoid mucoceles especially in recurrent cases and have a low threshold for surgery even in patients with stage 1 orbital complications with ethmoid mucoceles. Although lack of evidence regarding the relation between immunodefficiency and recurrent sinusitis, it should be kept in mind that immunodeficiency might be found in cases of recurrent pediatric rhinosinusitis and their orbital complications as in the aformentioned patient.

We gathered our treatment algorithm in Fig. 5. After diagnosis patients are classified according to the Chandler's classification system. In the presence of abscess surgical therapy is almost totally implemented. In the presence of preseptal or orbital cellulitis medical therapy

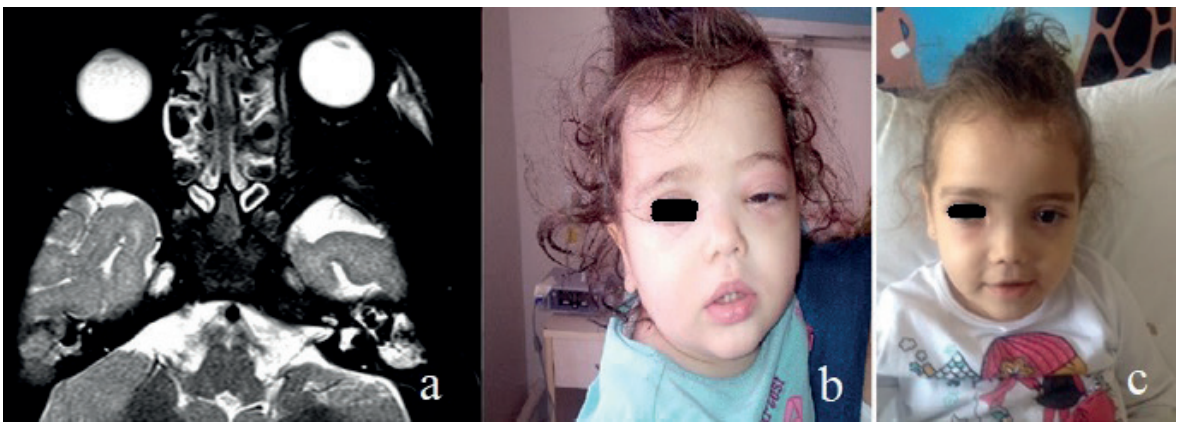

Fig. 3. MRI and photos of one year-old-girl diagnosed with left sided preseptal cellulitis in first admission (a: pretreatment MRI, b: pretreatment photo, c: The 6th day of treatment).

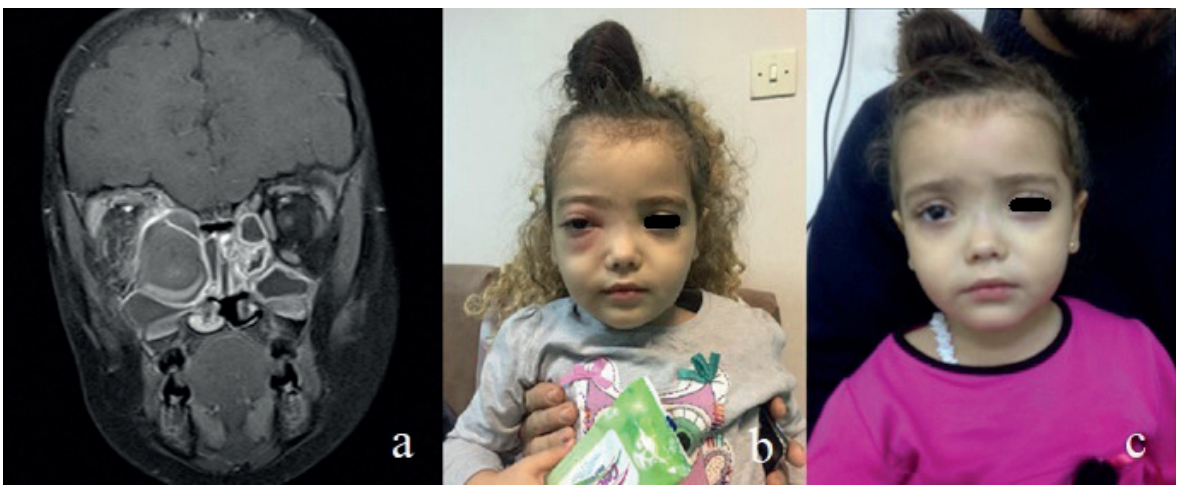

Fig. 4. Same girl's MRI of 3-year-old girl diagnosed with right sided ethmoid mucocele in second admission (two years later) (a: preoperative MRI, b: postoperative first day, c: postoperative 4th day).

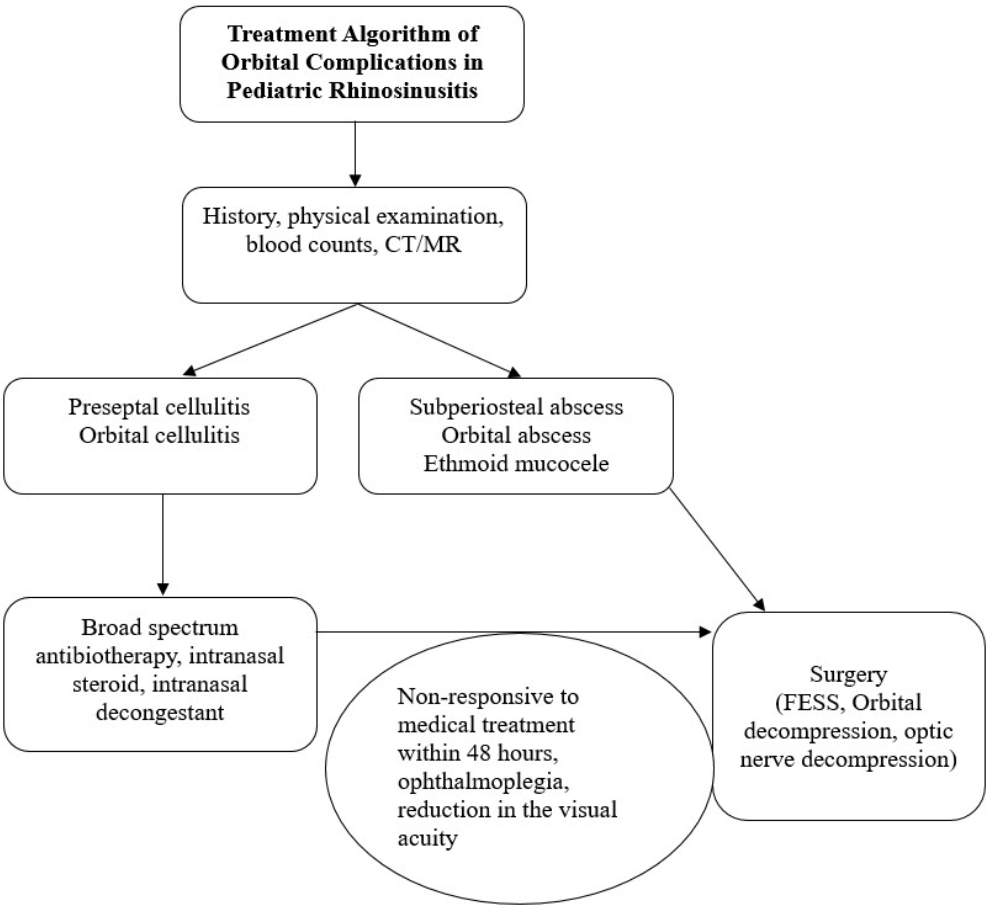

Fig. 5. Treatment algorithm regarding acute rhinosinusitis of pediatric patients with orbital complications.

is initiated. If the patient is non-responsive to medical therapy, if there is opthalmoplegia or decrease in visual acuity surgical therapy is required.

Morbidity and mortality of orbital complications could significantly be reduced by using appropriate treatment methods and an prompt diagnosis. With reference to the results of this study medical therapy was sufficent in group 1 and 2 patients. Additionally eventhough medical therapy responsive subperiosteal abscesses are amenable to medical therapy, most radiologically proven abscesses require surgical intervention especially when there is loss in vision, limitation in ocular movement or unresponsiveness to aggressive medical therapy. As in the recurrent case, eventhough ethmoid mucoceles accompany only a small percentage of patients with preseptal edema, surgical treatment needs to be considered especially in recurrent cases. It is suggested that patients with chronic or recurrent infections and complications should undergo further investigation of their immunological status as the patient with IgG deficiency in this study. We hope findings of this study might help and guide both otolaryngologists and pediatricians managing this serious complication of rhinosinusitis.

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Original Article

Circulating Epstein-Barr virus DNA and cell-free DNA in pediatric lymphomas

Bilgehan Yalçın[1], Tezer Kutluk[1,2], Serpil Kahraman Ağbaba[2], Çetin Demir[2], Beril Talim[3]

[1]Department of Pediatric Oncology, Hacettepe University Cancer Institute and Faculty of Medicine; [2]Drug Resistance Laboratory, Hacettepe University Oncology Hospital, [3]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background and objectives. Quantification of serum Epstein–Barr virus (EBV) DNA and/or cell-free total DNA (cf-DNA) may become valuable sources for prognosis evaluation and monitoring treatment response in lymphomas. We aimed to investigate their roles as potential markers in pediatric Hodgkin (HL) and non-Hodgkin lymphomas (NHL).

Method. Between the years 2005–2008, 34 patients with HL and 45 with NHL were prospectively included. Serum samples were collected upon diagnosis, after 1-3 and 4-6 months and at the end of treatment, or at disease recurrence. RT-PCR determination of cf-DNA and EBV DNA were performed using amplification of BAMH1W region of EBV genome and of human β-globin gene. Results were analyzed for correlation with clinical and pathological characteristics.

Results. Median ages were 8.9 years for HL and 8.8 years for NHL cases. Twenty-three healthy children cured from various childhood cancers served as the control group. In the controls, median serum EBV DNA copy number was '0' and median serum cf-DNA level was 50 ng/ml. At initial diagnosis, serum EBV DNA copy numbers were elevated in 20/34 HL and 8/45 NHL cases ($p<0.001$). Median serum EBV DNA copy numbers were 15987/mL (125-6032075) and 25162 (1475-1214550) for HL and NHL cases, respectively ($p= 0.9$). Median serum cf-DNA levels were 435 ng/ml (2.3-17306) in HL and 700 ng/ml (4.9-14009) in NHLs ($p= 0.12$). Serum EBV DNA copy numbers and cf-DNA levels decreased significantly after induction treatment and in the follow-up. In 10/13 NHL cases with a relapse, marked elevations were detected in serum cf-DNA levels at recurrences. No significant differences were detected between median cf-DNA levels according to disease stages, response status to treatment or presence of recurrent disease.

Conclusion. Serum EBV DNA copy numbers and cf-DNA levels were elevated at initial diagnosis in both HLs and NHLs and decreased parallel to treatment response. In NHL cases, remarkable elevation of cf-DNA levels at recurrences indicated that cf-DNA levels might be useful in the follow-up of pediatric NHLs.

Key words: Hodgkin lymphoma, non-Hodgkin lymphoma, serum Epstein-Barr virus, serum cell-free DNA, children.

Tumor-derived DNA is elevated in the serum of patients with cancer. The quantification of circulating Epstein Barr virus (EBV) DNA is reported to have an important role in the diagnosis and management of EBV-associated lymphoid malignancies.[1-6] Circulating cell-free total DNA (cf-DNA) has been studied in a wide range of physiological and pathological conditions, including pregnancy, trauma, inflammatory disorders and malignancies.[7,8] Elevated levels of cf-DNA have been reported in many tumour types including pediatric cancers.[8-11] Both serum EBV DNA and/or cf-DNA may become valuable sources for prognosis evaluation in pediatric lymphomas

✉ Bilgehan Yalçın
yalcinb@hacettepe.edu.tr

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as well as for monitoring treatment response and early identification of recurrences. Few studies have investigated both circulating EBV DNA and total cf-DNA in Hodgkin (HL) and non-Hodgkin lymphomas (NHL).[12-14] In this study, we aimed to investigate the value of these molecular markers for pediatric lymphomas.

Material and Methods

During the period between June 2005-November 2008, 34 patients with HL and 45 patients with NHL who were treated with similar standart treatment protocols were prospectively included in the study. Twenty-three healthy children who were cured from various childhood solid cancers other than lymphomas or EBV-related malignancies and were under regular follow-up served as the control group (male/female: 15/8; median age 8.3 years, range 1.7–17.9) to determine the normal range of circulating EBV DNA copy numbers and total cf-DNA. The study was approved by the Ethical Review Board of Hacettepe University (HEK 05/124) and informed consents were obtained from the patients and controls and their parents according to institutional guidelines.

Blood samples were collected from patients upon diagnosis before treatment started, in the follow-up at the end of the induction chemotherapies (1-3 months after diagnosis), at the end of the post-induction chemotherapies (4-6 months after diagnosis), and also at the end of scheduled treatments or at the end of first year after diagnosis; and for some patients when tumor recurrence or progression were suspected or documented.

Serum samples were stored at -20^{0}C and were thawed at room temperature at the time of study. DNA was extracted from 200 µL of serum with the QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the blood and body fluid protocol.

Quantitative real-time Polymerase chain reaction (PCR) determination of total cf-DNA and EBV DNA were performed using a Rotorgene PCR cycler (Qiagen, Germany). The assay was based on amplification of BAMH1W region of Epstein–Barr virus genome and of the β-globin gene of human genome fragments as a housekeeping gene to quantify total cf-DNA. Each PCR run was performed in duplicate and the mean values of results were calculated. Reactions were performed using universal PCR conditions.

Serial dilutions of human genomic DNA (5-log dilution from 50 000 to 5 copies/mL) extracted from the EBV-positive Namalwa cell line were used to establish a standard calibration curve. The limit of detection was five copies. Results were expressed as copies of EBV/mL of serum. Each cell was estimated to contain 6.6 pg of DNA and a conversion factor of 2 copies per cell for β-globin gene was used in order to quantify total cf-DNA levels as ng/ml. The final results were presented in terms of copies/mL or ng/mL in the original serum sample. The results were interpreted for investigating the changes in serum EBV DNA copy numbers and cf-DNA levels and also their correlation with the clinical and pathological characteristics of the patients with HL and NHL. Amplification data were collected and analyzed by Sequence Detection System software (Applied BioSystems, Foster City, CA, USA).

Immunohistochemistry (IHC) for EBV detection was performed on formalin-fixed paraffin-embedded tissues from diagnostic biopsies with monoclonal antibodies against LMP-1.

The distribution of various variables between patient groups were compared using X^2 test. The mean values in the subgroups were compared using *t*-test, and the median values were compared using Wilcoxon, Mann-Whitney-U or Kruskal-Wallis tests. Correlation between sets of data were analysed with Pearson test and linear regression analysis. Overall survival (OS) and event-free survival (EFS) rates were calculated by the Kaplan–Meier method[15] and differences in survival were compared using the log-rank test. In every instance, a p value < 0.05 was considered statistically significant.

For the statistical analyses, PASW Statistics for Windows software (Version 18.0. Chicago: SPSS Inc) was used.

Results

The median ages were 8.9 years (3.5-17.8; Male/Female: 25/9) for cases with HL and 8.8 years (1.9-16.5; Male/Female: 37/8) for cases with NHL, respectively. The clinicopathological characteristics of all cases are given in Table I. Histopathological subgroups were as follows: Hodgkin lymphomas: mixed cellularity (n= 27), nodular sclerosis (n= 8), lymphocyte depletion (n= 2), not specified (n= 7); non-Hodgkin lymphomas: mature B-cell (n= 20), lymphoblastic (n= 15), anaplastic large cell (n= 5), and diffuse large B-cell (n= 5). In the control group, the median serum EBV DNA copy number was '0' and median serum cf-DNA level was 50 ng/ml which were parallel to those reported for healthy individuals. Serum EBV DNA copy numbers >0 and serum cf-DNA levels >50 ng/ml were accepted as elevated.

Serum EBV DNA copy numbers

At initial diagnosis, serum EBV DNA copy numbers were elevated in 20/34 (59%) of HL and 8/45 (18%) of NHL cases ($p< 0.001$). Mean serum EBV DNA copy numbers were 451446/ml (-/+ 1337709) in HLs and 267034/mL (-/+ 431777) in NHLs; the median values were 15987/mL (125-6032075) and 25162 (1475-1214550) for HL and NHL cases, respectively ($p= 0.9$).

Table I. Clinicopathological characteristics of children with Hodgkin and non-Hodgkin lymphomas.

		Hodgkin lymphoma (n)	Non-Hodgkin lymphoma (n)	Total (n)
Gender	Male	25	37	62
	Female	9	8	17
	Total	34	45	79
	p	0.35		
Stages of disease	Localized (I/II)	16	6	22
	Disseminated (III/IV)	18	39	57
	Total	34	45	79
	p	0.001		
Serum LDH	Normal-low	20	19	39
	Elevated (>500)	13	25	38
	Total	33	44	77
	p	0.13		
Tumor tissue EBV LMP1 (by IHC)	Negative	8	21	29
	Positive	22	0	22
	Total	30	21	51
	p	<0.001		
Serum EBV DNA levels (copies/mL)	Elevated	20	8	28
	Negative ('0')	14	37	51
	Total	34	45	79
	p	<0.001		
Serum cell-free DNA Levels (ng/ml)	Elevated (>50)	30	41	71
	Low (≤50)	4	4	8
	Total	34	45	79
	p	0.67		

LDH: lactate dehydrogenase, EBV: Epstein-Barr virus, LMP1: latent membrane protein 1, IHC: immunohistochemistry.

In 30/34 HL cases immunohistochemistry for Epstein-Barr virus Latent Membrane Protein (EBV LMP1) was studied in the tumor samples, 22 samples (73%) were positive and 8 were negative. Serum EBV DNA copy numbers were elevated in 16/22 (72.7%) cases positive for tumor tissue EBV LMP1 and in 2/8 (25%) of cases negative for tumor tissue EBV LMP1 (p= 0.02). Tumor tissue EBV LMP1 was negative in all 21 NHL cases studied.

In both HL and NHL cases, elevated serum EBV DNA copy numbers decreased significantly following induction chemotherapies and in the follow-up. Fig. 1 (a, b) shows the elevated median serum EBV DNA copy numbers at initial diagnosis and decreases in the follow-up periods. In 5/34 HL lymphoma cases and in 13/45 NHL cases disease recurrences occurred. No significant increases were detected in the follow-up serum EBV DNA copy numbers of cases who experienced disease recurrences.

In both HL and NHL groups, serum median EBV DNA copy numbers did not differ significantly when the cases were compared according to gender, living in rural or urban towns, initial serum lactate dehydrogenase (LDH) levels being low or high, presence or absence of B symptoms, and the initial chemotherapy response statuses.

Initial serum median EBV DNA copy number was significantly higher in HL cases with advanced stage disease (stages III or IV, 1376/mL) compared to cases with localized disease (stages I and II, 109/mL) (p= 0.01). In HL cases, there was no significant difference between the cases according to the histopathological subtypes for elevation of serum EBV DNA copies. In NHL cases, no significant difference was seen for elevation of serum EBV DNA copies between the groups according to histopathological subtypes, disease stages, and other clinical or laboratory characteristics.

Serum cf-DNA levels

Mean serum cf-DNA levels were 1290 ng/mL (-/+ 3150) in HL and 1924 ng/mL (-/+ 700) in NHLs (p= 0.35), and median levels were 434 ng/mL (2.3-17306) in HL and 700 ng/mL (4.9-14009) in NHLs (p= 0.12). In the control group, the mean and median serum cf-DNA levels were 82 and 50 ng/mL (7.8-386), respectively. The differences of median values of serum cf-DNA levels in the controls and study patients were significant (p< 0.001 for both HLs and NHLs). For all HL and NHL cases, a significant correlation was seen between the serum cf-DNA levels and serum LDH levels at initial diagnoses (p= 0.001; Pearson R: 0.368)

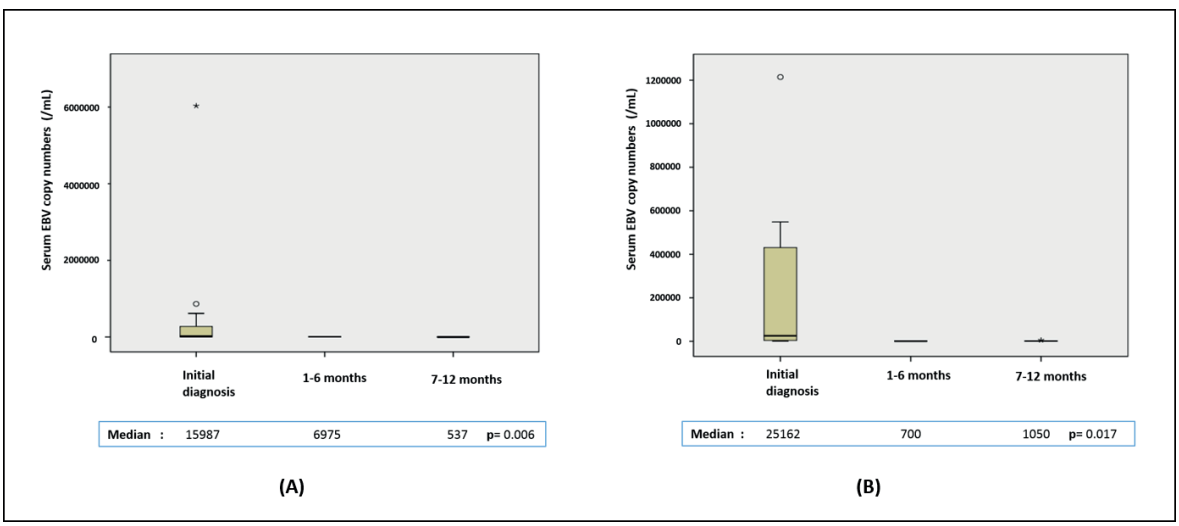

Fig. 1. Serum EBV DNA levels in 34 Hodgkin (a) and 45 non-Hodgkin lymphoma cases (b).

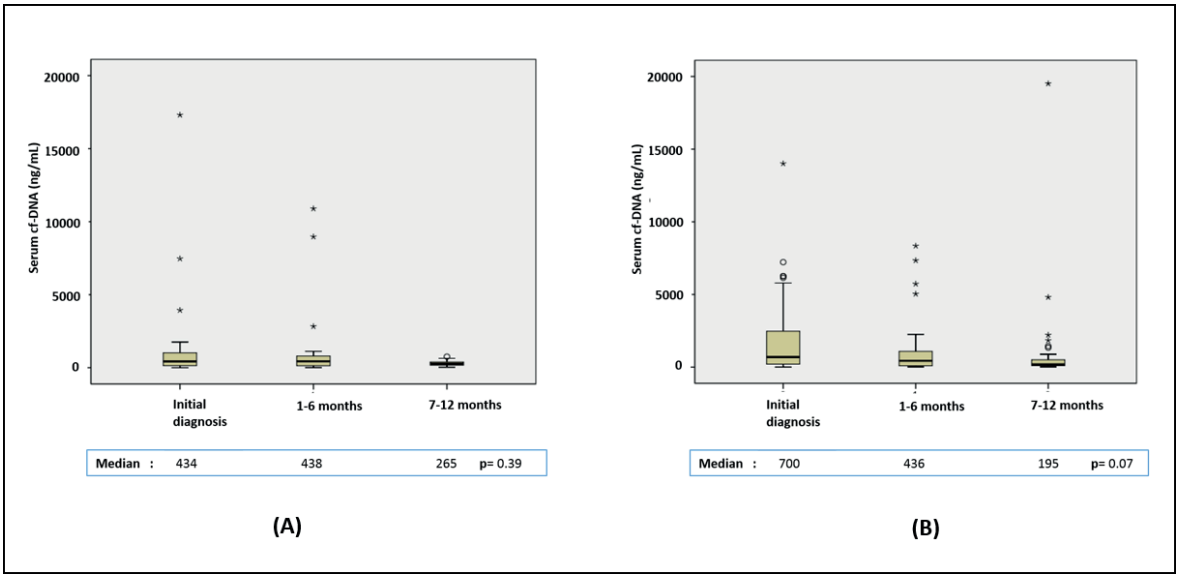

Fig. 2. Serum cf-DNA levels in 34 Hodgkin lymphoma (a) and in 45 non-Hodgkin lymphoma cases (b).

Considering all HL and NHL cases, serum cf-DNA levels decreased at the end of induction chemotherapies and in the follow-up. In HL cases, the prospective changes in serum cf-DNA levels were less significant compared to NHL cases. Fig. 2 (a, b) shows the elevated serum cf-DNA levels at initial diagnosis and decreases in the follow-up periods of cases with HL and NHL. In NHL cases who did not have any recurrence of their disease, the decrease in the serum cf-DNA levels was much more significant (p= 0.005). The course of serum cf-DNA levels in the follow-up of some individual patients with HL or NHL who experienced recurrence of their disease are shown in Fig. 3 (a-f). Ten of 13 NHL cases and 1/5 HL cases who had experienced disease recurrences had simultaneous serum cf-DNA data. In 10 NHL cases the median serum cf-DNA level was 4133 ng/mL at initial diagnosis and decreased to 283 ng/mL at the end of induction therapies (p= 0.047) which increased to 1079 ng/mL when recurrences were detected (p= 0.013). In cases with or without relapses, median serum cf-DNA levels at initial diagnosis were 569 ng/mL and 406 ng/mL (p= 0.77) for HL cases, and 1039 ng/ml and 699 ng/ml (p= 0.44) for NHL cases.

In 13/34 HL cases and 11/45 NHL cases, elevated serum cf-DNA levels were detected in the follow-up with no evidence of recurrence or progression (median, 469 ng/ml) which were lower than those detected in the relapsed cases at the time of recurrences (median, 838 ng/ml) (p= 0.013).

In all lymphoma cases and in HL and NHL cases separately, the differences in the median serum cf-DNA levels were not significant when the cases were compared according to their disease being localized or disseminated, serum LDH levels being low or high, initial chemotherapy response statuses and the presence or absence of recurrent disease. In both HLs and NHLs, the differences in the median serum cf-DNA levels according to histopathological subtypes were not significant.

Survival Analysis

At a median of 8 years follow-up, 64 cases were alive and under regular follow-up, 10 cases were lost to follow-up and 8 cases died. Five-year event-free (EFS) and overall survival (OS) rates were 82.1% and 97% for all HL cases and 68.1% and 86.4% for all NHL cases. In HL patients, five-year EFS rate was 78.6% in cases negative for serum EBV DNA and 79.4% in cases with elevated serum EBV DNA copy numbers (p= 0.8); five-year EFS rates were 75% and 82.8%

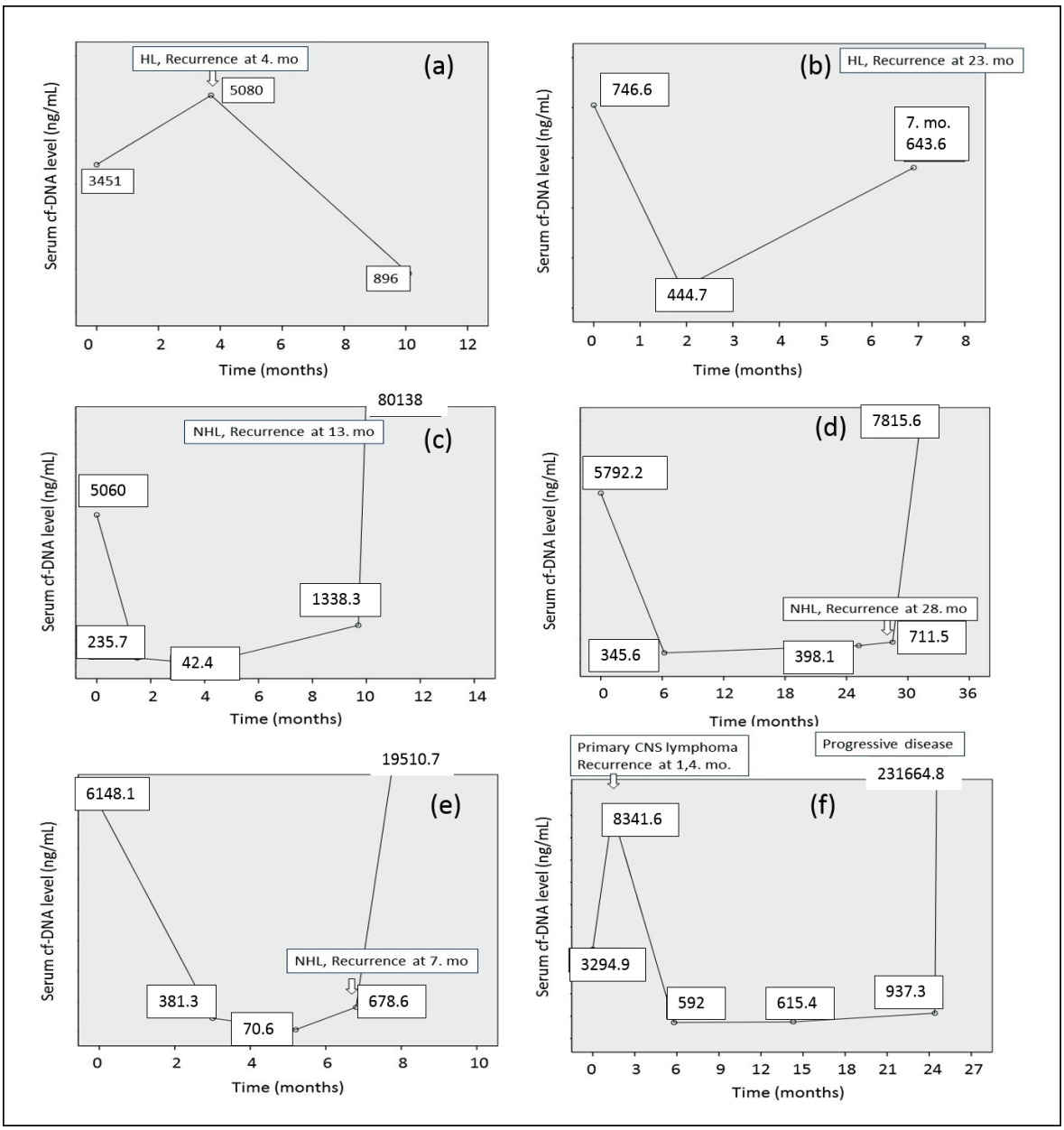

Fig. 3. The course of serum cell-free DNA (cf-DNA) levels in selected Hodgkin (a & b) and non-Hodgkin lymphoma (c - f) cases who experienced recurrences in the follow-up (HL: Hodgkin lymphoma, NHL: non-Hodgkin lymphoma, CNS: central nervous system, mo.: month).

in cases with low serum cf-DNA and elevated serum cf-DNA levels (p= 0.9), respectively. In NHL patients, five-year EFS rates were 64.4% in cases negative for serum EBV DNA and 85.7% in cases with elevated serum EBV DNA copy numbers (p= 0.2); five-year EFS rates were 66.7% and 68.6% in cases with low serum cf-DNA and elevated serum cf-DNA, respectively (p= 0.9).

Discussion

A proportion of childhood lymphomas including HL and Burkitt's lymphoma is associated with EBV and circulating EBV DNA has been detected in the plasma/serum of EBV-positive lymphoma cases. Circulating cell-free EBV DNA has been shown to be useful for early

detection, prognostication and monitoring of treatment response of nasophayrngeal carcinoma and also is used to guide disease stratification and treatment strategies.[16]

Among patients from the United States and most parts of Europe, approximately 30-50% HL cases have tumor cells (Hodgkin Reed-Sternbeg - HRS cells) that harbor the EBV virus.[17] In contrast, in Turkey and in some countries from Africa and Asia a very high EBV association has been found, with the percentage approaching 100%.[18-20] Cavdar et al.[18] from Turkey reported positivity for EBV by serological and IHC methods in nearly 75% of children with HL which is higher than reported from Western countries. The rate of positivity in our study for EBV LMP1 by IHC in HL cases was comparable to their results. Çavdar et al. also reported that in our country children were exposed to EBV at a younger age due to poor living conditions.[18]

Serum EBV DNA copy numbers

In our study, nearly 3/4 of HL tumor samples were positive for EBV LMP1 by IHC; 72% of cases with positive IHC had elevated serum EBV DNA compared to 25% of cases with negative IHC. Similar results have been reported by others.[21] The strong correlation between the presence of EBV DNA in involved lymph node biopsies and blood samples might suggest that HRS cells are the source of EBV viral DNA. Spacek et al.[2] reported that circulating free EBV DNA most likely represents tumor derived viral DNA and thus corresponds to disease activity in EBV-positive HL.

In our patients, serum EBV copies were elevated in 59% of HL cases at initial diagnosis and decreased significantly following induction chemotherapies and in the follow-up as shown in Fig. 1 (a, b). However, no significant increases were detected in the EBV DNA copy numbers of cases who experienced disease recurrences.

In several similar studies, circulating EBV DNA has been shown to be elevated at diagnosis and decreased after treatment and was proposed as a biomarker for disease monitoring.[1,3,22] Thus, our results may indicate that serum EBV DNA can be a parameter to monitor treatment response in EBV-associated pediatric HL. Circulating EBV DNA copy numbers might be undetectable at the time of recurrence in some cases with EBV-related malignancies. The results of our study need to be verified in further studies with higher number of pediatric lymphoma cases.

In our HL cases, the presence of constitutional 'B-symptoms' was not associated with serum EBV DNA viral load at diagnosis. Spacek et al.[2] also reported no association of 'B-symptoms' with plasma EBV DNA viral load. Serum median EBV DNA copy number was significantly higher in our HL cases with advanced disease which supported the results reported by Musacchio et al.[23] but contradicted with those of Spacek et al.[2] and Gandhi et al.[1]

Two separate studies reported that elevated pretreatment blood EBV DNA was associated with poor prognosis in lymphomas.[24,25] However, we couldn't show any prognostic significance of serum EBV positivity for survival in our HL cases.

Studies examining the serum-plasma EBV status in EBV-related NHLs are rare. In a wide range of EBV–associated lymphoid malignancies, circulating EBV DNA copy numbers have been found to correlate with disease activity and reported to be a useful tumor marker.[4,5] In 8/45 of our NHL cases who had elevated serum EBV DNA copy numbers, the numbers decreased significantly following chemotherapy as a response to treatment. However, we didn't find any correlation with histopathological subtypes or disease stages.

Lei KI, et al.[4] reported that plasma EBV DNA was elevated in NHL cases and correlated well with the therapeutic response. In 2013, Kabyemera et al.[6] reported that EBV load in blood might be a diagnostic and prognostic marker for the onset and monitoring of NHL in African childen.

In our NHL cases who had disease recurrences, no significant increases were detected in

circulating EBV DNA copy numbers. Serum EBV DNA copy numbers correlated well with response to chemotherapy but our results did not support its use as a follow-up marker for disease monitoring and detection of recurrences.

Machado et al.[12] reported that circulating EBV DNA was elevated in 7/30 pediatric B-cell NHLs and decrease of EBV viral load was associated with therapy response.

Our results indicated that serum EBV DNA copy numbers can be used as a biomarker of response to treatment in EBV associated HL and NHLs. Since the copy numbers were not elevated in cases with a recurrence of disease, their value for disease monitoring might not be proposed opposite to nasopharyngeal carcinomas.

In our study, serum EBV DNA copy numbers were elevated in 8 cases among all NHL cases. So, it was not possible to perform further subgroup analysis depending on the subtypes of lymphomas.

Serum cf-DNA levels

Circulating cf-DNA can be found in small amounts in serum of healthy individuals at concentrations between 0 and 100 ng/ml of blood with an average of 30 ng/ml.[7,9] The median level was 50 ng/mL in our control cases which was similar to these values. In recent years, many studies reported the significance of cf-DNA in the circulation for different cancer types like lung, ovarian and gastrointestinal cancers.[8,10] Kurihara et al.[11] investigated circulating cf-DNA in 44 children with solid tumors who underwent surgical intervention and they concluded that cf-DNA levels were significantly correlated with disease stages. There are few reports available for patients with lymphomas.[12-14,26]

In our study, mean and median serum cf-DNA levels were significantly elevated in both HL and NHL cases compared to controls, the levels being higher in NHLs. Also, the correlation of serum LDH levels with cf-DNA levels was significant. Lactate dehydrogenase is a strong prognostic indicator in lymphomas, elevated levels are correlated with advanced stage and tumor load which indicate poorer prognosis.[27] Hohaus et al.[13] reported that increased levels of plasma DNA were associated with advanced stage disease, presence of B symptoms and elevated LDH levels in adult lymphomas. Our study results are in accordance with theirs which might indicate a common mechanism for release of LDH and cf-DNA from the tumor tissues, and also suggest that elevated cf-DNA levels may reflect tumor load.

In our study, serum cf-DNA levels decreased at the end of induction therapies parallel to a decrease in LDH levels being more prominent in NHLs. When the decrease in cf-DNA levels is not prominent or levels remain high, it might indicate the presence of residual or refractory disease. In 10 NHL cases with recurrences, the significantly lowered serum cf-DNA levels at the end of induction therapies were elevated remarkably at the time of recurrences. In some patients with recurrences, serum cf-DNA levels were found elevated long before the detection of recurrences (Fig. 3).

Machado et al.[12] reported that cf-DNA levels were significantly elevated at diagnosis which declined at the end of treatment. Similarly, Schwarz et al.[28] reported that in children with lymphoblastic leukemia, high levels of cf-DNA were detected in the plasma at diagnosis, decreased rapidly after therapy in few days.

In nearly 1/3 of our lymphoma cases increases in cf-DNA levels were seen with no evidence of recurrence or progression which were less marked compared to increases in cases with recurrences. The release of DNA may also involve other unknown events and increases in the follow-up should be evaluated cautiously with other variables.

Primerano et al.[29] investigated plasma levels of cf-DNA in a large series of pediatric HLs and reported significantly higher levels compared to controls and proposed that levels of plasma cf-DNA might constitute a non-invasive tool in management of HL patients. In 201 pediatric

lymphoma cases, Mussolin et al.[14] found no significant relationship between lymph node histology and cf-DNA levels and no correlation between cf-DNA and B-symptoms, LDH levels or bulky disease. We didn't detect any significant difference between the median serum cf-DNA levels in HLs and NHLs according to histopathological subtypes.

Our results indicate that, although an increased DNA concentration in blood is not specific for a defined disease, quantitative analysis of cf-DNA as a noninvasive approach may have a diagnostic value and might be used in the follow-up of pediatric lymphomas in combination with other clinical and laboratory parameters.

In conclusion, serum EBV DNA copy numbers can be used as a biomarker of response to treatment in pediatric lymphomas. Since the copy numbers were not elevated in cases with a recurrence of disease, their value for disease monitoring might not be proposed. Further prospective studies are required to determine the value of serial circulating EBV DNA monitoring as a predictor of relapse. Serum cf-DNA levels were elevated significantly at initial diagnosis in both HL and NHL cases. Significant decreases were observed in cf-DNA levels when the cases entered remission. Remarkable elevation in serum levels at recurrences in our NHL cases indicated that serum cf-DNA levels can have importance in the follow-up of pediatric NHLs.

Acknowledgement

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Original Article

Intratracheal administration of budesonide with surfactant in very low birth weight infants to prevent bronchopulmonary dysplasia

Minji Heo, Ga Won Jeon

Department of Pediatrics, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea.

ABSTRACT

Background and objectives. Respiratory distress syndrome (RDS) is a major cause of early postnatal death in preterm infants. Bronchopulmonary dysplasia (BPD) is one of the most fatal chronic respiratory complications of preterm infants after management of RDS. Anti-inflammatory therapy with corticosteroid is one of the effective treatments to prevent BPD. However, systemic administration of corticosteroid is not recommended because of long-term adverse effects. We studied the effect of early intratracheal instillation of budesonide with surfactant in preterm infants with severe RDS.

Method. Very low birth weight infants (VLBWIs) weighing less than 1,500 g who were admitted to the neonatal intensive care unit (NICU) of Busan Paik Hospital between January 2018 and December 2018 and diagnosed with severe RDS were enrolled. The treatment group was given a mixture of budesonide and surfactant (calfactant) while the control group was given surfactant (calfactant) only.

Results. Surfactant re-dosing, duration of mechanical ventilation, BPD, mortality, and retinopathy of prematurity (≥ stage 2) were not different between the two groups though there were decreasing trends in the treatment group compared to those in the control group. The duration of hospital stay was longer in the control group with statistical significance.

Conclusion. Early intratracheal administration of budesonide with surfactant in preterm infants with severe RDS might decrease BPD and mortality without disturbing surfactant function. Further studies with different preparations of surfactants with a large number of preterm infants are required.

Key words: bronchopulmonary dysplasia, budesonide, preterm infant, pulmonary surfactant, respiratory distress syndrome.

Respiratory distress syndrome (RDS) is a major cause of early postnatal death in preterm infants. Bronchopulmonary dysplasia (BPD) is one of the most fatal chronic respiratory complications in preterm infants after management of RDS. The incidence of BPD has not decreased despite advances in neonatal care of RDS in the post-surfactant era.[1] BPD is a lung injury syndrome. Its pathogenesis is multifactorial. Inflammation is an important pathogenetic factor in the development of BPD.[2] Anti-inflammatory therapy with corticosteroid is one of the most effective treatments to prevent BPD.[3] However, systemic corticosteroid can cause long-term adverse effects such as poor somatic growth, small head circumference, and neurodevelopmental disabilities such as neuromotor deficits, and cognitive deficits.[4] Thus, systemic administration of corticosteroid is not recommended.[5]

If corticosteroid can be used directly to lungs, then systemic adverse effects can be decreased and local anti-inflammatory effect can be

✉ Ga Won Jeon
iamgawon@hanmail.net

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increased. However, there have been limitations to deliver corticosteroid to distal lungs of newborn babies even if corticosteroid is inhaled as aerosol particles.

Budesonide is a strong corticosteroid with a local anti-inflammatory effect commonly used as a nebulizing suspension for treating bronchial asthma in children.[6] However, inhalation of budesonide as aerosol particles with metered dose inhaler to newborn babies is technically difficult. Budesonide has to be deposited in lung tissues by inhalation. It needs to be successfully distributed to lung tissues and then dissolved in lung cells of newborn babies. Inhalation of budesonide to newborn babies has been shown to be ineffective because this method cannot deliver budesonide to distal lungs.[7-9] Thus, an alternative lung-targeted delivery method is needed. Some researchers have studied intratracheal administration of budesonide. However, intratracheal administration of budesonide alone is ineffective in decreasing inflammation of lungs.[10] One of the suggested methods to deliver budesonide to distal lungs is intratracheal administration of budesonide with vehicle.

Pulmonary surfactant is a mixture of phospholipids and surfactant proteins (SPs).[11,12] Phospholipids bilayers incorporated with SPs are typical structures of surfactant. These layers called surface films play an important role in adsorption and spreading of surfactant into distal lungs with excellent solubility related to the rich lipid content that can lower surface tension.[13] Intratracheal administration of drug with surfactant as a vehicle markedly facilitates the distribution and spreading of the drug compared to administration of the drug alone.[14-16] Thus, surfactant has been suggested as a vehicle to deliver drugs to distal lungs.[17] Huang et al.[18] revealed that the administration of budesonide with surfactant can facilitate pulmonary distribution of budesonide compared to budesonide alone using fluorescent dye in mice. The objective of the present study was to determine the effect of early intratracheal instillation of budesonide with surfactant in preterm infants with severe RDS for alleviating BPD.

Material and Methods

The protocol of this study was reviewed and approved by the Institutional Review Board of Inje University Busan Paik Hospital (identification code: 19-0019) in accordance with the Declaration of Helsinki. A waiver of consent was granted for chart review without patient contact. Very low birth weight infants (VLBWIs) weighing less than 1,500 g who were admitted to the neonatal intensive care unit (NICU) of Busan Paik Hospital between January 2018 and December 2018 and diagnosed with severe RDS were enrolled. VLBWIs with severe RDS on chest radiography who required mechanical ventilator with fractional inspired oxygen (FiO_2) of > 0.5 were included. VLBWIs with chromosomal abnormality, congenital cardiac anomaly, or congenital pulmonary anomaly were excluded as these conditions could be confounding factors by interfering with pulmonary function and survival, making them unsuitable for evaluating the efficacy or safety of budesonide with surfactant. Included infants were allocated to two groups according to the medicine instilled: treatment group, surfactant and budesonide; and control group, surfactant only.

Study protocol

Clinical data were collected retrospectively from medical records. The diagnosis of RDS and the decision to perform surfactant replacement were left to the attending physician based on disease severity according to chest radiography and assisted ventilation.[19]

Surfactants were instilled into the trachea via an endotracheal tube using an orogastric tube. The treatment group was given a mixture of 0.25 mg/kg (1 mL/kg) of budesonide (Pulmicort nebulizing suspension, Astra Zeneca, Lund, Sweden) and 105 mg/kg (3 mL/kg) of calfactant (Infasurf®, ONY, Inc., Amherst, NY, USA) while the control group was given 105 mg/

kg of calfactant only. The concentration ratio of calfactant to budesonide was 35 mg: 0.25 mg or 140:1. The dosage of budesonide was determined according to previous studies.[20,21]

Infant demographic factors included gestational age, birth weight, gender, small for gestational age (SGA), Apgar score, and clinical risk index for babies (CRIB) II score. Maternal demographic factors included antenatal corticosteroids therapy, maternal gestational diabetes mellitus (GDM), maternal pregnancy-induced hypertension (PIH), and histologically confirmed chorioamnionitis. Outcomes associated with BPD included doses of surfactant, total duration of mechanical ventilation, duration of invasive ventilation, BPD, severity of BPD, and mortality. Outcomes associated with prematurity included patent ductus arteriosus (PDA), high grade intraventricular hemorrhage (IVH, ≥ grade 3), periventricular leukomalacia (PVL), high stage retinopathy of prematurity (ROP, ≥ stage 2), necrotizing enterocolitis (NEC, ≥ stage 2), and duration of hospital stay.

SGA was defined as a birth weight less than the tenth percentile on fetal-infant growth chart for preterm infants.[22] BPD was defined as an oxygen dependency at 36 weeks post-menstrual age with oxygen treatment for at least the first 28 days of life. BPD was subdivided to three groups according to its severity: mild BPD, breathing room air at 36 weeks post-menstrual age or discharge; moderate BPD, need for < 30% O2 at 36 weeks post-menstrual age or discharge; and severe BPD, need for ≥ 30% O2 with or without positive pressure ventilation or continuous positive pressure at 36 weeks post-menstrual age or discharge.[23] Sepsis was limited to positive blood culture with clinical signs of systemic infection.[24]

Statistical analysis

For nominal variables, Chi-square test or Fisher's exact test was performed. For continuous variables, Student's *t*-test or Mann-Whitney *U* test was performed. All statistical analyses were performed using SAS Enterprise Guide 3.0 (SAS Institute, Cary, NC, USA). *P*-values < 0.05 were considered significant. Data are given as mean ± standard deviation.

Results

Demographic factors

Among a total of 68 VLBWIs who were admitted to the NICU of Busan Paik Hospital during the period of enrollment (January 2018 through December 2018), 34 VLBWI who were diagnosed as severe RDS were enrolled: 16 in the treatment group (budesonide + surfactant) and 18 in the control group (surfactant). There were no significant differences in gestational age, birth weight, gender, SGA, Apgar score at 1 and 5 minutes, CRIB II score, antenatal corticosteroids, maternal GDM, maternal PIH, or chorioamnionitis between the treatment group and the control group (Table I).

Gestational age was $28^{+4} \pm 1^{+6}$ weeks (range: 25^{+2} - 32^{+0} weeks), and $28^{+2} \pm 2^{+0}$ weeks (range: 25^{+0} - 33^{+4} weeks), and birth weight was 1055 ± 247 g (range: 460 - 1400 g), and 1032 ± 255 g (range: 615 - 1400 g) in treatment group and control group, respectively.

BPD and mortality

Surfactant re-dosing was not different between the two groups (treatment group: 5 (31.3%); control group: 8 (44.4%); $p = 0.429$) (Table II). Total duration of mechanical ventilation (treatment group: 14.7 ± 15.7 days; control group: 26.3 ± 26.7 days; $p = 0.162$), and duration of invasive ventilation (treatment group: 10.6 ± 13.2 days; control group: 19.0 ± 25.2 days; $p = 0.266$) were not different between the two groups, though there were decreasing trends in the treatment group compared to those in the control group (reduction of 11 days and 8 days, respectively).

The incidence of BPD, mortality rate, and BPD or death were not different between two groups. There were decreasing trends of BPD

Table I. Demographic factors.

	Treatment group (n= 16)	Control group (n= 18)	p value
Gestational age, week^{+day}	$28^{+4} \pm 1^{+6}$ (25^{+2} - 32^{+0})	$28^{+2} \pm 2^{+0}$ (25^{+0} - 33^{+4})	0.641
Birth weight, g	1055 ± 247 (460 - 1400)	1032 ± 255 (615 - 1400)	0.791
Male, n (%)	8 (50.0)	9 (50.0)	1.000
SGA, n (%)	4 (25.0)	5 (27.8)	0.855
Apgar score at 1min	4.25 ± 1.53	4.44 ± 1.65	0.725
Apgar score at 5min	6.81 ± 1.05	6.67 ± 1.19	0.708
CRIB II	7.50 ± 2.71	7.56 ± 2.77	0.953
Antenatal corticosteroids, n (%)	16 (100.0)	18 (100.0)	
Maternal GDM, n (%)	0 (0)	2 (11.1)	0.169
Maternal PIH, n (%)	3 (18.8)	1 (5.6)	0.233
Chorioamnionitis, n (%)	7 (43.8)	5 (27.8)	0.331

SGA: small for gestational age, CRIB: clinical risk index for babies, GDM: gestational diabetes mellitus, PIH: pregnancy induced hypertension.

Table II. Bronchopulmonary dysplasia and mortality.

	Treatment group (n= 16)	Control group (n= 18)	p value
Surfactant re-dosing, n (%)	5 (31.3)	8 (44.4)	0.429
Total duration of mechanical ventilation, days	14.7 ± 15.7	26.3 ± 26.7	0.162
Duration of invasive ventilation, days	10.6 ± 13.2	19.0 ± 25.2	0.266
BPD, n (%)	5 (33.3)	8 (53.3)	0.269
BPD (moderate to severe), n (%)	2 (13.3)	4 (26.7)	0.361
BPD (severe), n (%)	0 (0.0)	2 (13.3)	0.143
Death, n (%)	1 (6.3)	4 (22.2)	0.189
BPD or death, n (%)	6 (37.5)	11 (61.1)	0.169

BPD: bronchopulmonary dysplasia.

(treatment group: 33.3%; control group: 53.3%; $p = 0.269$), mortality rate (treatment group: 6.3%; control group: 22.2%; $p = 0.189$), and BPD or death (treatment group: 37.5%; control group: 61.1%; $p = 0.169$). However, we couldn't find statistical differences.

There was no severe BPD in the treatment group whereas 13.3% had severe BPD in the control group

Outcomes Associated with Prematurity

Incidences of ligation of PDA, high grade IVH (≥ grade 3), PVL, and NEC (≥ stage 2) were similar between the two groups (Table III). Incidence of high stage ROP (≥ stage 2) was not different between the two groups, though there was a decreasing trend in the treatment group compared to the control group (treatment group: 12.5%; control group: 33.3%; $p = 0.166$). The duration of hospital stay was longer in the control group with statistical significance (treatment group: 73.2 ± 19.5 days; control group: 91.9 ± 20.3 days; $p = 0.018$).

Discussion

Budesonide has a structure similar to cholesterol, a sterol component of mammalian cell membrane. Thus, budesonide is lipid

Table III. Outcomes associated with prematurity.

	Treatment group (n= 16)	Control group (n= 18)	p value
Ligation of PDA, n (%)	1 (6.3)	3 (16.7)	0.347
IVH (≥ grade 3), n (%)	1 (6.3)	1 (5.9)	0.965
PVL, n (%)	1 (6.3)	0 (0.0)	0.295
ROP (≥ stage 2), n (%)	2 (12.5)	5 (33.3)	0.166
NEC (≥ stage 2), n (%)	0 (0.0)	0 (0.0)	
Hospital stay, days	73.2 ± 19.5	91.9 ± 20.3	0.018 *

PDA: patent ductus arteriosus, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia, ROP: retinopathy of prematurity, NEC: necrotizing enterocolitis.
*p-values <0.05.

soluble. It has strong affinity to airway and lung tissues. After budesonide is absorbed in lung tissue, it can conjugate with fatty acid and make fatty acid esters of budesonide. This conjugated form of budesonide is lipophilic, thus prolonging intracellular retention with very slow release of free budesonide. Other corticosteroids such as fluticasone propionate or beclomethasone dipropionate could not make fatty acid esters, which is why budesonide has longer pharmacological effects in airway tissues than other corticosteroids.[25,26] This is also the reason why topically inhaled budesonide has markedly better uptake and retention in airway tissues than non-inhaled type of corticosteroid.

We calculated the dosage of budesonide according to a previous study reporting that if the concentration ratio of surfactant and budesonide was 50:1 or more, then budesonide would not disturb the role of surfactant to decrease surface tension.[20] Therefore, calfactant (105 mg/kg, 35 mg/ml) and budesonide (0.25 mg/kg, 0.25 mg/ml) at a concentration ratio of 35 mg:0.25 mg or 140:1 (>50:1) was chosen so that surfactant function would not be disturbed by budesonide.

Systemically administered corticosteroid can cross the blood brain barrier and result in high concentrations in brain tissues, especially in hippocampus which contains high density of corticosteroid receptors.[3] Corticosteroid in brain tissues can change cerebral blood flow abnormally. It can cause ischemia and exert adverse effects on brain growth, especially in the hippocampus which is a critical area for memory and learning. This is why systemic administered corticosteroid can cause neurodevelopmental side effects.

To improve the local effect and decrease the systemic side effect of steroid, another route of drug delivery has been studied. Shah et al.[27-29] have reviewed the effect of inhaled versus systemic corticosteroids in preterm infants to prevent BPD with meta-analysis over three times. There were differences between study protocols. However, most trials administered inhaled budesonide or beclomethasone dipropionate by metered dose inhaler and a spacer device or intravenous dexamethasone. They found that there were no significant differences in effectiveness or side effects between inhaled and systemic corticosteroids. All reviewed randomized controlled trials did not use surfactant as a vehicle. They administered inhaled corticosteroid with metered dose inhaler, a well-known ineffective delivery system of drugs to the airway and lung in preterm infants. They suggested that a better mode of delivering inhaled corticosteroid could be more effective without increasing side effects.

Researchers have investigated intratracheal administering steroid with surfactant as a vehicle in animal models. Fajardo et al.[30] have revealed that intratracheal instillation of budesonide with surfactant is more effective than intratracheal

budesonide alone or inhaled budesonide in ventilated rabbit. Chen et al.[31] have administered intratracheal high dose dexamethasone (0.5 mg/kg) with surfactant (beractant, 50 mg/kg or 100 mg/kg) in rats with acute lung injury. They concluded that intratracheal administration of high dose dexamethasone with surfactant could alleviate inflammation of lungs compared to intratracheal administration of surfactant alone. In another animal study with rats, intratracheal administered low dose dexamethasone (0.04 mg/kg, 1 mL/kg) with surfactant as a vehicle (beractant, 10 mg/kg, 5 mL/kg, diluted to 2 mg/mL) improved the distribution of dexamethasone to peripheral lungs compared to the use of saline as a vehicle. It also decreased circulating concentration of dexamethasone compared to systemic administration of dexamethasone.[32] They suggested that the most effective mode of delivering corticosteroid could be intratracheal instillation. Yang et al.[33] have reported that intratracheal administered budesonide (0.5mg/kg) with surfactant (beractant, 100mg/kg) in neonatal piglets can improve oxygenation and histological lung injuries during acute period (in four hours) compared to no treatment, although it has no difference compared to treatment with surfactant alone. However, budesonide did not disturb surfactant function of decreasing surface tension. They reported updated results[34] that intratracheal administration of budesonide (0.25 mg/kg) with surfactant (beractant, 100 mg/kg) in neonatal piglets with severe RDS could improve oxygenation and histological lung injuries over 24 hours constantly. Differences from their previous study were suggested by too short observation periods such as four hours to alleviate oxygenation and histological changes. Considering that BPD is a lung injury syndrome with chronic and multifactorial pathogenesis, observation over a longer period might be more reliable for evaluating the effectiveness of drugs to alleviate BPD.

In another study by Ricci et al.[10], intratracheal administration of 0.25 mg/kg (1 mL/kg) budesonide with 200 mg/kg (2.5 mL/kg) surfactant improved tidal volume, gas exchange, and lung compliance in premature rabbits. They used poractant alfa as a vehicle, not beractant. The concentration ratio of poractant alfa to budesonide was 80 mg: 0.25mg or 320:1 (≥ 50:1). Thus, surfactant function might not be disturbed by budesonide. Also, administration of budesonide with surfactant decreased lung inflammation compared to budesonide or surfactant alone. This meant that budesonide did not disturb surfactant function, and surfactant can be a good vehicle to deliver budesonide to distal lungs to alleviate BPD. Roberts et al.[35] have reported that, when budesonide with surfactant is administered intratracheally in premature lambs, budesonide and its metabolites (16α-hydroxy prednisolone, or budesonide-palmitate) could not be detected in the brain tissue. This means that intratracheally administered budesonide with surfactant is deposited in the airway and lung tissues mostly. It is less absorbed systemically, showing low plasma concentration. It could not cross the blood brain barrier, resulting in minimal neurologic side effects with only local anti-inflammatory effect in airway tissue

Yeh et al.[20] have conducted a prospective randomized controlled trial (RCT) with intratracheal instillation of 0.25 mg/kg (1 mL/kg) budesonide with 100 mg/kg (4 mL/kg) surfactant (beractant) in 116 VLBWIs with severe RDS. They reported that this therapy could decrease the incidence of BPD and mortality and increase survival without BPD compared to surfactant only therapy without significantly increasing adverse events. In a follow up study[21] of this trial at the age of two to three years of these enrolled preterm infants (67 VLWBIs, 35 budesonide treated and 32 control), there were no significant differences in the incidence of hospital admission by respiratory causes or physical growth such as body weight, height, or head circumference between budesonide with surfactant treated group and surfactant only treated group. There were no significant differences in Mental Development Index (MDI) or Psychomotor Development Index (PDI) by Bayley Scales of Infant Development

(BSID) II between budesonide with surfactant treated group and surfactant only treated group either. Authors concluded that there were no long-term adverse events associated with intratracheal instillation of budesonide with surfactant. In a prospective multicenter RCT,[36] 265 VLBWIs in three tertiary NICUs in USA and Taiwan were enrolled. Budesonide (0.25 mg/kg, 1 mL/kg) with surfactant (beractant, 100 mg/kg, 4 mL/kg) treatment decreased the incidence of BPD or mortality compared to surfactant (100 mg/kg) only treatment (66% to 42%, $p < 0.001$). Also, levels of interleukins (IL-1, -6, and -8) in tracheal aspirates were lower in budesonide with surfactant treatment group compared to those in surfactant only treatment group, indicating that budesonide could ameliorate inflammation. Physical growth and neurodevelopmental impairment were similar between the two groups in a follow up study at the age of two to three years of these enrolled preterm infants. Researchers reported that intratracheal administration of budesonide with surfactant decreased BPD or mortality without significantly increasing acute or long-term adverse effects.

The differences of this study from Yeh's studies[20,21,36] are that we used different preparation of surfactant (calfactant in this study vs. beractant in Yeh's studies), and different concentration ratio of surfactant to budesonide (140:1 in this study vs. 100:1 in Yeh's studies).

We couldn't find differences in the BPD, mortality rate, BPD or mortality, ventilator duration, and high stage ROP between the two groups. There were decreasing trends of those parameters in the treatment group compared to the control group without statistical differences. Hospital stay was decreased in the treatment group compared to control group with statistical significance. It might be due to decreasing trends of BPD and ventilator duration in the treatment group. The limitation of this study is that the small number of enrolled patients to prove statistical differences.

In conclusion, early intratracheal administration of budesonide and surfactant in preterm infants with severe RDS might decrease BPD and mortality without disturbing surfactant function. Further prospective randomized controlled studies with different preparations of surfactants with a large number of preterm infants are required. To the best of our knowledge, this is the first study administering budesonide with calfactant in preterm infants having severe RDS.

The protocol of this study was reviewed and approved by the Institutional Review Board of Inje University Busan Paik Hospital. A waiver of consent was granted for chart review without patient contact.

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Original Article

Diverse clinical characteristics of *Aspergillus* growth in patients with cystic fibrosis

Nagehan Emiralioğlu[1], Deniz Doğru[1], Özlem Doğan[2], Dolunay Gülmez[2], Sevtap Arıkan Akdağlı[2], Sanem Eryılmaz Polat[1], Gökçen Tuğcu[1], Mina Gharibzadeh Hızal[1], Ebru Yalçın[1], Uğur Özçelik[1], Burçin Şener[2], Nural Kiper[1]

Departments of [1]Pediatric Pulmonology and [2]Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background and objectives. Patients with cystic fibrosis (CF) have a varying spectrum of clinically significant *Aspergillus* disease in addition to allergic bronchopulmonary aspergillosis (ABPA). Here we aimed to review the different clinical phenotypes related with *Aspergillus* growth on the airway culture of patients with CF, we also aimed to investigate the effect of *Aspergillus* growth on lung function tests.

Method. The medical records of 100 patients with CF who had *Aspergillus* growth on airway culture within the period of April 2001 and June 2016 were retrospectively analyzed. Age, gender, symptoms, physical examination findings, pulmonary function tests, the diagnosis of ABPA, and airway culture results were recorded for every visit. Patients with *Aspergillus* growth on airway cultures were classified into different groups as ABPA, *Aspergillus* sensitization, *Aspergillus* colonization and *Aspergillus* bronchitis.

Results. Medical records of 83 patients and 147 sputum cultures were attained from 100 patients. The mean age of the patients was 17.6±7.6 years and the mean age of the first *Aspergillus* growth in sputum culture was 12.5±6.7 years. At first isolation, *Aspergillus fumigatus* SC was the most common *Aspergillus* SC in sputum (76.3%) and 14.5% of these patients required hospitalization. *Aspergillus* sensitization was diagnosed in 3.6% (n= 3) of the patients. *Aspergillus* colonization was diagnosed in 18.1% (n= 15) of all patients and led to a decline in FEV1%, FVC% and FEF25-75% which was not statistically significant, furthermore. ABPA was detected in 9.6% (n= 8) of all patients and led to a statistically significant decline in FEV1% (p= 0.02); nonsignificant decline in FVC% and FEF25-75%. *Aspergillus* bronchitis was detected in 43.4% (n= 36) of all patients and led to nonsignificant decline in FEV1%, FVC% and FEF25-75%.

Conclusion. ABPA is recognized as the most common *Aspergillus* associated disorder in CF patients and is related to deteriorated pulmonary function tests; however *Aspergillus* colonization and bronchitis may also be associated with worsening lung function.

Key words: cystic fibrosis, aspergillus colonization, aspergillus sensitization, ABPA, aspergillus bronchitis.

Cystic fibrosis (CF) is a systemic chronic disease and abnormalities in CFTR (Cystic fibrosis transmembrane conductance regulator) function affect interactions between the epithelial surface and microorganisms such as fungi.[1] Conidial spores are usually cleared by airway epithelium; however, in CF due to disruption of epithelial barrier and germination of the fungi induce inflammatory response. Increased mucus viscosity and lung injury result in an ongoing cycle of infection, inflammation and pulmonary damage.[1,2] *Aspergillus*, the most commonly detected filamentous fungi in CF induces this abnormal pulmonary inflammation.[2] The main *Aspergillus*- associated clinical manifestations

✉ Nagehan Emiralioğlu
drnagehan@yahoo.com

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in CF are Allergic Bronchopulmonary Aspergillosis (ABPA), *Aspergillus* sensitization, *Aspergillus* colonization, *Aspergillus* bronchitis and, rarely aspergilloma and invasive disease.[1]

ABPA is a hypersensitivity response to *Aspergillus* antigens; the prevalence of ABPA changes from 2 to 8%, and lung function has been shown to deteriorate over time related with ABPA. The increasing prevalence of *Aspergillus* sensitization has also been reported from 20 to 65% recently and separate from CF-ABPA, *Aspergillus* sensitization has significant clinical effects on lung function and patient morbidity.[1,3,4] The term 'colonization' defines the presence of a microorganism which causes neither symptoms nor immunologic response. *Aspergillus* bronchitis was first described by Shoseyov et al.[5] for patients with CF who had respiratory exacerbations non-responsive to appropriate antimicrobial therapy, cultured *Aspergillus* from sputum and responded to antifungal therapy. Few studies have addressed the role of *Aspergillus* on lung function in CF. However, the effect of colonization and *Aspergillus* bronchitis on lung function other than ABPA is not clear.[1]

Recommendations for the treatment of ABPA in patients with CF are now clearly described; however, the pathogenic role of *Aspergillus* and the benefit of treatment of an *Aspergillus* bronchial infection remain to be clarified in clinical conditions other than ABPA. There is also no consensus on the antifungal therapies of CF patients presenting with persistent *Aspergillus*-positive cultures or those with sensitization to *Aspergillus*.[6-8]

Here we aimed to review the different clinical phenotypes related with *Aspergillus* growth on the airway culture of patients with CF and we also aimed to evaluate the effect of *Aspergillus* growth on lung function of these patients. Our second aim was to review the risk factors affecting the growth of *Aspergillus* in airway culture of patients with CF.

Material and Methods

Subjects

In this retrospective cohort study, medical records of children with a diagnosis of CF (two positive sweat chloride tests (≥60 mmol/L) and/or genotype confirmation) treated at our tertiary care centre between April 2001 and June 2016 who had *Aspergillus* growth in sputum culture were evaluated.

Age, gender, symptoms, physical examination findings, pulmonary function tests including Forced expiratory volume in the first second (FEV1), Forced vital capacity (FVC), Forced expiratory flow between 25% and 75% of the FVC (FEF25-75), and airway culture results were recorded for every visit. "Increase in symptoms" were defined as increased cough, sputum, dyspnea or fever; new physical examination findings were also defined newly detected lung sounds like rale or rhonchi that were not defined on clinical follow-up of the patient. Immunological data included total immunglobulin E (IgE), specific anti - *A. fumigatus* IgE in all patients and specific anti - *A. fumigatus* IgG only in two patients as it is not routinely performed in our hospital. Skin prick test reactivity to *Aspergillus* antigen and treatment modalities were also recorded in all patients. Patients were followed every three months. Spirometric data were recorded for three years.

Microbiological methods

Airway cultures were taken at each visit to our centre from all patients. Respiratory cultures of the patients were inoculated onto 5% sheep blood agar, MacConkey agar, chocolate agar with bacitracin, mannitol salt agar and *Burkholderia cepacia* selective agar. Bacterial isolates were identified by conventional methods and automated bacterial identification systems (BD Phoenix, USA; or VITEK 2, bioMerieux, France). Mycological cultures were plated on Sabouraud dextrose agar (SDA) and incubated at 35 ± 2°C and 25 ± 2°C for seven

days. Identification of *Aspergillus* strains at species complex (SC) level were performed using conventional mycological methods.[9]

Definitions

Patients with *Aspergillus* growth on airway cultures were classified into different groups according to criteria defined by Baxter et al. and Shoseyov et al.[5,10,11] As *Aspergillus* specific IgG testing is not available in our hospital, we could not use *Aspergillus* specific IgG for this classification.

1- *Aspergillus* colonization: Two or more *Aspergillus fumigatus* SC positive cultures in any 12 months during the study period without elevation of total IgE and *A. fumigatus* specific IgE.

2- *Aspergillus* sensitization: Elevation of total IgE and *A. fumigatus* specific IgE without fulfilling the diagnostic criteria of ABPA. *A. fumigatus* specific IgG is not elevated. Because of missing data of *Aspergillus* specific IgG in our cohort, we defined sensitization depending on clinical criteria, total IgE and *A. fumigatus* specific IgE.

3- *Aspergillus* bronchitis: Due to missing examinations of sputum galactomannan, *Aspergillus* specific DNA and serum specific IgG in our cohort, *Aspergillus* bronchitis was defined according to these criteria; clinical deterioration with positive sputum cultures for *Aspergillus*, no antibiotic treatment response, total serum IgE level <200 kU/l, no observation of new pulmonary infiltrates and appropriate antifungal treatment response with the exclusion of ABPA.[5,12] Because of missing data of *Aspergillus* specific IgG; differential diagnosis with colonization was made depending on clinical deterioration of these patients.

4- ABPA diagnosis was based on the criteria published in the ABPA consensus paper that included five or more of the following: (a) Acute or subacute clinical deterioration not attributable to another etiology; (b) Total serum IgE concentration higher than 500 IU/ml; (c) Skin prick test reactivity to *Aspergillus*; (d) Presence of serum IgE antibodies to *A. fumigatus* (higher than 0.35 kU/l); (e) Precipitins or IgG antibodies to *A. fumigatus*; (f) New or recent pulmonary infiltrates, mucus plugging, or bronchiectasis with no response to antibiotics and physiotherapy.[13]

This study was approved by the local institutional review board in 2018 with the number GO/18/271-43.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to determine whether the variable had a normal distribution. Means and standard deviations (SD) were calculated for continuous and numbers (percentages) for categorical data. More than two independent groups were compared using One-Way Anova and Tukey post-hoc test for normal continuous variables or Kruskal–Wallis test and Bonferroni adjusted Mann–Whitney U test for non-normal continuous variables. Mann-Whitney U test was used to compare continuous variables between independent two groups. Chi squared test was performed to compare proportions between groups. Logistic regression was used to analyse the effects of potential factors on *Aspergillus* growth. Repeated measures of variance analyses were used for yearly lung function test changes of patients. Mean FEV1 and FVC decline were expressed as the annual average change in FEV1% and FVC% during one year period by simple regression analysis. Statistical tests were two-sided and statistical significance was accepted at $p < 0.05$.

Results

There were 100 patients with *Aspergillus* growth in airway culture during the study period. From these, medical records of 83 patients and 147 airway cultures of these patients were attained. The mean age of all patients was 17.6 ± 7.7 years and the mean age of the first *Aspergillus* growth

in airway culture was 12.5 ± 6.7 years. Male to female ratio was 47/36. 26.5% (n= 22) of patients have F508 homozygous deletion, 16.8% (n= 14) of patients have F508 heterozygous deletion and 56.4% (n= 47) of patients have other CFTR mutations.

Fifty-three patients had *Aspergillus* growth more than once. Bronchiectasis was detected in 91.6% (n= 76) and pancreatic insufficiency was noticed in 100% (n=83) of the patients. Chronic *Pseudomonas aeruginosa* and chronic *Staphylococcus aureus* colonization were detected in 49.4% (n= 41) and 48.2% (n= 40) of patients, respectively.

At first isolation, *A. fumigatus* SC was the most common SC of *Aspergillus* in airway cultures (76.3%). 25.3% (n= 21) of the patients had *Aspergillus* growth once a time and asymptomatic at first isolation. The remaining 62 patients were classified into different groups. ABPA was diagnosed in 9.6% of patients (n= 8) and totally ABPA was diagnosed 24 times of these eight patients in this period. *Aspergillus* sensitization was detected in three patients and *Aspergillus* colonization was detected in 18.1% (n= 15) of the patients. *Aspergillus* bronchitis was detected in 43.4% (n= 36) of the patients. Table I shows the clinical characteristics of patients at first isolation according to diagnosis of ABPA, *Aspergillus* colonization, *Aspergillus* bronchitis and *Aspergillus* sensitization.

In three years of follow up, ABPA led to statistically significant decline in FEV1% (p= 0.02). Although decline in FVC% and FEF25-75% were also present; these findings were not statistically significant.

Aspergillus colonization and *Aspergillus* bronchitis also led to decline in FEV1%, FVC% and FEF25-75% which was not statistically significant in these patients furthermore. Table II shows the change of lung function tests within the time in patients with ABPA, *Aspergillus* colonization and *Aspergillus* bronchitis. Figure 1 and Figure 2 also show the graphic of the lung function tests within the groups of ABPA, *Aspergillus* colonization and *Aspergillus* bronchitis. The decline of FEV1% (p= 0.45) and FVC% (p= 0.10) were not statistically significant between the groups furthermore. We did not include the change of lung functions in the group with *Aspergillus* sensitization due to small number of patients in this group. The mean FEV1 decline within the groups of ABPA, *Aspergillus* bronchitis and *Aspergillus* colonization was 5.5%, 3.3%, 2.2% per year and the mean FVC decline within the groups of ABPA, *Aspergillus* bronchitis and *Aspergillus* colonization was 6.2%, 3.8%, 0.3% per year respectively in the following three years.

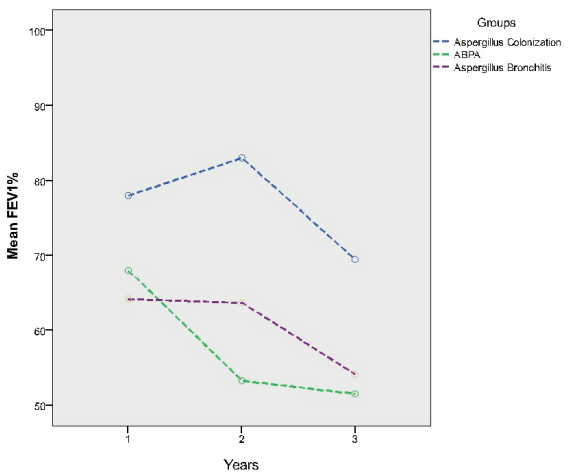

Fig. 1. Change of FEV1% in patients with ABPA, *Aspergillus* colonization and *Aspergillus* bronchitis.

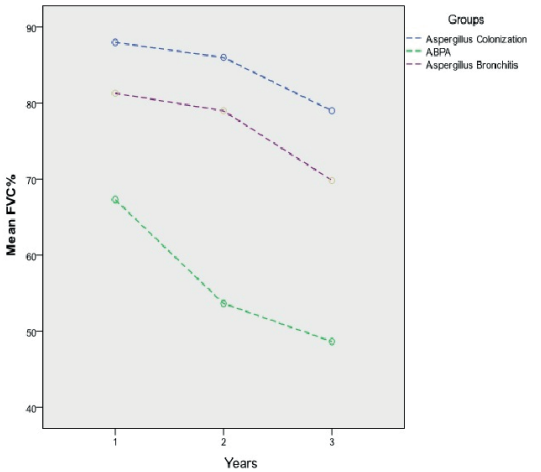

Fig. 2. Change of FVC% in patients with ABPA, *Aspergillus* colonization and *Aspergillus* bronchitis.

Table I. Demographic and clinical characteristics of patients with ABPA, *Aspergillus* colonization, *Aspergillus* bronchitis and *Aspergillus* sensitization.

	ABPA	*Aspergilllus* colonization	*Aspergillus* bronchitis	*Aspergillus* sensitization	p
Number of patients, n	8	15	36	3	
Age at last visit (years), Mean (±SD) (Min-Max)	18.6 (± 4.2) (13.5-24)	18.6 (± 4.5) (7.5-27)	18.4 (± 5.5) (6.5-29)	19.7 (± 5) (14.5-24.5)	0.95
Age at first *Aspergillus* isolation (years), Mean (±SD) (Min-Max)	13.6 (± 4.1) (8-21)	12.3 (± 6.9) (3-27)	13.6 (± 5) (5-27)	11.2 (± 5.1) (7.5-17)	0.71
CFTR mutation, n					0.66
F508 del homozygous	2	3	10	2	
F508 del heterozygous	1	5	4	1	
Other	4	7	22	0	
Increase in symptoms at first isolation, n (%)	7 (87.5%)	6 (40%)	23 (63.9%)	0	0.01
New physical examination finding at first isolation, n (%)	5 (62.5%)	5 (33.3%)	20 (55.6%)	0	0.10
Bronchiectasis, n (%)	8 (100%)	15 (100%)	35 (97.2%)	3 (100%)	0.70
Chronic *P. aeruginosa* colonization, n (%)	5 (62.5%)	9 (60%)	17 (47.2%)	3 (100%)	0.23
Chronic *S. aureus* colonization, n (%)	4 (50%)	8 (53.3%)	15 (41.7%)	1 (33.3%)	0.77
Pulmonary function tests at first isolation, Mean (±SD)					
FEV1%	55 (± 24)	76 (± 10)	60 (± 19)	64 (± 45)	0.22
FVC%	59 (± 22)	87 (± 11)	68 (± 20)	64 (± 10)	0.08
FEF25-75%	47 (± 24)	62 (± 25)	49 (± 23)	45 (± 13)	0.68
Aspergillus SC, n					0.004
A. fumigatus	2	13	27	1	
A. flavus	4	0	3	2	
A. terreus	1	0	3	0	
A. niger	0	0	2	0	
Aspergillus spp.	1	0	1	0	
Coinfection with other pathogens at first isolation, n					0.59
No growth	1	2	3	0	
S. aureus	2	4	12	0	
H. influenza	0	0	1	0	
S. pneumonia	0	1	0	0	
P. aeruginosa	3	4	8	3	
Acinetobacter	0	0	0	0	
S. aureus and *P. aeruginosa* coinfection	2	4	12	0	
Inhaled antibiotic at first isolation, n					0.90
Inhaled tobramycin	4	5	11	1	
Inhaled colimycin	0	1	1	0	
Treatment at first isolation, n					0.001
Oral antifungal treatment	6	12	32	3	
Intravenous antifungal treatment	2	0	0	0	
Steroid treatment	8	0	0	0	
Hospitalization, n	3	2	7	0	0.42

ABPA: allergic bronchopulmonary aspergillosis, CFTR: cystic fibrosis transmembrane conductance regulator gene, FEV1: forced expiratory volume in the first second, FVC: forced vital capacity
FEF25-75: forced expiratory flow between 25% and 75% of the FVC.

Table II. Lung functions of patients in different groups.

Lung function test	*Aspergillus* bronchitis (n= 12)	p	*Aspergillus* colonization (n= 6)	p	ABPA (n= 5)	p
FEV1%, mean±SD		0.41		0.09		0.02
First year	64.1 ± 18.4		83 ± 10		68 ± 17	
Second year	63.6 ± 19.4		73.6 ± 8		53.2 ± 12.5	
Third year	54.1 ± 23.5		76.3 ± 7.7		51.5 ± 14.6	
FVC%, mean±SD		0.31		0.14		0.22
First year	81.3 ± 11.9		85.6 ± 0.5		67.3 ± 23.5	
Second year	79 ± 9.9		76 ± 7.2		53.6 ± 13.2	
Third year	69.8 ± 21.5		84.6 ± 5		48.6 ± 17.7	
FEF25-75%, mean±SD		0.41		0.26		0.12
First year	49.8 ± 22.3		62.3 ± 10.5		56 ± 17	
Second year	42 ± 18.8		47.3 ± 5.5		35 ± 7.7	
Third year	39.8 ± 20.1		58.3 ± 10.6		40 ± 13.7	

ABPA: allergic bronchopulmonary aspergillosis, FEV1: forced expiratory volume in the first second, FVC: forced vital capacity, FEF25-75: forced expiratory flow between 25% and 75% of the FVC.

The risk of *Aspergillus* growth in airway cultures was not affected significantly by the age, CFTR mutation, symptoms, physical examination findings, radiological findings, sputum culture results and inhaled antibiotic treatment in logistic regression analysis.

Discussion

A. fumigatus SC is one of the main fungal subgroup found in CF airways and results in different clinical states and wide range of host responses affecting the progression of CF lung disease. In this retrospective cohort, we reviewed different clinical phenotypes of *Aspergillus* growth on the airway culture of patients with CF classified *Aspergillus* colonization, *Aspergillus* bronchitis, *Aspergillus* sensitization and ABPA.[7,11]

The prevalence of *Aspergillus* colonization ranges from 10 to 57% with increasing age in CF patients.[14] de Vrankrijker et al.[15] reported a cohort of 259 CF patients and found 61 (23.5%) of these patients had *A. fumigatus* colonization according to the criteria of having more than 50% positive sputum cultures in a given year. The prevalence of *Aspergillus* colonization in pediatric CF patients is not well established yet. In our cohort we reported 18.1% of patients with *Aspergillus* colonization. Saunders et al.[16] also reported 22% of children fulfilled the criteria for *A. fumigatus* colonization within eight years of study period. The prevalence of ABPA in patients with CF varied from 3 to 25%, and the prevalence of *Aspergillus* sensitization varied from 20 to 65% in a metaanalysis by Maturu et al.[17] Our cohort revealed 9.6% of ABPA patients. In our cohort we had three patients with sensitization. Baxter et al.[10] and Shoseyov et al.[5] defined *Aspergillus* bronchitis as repeatedly *Aspergillus* growth in sputum samples without hypersensitivity to *Aspergillus* and with persistent respiratory symptoms and no response to antibiotics in patients with CF. Baxter et al.[10] reported 30% of patients with *Aspergillus* bronchitis in their adult cohort. In our cohort we also reported 43.4% of patients with *Aspergillus* bronchitis similar to these results.

Few studies have reported the association between age and risk of *Aspergillus* isolation. Saunders et al.[16] reported that the isolation of *A. fumigatus* was common in children older than 10 years and most of the older children

eventually became persistently colonized. We also found that the mean age of the first *Aspergillus* growth in airway culture was 12 ± 6.6 years similar to this study. In our cohort the risk of *Aspergillus* colonization was not affected significantly by age, CFTR mutation, symptoms, physical examination findings, radiological findings, sputum culture results and inhaled antibiotic treatment. Colonization of the airways by *A. fumigatus* usually develops after chronic colonization with *P. aeruginosa*. Noni et al.[18] concluded that their patients with colonization were not found to have significantly higher *P. aeruginosa* colonization and inhaled antibiotic treatment rates similar to our study. However Bargon et al.[19] suggested that prophylactic antibiotics may cause *A. fumigatus* colonization. Another study by Burns et al.[20] concluded the use of inhaled tobramycin increased *A. fumigatus* isolation in the treatment group, at the end of the study period. According to our findings, it is difficult to say whether there is a causal relationship between the use of inhaled antibiotics and *A. fumigatus* colonization. However most of our patients were using inhaled tobramycin at first isolation of *Aspergillus*.

There are few studies investigating the role of *A. fumigatus* on lung function in CF, and these studies have not shown significant lung function decline in patients colonized with *A. fumigatus*.[21] Bargon et al.[19] found no significant association between *A. fumigatus* colonization and lung function, in their adult CF patients. Although the decline of FEV1, FVC and FEF25-75 in our cohort with *Aspergillus* colonization, these findings were not statistically significant. Other retrospective cohort analysis on 163 patients with *Aspergillus* colonization also did not find any differences regarding lung function decline during the study period.[22] A causal relationship was not shown, but this study suggests that *A. fumigatus* may influence lung functions and risk of pulmonary exacerbations. However, in another cross-sectional study with 7010 CF patients from the European Epidemiologic Registry of Cystic Fibrosis, *Aspergillus* colonization was associated with impaired lung functions.[23] In Canada, a retrospective cohort study of pediatric non-ABPA CF patients showed that two or more respiratory samples positive for *A. fumigatus* in any given year was associated with a significant reduction in FEV1 and a significant increase in pulmonary exacerbations requiring hospitalization compared with pediatric CF patients without *A. fumigatus* in respiratory samples.[22]

Noni et al.[18] showed that *A. fumigatus* chronic colonization and lung function decline may have a causal relationship. Patients with *A. fumigatus* chronic colonization had significantly lower lung function in a seven-year prospective study period. Also baseline FEV1 was statistically different between groups before colonization and this may have led to the chronic colonization in these patients. Saunders et al.[16] also reported that *A. fumigatus* colonization may be associated with worse lung functions.

Aspergillus sensitization and its association with poorer lung function in CF lung disease was investigated by a number of studies.[4,8] Baxter et al.[10] revealed that CF patients with *Aspergillus* sensitization showed a significantly higher FEV1 decline over two years compared to the group of patients without sensitization. We could not estimate the effect of *Aspergillus* sensitization on lung functions, because of the small number of patients in our cohort. Furthermore, Fillaux et al.[3] reported that, ABPA, *Aspergillus* sensitization and persistent carriage have an impact on pulmonary functions in CF patients. *Aspergillus* bronchitis is also associated with lung function decline in patients with CF.[1,5] *Aspergillus* bronchitis led to decline in FEV1%, FVC% and FEF25-75% in our cohort which is not statistically significant.

Kraemer et al.[24] showed a significant negative effect of ABPA on FEV1 in a retrospective study with 122 mostly pediatric CF patients. Baxter et al.[10] also reported, CF patients with ABPA had significant FEV1 decline over a two year study period compared to other patients without

ABPA. Our findings also showed that the most prominent lung function decline was in patients with ABPA which was not statistically significant as shown in Figure 1.

Aspergillus colonization and infection are treated with antifungal agents such as azoles, however ABPA is usually treated with corticosteroids additionally with itraconazole or voriconazole.[6,25] There is only one published prospective, randomized, controlled study investigating the effect of antifungal therapy on pulmonary outcomes in CF patients.[26] Due to the retrospective nature of our study we have limited results about the effect of antifungal therapy on pulmonary outcome.

Our findings are limited by the retrospective nature of data collection and low numbers of the patients and the lack of longitudinal data for *Aspergillus* classification stages in the relevant subgroups. Also because this is a retrospective study of CF patients with *Aspergillus* growth, we could not compare the results with other CF patients as a control group. It can be assumed that patients can change within the different classification stages and that the classification status is probably not stable. We could not classify the patients depending on immunological results due to missing examinations of *Aspergillus*-specific IgG in these patients, we also defined sensitization, *Aspergillus* bronchitis, and colonization depending on the clinical criteria. Also due to a small number of patients having performed spirometry, evaluation of the lung function tests within years is difficult to estimate the prognosis of colonization, sensitization and ABPA in CF patients.

In conclusion, our study provides evidence for the significant effect of *Aspergillus* colonization on lung functions and emphasizes that chronic colonization should be considered pathogenic in CF patients. However, the most important question that requires addressing is the clinical significance of the fungi detected in CF and whether eradication needs consideration. Prospective controlled studies with treatment arms are an important requirement for this field to progress.

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Original Article

Awareness assessment for parents of children with congenital heart diseases regarding fetal echocardiography

Shaimaa Rakha

Department of Pediatric Cardiology, Mansoura University Faculty of Medicine, El Gomhouria St, Mansoura, Egypt.

ABSTRACT

Background and objectives. The high recurrence rate of congenital heart disease (CHD) in siblings was the rationale for recommending fetal echocardiography. However, in a developing country like Egypt, there are limited fetal echocardiographic examinations under this category. The study was conducted to assess knowledge about fetal echocardiography amongst parents of children with CHD.

Method. A questionnaire survey was conducted in a tertiary pediatric hospital from June to December 2018. The study included parents having children with CHD follow up in the outpatient clinic or admitted in the pediatric cardiology ward. The questionnaire included demographic data of parents regarding sex, age, education, and residence. It also includes a detailed inquiry about parents' knowledge regarding the availability, safety, and value of fetal echocardiography.

Results. Participants were 200 parents, mostly mothers 159 (79.5%). The median age of parents included in the study was 33.5 (29-40) years. Regarding awareness, 134 (67%) did not know any prenatal diagnostic investigation for CHD, 46 (23%) knew fetal echocardiography, and 20 (10%) named other tools. Nevertheless, 34% of parents thought they would need fetal echocardiography in a subsequent pregnancy. Although 178 (89%) of parents thought it might be a safe investigation, 33% did not think it will have additional benefits over postnatal echocardiography. The age of the patient and age of the participating parent were the only statistically significant predictors for parents knowledge on fetal echocardiography existence with p= 0.008, 95% CI=1.039-1.282 and p= 0.015, 95% CI = 0.864-0.984, respectively.

Conclusion. Parents of children with CHD have significant knowledge gaps regarding Fetal Echocardiography. Our findings suggest that the current parent counseling is inadequate and needs further focus, especially in developing countries, to promote parents' understanding of the prenatal cardiac diagnosis. Data on fetal echocardiography should be clarified at the initial diagnosis of pediatric CHD.

Key words: awareness, parents, congenital heart disease, fetal echocardiography.

Congenital heart diseases (CHD) are the most prevalent anomaly with an incidence of 8 per 1000 live births.[1,2] Fetal echocardiography is considered the standard imaging technique used for the diagnosis and assessment of CHDs in prenatal life with high sensitivity and specificity.[3,4] It also provides details about the anatomy and functions of the fetal heart. Commonly, it is performed between 18 weeks and 22 weeks of gestational age.[5] Fetal echocardiography is considered essential for the identification of CHDs, timely interventions, and counseling of parents.[6] Furthermore, accurate diagnosis of fetal heart lesions offers several benefits, including improvements in outcomes of critical neonatal cardiac anomalies.[7]

The recurrence risk of cardiac anomalies in siblings with unaffected parents is 2-6%.[8-10] The risk for recurrence is higher if more than one sibling is affected.[11,12] Besides, some lesions might have more tendency for recurrence with risk up to 8% such as hypoplastic left heart syndrome (HLHS).[13,14] The concordance for most lesions

✉ Shaimaa Rakha
drshimaarhaka@mans.edu.eg

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were found to be <50%, while exact concordance for CHDs may be around 20-35%.[15,16] Hence, fetal echocardiography is indicated with family history of CHD, especially in a sibling. According to the American Heart Association scientific statement on fetal cardiology, it is recommended with a level of evidence I/B.[16] However, fetal echocardiographic examinations under this category of indications are still limited, particularly in a developing country like Egypt. The counseling for the parents of children with CHD about antenatal diagnosis and its benefits is considered a fundamental step in the management of pediatric CHD. Therefore, the current study was conducted to assess the knowledge gaps about fetal echocardiography amongst parents of children with CHD to determine the success in the counseling process of parents regarding that point.

Material and Methods

A questionnaire survey was conducted from June to December 2018 in a tertiary pediatric hospital, Mansoura University Children Hospital, Egypt. The study was approved by the institutional review board of Mansoura University, Faculty of medicine with number of R.18.11.334 on 11/2018. The included participants in the study were the parents of children with CHD followed up in the pediatric cardiology outpatient clinic or admitted in the pediatric cardiology ward. All enrolled parents were informed about the study and written consent was obtained. Parents of pediatric patients with acquired heart diseases or arrhythmia were excluded from the data analysis. The patients who were visiting the hospital as newly diagnosed cases were not included. Patients accompanied to the hospital with non-legal guardians like grandparents and other relatives were excluded.

Before we began the questionnaire, demographic and clinical data of the patients were collected from their hospital records such as age, gender, primary cardiac lesion, and associated syndromes. Parents were approached in the waiting room of the outpatient area or the patient's room on the inpatient floors. The questionnaire was conducted with face to face interview with the parents at the end of the outpatient visit or during the hospital stay with further clarification using phone if necessary. A pilot study was carried out with parents to ensure that the questions were easily understood. The survey included questions about parents' demographics, such as sex, age, employment, education, and residence. We asked the parents about having a child or pregnancy after the currently affected and if fetal echocardiography was performed. Moreover, it included a detailed inquiry about parents' awareness regarding knowledge of availability, safety, appropriate time, and value of fetal echocardiography. The parent questionnaire is demonstrated in Table I.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 25 (SPSS, Inc, an IBM Company, Chicago, IL, USA). Data are expressed as frequency and percentage or median and interquartile range according to the variable. Logistic regression analysis was used to identify significant determinants of awareness about fetal echocardiography. A probability value of $p < 0.05$ was considered significant. Microsoft Office Excel 2013 (Microsoft, Inc., Redmond, WA, USA) was used for formulating tables and graphs.

Results

A questionnaire survey was conducted in a tertiary pediatric hospital from June to December 2018. During six months, 200 parents of patients with CHD accepted to participate in our study. The demographic information for the study cohort is enlisted in Table II. The median age of the child with CHD was 5 (1.5-11) months and most frequently being females 106 (53.0%). Most of the patients had acyanotic CHD 164 (82%), and136 (68%) of the cases did not require catheter or surgical intervention. Also, most of the patients were non-syndromic 180 (90%), with Down syndrome, the most frequently associated syndrome found in 11(5.5%).

Table I. Parents questionnaire used in the study.

- Age of the participating parent
- The participating parent
  - Father
  - Mother
- Parent Education
  - None schooled
  - Middle school
  - High school or equivalent education
  - University graduate
  - Postgraduate education
- Parent Residence
  - Urban
  - Rural
- Parent Occupation
  - Employed
  - Non- Employed
- Do you know any investigation used to detect heart disease antenatally?
  - No
  - Yes (mention?)
- Did you hear about fetal echocardiography?
  - No
  - Yes (mention the source?)
- Did you have a pregnancy after the affected child?
  - No
  - Yes (did you have fetal Echo? Any detected CHD?)
- Do you think in the subsequent pregnancy you (your partner) will need a fetal Echocardiography?
  - No
  - Yes
- When do you think the best time during pregnancy is to have a fetal Echocardiography?
- Do you think the fetal Echocardiography is risky for the fetus and Mother?
  - No
  - Yes
  - Unknown
- Do you think the antenatal diagnosis of congenital heart diseases is beneficial?
- Do you think the fetal Echocardiography is risky for the fetus and Mother?
  - No
  - Yes (how)

On analyzing the parents' demographics, the median age of participating parents at the time of the survey was 33.5 (29-40) years. Mothers were more commonly enrolled (162 mothers versus 38 fathers). Regarding education, the majority of parents were high school graduates 71 (35.5%) followed by middle-schooled parents 56 (28%). Furthermore, most of the study subjects were residents in rural areas (145 (72.5%)).

Diagnoses of the primary CHD in the patients whose parents were included in the study are demonstrated in Table III. The most frequent cardiac lesion in the children was Ventricular Seotal Defect (VSD) followed by Pulmonary stenosis (PS); 64 (32%) and 33(16.5%), respectively.

Table IV demonstrates the parents' extent of awareness regarding prenatal cardiac diagnosis.

Table II. Baseline characteristics of the patients and their parents.

Variable	N(%) or median (IQR)	
Age of the patient in Months	5 (1.5-11)	
Sex of patients	Male	94 (47.0 %)
	Female	106 (53.0%)
Patient CHD category	Cyanotic	36 (18%)
	Acyanotic	164 (82%)
Patient with surgical or catheter intervention	None	136(68%)
	Surgery	34(17%)
	Catheter intervention	30(15%)
Patient with a confirmed syndrome	Non syndromic	180 (90%)
	Down syndrome	11(5.5%)
	Noonan syndrome	3(1.5%)
	Elis Van Creveled syndrome	2(1%)
	Alagille syndrome	2(1%)
	Williams syndrome	1(0.5%)
	LEOPARD syndrome	1(0.5%)
Age of the participating parent in years	33.5 (29-40)	
The parent giving the questionnaire	Mother	162 (81%)
	Father	38 (19%)
Education	Non-schooled	35(17.5%)
	Middle schooled	56(28%)
	High schooled	71(35.5%)
	University graduate	36(18%)
	Postgraduate education	2 (1%)
Residence	Urban	56 (28%)
	Rural	145 (72%)
Occupation	Housewife or unemployed	146(73%)
	Employed	54(27%)

CHD: congenital heart disease, IQR: interquartile range, N: number of cases.

On asking the parents about the availability of a diagnostic investigatory tool for antenatal diagnosis of cardiac disorders, 66 (33%) of parents confirmed that information but only 46 (23%) knew fetal echocardiography while 20 (10%) gave other answers like the conventional prenatal ultrasound, 3D, 4D ultrasound and others did not name the tool as shown in Figure 1. Regarding the information source, 33 (17.5%) were from pediatric cardiologist counseling, followed by family and friends 9 (4.5%) and obstetrician 7 (3.5%). The other sources included general pediatrician, other patients' parents, the internet, a doctor relative, and other maternal treating physicians like Diabetes specialist for a diabetic mother. (Fig. 2). A sibling was born after the currently affected child in 45 (22.5%) of the families and 8/45 had CHD as well. As a result, the recurrence rate of CHD in our cohort is 17.77%. Out of the 45 subsequent siblings or pregnancies, only 12 (26.67%) received a fetal echocardiography.

The appropriate timing for fetal echocardiography was not known in 172 (86%), whereas 14 (7%) thought it is the 4th or the 5th month of gestation. Moreover, 178 (89%) of the parents thought it would be safe to have fetal echocardiography while 8 (4%) thought it might be risky to the fetus.

Table III. Diagnosis of the primary CHD in the patients whose parents participated in the study.

CHD type	N	%
Atrial septal defect	24	12
Ventricular septal defect	64	32
Patent ductus arteriosus	15	7.5
Atrioventricular septal defect (complete or partial)	10	5
Pulmonary stenosis	33	16.5
Aortic stenosis	9	4.5
Aortic coarctation	4	2
Tetralogy of Fallot	12	6
Double outlet right ventricle	11	5.5
Transposition of great arteries	4	2
Double inlet left ventricle	1	0.5
Pulmonary atresia/intact interventricular septum	2	1
Tricuspid atresia	1	0.5
Vascular ring	1	0.5
Congenital mitral regurgitation	4	2
Heterotaxy	1	0.5
Congenitally corrected transposition of great arteries	1	0.5
Truncus arteriosus	1	0.5
Anomalous pulmonary venous return	2	1
Total	200	100

CHD: congenital heart disease, N: number of cases.

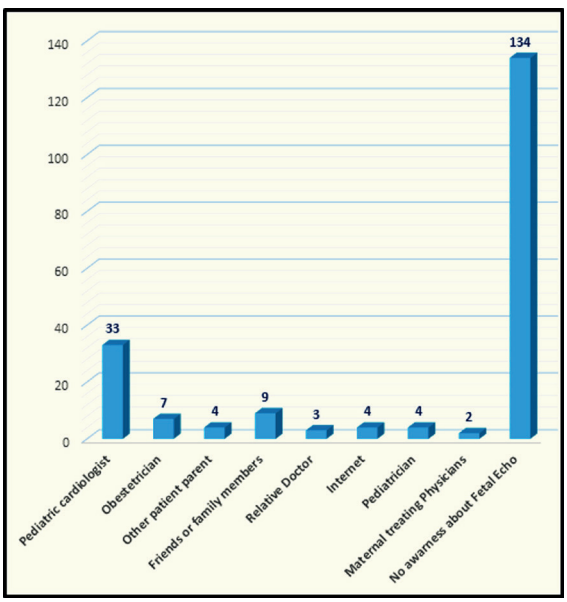

Fig. 1. The sources of parents' awareness of fetal cardiac diseases, diagnostic investigatory tool.

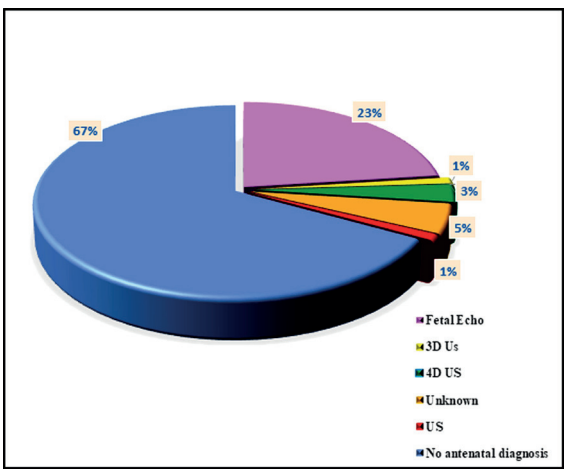

Fig. 2. Pie Chart showing the antenatal cardiac diagnostic investigations as mentioned by parents in percentages.

Table IV. Data demonstrating the parents' extent of awareness about prenatal cardiac diagnosis.

Parameter	Answer	N (%)
Investigations to diagnose fetal cardiac diseases	Yes	66(33%)
	No	134(67%)
A child or pregnancy after the patient	Yes(diagnosed CHD)	8(16%)
	Yes (no CHD)	37(18.5%)
	No	155(77.5%)
Fetal echo in the subsequent sibling	Yes	12/45
	No	33/45
Does fetal echocardiography have risks for mother or fetus?	Yes	8(4%)
	No	178(89%)
	Unknown	14(7%)
The recommended month of gestation for fetal echocardiography	3rd gestation month	11(5.5%)
	4th gestation month	4(2%)
	5th gestation month	10(5%)
	6th gestation month	1(0.5%)
	7th gestation month	2(1%)
	Unknown	172(86%)
Value of antenatal cardiac diagnosis	Yes	134(67%)
	No	64(32%)
	Unknown	2(1%)
Need of fetal echocardiography in the next pregnancy	Yes	121(60.5%)
	No	79(39.5%)

CHD: congenital heart disease, N: number of cases.

Table V. Benefits of antenatal CHD diagnosis from parents' perspective.

Value	N	%
Early diagnosis	70	35
Antenatal treatment	17	8.5
Prepare for postnatal complication	21	10.5
Exclude fetal cardiac disease	5	2.5
Check on fetal wellbeing	9	4.5
Prepare parents antenatal to accept the problem	7	3.5
Avoid antenatal complications or disease progression	2	1
Find the cause of the disease	3	1.5
Total	134	67

CHD: congenital heart disease, N: number of cases.

The benefits of antenatal CHD diagnosis, as explained by parents, are shown in Table V. Two-thirds of study participants confirmed that it would be of benefit to diagnose CHD in fetal life. Early diagnosis was confirmed to be of a significant benefit by 70 (35%) of parents, and 21 (10.5%) added that it would help to prepare for postnatal complications. Only 17 (8.5%) thought that antenatal cardiac therapy or intervention is possible.

A logistic regression analysis model was created to detect the correlation between patients or parents' demographic data and knowledge regarding the presence of fetal

echocardiography as summarized in Table VI. The age of the affected child with CHD and the age of the participating parent were statistically significant independent predictors for parents knowledge of fetal echocardiography with (p= 0.008, OR:1.15, 95%CI:1.039-1.282) and (p= 0.015, OR:0.92, 95%CI: 0.864-0.984) respectively. In other words, the knowledge of parents on the presence of fetal echocardiography was more likely with increase in the age of their affected child while that knowledge decrease with the increase in parents' age.

Discussion

The high recurrence risk of cardiac anomalies in siblings was the rationale behind the mandatory fetal echocardiography in the subsequent pregnancies for families having a child with CHD.[16] In the current study, we have assessed knowledge of parents with children having CHD regarding prenatal cardiac diagnosis from different domains. Despite recent advances in prenatal cardiac detection, the findings of the present study suggest that parents have essential knowledge gaps either in part or entirely.

In our study, only 23% of the included parents knew about fetal echocardiography which is considered a much higher percentage than a study from India which found that awareness in the parents of CHD children is only 2%.[17] Another domain is the extent of awareness, which is lacking at variable levels. Disappointingly, their knowledge appears to be quite deficient.

Regarding the timing, of 46 parents who knew fetal echocardiography, only 14 could roughly determine the standard appropriate time for conventional prenatal diagnosis (the fourth and, the fifth month of gestation). Unfortunately, parents who had a fetal cardiac diagnosis in previous pregnancy with complex CHD and the unfavorable outcome had the impression that antenatal cardiac assessment is unnecessary.

The significant knowledge gap among parents with CHDs in offsprings regarding prenatal cardiac diagnosis and fetal echocardiography

Table VI. Logistic regression analysis of possible determinants of parents' knowledge about fetal echocardiography.

Covariates	P value*	OR	95% C.I.	
			Lower	Upper
Age of the patient	0.008	1.154	1.039	1.282
Cyanotic patient	0.144	2.026	0.785	5.23
Intervention	0.706			
▫ *No intervention vs surgery*	0.808	1.143	0.388	3.374
▫ *Catheter intervention vs surgery*	0.618	0.691	0.162	2.952
Syndromic child with CHD	0.643	1.36	0.37	5.001
Parent giving questionnaire (Mother)	0.1	0.364	0.109	1.216
Age of parent giving questionnaire	0.015	0.922	0.864	0.984
Employment	0.263	0.529	0.174	1.611
Education	0.008			
▫ *Non-schooled vs postgraduate*	0.244	0.155	0.007	3.57
▫ *Middle -schooled vs postgraduate*	0.181	0.126	0.006	2.627
▫ *High-schooled vs postgraduate*	0.583	0.438	0.023	8.343
▫ *University graduate vs postgraduate*	0.967	0.938	0.046	19.294
Urban residence	0.397	1.424	0.628	3.226

CHD: congenital heart disease, CI: confidence interval, OR: odds ratio
*P value is significant if <0.05

could be attributed to a defect in the counseling process at the initial diagnosis. Currently, most of the developing countries suffer from a mismatch between patients' numbers and qualified medical personnel, thereby limiting the time for familial education.[17] Furthermore, pediatric cardiologists may not want to provide too much information at the initial diagnosis of CHD due to concern that families might be overwhelmed or shocked resulting in lack of the necessary information delivered in the care of fetal or newborn patients with CHD as suggested by Arya et al.[18] Besides, the fetal echocardiography cost might be one of the obstacles for women with low socioeconomic status in developing as well as some developed countries.[19,20]

However, to raise parents' awareness about the antenatal diagnosis of CHDs, improvements are required on variable levels. Pediatric cardiologists should be the first category to be addressed. Guidelines about the counseling process for families should include data about fetal echocardiography as a fundamental step in the management of neonatal and pediatric CHDs. The data regarding fetal echocardiography should be revisited in the follow up in the pediatric cardiology clinics. Furthermore, the general pediatrician should have the necessary information about the indications and benefits of prenatal cardiac diagnosis as a part of their basic training. Besides, health care professional auditing regarding knowledge of the fetal echocardiography is inevitable. In a study, the referral personnel were found to be responsible for the delayed referral of indicated pregnant cases for fetal echocardiography.[21]

Displaying posters, flyers, and brochures in the pediatric cardiology departments and outpatient clinics could be beneficial as well. In the new era of communication platforms like social media, general population awareness could be improved through medical awareness shows.

This study has a few potential limitations. The used methodology is liable for parents to recall bias in some questions, and the questionnaire has not been previously validated. Moreover, subjects enrolled in the study are a cohort of parents of patients in a single center. Therefore, it might not be entirely representable for that category. Further community based large scale studies might be required to assess public awareness as well regarding prenatal cardiac assessment.

In conclusion, parents of children with CHD have significant knowledge gaps regarding prenatal cardiac diagnosis and fetal echocardiography. Our findings suggest that current parent counseling and education are inadequate and needs to be improved to promote better understanding of availability, timing, safety, and prenatal-neonatal value of fetal echocardiography.

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Original Article

Clinical features and treatment of ruptured pulmonary hydatid cyst in children

Tunç Özdemir[1], Ali Sayan[1], Belce Candan[1], Gökhan Köylüoğlu[2]

[1]Department of Pediatric Surgery, University of Medical Sciences, Tepecik Training and Research Hospital, İzmir; [2]Department of Pediatric Surgery, Katip Çelebi University, Tepecik Training and Research Hospital, İzmir, Turkey.

ABSTRACT

Backgroud and objectives. Rupture is the main complication of the pulmonary hydatid cyst (HC). The aim of this study was to evaluate the clinical features and treatment of ruptured pulmonary HC.

Method. The medical records of children who had undergone surgery between January 1999 and December 2017 for pulmonary HC were retrospectively evaluated. One hundred forty seven of these patients had ruptured HC at the time of diagnosis. The gender, age at the time of diagnosis, sociogeographic status (i.e., from urban or rural population group), symptoms, affected lung region, cyst dimensions, preoperative complications, medical treatment duration, and associated morbidities were evaluated.

Results. The study included 649 patients with pulmonary HC. Mean age was 9,8 (2-17) years. Three hundred forty four patients were male and 305 were female. The most common symptoms were, cough accompanying mucopurulent sputum, hydropthysis and dyspnea in patients with ruptured HC. The diagnosis of all the patients were established in the light of the findings obtained from two-sided chest x-ray and CT of the thorax. Simple cystotomy via posterolateral thoracotomy was the treatment of choice. Cappitonage was not performed in any patients. Lung resection was performed only if there was an irreversible and disseminated pulmonary destruction.

Conclusion. Rupture of the pulmonary HC is the most common and also the most feared complication. Rupture may be either intrabronchial or intrapleural. Radiologic imaging is diagnostic. Rupture of the pulmonary HC must be considered as an emergent issue. Simple cystotomy amd removal of the laminated membranes are adequate treatment of choice. Meticulous closure of the bronchial openings is mandatory to avoid prolonged air leak. Cappitonage is unnecessary.

Key words: hydatid cyst, chest, pediatric.

Uncomplicated hydatid cyst (HC) is the most common parasitic disease of the lungs. It has an endemic distribution, especially in rural areas of developing countries. The most common form is Echinococcus granulosus, which gives rise to cysts primarily in the liver and lungs. The lungs are the second most frequently involved organ following the liver. The reported incidence of HC disease in Turkey is 20 per 100,000 people.[1]

✉ Tunç Özdemir
ozdemirtunc@yahoo.com

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The growth rate of the HC varies in different organs. Tissue elasticity probably plays a major role in limiting the growth rate. Growth in soft organs is faster than in dense organs. It is reported that liver cysts grow at a lower rate than lung cysts.[2,3] Negative intrathoracic pressure may result in rapid growth of a pulmonary cyst, whereas the compact tissue and hepatobiliary system in the liver probably limit HC growth.[4]

Generally, pulmonary HCs are asymptomatic. In some patients, symptoms such as dyspnea, coughing, nausea and vomiting related to mass effect may occur. Pulmonary HCs, may reach to giant diamaters in pediatric population because of the higher elasticity of the lungs in this age

group, which permits cyst expansion.[2] Rupture is the main complication of cysts especially in patients with large cysts and may be related to the presence of symptoms, and to morbidity and mortality. Rupture of a cyst into the pleural space or bronchus may even cause anaphylactic reactions.[5] Furthermore, obstruction of the tracheobronchial tree with cystic membrane can lead to suffocation.[6]

We herein report the clinical features and treatment approach of 649 pediatric patients with pulmonary HC. One hundred forty seven of the patients had ruptured pulmonary cysts.

Material and Methods

This research was reviewed and approved by institutional Ethics Comitee of University of Medical Sciences, Tepecik Training and Research Hospital (Reference number: 2019/03-12).

The medical records of 649 patients (2-17 years of age) who were referred to our tertiary center and had undergone surgery between January 1999 and December 2017 for pulmonary HC were retrospectively evaluated. The patients were divided into two groups. Patients in group I had uncomplicated pulmonary HCs (N: 502). Group II is consisted of the patients with ruptured pulmonary HC. The preoperative 2-sided chest x-rays and computed tomography (CT) scans of the thorax of all patients were assessed for the size, location, and complication of the cysts. Indirect hemagglutination (IHA) serology was also conducted. Demographic aspects of the patients at the time of diagnosis, sociogeographic status (i.e., from urban or rural population group), symptoms, affected lung region, cyst dimensions, preoperative complications, medical treatment duration, and associated morbidities of the two groups were statistically compared. Fisher's exact test was used for ratio comparison (number of boys and girls with cysts, number of patients from rural and urban areas, location and size of the cysts, mean hospital stay).

Results

In this retrospective study, 502 patients with non-complicated pumonary HC and 147 patients with ruptured pulmonary HC were treated surgically in our referral center from January 1999 to December 2017. Demographic characteristics of the patients were summarized in Table I. There were no statistically significant difference between two groups related to age, gender and sociogeographic status (Table I).

The presenting signs and symptoms of the patients from both groups are featured in Table II; some patients had more than one symptom. The most common symptom was cough accompanying mucopurulent sputum, being presented in 101 (68%) patients, followed by massive hydropthysis (38%). Dyspnea was another major complaint (34%) in group II. Two patients were admitted with respiratory distress and cardiopulmonary arrest because of endobronchial rupture. Both patients were reanimated and survived. Thirteen patients had pulmonary abscess formation because of delayed presentation (9%). Fourteen percent of the patients from group 1 was diagnosed with x-rays which were taken for different purposes (N: 70).

Table I. Demographic characteristics of two groups.

		Group 1	Group 2	P value
Age (years)		9.8	9.4	0.06
Gender	Male	53%	55%	0.05
	Female	47%	45%	0.05
Sociogeographic status	Urban	40%	41%	0.07
	Rural	60%	59%	0.07

Table II. Signs and symptoms of patients.

	(Group I) n(%)	N (Group II) n(%)	P value
Hydropthysis	0 (0)	56 (38.0)	0.0002
Cough-sputum	55 (10.9)	101 (68.7)	0.001
Dyspnea	12 (2.3)	51 (34.6)	0.006
Anaphylaxis	0 (0)	11 (7.4)	0.0004
Pneumothorax	0 (0)	23 (15.6)	0.0007
Pleural fluid	3 (0.5)	47 (31.9)	0.0002
Suffocation	0 (0)	2 (1.3)	0.001
Chest pain	22 (4.3)	32 (21.7)	0.0007

Combined lung and liver cyst were identified in 9 patients (7% of all patients). One patient had concomitant cranial HC. More than one cyst was present in 12 patients in group I (2%). Ten patients had ipsilateral multiple cysts, two had contralateral. Fourteen patients of group II had multiple HCs (9%). (8 ipsilateral, 6 contralateral). The difference between two groups was statistically significant (p= 0.007).

Locations of HCs regarding to pulmonary lobes were summarized in Table III. While, ratios of affected lung region according to lobes were similar, ruptured HCs were more prone to be located peripherally (p= 0.0001).

The diagnosis of all the patients were established in the light of the findings obtained from two-sided chest x-rays and CT of the thorax. In addition, all the patients were screened through abdominal ultrasound and cranial tomography. On chest x-rays water-lily sign or Camelot sign was present in the majority of the patients in group II. "Cumbo sign" and "Dry-cyst sign" were seen in CT imaging of patients with intrabronchial rupture. The ruptured cyst was in the left lung in 82 (55%) patients and the right in 65 (45%). Average cyst diameter was 6.4 cm in group I (4-12 cm) and 8.6 cm in group II (7.5-17 cm) (p= 0.004).

Serological tests (IHA) were positive (≥1/128) in 346 patients (69%) in non-complicated group and 96 patients (65%) in ruptured HC group. The difference between two groups were statistically insignificant (p= 0.05).

In non-complicated patients, routine benzimidazole compound 10 mg/Kg twice per day therapy was started and continued 7-14 days preoperatively. In group II, benzimidazole was started and surgical therapy was planned immediately.

Standard classic posterolateral thoracotomy was used. After entering the pleural cavity, the lung was spared from adhesions of chest wall and diaphragm. Then cysts were identified and surrounded by 5% povidone iodine irrigated abdominal lap to prevent seeding of possible ruptured laminated membrane. Pericyst wall was opened and the laminated membrane was delivered and enucleated. After removal of laminated membranes, bronchial openings were identified using intrapulmonary positive pressure maneuver and closed using 2/0

Table III. Affected lung region for both groups.*

	Right lower lobe	Left lower lobe	Left upper lobe	Right upper lobe	Right middle lobe	Peripherally located
Group I	175 (34.8)	100 (19.9)	90 (17.9)	91 (18.1)	46 (9.1)	76 (15.1)
Group II	49 (33.3)	31 (21.0)	29 (19.7)	26 (17.6)	18 (12.2)	98 (66.6)

*Data shown as: n (%)

polyglactin. The cavities were left open after closure of the bronchial openings. Cappitonage was not performed in any patients. Lung resection was performed only if there was an irreversible and disseminated pulmonary destruction (N:7 patients). None of the patients from group I required lung resection. The most common postoperative complication was prolonged air leak (N:5 patients, 4 in group II, 1 in group I). No further surgical intervention was necessary in these 5 patients. There was no mortality.

Mean hospital stay was 4,6 days (3-12) in group I, 7.4 days (5-21 days) in group II. The difference between two groups was statistically significant (p= 0.002).

Postoperative benzimidazole compound therapy was continued for 3 months for both groups (Albendazole, 10 mg/kg per day, administered twice daily). Medical treatment was started 5-14 days before surgery in group I. In group II, benzimidazole compound was started immediately and surgical intervention was done without delay. The patients were followed for 32 months postoperatively (28-62 months) with 6-months interval. Routine chest x-rays were taken during follow-up. No complications were observed except temporary atelectasis which dissolves spontaneously. No reccurence was detected during follow-up.

Discussion

Pulmonary HCs are generally asymptomatic. Cyst rupture may occur. Rupture of the cyst is the most common and also the most feared complication. The rate of spontaneous rupture was 22.6% (147 of 649 patients) in our study. This finding is consistent with the literature.[7]

The factors yielding perforation, such as pressure, size, and the central or peripheral location of the cysts, have been investigated in clinical studies.[8-10] Expanding HCs may result in high pressure due to the low volume of the thorax leading to perforation in childhood.[11] Önal et al.[11] concluded that, HCs located in the right middle lobe and lingula have high perforation and postoperative complication rates in children. Therefore they recommend surgical treatment as soon as possible in these patients to prevent the risk of rupture.[11] Yalin et al.[8] reported that there is positive correlation between the size and pressure of the cysts in liver HC. Yuksel et al.[12] argued that, as the pressure inside the cyst increases, the parenchyma surrounding the cyst becomes thinner. Therefore as the diameter of the cyst increases, the risk of direct rupture increases.[12] However, Kocer et al.[13] found no relationship between the size of the cyst and the risk of perforation. It was noted by Lewall et al.[14] that peripherally located HCs rupture directly into the pleural space frequently because the pericyst layer and lung tissue that surround the cysts are very thin. Peripherally and subpleurally located HCs and increased pressure inside the cysts lead to pleural necrosis, and these factors have an effect on rupture into the pleural space.[10] In our study, a majority of the ruptured cysts were located peripherally. Difference between two groups was statistically significant. In our study group, we found that, 33% of ruptured HCs are located at right lower lobe of lung. However, the statistical analysis did not appear significant between two groups of our study regarding to right lower lobe location.

Rupture may be either intrabronchial or intrapleural. Inrabronchial rupture of the HC is mostly presented as hydropthysis, cough, dyspnea or even suffocation leading to death. Rupture of the HC into the pleural space can cause spontaneous pneumothorax, hydro-pneumothorax, tension pneumothorax, empyema and pleural thickening. Bronchial and pleural perforation rates have been reported of 21.1% and 3.6%, respectively by Cangir et al.[15] Ruptured HCs have been reported to have greater postoperative morbidity and mortality than intact cysts.[7,16]

Surgical treatment may be difficult in ruptured HCs due to contamination and destruction of the lung tissue. Numerous surgical procedures have been described in the literature, which,

excision of entire cyst by enucleation (Barrett technique), excision of pericyst (Perez Fontana), cystotomy, capitonnage, wedge resection, segmentectomy and lobectomy.[17-19] The choice of surgical technique depends of perioperative findings. Barrett technique, is generally preferred on peripherally located small cysts, but extirpation is difficult to accomplish without rupturing the cyst.[18,19] Resection techniques are used only if parenchymal lesions seems irreversible.[17,18] Because the affected lung parenchyma in children has a great capacity for healing especially for children and young adults, pulmonary resection should not be routinely performed even in the complicated cysts.[17,18]

The capitonnage procedure, which is considered to reduce postoperative complications, may not be possible due to infection of the pericystic tissues. And either not necessary. Capitonnage procedure has not been carried out in our patients. Lung resection was necessary in 7 patients with an irreversible and disseminated pulmonary destruction. Resections were performed to patients whose cysts were located peripherally and achieved by stapling devices. No anatomic resections were performed.

Lessons learned from 649 patients with pulmonary HCs were;

- Both genders are affected equally. However, children who live in rural area are more prone to be affected than the children who live in urban area. There was no statistically significant difference between the patients with non-ruptured and ruptured pulmonary HCs.
- Mucopurulent sputum with forceful cough, hydropthysis and dyspnea are the major complaints in ruptured HCs. Massive hydropthysis may lead to suffocation and cardiopulmonary arrest. Therefore, ruptued HCs must be considered as an emergent issue.
- Majority of the ruptured pulmonary HCs were located peripherally. Right lower lobe was the mostly affected lung area.
- "Size does matter". Larger cysts are more prone to rupture than smaller ones.
- Radiologic imaging is diagnostic for ruptured pulmonary HCs. In chest X-rays water-lily sign or Camelot sign (A cyst membrane floating on cystic water with free air above) was present in the majority of the patients. "Cumbo sign" which is described as "Presence of air fluid level within the endocyst and the air crescent among the pericyst and endocyst" on CT was seen in patients with intrabronchial rupture (Fig. 1). "Dry-cyst sign" is also common.
- Simple cystotomy and removal of the laminated membranes are adequate treatment of choice. Meticulous closure of the bronchial openings is mandatory to avoid prolongead air leak. Cappitonage is unnecessary in the lung however, especially in peripherally located ruptured HCs, resection of irreversibly destructed lung tissue facilitates postoperative recovery. Anatomic resection of the lung lobes should not be done. Stapling devices may be used in simple lung tissue resections.

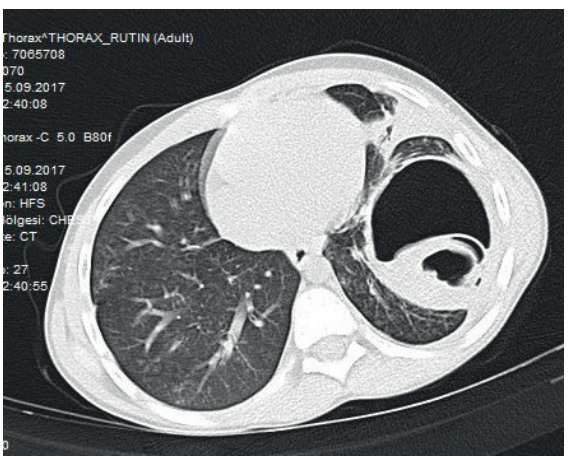

Fig. 1. Cumbo Sign of ruptured pulmonary hydatid cyst in CT.

- Medical treatment with benzimidazole compound, either albendazole or mebendazole, is mandatory in the treatment of pulmonary HC. In non-complicated patients, medical treatment must be started 5-14 days preoperatively.[20] In patients with ruptured pulmonary HC, benzimidazole compound should be started preoperatively and must be continued postoperatively. Owing to their hepatotoxicity, a 1-week to 2-week interval should be given between 3-week and 4-week cycles and treatment may last 3 to 6 months.

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Original Article

Bleeding characteristics and management of minor surgeries in rare bleeding disorders: report from a Turkish Pediatric Hematology Center

Sema Aylan Gelen, Nazan Sarper, Emine Zengin

Division of Pediatric Hematology, Department of Pediatric, Kocaeli University Faculty of Medicine, Kocaeli, Turkey.

ABSTRACT

Background and objectives. In this retrospective report the aim was to present the experience about bleeding characteristics and management of minor surgeries in rare bleeding disorders (RBDs).

Methods. Twenty-six patients were included; with Factor (F) V, FV+VIII, VII, FXI deficiency and afibrinogenemia. Six of the patients were asymptomatic.

Results. Fifty-three percent of the patients suffered from mucosal bleeding. Life-threatening bleedings were observed only in the patients with afibrinogenemia and good hemostatic control could only be provided with plasma-derived (pd)-fibrinogen concentrate. Twelve of the patients had undergone 17 minor surgeries. In the patients with FVII and FXI deficiencies with plasma F:C activity between 20-47%, there was a history of uneventful tooth extractions, circumcisions and a pilonidal sinus operation performed without any replacement treatment, whereas one patient with plasma F:C activity of FVII 47% had a history of poor hemostatic control during an adeno-tonsillectomy operation. Although some of these patients were asymptomatic to be on the safe side, minor operations were performed with preoperative administration of one dose of (pd)-fibrinogen concentrate to one afibrinogenemia patient, recombinant active FVII (rFVIIa) to 2 FVII deficient patients and fresh frozen plasma (FFP) to 3 FXI deficient and 1 FVII deficient patients plus postoperatively tranexamic acid (TXA) for 5-7 days. Only with one dose of the replacement therapy just before surgeries good hemostatic control was achieved and none of them had bleeding neither during nor after the surgeries.

Conclusion. We suggest that minor operations must be performed with preoperative replacement therapies plus 5-7 days of antifibrinolytics under close observation of the hematologist and the surgeon.

Key words: blood coagulation disorders, rare diseases, minor surgical procedures.

Rare bleeding disorders including factor (F) II, FV, combined FV and FVIII, FVII, FX, FXI, FXIII, and fibrinogen deficiencies represent 3-5% of all inherited coagulation deficiencies.[1-3] In addition to rarity of these deficiencies, the severity of the bleeding symptoms is heterogenous. Patients may suffer from mucocutaneous bleeding, but more severe bleeding like gastrointestinal (GIS) bleeding, central nervous system (CNS) bleeding, post-traumatic hemarthrosis and hematomas, or umbilical cord bleeding may be observed. Some patients are asymptomatic, but they may present with surgical or invasive procedure-associated bleeding.[3,4] Management of surgery is based on experience from case reports.[5] Bleeding history of the patient or family may determine the severity of the disorder. Plasma F:C activity is not always correlated with bleeding severity.[6]

Asymptomatic patients or patients with mild symptoms undergoing minor surgery are a dilemma for hematologists although there are some reports and guidelines about management of bleeding episodes and minor surgery.[3,5,7,8]

✉ Sema Aylan Gelen
semaylan@hotmail.com

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The bleeding history of young children who have had no haemostatic challenge may not predict the risk of bleeding in elective surgery. Here, bleeding characteristics and the management of minor surgeries is presented in children and adolescents.

Material and Methods

This retrospective study was approved by the Ethics and Research Committee of Kocaeli University (KÜ GOKAEK 2019/45). Written informed consents of the patients' legal guardians were obtained during diagnosis for use of patient data in scientific publications.

The hospital records of the patients with RBDs diagnosed and followed up between 1999 and 2018 were evaluated. Patient age at diagnosis, bleeding characteristics at presentation, medical history, plasma factor levels, management of the bleeding episodes and surgeries were documented.

Diagnosis was based on the bleeding history, bleeding symptoms of the patient, family history, prothrombin time (PT), activated partial-thromboplastin time (APTT), and plasma F:C levels. In cases of FVII deficiency, only PT; in FX, FV, Fibrinogen, FV+VIII deficiency both PT and APTT, in FXI deficiency only APTT is expected to be prolonged. All of the coagulation studies were performed in the local laboratory. For fibrinogen <100 mg/dl and for FV, FVII, FX, and FXI, <50% was considered as a deficiency.[7,8] Genetic analysis was performed only in the case of one patient with afibrinogenemia.

Clinical bleeding episodes were classified into four severity categories. Asymptomatic patients had no documented bleeding episodes. Grade I bleeding was defined as bleeding after trauma or drug ingestion. Grade II bleeding was defined as spontaneous minor bleeding, such as bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis and menorrhagia. Grade III bleeding was defined as spontaneous major bleeding, such as hematomas, hemarthrosis, CNS bleeding, GIS bleeding, and umbilical cord bleeding.[3]

Minor surgery often refers to the surgery which does not have a significant risk of large volume blood loss or blood loss into a confined anatomical space like major abdominal, intracranial, cardiovascular, spinal or major orthopaedic surgeries, that requires hemostatic support less than 5 consecutive days.[9,10]

SPSS version 22 statistical software was used for descriptive analysis. Quantitative data were given as mean ± standard deviation.

Results

There were 12 (46.2%) females and 14 (53.8%) males. The median age at diagnosis was 7.7 years (8 months-16.9 years). The mean follow-up time was 6.9 years (2 months-16.3 years). The median diagnosis age of the patients with severe bleeding phenotype (Grade III bleeding) was 3 years (8 months-16.9 years), and severe factor deficiencies (with plasma F:C activity <1% or undetectable) was 3,89 years (8 months-16.9 years).

FVII deficiency (n:12, 46.2%) was the most frequent RBD, followed by FXI deficiency (n:6, 23.1%), afibrinogenemia (n: 4, 15.4%), FV deficiency (n: 3, 11.5%), and FV+FVIII deficiency (n:1, 3.8%). There was consanguinity in 9 (34.6%) patients, and 7 (26.9%) patients had a family history of bleeding. Molecular analysis could be performed in patient 1 and mutation in FGB gene (FGB exon 4 C/T nt.5330/p.leu 195 pro mutation) was detected.

Four asymptomatic patients (23.1%) were diagnosed during preoperative coagulation screening. One patient was screened due to family history and another during a urinary tract infection. According to the bleeding severity, 3 patients (11.5%) presented grade I bleeding, 11 (42.3%) grade II bleeding, and 6 (23.1%) grade III bleeding. The most common bleeding symptom during follow-up of the patients was epistaxis (n: 11, 42.3%). Other bleedings were gum bleeding (n: 7, 26.9%), ecchymosis (n: 6, 23.1%), menorrhagia (n: 6, 50% of the adolescent girls), hemarthrosis (n: 4, 15.4%), hematuria (n:

1, 3.8%), postoperative bleeding (n: 1, 3.8%), intraabdominal bleeding (n: 1, 3.8%), CNS bleeding (n: 1, 3.8%), spontaneous splenic rupture (n: 1, 3.8%), and soft tissue hematoma (n: 1, 3.8%) (Table I).

Life-threatening bleedings were observed only in the patients with afibrinogenemia. Patient 1, Patient 2 and patent 4 with afibrinogenemia presented with grade III bleeding including spontaneous spleen rupture, intraabdominal bleeding and CNS bleeding respectively and good hemostatic control was provided with pd-fibrinogen concentrate. Patient 2 with afibrinogenemia had a sibling who died due to intracranial bleeding following trauma.

Prophylaxis with 30 mg/kg pd-fibrinogen weekly was administered only to patient 4 with afibrinogenemia, following spontaneous CNS bleeding.

As single factor concentrates, only rFVIIa and pd-fibrinogen were available in Turkey for our RBD patients with FVII deficiency and afibrinogenemia. The bleeding episodes of the 7 patients with FVII deficiency was controlled with rFVIIa. FFP and/or TXA were used for the bleeding episodes of the other patients. Bleeding episodes of the 3 patients with afibrinogenemia, one patient with FV deficiency and one patient with FVII deficiency required also packed red cell transfusions (Table I).

Seventeen minor surgical interventions were performed in 12 patients; 7 (41.2%) were tooth extractions, 7 (41.2%) were circumcisions, one (5.9%) was hernioplasty, one (5.9%) was pilonidal sinus operation and one (5.9%) was adeno-tonsillectomy. Seven operations (circumcisions, tooth extractions, pilonidal sinus operation and adeno-tonsillectomy) were performed before factor deficiency was diagnosed. In these surgeries performed without any replacement treatment, hemostasis was good except adeno-tonsillectomy operation (patient 23). In the elective surgeries performed after diagnosis of RBDs, preoperative rFVIIa, pd-fibrinogen or FFP replacement plus antifibrinolytic treatment for 5-7 days were started even if plasma F:C activity was 20-50%. Hemostatic control was good in all patients, and the replacement treatment was not continued after the surgeries in any of the patients. Management of minor surgical procedures is shown in Table II.

Discussion

This is a single-center study presenting 26 patients with RBDs, but a lengthy follow-up period gives useful data about bleeding characteristics. Seventeen minor surgical interventions in 12 of the patients were also presented. Similar to other reports from Turkey and the European network of RBDs, the most common disorder was FVII and FXI deficiency, followed by FV, FX, fibrinogen and FXIII deficiencies. FV+FVIII was rarely observed.[11-13]

As expected, in the present study, mucosal bleeding was common. Of the female adolescents, 50% suffered from menorrhagia. Hemarthrosis was observed in fibrinogen, FV, and FV+VIII deficiency.

Three of the four patients with afibrinogenemia had life-threatening bleeding. They suffered from subdural hematoma, intraabdominal bleeding, and severe scalp bleeding episode after minor traumas. Patient 1 even reported no trauma history on admission to the emergency unit with severe anemia and spleen rupture. The risk of splenic rupture is known to be high in congenital afibrinogenemia patients.[14] In the literature there are reported patients who have undergone splenectomy due to splenic rupture.[14-16] In our patient, because the diagnosis was known, splenectomy was not required as a result of rapid intervention with pd-fibrinogen concentrate.

These patients also required packed red cell transfusions. Pd-fibrinogen concentrate replacement and packed red cell transfusions were administered in the first 30 minutes before any imaging study and splenic rupture could be managed successfully. In the European Network of Rare Bleeding Disorders

Table I. Demographic features, bleeding characteristics and replacement treatment of patients with rare bleeding disorders.

Patient no	sex	Deficient factor	Age at diagnosis	Follow-up period	Plasma coagulation activity (%)	Presenting symptom	Bleeding sites during follow-up	Bleeding severity	Replacement treatment*
1	F	Fibrinogen	8 months	16.5 years	0	Venopunction site bleeding	Easy bruising, ecchymosis, menorrhagia, hemarthrosis, gum bleeding, epistaxis, spontaneous spleen rupture	Grade III	On demand
2	F	Fibrinogen	16.9 years	5.7 years	0	Intraabdominal bleeding	Gum bleeding, epistaxis, intraabdominal bleeding	Grade III	On demand
3	M	Fibrinogen	3.89 years	3.7 years	0	Ecchymosis	Ecchymosis	Grade II	No treatment
4	F	Fibrinogen	3 years	12.8 years	0	Posttraumatic scalp bleeding	Subdural hematoma, gum bleeding	Grade III	Prophylaxis
5	F	FXI	16.5 years	10 months	2	Prolonged APTT during preoperative coagulation screening	asymptomatic	No bleeding	No treatment
6	M	FXI	3.3 years	1.4 years	0.6	Prolonged APTT during preoperative coagulation screening	Ecchymosis	Grade I	On demand
7	M	FXI	5.1 years	2.2 years	14	Epistaxis	Epistaxis	Grade II	No treatment
8	F	FXI	14.8 years	2.5 years	0.1	Easy bruising	Easy bruising	Grade I	On demand
9	M	FXI	6.2 years	11.7 years	0.04	Prolonged APTT during preoperative coagulation screening	Gum bleeding	Grade II	On demand
10	M	FXI	5.4 years	8 years	22	Prolonged APTT during urinary infection	Asymptomatic	No bleeding	No treatment
11	F	FV+FVIII	11.2 years	7.3 years	FV 5 FVIII10	Hemarthrosis	Hemarthrosis, menorrhagia, epistaxis	Grade III	On demand
12	M	FV	1.7 years	2.4 years	3	Ecchymosis	Ecchymosis	Grade II	On demand
13	M	FV	1.9 years	8.5 years	3	Gum bleeding and hematoma	Hemarthrosis, gum bleeding, epistaxis	Grade III	On demand
14	F	FV	5.5 years	11.6 years	0.04	Hematemesis after vomiting	Easy bruising, menorrhagia, hemarthrosis, gum bleeding, epistaxis, soft tissue hematoma	Grade III	On demand
15	M	FVII	9.9 years	6.3 years	27	Prolonged PT during preoperative coagulation screening	Epistaxis	Grade II	On demand

*surgical interventions are not included.

Table I. Continued.

Patient no	sex	Deficient factor	Age at diagnosis	Follow-up period	Plasma coagulation activity (%)	Presenting symptom	Bleeding sites during follow-up	Bleeding severity	Replacement treatment*
16	F	FVII	14.7 years	10 months	29	Ecchymosis	Ecchymosis, menorrhagia, gum bleeding, epistaxis	Grade II	On demand
17	F	FVII	14.2 years	1.2 years	32	Prolonged PT during preoperative coagulation screening	Menorrhagia	Grade II	On demand
18	F	FVII	13.4 years	6.6 years	40	Prolonged PT during preoperative coagulation screening	Hematuria (nephrolithiasis)	No bleeding	On demand
19	M	FVII	8.3 years	8 years	28	Asympthomatic History of a sibling with FVII deficiency	Aymptomatic	No bleeding	No treatment
20	F	FVII	5.7 years	7.7 years	32	Prolonged PT during preoperative coagulation screening	asymptomatic	No bleeding	No treatment
21	M	FVII	8.5 years	7.8 years	20	Prolonged PT during preoperative coagulation screening	asymptomatic	No bleeding	No treatment
22	F	FVII	1.2 years	8 years	25	Ecchymosis	Ecchymosis	Grade II	On demand
23	M	FVII	7.3 years	8.6 years	47	Postoperative bleeding (adenotonsillectomy]	asymptomatic in daily life	Grade I	On demand
24	M	FVII	10.5 years	9.1 years	27	Epistaxis	Epistaxis	Grade II	No treatment
25	M	FVII	2.3 years	9.2 years	2	Epistaxis	Epistaxis	Grade II	On demand
26	F	FVII	11 years	10.2 years	28	Menorrhagia	Menorrhagia, epistaxis	Grade II	On demand

*surgical interventions are not included.

Table II. Management of minor surgeries of the patients with rare bleeding disorders.

Patient number	Deficient factor	Plasma coagulation activity (%)	Surgical procedure	Replacement therapy	Hemostatic control
1	Afibrinogenemia	0	Tooth extraction	Plasma-derived fibrinogen concentrate 100 mg/kg (1 dose pre-op) and TXA 20 mg/kg po thrice daily, for 5 days	Excellent
6	FXI deficiency	0.6	Tooth extraction	FFP (15 ml/kg pre-op) and TXA 20 mg/kg po thrice daily, for 5 days	Excellent
8	FXI deficiency	0.1	Tooth extraction	FFP (15 ml/kg pre-op) and TXA 20 mg/kg po thrice daily, for 5 days	Excellent
9	FXI deficiency	0.04	Circumcision	FFP (15 ml/kg pre-op) and TXA 20 mg/kg po thrice daily, for 5 days	Excellent
			Tooth extraction	FFP (15 ml/kg pre-op) and TXA 20 mg/kg po thrice daily, for 5 days	
10	FXI deficiency	22	Circumcision	No replacement history	Excellent
			Tooth extraction	TXA 20 mg/kg po thrice daily, for 5 days	Excellent
11	FV+FVIII deficiency	FV 5 FVIII 10	Tooth extraction	No replacement history (before diagnosis)	Excellent
15	FVII deficiency	27	Circumcision	rFVIIa (30 μg/kg pre-op) and TXA 20 mg/kg po thrice daily for 7 days	Excellent
17	FVII deficiency	32	Pilonidal sinus operation	No replacement history (before diagnosis)	Excellent
18	FVII deficiency	40	Circumcision	No replacement history (before diagnosis)	Excellent
21	FVII deficiency	20	Tooth extraction	no treatment	Excellent
			Circumcision	rFVIIa (30μg/kg pre-op) and TXA 20 mg/kg po thrice daily, for 7 days	Excellent
23	FVII deficiency	47	Adeno-tonsillectomy	No replacement history (before diagnosis)	poor
			Hernioplasty	FFP (15 ml/kg pre-op) and TXA 20 mg/kg po thrice daily, for 7 days	Excellent
			Circumcision	FFP (15 ml/kg pre-op) and TXA 20 mg/kg po thrice daily, for 7 days	Excellent
24	FVII deficiency	27	Circumcision	No replacement history before diagnosis	Excellent

study it is reported that 42.3% patients with afibrinogenemia and hypofibrinogenemia had a Grade III bleeding history. A strong association is reported between fibrinogen activity level and clinical bleeding severity.[17] As in Patient 4, prophylaxis with pd-fibrinogen concentrate is recommended in patients with a history of life-threatening bleeding, especially after central nervous system bleeding.[18]

The patient with FV+FVIII deficiency presented with hemarthrosis after trauma and also had epistaxis and menorrhagia episodes. Replacement with FVIII concentrate plus FFP is recommended but we could achieve hemostasis with FFP or TXA.[5]

The plasma F:C activity of six patients with FXI deficiency was 0.1-22%. Two were asymptomatic (F:C activity 2% and 22%) during diagnosis, whereas the others were mild mucosal bleeders. It is reported that there is a weak correlation between bleeding phenotype and F:C activity in FXI deficiency.[3] Despite F:C activity <1% in 3 FXI deficient patients, none of them had severe bleeding phenotype consistent with the literature.

In the present study, three patients showed FV deficiency with factor levels 3%, 3%, and 0.4%, respectively. In addition to the mucosal bleeding, these patients suffered from soft tissue hematoma and hemarthrosis. All bleeding episodes were successfully treated with FFP and TXA. Life-threatening bleeding episodes are very rare in FV deficiency.[19] None of our FV deficient patients experienced life-threatening bleedings.

Clinical findings of FVII deficiency were reported to be heterogeneous, ranging from asymptomatic patients to patients with serious and fatal bleeding.[7,20] Some polymorphisms were described within human F7 gene that affect both plasma FVII:C levels and plasma VII antigen levels. Patients with F:C level near to 50% may be carriers of these polymorphisms.[21] In the present study 5 of the 12 patients with FVII deficiency were diagnosed due to prolonged PT during preoperative coagulation screening. Another asymptomatic patient (Patient 19) with F:C activity 28% was diagnosed due to family history. Similar to the literature, the most frequent symptoms were epistaxis and menorrhagia.[20] Half of the female adolescents had menorrhagia, and one of them was treated with both rFVIIa and FFP plus TXA, and even required packed red cell transfusion.

Consistent with our patients, it is reported that, in FV, FVII and FXI deficiencies there is a weak correlation between the bleeding severity and F:C level.[3,8,20]

Packed red cell transfusion was required in 10-20% of patients with RBDs.[2] In the present study, packed red cell transfusion was administered to 19.2% of the patients during bleeding episodes.

A fibrinogen plasma level of 1–1.5 g/L is recommended for minor surgery; this level may be achieved by a 50-100 mg/kg dose. Due to long half-life, this dose may be repeated at 2-4 day intervals when required. For minor surgery, TXA 15-20 mg/kg may be sufficient.[22] In a phase III trial of pd-fibrinogen concentrate, three minor surgeries (radioisotope synovectomy, dental extraction, circumcision) were planned. Plasma preoperative fibrinogen levels of 100 mg/dl were achieved with a median 70.0 (65.8-102.6) mg/kg infusion and excellent hemostasis was achieved.[23] In our study, only one patient with afibrinogenemia had a minor operation (Patient 1, tooth extraction), and the procedure was without bleeding complication under preoperative one dose 100 mg/kg pd-fibrinogen concentrate plus TXA therapy and did not required any repeated dose.

In a post-marketing study of recombinant FXIa in bleeding episodes, surgery, and invasive procedures, the median infusion dose per episode was 18.0 U/kg (mean 18.7) and the median number of infusions per episode was 1.0. Hemostatic control was satisfactory.[24] Two of the patients with FXI deficiency (Patients 9 and 10) underwent circumcision with excellent hemostasis (one without any replacement; the

other with FFP and TXA administration). Four tooth extractions were performed with FFP and/or TXA with good hemostatic control. We used FFP instead of recombinant FXI concentrate, because it is not available in Turkey.

Some reports have shown that in patients with factor V plasma F:C activity 14.1 to 22.4%, single uncomplicated tooth extractions can be managed safely without replacement therapy. Mouth rinse with tranexamic acid, nonresorbable sutures, and a gelatin sponge provided satisfactory results.[25] None of the FV-deficient patients underwent any surgical procedure in the present study.

In the present study, a seven-year-old, FVII deficient patient with F:C activity of 47% had postoperative bleeding following adeno-tonsillectomy. He had no bleeding history. Hernioplasty and circumcision were managed successfully with FFP replacement. In this series patients' F:C activity were between 20-47%. Some minor operations could be managed without replacement treatment similar to the literature. Two patients with plasma FVII coagulation levels 0.6% and 7% were reported with an uneventful tooth extraction with only antifibrinolytic administration.[26] Surgical bleeding is reported in about one third of the cases with FVII deficiency.[27,28] Although evidence is slight, for the minor surgery TXA 15-20 mg/kg was sufficient.[8] Some authors suggest that the replacement with only rFVIIa on the day of surgery may be safe in both severe and mild cases due to accumulation of the hemostatically active factor within the extra-vascular space.[29,30] In the STER study also, in oral surgery, the replacement therapy was given only on the day of the operation and good hemostasis was achieved. Concomitant medication was antifibrinolytics. In the STER study, all enrolled patients had FVII coagulant activity levels of ≤20% of normal.[31] In this series also to be safe, pre-operative replacement therapy was administered to patient 21 before circumcision although he was asymptomatic. In a study aiming to explore the relationship between F:C activity level and the clinical bleeding severity, it was shown that F:C activity levels that were necessary for patients to remain asymptomatic were: fibrinogen > 100 mg dl, FV 12 U dl, combined FV + VIII 43 U dl, FVII 25 U dl, FX 56 U dl, FXI 26 U dl and FXIII 31 U dl.[11]

Due to the absence of any correlation between plasma F:C activity and surgical bleeding in FV, FVII and FXI deficiency[3,8,11,20]; we suggest that for the safety of the patients, preoperative replacement treatment should be given regardless of the plasma F:C activity in these patients.

In this series a patient with FV+VIII deficiency had a history of uneventful tooth extraction without any replacement therapy. In the treatment of the bleeding episodes and surgery replacement treatment with FFP, rFVIIIa and desmopressin is recommended.[31,32]

FFP is a blood component that is easily available and relatively cheap. But there is still a risk of blood-borne infections, volume overload, and transfusion related acute lung injury.

Due to the rarity of RBDs and the heterogeneity of the clinical presentationsthe treatment during bleeding episodes and the management of the surgical interventions can be challenging for physicians. Some minor surgeries can be managed even without replacement therapy. The minor surgeries of RBDs can be managed safely with FFP or FVIIa or pd-fibrinogen concentrate, in addition to TXA. F:C activity of about 15-20% is safe for minor surgery.

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Original Article

Neuropsychological outcome in cases with acute disseminated encephalomyelitis

Seda Kanmaz[1], Sezen Köse[2], Cenk Eraslan[3], Erdem Şimşek[1], Hepsen Mine Serin[1], Sanem Yılmaz[1], Gül Aktan[1], Hasan Tekgül[1], Hatice Serpil Erermiş[2], Sarenur Gökben[1]

[1]Division of Child Neurology, Department of Pediatrics, [2]Child and Adolescent Psychiatry, [3]Neuroradiology, Ege University Faculty of Medicine, İzmir, Turkey.

ABSTRACT

Background and objectives. Acute disseminated encephalomyelitis (ADEM) is an immune-mediated, inflammatory and demyelinating disorder of the central nervous system. There have been a few studies in recent years on the fact that these cases have neurocognitive impairment. The purpose of this study is to evaluate the neurocognitive outcome and quality of life in cases with ADEM.

Methods. Eleven cases who were on follow-up between 2008 and 2017 were included in the study, systemic, neurological and psychiatric examinations were done. All magnetic resonance images were re-evaluated. The neuropsychiatric evaluation was performed by clinical examination and psychometric scales; (1) The Pediatric Quality of Life Inventory 4.0, (2) Child Behavior Checklist, (3) Children's Depression Inventory, (4) The Wechsler Intelligence Scale for Children-Revised and (5) Continuous Performance Test. The cases in our study underwent neuropsychiatric evaluation 3-42 months after the diagnosis of ADEM had been established.

Results. Nine cases (81.8%) fully recovered without neurologic deficit. One case (9.1%) had a psychiatric disorder. During follow-up, cognitive and psychiatric problems were encountered in half of the cases (54.5%). Most of the cases with basal ganglia involvement (80%) displayed attention deficit and cognitive problems.

Conclusion. In particular, cases with basal ganglia involvement should be followed carefully in terms of attention and cognitive problems.

Key words: ADEM, neuroimaging, neuropsychiatric disorders.

Acute disseminated encephalomyelitis (ADEM) is an immune-mediated, inflammatory and demyelinating disorder of the central nervous system (CNS). It is characterized by multifocal neurological symptoms and encephalopathy and is often triggered by an infection or vaccination.[1,2] It is more common in males and in children aged 5 to 8 years with an incidence of 0.3-0.6/100,000.[3] The diagnosis of ADEM is based upon clinical and abnormal craniospinal magnetic resonance imaging (MRI) findings. Cranial MRI findings are characterized by diffuse, large, poorly marginated lesions in the white matter, best observed in T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, but the gray matter can also be involved.[4] It has been reported that the size or location of the lesions at the beginning are not related with the outcome.[5-7] Although most cases recover completely, residual neurological problems (motor deficit, dysphasia, reduced visual acuity, ataxia, and epilepsy) can be observed in some cases.[5,8] In recent years, neuropsychiatric symptoms such as cognitive impairment, behavioral disorders, and attention deficit have been reported in cases diagnosed with ADEM, but there are only a few studies in this subject.[9-15] The purpose of this study is

✉ Seda Kanmaz
drsedakanmaz@gmail.com

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to evaluate the neurocognitive outcome and quality of life in cases with ADEM, also to examine the relationship between the data and the clinical and radiological features.

Material and Methods

Participants

Twenty seven cases were diagnosed with ADEM and treated between January 2008 and December 2017 in the Child Neurology Department at Ege University Hospital. Diagnosis of ADEM depending on both clinical (multifocal CNS involvement besides encephalopathy) and radiological findings was established. During follow-up five cases showed new attacks, they were diagnosed with multiple sclerosis or neuromyelitis optica spectrum diseases, depending on clinical, radiological and biochemical findings. All of them were excluded. The cases with the inclusion criteria described below were recruited from 22 cases with ADEM.

The study inclusion criteria were:

1) Cases who were diagnosed with monophasic ADEM.
2) Cases who were aged 6-16 years at the time of study for the availability of normative data for the neuropsychologic tests.
3) Cases who were followed up at least for the first three months after the ADEM diagnosis.

Five cases who were diagnosed with ADEM previously could not be reached since their contact details had changed. Six of 22 cases with ADEM were excluded since they were younger than 6 years or older than 16 years at the time of study. Eleven cases were enrolled in the study.

Procedures

The cases were contacted by telephone and were invited to our clinic for neurocognitive and psychiatric evaluation. The case's complaints were inquired by the pediatric neurologist, systemic and neurological examinations were performed. Gender, age at the time of diagnosis, presenting symptoms, duration of treatment and follow-up duration were recorded from the case files. Craniospinal MRI scans performed at beginning of the illness were available for all the cases. Follow-up of neuroimages were obtained between 3rd month-2nd years. First and last follow-up MRI scans of the cases were re-evaluated by the neuroradiologist. Radiological assessment was recorded according to the criteria described below:[16]

1) Areas of involvement: white matter, gray matter, deep gray matter (basal ganglia, thalami), brain stem, spinal cord
2) Enhancement administration of gadolinium
3) The presence of mass effect

Follow-up MRI findings were described as complete resolution (disappearance of all demyelinating lesions) and partial resolution (decrease in size or signal intensity of demyelinating lesions).

Cognitive and Neuropsychological Assessment

Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime Version (K-SADS-PL) is a semi-structured interview used to determine psychopathology in children and adolescents according to DSM-IV-TR criteria.[17] The Turkish adaptation study was conducted by Gökler et al.[18] The clinician-rated the score according to the interview conducted with the parent and the child and finally achieved summary ratings using the best clinical judgment.

Wechsler Intelligence Scale for Children-Revised (WISC-R) is a standardized intelligence test developed by Wechsler (1974) for children which includes two parts; verbal and performance subtests. The total intelligence quotient (IQ) score is calculated by summing verbal and performance scores. The Turkish standardization was conducted by Savaşır and

Şahin (1995) for children between 6-16 years of age.[19] Total IQ scores less than 70 was accepted as intellectual disability. Total IQ scores ranged between 70-79 and 80-89 were accepted as borderline intellectual functioning and below average intelligence, respectively. Wechsler (1974) stated that difference between verbal and performance IQ scores of at least 15 points is required to necessitate deeper investigation.[20]

Children's Depression Inventory (CDI) was developed by Kovacs (1985), and it is a 27-item self-report questionnaire used to evaluate depressive symptoms of children aged 6-17 years old. The cut-off point for depression is 19; scores higher than 14 can be used for evaluating the children who may be at risk for developing depression. Turkish validation was conducted by Öy for children older than 7 years.[21]

Child Behavior Checklist (CBCL) is a parent-rated scale developed to assess competence and problem behaviors in children by Achenbach and Edelbrock.[22] The Turkish version of the CBCL contains 20 competence items and 118 problem items for children aged between 4 and 18 years. The competence scales of the CBCL are Activity, Social, and School. From these three scales, a Total Competence score is obtained. The problems scored with the checklist are Aggressive Behavior, Anxious/Depressed, Attention Problems, Delinquent Behavior, Social Problems, Somatic Complaints, Thought Problems, and Withdrawn/Depressed. The 118 problem items describe a wide array of problems that are rated on a 3-point scale. Parents score each item as 0, 1, or 2 (not true, somewhat true, or very true, respectively). Scores of all problem scales constitute the total problem and internalizing and externalizing problem scores. A higher score represents higher severity. Children who score 0-66 are considered to be without behavioral or developmental problems.

The Pediatric Quality of Life Inventory 4.0 (PedsQLTM 4.0) is one of the measures that evaluate health-related quality of life (HRQoL) in 2-18-year-old children and adolescents and was developed by Varni, Seid, and Rode. It is a 23-item questionnaire, and takes approximately 7-10 min to complete. It examines four distinct areas of health-related functioning: physical functioning, emotional functioning, social functioning, and school functioning. The latter three scales are combined to determine a broad psychosocial summary score. The scale is composed of a child self-report and parallel parent proxy report that assess parents' perception of their children's HRQoL for ages 5-7, 8-12, and 13-18 years. The response scale is a 5-point Likert scale. The items are reverse scored between 0 and 100, where the answer ''0 = never a problem'' scores 100 and ''4 = almost always a problem'' scores 0, so that the higher the total PedsQLTM 4.0 score, the better the HRQoL.[23] Turkish translation, reliability, and validity studies for 2-18 years of children have been carried out.[24,25]

The Conners Continuous Performance Test Third Edition™ (Conners CPT 3™) is a computer-based program for measuring attention-related problems in individuals aged eight years and older. By indexing the respondent's performance in areas of inattentiveness, impulsivity, sustained attention, and vigilance, the Conners CPT 3 can aid in the assessment of Attention-Deficit/Hyperactive Disorder (ADHD) and other neurological conditions related to attention. The Conners CPT 3 provides objective information about an individual's performance in attention tasks. The administration of the test takes 14 minutes. The stimuli consist of letters; non-targets (the letter X) to targets (all other letters). The children are asked to press the space bar or click the mouse for every letter that appears on the screen, except the letter X. Conners CPT3 reports the results on omission and commission rates, overall hit reaction time (Hit RT), overall hit reaction time standard error (Hit RT SE), variability, detectability (d'), response style (β), perseverations, hit reaction time by block change (Hit RT Block Change), hit reaction time standard error by block change (Hit SE Block Change), hit reaction time by interstimulus interval change (Hit RT ISI Change), and hit

reaction time standard error by interstimulus interval change (Hit SE ISI Change). Conners CPT3 measures are grouped into indicators of inattentiveness, impulsivity, sustained attention and vigilance. For children under the age of 8 years The Kiddie version of the test was used (The Kiddie CPT 2). In the Kiddie version the targets are pictures of objects that are familiar to preschool children such as house or boat, and the non-target stimuli is a ball.[26]

Informed consent was obtained from the cases and their parents. This study was approved by the local ethics committee (18-10.1/44-16.10.2018). Statistical analysis was descriptive.

Results

Neurological Outcome

Six cases were male and five were female. The mean age at the time of diagnosis was 7.36±4 (1-15) years. Three cases were diagnosed with ADEM before the age of five years and eight cases were diagnosed after the age of five years. All cases were polysymptomatic. At the time of diagnosis, the most common findings were altered state of consciousness (n= 8, 72.7%), headache (n= 7, 63.6%) and ataxia (n= 6, 54.5%), respectively. Lumbar puncture was performed in seven cases; cerebrospinal fluid protein level was elevated in one case and four cases had pleocytosis. Cerebrospinal fluid oligoclonal band and anti-myelin oligodendrocyte glycoprotein antibody were evaluated and found as negative in six and three cases, respectively. The mean duration of follow-up was 25 ± 22 (3-42) months. Four cases were followed-up for less than one year, whereas seven were followed-up for more than one year. Nine cases (81.8%) fully recovered without neurologic deficit during the follow-up period. One case who had a history of respiratory arrest at the time of initial diagnosis and who was being followed for three months had nasal speech and one case had urinary incontinence. Clinic characteristics of the cases are shown in Table I.

Radiologic outcome

At the time of initial diagnosis, ten cases had (90.9%) white matter, six cases (54.5%) had cortical, seven cases (63.6%) had deep gray matter (5:basal ganglia, 6:thalami) six cases (54.5%) had brain stem, one case (9.1%) had cerebellar and four cases (36.4%) had spinal involvement. Mass effect was not found in any of the cases. Seven cases (63.6%) displayed complete resolution of demyelinating lesions but four cases (36.4%) showed decrease in size of the lesions (Table I-II). Only one of four cases having partial resolution had more than one year of follow-up.

Psychiatric and psychometric outcome

In the psychiatric evaluation of 11 cases, 9 (81.8%) had no psychiatric disease, whereas one case (case no 5, female, 16 years old) had a depressive disorder, generalized anxiety disorder (GAD) and social phobia (SP), and one case (case no 3, male, 7 years old) had an articulation disorder.

The case having depressive disorder, GAD and SP was diagnosed with ADEM at the age of 13 years 8 months. Although she had some psychiatric problems, her last neurologic examination and cranial MRI were normal. She has been followed for 30 months since ADEM. When we evaluated this case she was suffering from depression, GAD and SP since 8 months and was taking antidepressant medications. Although medical treatment led to a significant remission of depressive symptoms, GAD and SP disorders were still ongoing. Her CDI score was 33. Although her psychiatric history revealed that she was socially anxious, avoidant and sensitive to separation during childhood, her clinical functionality was not impaired until the age of 15 years, when her sister got married and moved to another city our case had felt lonely. Our case and her mother described that she was fond of her sister. Although psychosocial stressors might cause depressive disorders, the role of ADEM in psychiatric vulnerability is not clear.

Table I. Clinical characteristics, psychiatric and psychometric evaluation results of patients and MRI findings.

	Case 1 6 years male	Case 2 8 years male	Case 3 7 years male	Case 4 15 years female	Case 5 16 years female	Case 6 8 years female	Case 7 9 years male	Case 8 9 years female	Case 9 9 years male	Case 10 6 years female	Case 11 12 years male
Age at diagnosis (year)	6	4	7	15	14	8	7	1	7	6	9
Follow-up time (Month)	4	42	3	3	30	40	15	72	24	3	39
Clinical findings	Headache Fever Dizziness Visual impairment Encephalopathy Ataxia	Fever Encephalopathy Paresia	Headache Fever Dizziness Visual impairment Encephalopathy Ataxia	Paresia	Headache Meningismus Paresia	Headache Fever Paresia	Headache Encephalopathy	Encephalopathy	Ataxia Vomiting Encephalopathy Paresia	Fever Encephalopathy Paresia Diplopia	Fever Encephalopathy Paresia Diplopia
Follow-up neurologic deficit	Urinary incontinence	-	Nasal Speech	-	-	-	-	-	-	-	-
Location of MRI lesions at the time of illness	Cortex White Matter Spinal Cord	White Matter Basal Ganglion Thalami Brain Stem Cerebellum	White Matter Basal Ganglion Brain Stem Spinal cord	White Matter Thalami	Cortex White Matter Spinal Cord	White Matter Basal Ganglion Thalami Brain Stem Spinal Cord	Cortex Thalami	Cortex White Matter	Cortex White Matter Basal Ganglion Thalami Brain Stem	White Matter Brain Stem	Cortex White Matter Basal Ganglion Thalami Brain Stem
Follow-up MRI / Time	PR / 3rd month	CR / 2nd year	PR / 3rd month	PR / 3rd month	CR / 1st year	CR / 1st year	CR / 1st year	PR /2nd year	CR / 1st year	CR / 3rd month	CR / 2nd year
WISC-R scores											
VIQ	97	128	104	132	116	97	97	107	104	116	96
PIQ	74	131	104	131	101	116	101	94	105	123	114
TIQ	85	133	104	135	110	107	99	101	105	121	105
PedsQoL scores											
PHTS	93.7	88.3	68.3	75	58.3	88.3	94.1	90	75.8	81.6	67.5
PSHTS	95.3	89.1	57.8	60.9	73.4	82.8	100	92.5	87.5	95.3	60.9
Emotional functioning	80	85	65	80	60	100	80	95	75	75	75
Social functioning	100	95	50	100	100	100	100	95	55	85	75
School functioning	-	85	85	55	55	100	95	80	100	80	45
STS	94.4	89.6	64.6	70	63.5	86.4	96.1	92.9	79.8	85.6	64.1

MRI: magnetic resonance imaging PR: partial resolution, CR: complete resolution, WISC-R: wechsler intelligence scale for children-revised, VIQ: verbal intelligence quotient, PIQ: performance intelligence quotient, TIQ: total intelligence quotient, PedsQoL: The pediatric quality of life inventory, PHTS: physical health total score, PSHTS: psychosocial health total score, STS: scale's total score, CPT: continuous performance test HRT: hit reaction time, HRT SD: hit reaction time standard deviation HRT ISI: hit reaction time interstimulus interval change, CBCL: child behavior checklist, CDI: children's depression inventory.

Table I. Continued.

	Case 1 6 years male	Case 2 8 years male	Case 3 7 years male	Case 4 15 years female	Case 5 16 years female	Case 6 8 years female	Case 7 9 years male	Case 8 9 years female	Case 9 9 years male	Case 10 6 years female	Case 11 12 years male
CPT scores				-	-				-		-
Detectability	47	62	52			38	44	43		43	
Error type											
Ommisions	50	74	50			45	42	44		43	
Commissions	42	57	50			35	43	43		52	
Perseverations	45	57	49			46	50	46		48	
Reaction time statistics											
HRT	63 (slow)	50	54			56	59	54		49	
HRT SD	54	65	50			45	42	49		42	
Variability	55	61	53			47	44	52		45	
HRT Block change	43	37	62			53	50	34		40	
HRT ISI change	58	61	30			42	50	57		55	
CBCL scores											
Withdrawn	64	50	50	68	54	53	50	50	50	57	50
Somatic complaints	61	50	56	51	50	50	50	54	50	54	59
Anxious/depressed	72	50	52	58	64	52	50	52	50	57	53
Social problems	73	50	52	50	50	50	50	50	50	57	59
Thought problems	76	50	57	50	63	50	50	50	50	50	50
Attention problems	69	50	50	50	57	50	50	50	50	50	57
Delinquent behavior	73	50	54	61	50	50	50	50	50	57	50
Aggressive behavior	77	50	50	50	53	50	50	50	50	53	51
Internalizing problems	77	37	49	54	57	46	37	42	35	55	53
Externalizing problems	71	43	51	60	58	51	43	48	40	57	51
Total Behavior problems	75	38	46	49	51	42	35	40	30	54	50
CDI score	5	2	2	9	33	6	4	7	2	8	12
Psychiatric diagnosis	No	No	No	No	Depressive Disorder Generalized Anxiety Disorder Social Phobia	No	No	No	No	No	No

MRI: magnetic resonance imaging PR: partial resolution, CR: complete resolution, WISC-R: wechsler intelligence scale for children-revised, VIQ: verbal intelligence quotient, PIQ: performance intelligence quotient, TIQ: total intelligence quotient, PedsQoL: The pediatric quality of life inventory, PHTS: physical health total score, PSHTS: psychosocial health total score, STS: scale's total score, CPT: continuous performance test HRT: hit reaction time, HRT SD: hit reaction time standard deviation HRT ISI: hit reaction time interstimulus interval change, CBCL: child behavior checklist, CDI: children's depression inventory.

Table II. Assessment of the relation of the MRI to psychiatric symptoms.

	Case	At the time of illness MRI						Follow-up MRI	Psychiatric symptoms				
		Cortex	White matter	Basal ganglia	Thalami	Brain steam	Cerebellum	Spinal cord		Attention deficit	Behavioral problem	Psychiatric diagnosis	Low performance IQ	Low verbal IQ
Psychiatric symptom +	1	+	+	-	-	-	-	+	PR	+	+	-	+	-
	2	-	+	+	+	+	+	-	CR	+	-	-	-	-
	3	-	+	+	-	+	-	+	PR	+	-	-	-	-
	5	+	+	-	-	-	-	+	CR	-	-	+	+	-
	6	-	+	+	+	+	-	+	CR	-	-	-	-	+
	11	+	+	+	+	+	-	-	CR	-	-	-	-	+
Psychiatric symptom -	4	-	+	-	+	-	-	-	PR	-	-	-	-	-
	7	+	-	-	+	-	-	-	CR	-	-	-	-	-
	8	+	+	-	-	-	-	-	PR	-	-	-	-	-
	9	+	+	+	+	+	-	-	CR	-	-	-	-	-
	10	-	+	-	-	+	-	-	CR	-	-	-	-	-

PR: partial resolution, CR: complete resolution.

The male case having articulation disorder was diagnosed with ADEM at the age of 7 years and was followed for 3 months. Although articulation disorder was observed during his psychiatric interview, his family reported that it was found before the ADEM attack.

According to the results of the WISC-R intelligence test, only one case (Case1) had borderline intellectual functioning, other cases had average or higher scores (Table I). Two cases (Case 1 and Case 5) demonstrated a lower performance IQ (PIQ) score than verbal IQ (VIQ) score. Case 1 received the lowest score in the coding subtest of PIQ evaluation. Two of the other cases (Case 6 and Case 11) demonstrated lower VIQ score than PIQ score. They received the lowest scores in the information subtest and they also had low scores in coding and block design subtests of PIQ which were determinant of reduced attention and visuospatial/ visuomotor skills. Regarding this result, attention problems were found in 4 of 11 cases (36.4%).

The CPT scores of cases are presented in Table I. Seven of eleven cases completed the CPT test. Four cases did not accept to participate in the tests. The T-scores of CPT of those four cases were within the typical range. Two cases (Case1,3) showed only one atypical T-score means and responded more slowly and one case (Case 2) scores showed some atypical T-scores in sustained attention, vigilance and inattentiveness dimensions of attention.

Although CPT results were compatible with attention problems in those cases, their psychiatric evaluations, parent and teacher report forms did not support clinical ADHD diagnosis according to DSM-IV-TR.

Child behavior checklist scores of ten cases were found to be within normal limits, whereas anxiety/depression, social problems, thought problems, attention problems, delinquent behavior, aggressive behavior and internalizing, externalizing and total behavior problem scores were high in one case (Case 1). Although an

emotional-behavioral problem was defined in the CBCL scale for this case, no active clinical psychopathology was determined on psychiatric examination according to DSM-IV-TR. This case was diagnosed with ADEM three months prior and has been within the early follow-up period. For this reason these problems were considered to be associated with the acute course of the disease.

Quality of life scale for children; the mean total scale score, total physical health score, and total psychosocial health score are presented in Table I. Age at time of diagnosis, duration of follow-up, and initial and control MRI findings of cases were compared with mean PedsQoL scores; however, no statistically significant correlation was found ($p > 0.05$).

The relationship between first MRI findings and presence of neuropsychological symptoms was also evaluated. The rate of neuropsychological symptoms were the highest in cases that had basal ganglia involvement when compared to cases with cortical, thalamic and brainstem involvement respectively (80% vs 50%, 50%, 66%). The rate of partial resolution of lesions in cases with or without neuropsychological symptoms were 33% and 40% respectively.

Discussion

ADEM is an immune-mediated, monophasic demyelinating disorder of the CNS with a good outcome. Less than 10% of cases may have significant neuropsychiatric morbidity such as motor deficits, focal epilepsy, decreased visual acuity and decline in neurocognitive functions.[27] In our study, most cases (81.8%) fully recovered without neurological deficit. One case had nasal speech and one case had urinary incontinence. However recent studies have reported that the cases may display permanent sequelae such as behavioral and attention deficit disorders.[3,9,15,27] These studies showed similar results and are outlined in Table III.[10-12,15] In our study, psychiatric/neurocognitive impairment was detected in six (54.5%) cases, two of them also had neurological deficits. One case (9.1%) had a psychiatric diagnosis (depressive disorder, generalized anxiety disorder, social phobia), one case (9.1%) had behavioral problems, two cases (18.2%) had lower PIQ scores than VIQ scores, two cases (18.2%) had lower VIQ scores than PIQ scores. Three of seven (42.9%) cases displayed impaired attention based on CPT.

Attention problems were described in 11-58% of cases following ADEM in different studies.[27-31] In our study, two families complained about the lack of attention in their children; but, objective psychiatric examination, family and teacher interviews were not compatible with ADHD. On the other hand, three of 7 (42.9%) cases had atypical T-scores in sustained attention, vigilance and inattentiveness dimensions of attention in CPT, our results are compatible with literature data. Although three cases did not fulfill the DSM-IV for ADHD, their parents were given information about the results of the CPT and suggested to follow their children for the symptoms of attention deficits.

The frequency of neurocognitive disorders including borderline IQ, attention deficit, decreased executive functions and psychiatric symptoms following ADEM were also reported between 4-66%.[10,12,27-33] The results of studies about the relationship between neurocognitive outcomes and the duration of follow-up are conflicting. Some reports point out that longer duration of follow-up is associated with better outcomes and resolving of abnormal neurocognitive findings.[27,32] Our cases underwent neuropsychiatric evaluation 3-42 months after the diagnosis of ADEM. In six cases neuropsychiatric symptoms were detected, four of them were followed up longer than one year, besides two of them were followed less than one year.

We detected reduced attention and visuospatial/visuomotor skills in 4 of 11 cases. Two of the them (Cases 1 and 5) demonstrated lower PIQ score than VIQ score, similar to Hahn et al.s'[10] study, indicating a cognitive profile of reduced

Table III. Review of the literature on neuropsychological outcome after acute disseminated encephalomyelitis.

Study	Year of publication	Number of patients	Follow-up Time (year)	Conclusion
Hahn et al. (10)	2003	6	3.5 ± 1.05	*66% (4/6) lower performance IQ (poorer visuospatial /visuomotor function)
				* Mild cognitive deficits in all children in at least one subcategory
				*No correlation between cognitive outcome and the location or extent of acute or residual MRI signal changes
Jacobs et al. (11)	2004	10 <5 years	3.9 ± 2.2	*Onset of ADEM <5 years associated with lower IQ, lower spelling reading scores and higher behavioral and emotional problems
		9 >5 years	2.2 ± 1.9	*The existence of deficits more than 3 years after ADEM
Rostasty et al. (28)	2009	12	6.9 ± 4.6	*25% IQ below average
				*58% attention, 16% memory, 25% school performance, 25% visuospatial skills and 25% impulse control impairment
Deery et al. (29)	2010	9	2.2 ± 1.7	*11% attention, 11% cognitive flexibility mildly impaired
Kuni et al. (12)	2012	19	5.4	*16% cognitive impairment
				*In the verbal skills, visual memory, attention, and information processing speed tests patients with ADEM had significantly lower scores than control group
				*No association between younger age of onset and cognitive impairment
				*Older age of onset correlated with worse visual memory and verbal skills
Parrish et al. (13)	2013	13	4.8 ± 4.1	*Greater parent-reported fatigue and depression in ADEM patients than in healthy controls
Suppiej et al. (33)	2014	22	6.8 ± 3.7	*Neurocognitive test mean scores were in average range
				*27% significant impairment (4/6 attention impairment)
				*Older age of onset associated with better language performance
				*Longer follow-up associated with better neuropsychological outcome
				*No association between neuropsychological findings and MRI lesions at onset and MRI outcome
Beatty et al. (30)	2016	23	3.9 ± 3.7	*4% IQ below average
				*54% attention, 22% processing speed, 22% delayed verbal recall, 18% visuomotor integration, 9% expressive naming, 4% auditory working memory impairment
				*Earlier age of onset associated with poorer sustained attention
				* No association between neuropsychological findings and MRI residual lesion volumes
				*T2 larger lesion volume associated with a variety of self-reported mood and behavior concerns
				*T1 larger lesion volume associated with depression, hyperactivity, and sense of inadequacy

Table III. Continued.

Study	Year of publication	Number of patients	Follow-up Time (year)	Conclusion
Shilo et al. (27)	2016	43	5.5 ± 3.5	*12% IQ below average *44% attention-deficit hyperactivity disorder, 32% behavioral problems, 21% learning disabilities *Older age at diagnosis associated with abnormal CBCL and behavioral problems *No association between poor-long term outcome and location of MRI lesions or full resolution in follow-up exam
Sadek et al. (34)	2016	18	0,5	*50% IQ below average, 25% mild intellectual disability
Iype et al. (31)	2018	102	1-10	*25.3% attention deficit, 13.1% behavioral problems, 22.2% poor learning skills *Poor learning skills associated with polyneuropathy and behavioral abnormality associated with tumefactive demyelination
Our study	2019	11		*18% low performance IQ score, 18% low verbal IQ score *42% (3/7) attention deficit *9% abnormal CBCL and behavioral problems *9% with a psychiatric diagnosis *54% with psychiatric symptoms

visuospatial/visuomotor skills relative to verbally mediated skills.

Jacobs et al.[11] emphasized that the children who were diagnosed with ADEM before 5 years of age had more severe behavioral and emotional problems. Beatty et al.[30], reported a psychiatric abnormality in 20-40% of 23 cases and pointed out that having the disease at a younger age is a risk factor for intellectual disability and behavioral disorders. White matter, which is the most commonly affected area in ADEM, shows the fastest development in early childhood, and axonal myelinization and synaptic density are closely correlated with functional development.[29] In particular, development of axonal myelinization and synaptic density were prominent between 3-7 years.[34] For this reason, the relationship between the age at the diagnosis of ADEM and the neurocognitive status is significant, and there is another study suggesting that cases diagnosed at a younger age have poorer outcomes in terms of speech function.[32] In our study, their first diagnostic ages of cases with neuropsychiatric symptoms ranged between 4-14 years, one of them was younger than 5 years.

In our group, we found that basal ganglia involvement at the beginning of the disease might be associated with attention problems at follow-up. In previous studies, any relationship between late neurocognitive dysfunction and lesion localizations have not been reported.[10,27,32] In our study, attention deficit or cognitive symptoms were most frequent in cases with basal ganglia involvement. There are many studies pointing out abnormalities of basal ganglia in children with ADHD.[35-37] Similarly, low verbal IQ scores and language problems have been reported in children with basal ganglia lesions.[36-39] In accordance with the literature, we couldn't find any relationship between neuropsychiatric symptoms and resolution of lesions on follow-up MRI.[27,30,32]

The limitations of this study are: (1) inclusion of a small sample size, (2) lack of a control group, (3) lack of application of CPT to all

cases. Despite the limited number of cases in our study, comprehensive cognitive and psychiatric evaluations were performed. In conclusion, psychosocial and cognitive problems were detected in 54.5% of cases with ADEM. In particular, cases with basal ganglia involvement should be followed carefully in terms of attention and cognitive problems.

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Original Article

Muscle strength and joint health in children with hemophilia: a cross-sectional study

Necati Muhammed Tat[1], Ayşe Merve Tat[1], Filiz Can[2], Bülent Antmen[3], Ahmet Fayik Öner[4]

[1]Department of Physiotherapy and Rehabilitation, Necmettin Erbakan University Faculty of Health Sciences, Konya; [2]Department of Physiotherapy and Rehabilitation, Hacettepe University Faculty of Health Sciences, Ankara; [3]Department of Pediatric Hematology and Oncology, Acıbadem Hospital, Adana; [4]Department of Pediatric Hematology and Oncology, Yüzüncü Yıl University Faculty of Medicine, Van, Turkey.

ABSTRACT

Background and objectives. We aimed to evaluate joint health in children with hemophilia (CwH) and to investigate the effects of hemarthrosis on the musculoskeletal system.

Method. Forty-one CwH aged between 6-18 years participated in the study. Joint health status was evaluated according to Hemophilia Joint Health Score (HJHS). Pain intensity level was assessed in resting and in activity using Visual Analog Scale. Range of motion was measured with goniometer and muscle strength was assessed with digital dynamometer. Arthropathic joints were examined in three groups named knee, elbow and ankle.

Results. Physical examination revealed arthropathy findings to be found in 29 knee, 19 elbow and 18 ankle joints. The median of flexion angle of the affected side were 120°, 122° and 12° for the knee, elbow and ankle and extension losses of these joints were 5°, 7° and 0, respectively. In CwH having knee and elbow arthropathy, index joint HJHS was found to be significantly higher than those with ankle arthropathy ($p < 0.01$). The flexor and extensor muscle strength significantly decreased in 11 CwH with unilateral elbow arthropathy compared to the non-arthropatic side ($p < 0.05$). In 15 CwH with unilateral ankle arthropathy decreased in the extensor muscle strength (plantarflexors) ($p < 0.05$). Extension loss showed a good correlation with index HJHS of elbow, knee and ankle joints, respectively. ($r_s = 0.599, 0.576, 0.606$, $p < 0.01$). We observed that the muscle strength of elbow flexors/extensors and ankle extensors were significantly decreased compared to the non-arthropathic side. However this situation was not detected in knee joint despite having highest index HJHS.

Conclusion. Our findings indicate that hemarthrosis may cause more muscle strength loss in the upper extremity than the lower extremity. Furthermore, extension loss was found to be an important parameter in physical examination of hemophilic arthropathy. Musculoskeletal system should be evaluated comprehensively at regular intervals and when necessary rehabilitative treatment should be planned.

Key words: hemophilia, arthropathy, muscle strength, range of motion, physical examination.

Hemophilia is a rare hereditary bleeding disorder that occurs in partial or complete deficiency of clotting factor VIII (in hemophilia A) and factor IX (in Hemohilia B).[1,2] Severity and frequency of bleedings are closely related to the factor levels.[3] Severe hemophilia is defined as factor basal level less than 1%, moderate hemophilia between 1-5% and mild hemophilia more than 5%.[1,4] Severe hemophilia is characterized by spontaneous musculoskeletal bleedings that occur mostly in the joints and is called hemarthrosis.[5] Recurrent hemarthrosis leads to a vicious cycle in the joint which leads to synovial hypertrophy and damage to the cartilage, followed by hemophilic arthropathy of the joint. It causes irreversible joint destruction due to the progression of deformation in the joint.[6]

✉ Necati Muhammed Tat
necatimuhammedtat@gmail.com

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Nowadays, the primary aim of hemophilia treatment is the prevention of hemophilic arthropathy.[7] Despite new developments in drug therapies and advances in gene therapy which have resulted in increased life-expectancy and quality of life for hemophilic individuals, uncertainties about how to rehabilitate existing joint damage of individuals with musculoskeletal problems and difficulties in accessing expert physiotherapists in hemophilia are currently the biggest challenges.[7,8] In terms of International Classification of Functioning (ICF), Disability and Health developed by WHO, it is known that muscle weakness and joint limitation in hemophilia negatively affects activity and participation, therefore, it is important to evaluate muscle strength and joint health conditions in children with hemophilia (CwH).

Hemophilia Joint Health Score (HJHS) is routinely used in assessing the prognosis of the disease and the effectiveness of the treatment. HJHS was designed to monitor the disturbances in mild or non-mild joints in CwH who received prophylactic treatment between 4-18 years of age.[9] HJHS was more sensitive than X-ray and safe in detecting early changes in joints.[9,10] The assessment of joint health in CwH should be routinely performed every six months with physical examination and every once a year with radiological assessment.[11,12]

In previous studies in hemophilia, the total score of HJHS was used in the evaluation of musculoskeletal disorders. However, the relationship between the dynamometric measurement of muscle strength and the index joint scores of HJHS was examined separately in our study. The aim of this study was to evaluate joint health in CwH and to investigate the effects of hemarthrosis on musculoskeletal system.

Material and Methods

This study was an observational cross-sectional study including 41 CwH aged between 6-18 years old and was conducted between February and April 2018 at Yuzuncu Yil University Department of Pediatric Hematology. The exclusion criteria of the study was the history of bleeding in any joint in the last two weeks, having any disease related to connective tissue, having a neurological disease or cognitive impairment and having undergone surgery related to joints. CwH who had participated in no regular physical activity and sports were included in the study.

In order to carry out the study, the approval of the required ethics committee was obtained with the numbered 03/31.01.2018 of the Ethics Committee of the Yuzuncu Yil University. Verbal consent was obtained from participants and written consent was obtained from the parents of all children.

Assessments

Hemophilia Joint Health Score (HJHS)

HJHS is a haemophilia-specific assessment method that assesses disorders occurring in six key index joints in its current version. The index joints are hinged joints such as knee, elbow and ankle joint with an excessive synovial fluid content and they are exposed to more mechanical stresses.[13] Swelling, duration of swelling, muscle atrophy, crepitus during motion, flexion loss, extension loss, joint pain and muscle strength were evaluated in eight sub-headings parameters.[8,9]

Range of motion (ROM) (flexion and extension loss) and muscle strength may reflect situation of joint function and structure. Others such as crepitation, swelling, duration of swelling may involve changes in the joint which do not correlate with disability.[11] The maximum damage score for each index joint is 20 points per joint. The last subtitle of the HJHS was the global gait score and its maximum score was 4 points. The maximum total HJHS score is 124 points.[8,9] High scores indicate poor joint health. The HJHS score of the index joints and total HJHS score of the participants were recorded.

Physical Examination

Physical examination which is a practical and inexpensive assessment method of joint, is often used to measure structural and functional joint damage.[8] Physical examination was performed by the same physiotherapist who was non-blind to the study, because this is a very new area of specialization for physiotherapists and there were no other experienced physiotherapists to evaluate CwH in our department. Evaluation of the joint ROM were performed using the same standardized goniometric measurements. The flexion angles and extension loss of the knee and elbow joints, the dorsiflexion and plantarflexion angles of the ankle joints were measured for all patients.[14] Pain intensity levels were evaluated both on rest and activity by using visual analogue scale (VAS). This consists of a 10 centimeter straight line which should be marked by the patients according to the pain intensity level. A level of ten centimeters shows severe pain intensity while zero centimeters indicate no pain on the line.[15]

The evaluation of muscle strength was performed using a digital dynomometer. The strength of the extensor and flexors muscles of the knee joint were measured during sitting position with hip and knee in 90° flexed position by applying resistance over the malleolus.[16,17] The strength of the extensor and flexor muscles of the elbow joint was measured while the elbow joint was flexed at 90° by applying the forearm resistance in the sitting position. To measure the strength of the dorsiflexors and plantar flexors of the ankle the joint lower legs of the patient were stabilized in the supine position and resistance was given from the metatarsal head.[17] The average muscle strength was recorded with the digital dynamometer as pound (1 pound = 0.4535kg).

Arthropathic joints were examined in three groups named as knee, elbow and ankle.

Statistical Analysis

Statistical analysis were performed using the SPSS software version 22. The variables were investigated using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirrov / Shapiro-Wilk tests) to determine whether or not they were normally distrubuted. Descriptive analysis were presented using means and standard deviation for normally distrubuted variables and median and minimum-maximum values (Physical charesteristics of participants). In the event the data did not show normal distribution between the groups, median values and minimum-maximum values were expressed and the non-parametric Kruskal-Wallis test was used. The correlation coefficients and their significance were calculated using the Spearmen test. In the cases of unilateral arthropathy in the groups, the dependent variables were presented using means and standard deviations for normally distrubuted variables and were compared with the Student t-test while not normally distrubted variables were compared with the Mann Whitney U test. A p-value of less than 0.05 was considered to show a statistically significant result.

Results

Of the 41 CwH, 39 had hemophilia A, 2 had hemophilia B and the the phenotype of 37 (90%) were severe and of 4 (10%) were mild. Three patients (7%) had inhibitor. The mean age (12.8 ± 3.7), height (146.2 ± 22.8) and body weight (43.0 ± 19.4) of the 41 CwH who participated in the study. Of these patients 21 CwH having arthropathy showed more than one index joint. Arthropathy was not found in the index joints of 6 participants.

The index joints HJHS, total HJHS, resting and activity pain levels of the participants are presented in Table I.

Physical examination revealed arthropathy in 29 knees, 19 elbows and 18 ankle joints groups and these joints were examined in all three groups.

There was no statistically significant difference between the ages (p=0.429), height (p=0.270) and

Table I. HJHS and VAS scores of all participants.

Parameters	Median (Min-max)
HJHS-Right Elbow	0 points (0-9 points)
HJHS-Left Elbow	0 points (0-7 points)
HJHS-Right Knee	2 points (0-13 points)
HJHS-Left Knee	2 points (0-11 points)
HJHS-Right Ankle	1 points (0-7 points)
HJHS-Left Ankle	0 points (0-3 points)
Total HJHS	9 points (2-32 points)
Activity VAS	3 cm (0-8 cm)
Resting VAS	0 cm (0-9 cm)

HJHS: hemophilia joint health score, VAS: visual analog scale.

body weights (p= 0.134) when the CwH were divided into classes according to arthropathic joints. Activity VAS (p= 0.446) and resting VAS (p= 0.760) were used to assess pain intensity level and no statistically significant difference was found between the groups. In CwH with knee and elbow arthropathy, index joint HJHS was found to be significantly higher than those with ankle arthropathy (p= 0.002). HJHS scores and physical assessment results (median values, minimum-maximum) according to the joints are given in Table II. Our study found that arthropathy developed in joints as a result of recurrent bleeding episodes showed a decrease in flexion angle and an increase in extension loss. Physical examination of groups showed in Table III.

Table IV showed the comparision of muscle strength in unilateral arthropathy. In 11 CwH with unilateral elbow arthropathy flexor (p= 0.041) and extensor (p= 0.021) muscle strength was significantly reduced compared to non-arthropathic side. In 19 CwH with unilateral knee arthropathy the extensor (p= 0.182) muscle group and the flexor (p= 0.385) muscle strength decreased in the affected joint but was statistically not significant. In 15 CwH with unilateral ankle arthropathy, the decrease in the dorsiflexor muscle strength (p= 0.191), which functions as the flexion of the joint, was not statistically significant while the decrease in the plantar flexor muscle strength acting as extension was significant (p= 0.040).

Extension loss was moderately correlated with elbow, knee and ankle HJHS (respectively r_s=0.599, 0.576, 0.606, p <0.01) and is displayed in Fig. 1. Flexion angle showed a negative moderate correlation with flexor muscle strength (r_s=0.523, p <0.05) and was strongly

Table II. Physical characteristics, HJHS and VAS scores of participants according to arthropatic joints.

	Knee (n= 29)	Elbow (n= 19)	Ankle (n= 18)	p value
Age (years)	14 (7-18)	16 (9-18)	12 (7-18)	0.429
Height (cm)	150 (107-180)	170 (121-176)	139 (107-179)	0.270
Weight (kg)	39 (17-76)	60 (21-75)	32 (17-71)	0.134
Resting VAS (cm)	0 (0-9)	0 (0-5)	0 (0-9)	0.760
Activity VAS (cm)	4 (0-7)	5 (0-8)	4 (0-7)	0.446
HJHS (Index joints)	5 (2-13)	5 (1-9)	3 (1-7)	0.002*

*Kruskall-Wallis test, p<0.05, Median (Minimum-Maximum) Values
HJHS: Hemophilia joint health score, VAS: visual analog scale.

Table III. Physical examination of groups.

	Knee (n= 29)	Elbow (n= 19)	Ankle (n= 18)
Flexion angle (°)	122 (90-135)	120 (90-145)	12 (7-20)
Loss of extension (°)	5 (0-10)	7 (0-35)	0 (0-14)
Flexor muscle strength (lbs)	17.7 (7.3-27.5)	16.8 (8.8-30.1)	13.4 (5.1-18.4)
Extansor muscle strength (lbs)	16.6 (8.1-32.2)	12.1 (7.8-17.3)	14.0 (7.7-24.5)

Median (Minimum-Maximum) Values

Table IV. Comparision of muscle strength in unilateral arthropathy.

Joints	Muscle Strength	Affected Side (pound)	Non-Affected Side (pound)	t/z	p
		Mean±SD	Mean±SD		
Knee joint	Flexors	16.9 ± 6.5	18.7 ± 6.3	t=-0.880	0.385
(n=19)	Extensors	18.9 ± 6.2	21.8 ± 6.9	t=-1,362	0.182
Elbow joint	Flexors	17.5 ± 3.8	21.2 ± 4.1	t=-2.189	0.041*
(n=11)	Extensors	11.6 ± 2.5	14.4 ± 4.8	t=-2.499	0.021*
Ankle joint	Dorsiflexors	13.4 ± 3.3	16.4 ± 5.2	z=-1.307	0.191
(n=15)	Plantarflexors	15.6 ± 4.5	18.7 ± 4.2	z=-2.054	0.040*

* Student's t-test was used knee and elbow joint, Mann-Whitney U test was used in ankle joint p<0.05

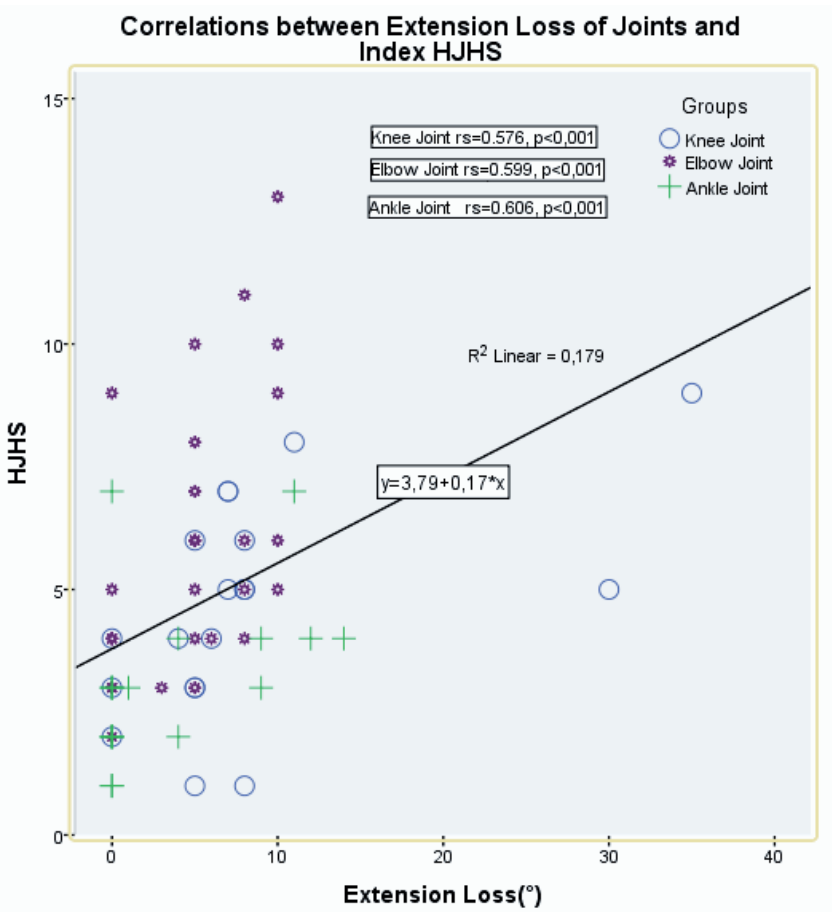

Fig. 1. Correlations between extension loss of joints and index HJHS.
HJHS: Hemophilia Joint Health Score

correlated with extensor muscle strength (r_s= 0.711, p <0.01) in elbow arthropathy. HJHS showed a negative moderate correlation with flexion angle (r_s= 0.621, p <0.001) and extensor muscle strength (r_s= 0.517, p <0.01), a negative weak correlation with pain on rest (r_s= 0.410, p <0.05) in knee arthropathy. In ankle arthropathy dorsiflexion angle showed a negative moderate correlation with pain on rest (r_s= 0.637, p <0.005) and pain on activity (r_s= 0.677, p <0.005).

Discussion

Recurrent joint bleedings are the hallmark of hemophilia that leads to progressive changes in the musculoskeletal system over the years. Our study supports the finding that hemarthrosis leads to decreased joint mobility and muscle strength in CwH. Joint health status can be improved by correct treatments in the long-term after frequent evaluations.

In the literature hemophilia A is more prevalent than hemophilia B and approximately 85% of patients have been reported to be hemophilia A.[2] The distrubution of hemophilic patients in our study was in accordance with the results of the literature. Inhibitory development in our study was 7%, Turkey Inhibitor Screening Project which was completed in 2010 found 13% inhibitor development rate in severe hemophilia patients. In our study inhibor results are consistent with the literature.

In this study we observed that the most common arthropathic joints in CwH were knee, elbow joint and ankle joint, respectively. Hemophilic arthropathy was commonly seen in knee joint due to lack of internal bone stability, three-dimensional movements, load bearing function and possible reasons such as exposure to trauma.[5] Stephensen et al.[13] showed that knee joint was the most common bleeding joint in individuals who cannot access prophylactic treatment routinely.[7] In the adolescent period, elbow joint has been damaged more because of a wider range of motion in daily living activities and frequent use. Deschamps et al.[18] showed that the ankle joint was mostly affected due to easy access to prophylactic treatment. In our country prophylaxis treatment has been widespread since 2010. In this study, ankle arthropathy was more common in the younger age groups, whereas knee and elbow joint arthropathy increased with age.

Soucie et al.[19] showed that healthy male individuals aged between 9-19 years normative values of knee flexion, elbow flexion and ankle dorsiflexion were 142°, 148° and 16°, respectively. In our study, median values of arthropathy joints were 122°,120° and 12°, respectively. Cuesta-Barriuso et al.[15] found that dorsiflexion and plantarflexion angles were 7 degrees and 37 degrees in patients with hemophilic arthropathy of the ankle aged between 20-44 years and those angles were far below the normative values. Goto et al.[17] found that arthropathy in knee and elbow joints as the severity of the disease progress, ROM in both flexion and extension showed significant decreases. As haemophilic arthropathy severity progresses osteophytis and osteochondral cysts form so that joint surface becomes irregular and consequently lead to narrowing of the joint space. These changes may resulted with decreases in joint ROM.

The index joint HJHS was significantly higher in hemophilic individuals having knee and elbow arthropathy than in those having ankle arthropathy ($p < 0.01$). In a study Tusell et al.[20] assessed patients with clinical examination and radiological evaluation and they revealed that the most effected joint and highest score was found in knee by clinical examination and in ankle joint by radiological score.[20] The reason why ankle arthropathy is low in HJHS may result from the low proportional loss of the ankle ROM relatively to those of the knee and elbow joints. Hence, the highest radiographic score of ankle arthropathy has been considered that it is not less severe than knee and elbow arthropathy.

In the current study when the unilateral arthropathy of the knee, elbow or ankle joints were compared with the non-arthropathic side, it was seen that the muscle strength of the elbow joint on the arthropathic side was significantly less than the non-arthropathic side. These results were found in accordance with the literature. Falk et al.[21] evaluated muscle strength of hemophilic children using an isokinetic device and reported that elbow flexor and extensor muscle strength significantly decreased in the hemophilia group. We thought that muscle strength may vary depending on the use of the elbow joint in activities. CwH

with elbow arthropathy mostly protects elbow joints and they don't use involved elbow joint movement frequently. They can compensate the elbow movements with the uninvolved elbow joint during activities. Therefore, decreases in muscle strength in studies may be related with inactivity or disuse of the joint movement due to fear of pain and re-bleeding.

The knee flexor and extensor muscle strength decreased in the arthropatic side but decreased in flexors and extensors muscle strength was statistically not significant in our study. As the knee joint is used continously in gait patern, it is expected that significant loss of muscle strength cannot occur. Similar results were found in the study conducted by Goto et al. and they found the relationship between joint function and severity of arthropathy.[17] Lobet et al.[22] showed in their study of isokinetic measurement of ankle joint arthropathy, that CwH have no significant difference in muscle strength compared to their healthy peers. In our study, the decrease in dorsiflexor muscle strength was not significant, but the decrease in plantar flexors muscle strength was significant. This observation may be due to the very simple change in load distribution during the gait cycle especially the heel off phase. Lower extremity muscle strength is very important in many daily life activities and reflects the functional capacity of the hemophilics. Weakness of the knee extensors is known to be characteristic of adult severe hemophilia, and in our study there was no significant difference in the CwH compared to the side without arthropathy. Knee muscles is used continuously in daily life activities such as standing, walking, squatting and the joints are always exposed to some loads. Thus, the muscles of knees cannot be inactive and having more stimulus in daily activities.

One of the limitation of this study was that radiological imaging could not be performed to evaluate joint health of CwH. If radiological imaging methods could have been used in the study, more detailed data could have been obtained for hemophilic arthropathy. The other limitation of this study was that we could not to assess functional independence and quality of life of the patients although both of them are very important musculoskeletal outcome tools. Turkish validation-reliability of these tests have not been completed yet, therefore we could not use them.

In summary, we observed that muscle strength was significantly decreased in CwH especially elbow flexors/extansors and ankle extansors compared to the non-arthropathic side but not in knee muscles despite having the highest index HJHS scores. Our findings revealed that hemarthrosis cause more muscle strength loss in the upper extremity than the lower extremity. Therefore, both upper extremity and lower extremity muscles should be strengthened from early ages in hemophilia. Furthermore extension loss was found to be another important parameter in physical examination of arthropathy and may be the cause of a decrease in the muscle strength in the hemophilic arthropathy. The musculoskeletal system should be evaluated comprehensively at regular intervals and when necessary, rehabilitative treatment should be planned.

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Original Article

Coronary artery fistulae and treatment in children

Kahraman Yakut[1], N. Kürşad Tokel[1], Birgül Varan[1], İlkay Erdoğan[1], Murat Özkan[2]

Departments of [1]Pediatric Cardiology and [2]Cardiovascular Surgery and Başkent University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background and objectives. In this study, we aimed to review the treatment options and long-term problems of patients who were diagnosed with coronary artery fistulae (CAF) in our institution. We also tried to determine the most appropriate time for treatment of this condition.

Method. From 2000 to 2018, the medical records of 56 patients (33 males and 23 females) who had CAF diagnoses were retrospectively reviewed.

Results. The mean age of the patients at the time of diagnosis was 3.9 ± 4.6 years (range, 1 month to 18 years) and the mean duration of the follow-up period was 7.4 ± 4.5 years (range, 1 year to 17.5 years).

The right coronary artery (RCA) was the most common origin site for CAF, the left main coronary artery (LMCA) was the second most common origin site whereas the left anterior descending coronary artery (LAD) was the third most common origin site. Catheter angiography showed that right ventricle (RV) was the site of termination for CAF in 23 patients (41.1%) while the CAF drained to the pulmonary artery in 16 patients (28.6%). Transcatheter intervention was performed in ten patients, while CAF were corrected surgically in five patients. Transcatheter intervention was initially attempted in two out of the five surgically-treated patients, but the procedure was unsuccessful. A vascular plug was deployed in six patients, a platinum coil was used in three patients, and a platinum coil with tissue adhesive was placed in one patient using a catheter. Early complications were seen in two patients during transcatheter intervention and in one patient during surgery. There were no instances of death or late complications in patients treated surgically or via transcatheter.

Conclusions. Coronary artery fistulae are usually asymptomatic, and medical therapy with long term follow up is the first line treatment. Fistulae that cause hemodynamically significant shunting, chamber enlargement, or visible symptoms should be closed at an early age. This study shows that transcatheter closure is a safe treatment option for CAF that may be performed with high success. Also, it should be known that surgery may be performed effectively with low rates of complications. Because complications can develop in treated and untreated patients of all ages, follow-up should occur during the patient's lifetime.

Key words: coronary artery fistulae, angiography, transcatheter closure, surgery treatment.

Coronary artery fistulae are rarely seen, and they are usually asymptomatic.[1-5] The clinical relevance of CAF focuses mainly on the mechanism of 'coronary steal phenomenon', which causes myocardial functional ischaemia even in the absence of stenosis. Common symptoms of this phenomenon are angina, dyspnoea during physical exertion, and heart palpitations.[2-5] The suggested diagnostic approach is guided by the patient's symptoms and consists of a number of instrumental examinations such as the electrocardiogram, treadmill test, echocardiography, computed tomography and coronary angiography. Bacterial endocarditis is rarely observed in patients with CAF.[6,7] Small, asymptomatic fistulae can be followed by echocardiography, while treatment of symptomatic or large asymptomatic fistulae is recommended.[2-5,8,9] Coronary angiography is required to plan

✉ Kahraman Yakut
kahramanyakut@gmail.com

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optimal management of CAF. Invasive treatments, such as transcatheter or surgical approaches, are usually a reasonable choice for selected patients. The long-term results of these strategies are similar. Transcatheter closure is the treatment of choice of our clinic; however, surgery is performed in patients with fistulae when transcatheter closure is not feasible. Both treated and untreated patients should have a lifelong follow-up period.

Material and Methods

The demographic features, clinical findings, electrocardiographic, echocardiographic and angiographic data from 56 patients (33 males, 23 females) with CAF were reviewed, and the clinical courses of treated patients were examined. The approval for the study was obtained from our university ethics committee (KA18/208-26.06.2018). Associated lesions with CAF were described. Patients who had CAF associated with abnormalities related to the origin of the coronary artery, right ventricle-dependent coronary artery circulation, complex congenital heart defects (CHD) and patients with acquired CAF were excluded from this study. Coronary artery dilation, coronary steal syndrome, cardiac chamber enlargement, an increase in the ratio of shunt-induced flow (Qp/Qs>1.5), left ventricle hypertrophy and congestive heart failure were major treatment indications. Patients were followed regularly, and their condition was evaluated using electrocardiography and echocardiography. Two-dimensional (2D) and colour doppler images were obtained from parasternal long and short axis, apical four chamber, subcostal and suprasternal views with variable frequency transducers using the Vivid™ q cardiovascular ultrasound system (GE Healthcare 9900 Innovation Drive Wauwatosa, WI 53226 USA). The coronary artery was considered to be dilated when its diameter was measured by using echocardiography higher than +2 standard deviation (SD). The Artis zee biplane system (Siemens Healthcare Headquarters Siemens Healthcare GmbH Henkestr. 127 91052 Erlangen Germany) was used for catheter angiography.

Statistical analysis

Statistical analysis was performed using the SPSS software version 21 (IBM Analytics, Armonk, New York, USA). Descriptive statistics were expressed as mean ± standard deviation (SD) and frequency.

Results

The mean age at the time of diagnosis was 3.9 ± 4.6 years (range, one months to 18 years) and 5.6 ± 4.5 (range, 6 months to 14.5 years) years at the time of transcatheter treatment (Table I). The mean follow-up period was 7.4 ± 4.5 years (range, one to 17.5 years), while treated patients were followed for a mean duration of 8.3 ± 4.4 years (range, 2-15.5 years). Coronary artery fistulae were revealed during echocardiographic examination in 32 (57.1%) patients with murmur, while 15 (26.8%) patients were diagnosed previously with other CHD's, five (8.9%) patients experienced cyanosis, and four (7.1%) patients had chest pain. Although 24 patients (42.9%) had associated lesions, interventionally or surgically-treated patients had no associated CHD's patients who were treated with either surgical or transcatheter techniques did not have an associated intracardiac defect. Ventricular septal defect, atrial septal defect and tetralogy of Fallot were common types of associated lesions (Table II). For 17 patients (30.4%), the RCA was the most common site of origin for CAF. Meanwhile 11 patients (19.6%) had CAF that originated at the LMCA, and 11 patients (19.6%) had CAF originating at the LAD. Coronary artery fistulae originated from the circumflex coronary artery (Cx) in five patients (8.9%), while one patient had CAF that originated in both the RCA and LAD. The termination site of fistulae was identified by catheter angiography. In 23 patients (41.1%), the CAF terminated in the RV, while the left ventricle (LV) was the termination site in only one patient (1.8%). Other termination

Table I. Demographic characteristics of 15 patients treated with transcatheter and/or surgery.

Diagnosis	Treatment age	Qp/Qs	Tract of	Treatment	Complication	Device	Outcome
CAF	14.5 years	1.3	RCA-RV	Transcatheter	No	Coils	*Survive*
CAF	1 years	1.8	Cx-RV	Transcatheter	No	Vascular plug	*Survive*
CAF	10 years	1	RCA-LA	Transcatheter	No	Vascular plug	*Survive*
CAF	10 years	1.4	RCA-RA	Transcatheter	No	Vascular plug	*Survive*
CAF	4 years	1.2	RCA-RA	Transcatheter	No	Vascular plug	*Survive*
CAF	5.5 years	1.6	RCA-RV	Transcatheter	No	Vascular plug	*Survive*
CAF	5.5 years	1.4	LAD-RV	Transcatheter	No	Coils	*Survive*
CAF	3 years	1.8	RCA-RV	Transcatheter	Coil embolization	Coils	*Survive*
CAF	2 years	1.4	LMCA-RV	Transcatheter	Myocardial infarction	Coils+Tissue adhesive glue	*Survive*
CAF	6 months	3.2	RCA-RV	Transcatheter	No	Vascular plug	*Survive*
CAF	9 years	1	Cx-LA	Surgical (unsuccessful transcatheter)	No	-	*Survive*
CAF	6 months	2.4	RCA-RV	Surgical (unsuccessful transcatheter)	No	-	*Survive*
CAF	10 months	1.6	LMCA-RV	Surgical	RBBB	-	*Survive*
CAF	4 years	1.7	RCA-RA	Surgical	No	-	*Survive*
CAF	5 years	1	LMCA-LV	Surgical	No	-	*Survive*

CAF: coronary artery fistula, Cx: circumflex coronary artery, LA: left atrium, LAD: left anterior descending coronary artery, LMCA: left main coronary artery, LV: left ventricle, RA: right atrium, RBBB: right bundle branch block, RCA: right coronary artery, RV: right ventricle, Qp/Qs ratio: pulmonary to systemic blood flow ratio.

Table II. Additional congenital heart diseases associated with coronary artery fistula.

Congenital heart disease	N	%
TOF	4	7.1
VSD, ASD, PS	3	5.4
VSD, DCRV	3	5.4
ASD	3	5.4
PDA	2	3.6
BAV, AS, CoA	1	1.8
DSM, DAA	1	1.8
BAV, AS	1	1.8
VSD, PDA	1	1.8
VSD, ASD	1	1.8
DCRV	1	1.8
VSD	1	1.8
TA	1	1.8
PS	1	1.8
Total	24	42.9

AS: aortic stenosis, ASD: atrial septal defect, BAV: bicuspid aortic valv, CHD: congenital heart disease, CoA: coarctation of the aorta, DAA: double aortic arch, DCRV: double chamber right ventricle, DSM: discrete subaortic membrane, PDA: patent ductus arteriosus, PS: pulmonary stenosis, TA: truncus arteriosus, TOF: tetralogy of Fallot, VSD: ventricular septal defect.

sites were the pulmonary artery in 16 patients (28.6%), the right atrium (RA) in three patients (5.4%), and the left atrium (LA) in two patients (3.6%) (Table I). Cardiac catheterisation wasn't performed in four patients because they had small and asymptomatic fistulae. These patients were followed regularly. Initially small fistulae were evaluated by cardiac catheterisation. However, small fistulae couldn't be evaluated by catheterisation in seven (12.5%) patients because selective coronary angiography was not performed. Cardiac catheterisation was not performed in the last four patients with small and asymptomatic fistulae, and instead they were followed periodically using echocardiography. Small fistulae grew over time in three patients; two of these patients were treated with transcatheter occlusion while one underwent surgery (12.5 years, 5 years and 4.5 years after receiving their diagnoses respectively). Fifteen patients (26.8%) were treated by either transcatheter or surgical means. Forty-one patients (73.2%) needed no treatment, since their fistulae were small, asymptomatic, and had low shunting. The mean pulmonary to systemic blood flow (Qp/Qs) ratio among treated patients was 1.6 (min: 1, max: 3.2). The most common treatment indication was coronary artery dilation, which was found in 28.6% of patients, followed by coronary steal syndrome in 16% of patients. Other indications included the enlargement of cardiac chambers among 14.3% of patients, a Qp/Qs ratio greater than 1.5 in 12.5% of patients, and the presence of congestive heart failure symptoms in 9% of patients. A transcatheter approach was preferred to treat CAF in ten patients. Transcatheter treatment failed in two patients who had tortious and narrow fistulae because the device could not be placed distal to the fistulae. Instead, surgical correction was performed in these patients.

Coronary artery fistulae were occluded interventionally in six patients using vascular plugs (Fig. 1) (Cera™ Lifetech Scientific

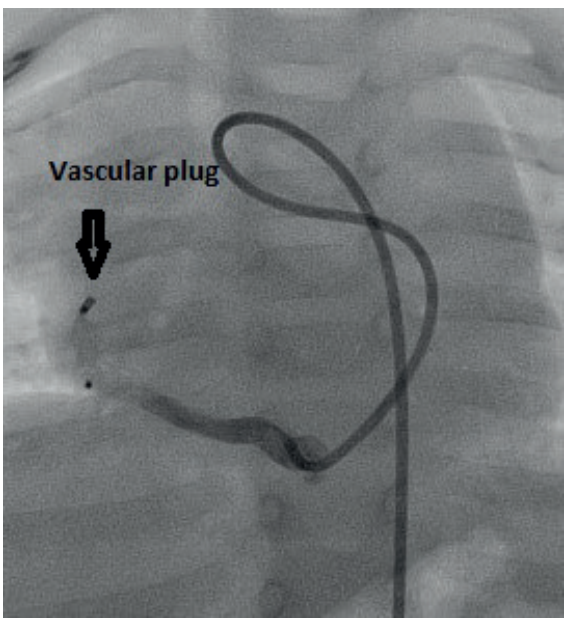

Fig. 1. A 6x7 mm vascular plug (Cera™ Lifetech Scientific Corporation, China) placed in the distal part of the dilated circumferential coronary artery of a one year old child is seen. No residual shunt was observed in the control angiogram.

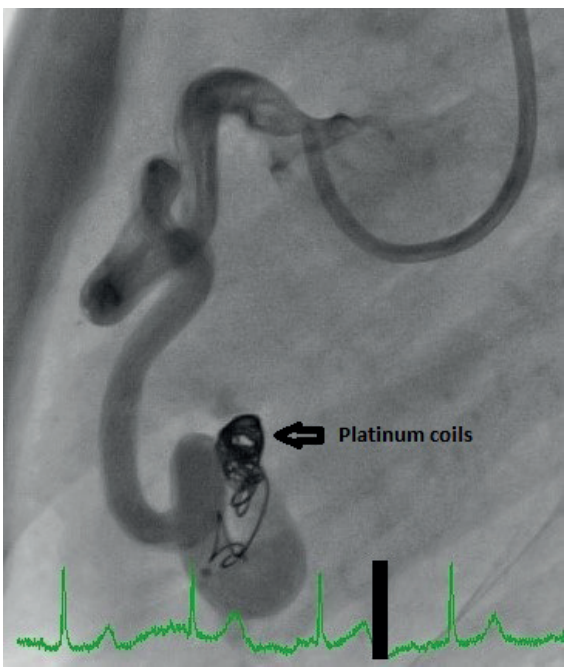

Fig. 2. In a 14.5 year-old patient, five platinum coils placed in the right coronary artery from the site where the coronary artery is dilated and curved and the distal part drained in the right ventricle is seen. No residual shunt was observed in the control angiogram.

Corporation, China), (Amplatzer™ Vascular Plug, St. Jude Medical, USA). Platinum coils (Boston Scientific. USA) were used to occlude the CAF in three patients (Fig. 2), while the platinum coil plus tissue adhesive glue was used in one patient. Five patients were successfully treated with surgery, and transcatheter treatment attempted in two patients before surgery A large tunnel-like structure between the LCA and RV in one patient, between the RCA and RV in one patient, and between the RCA and RA in other two patients were observed. Direct surgical corrections were performed in these four patients. Different techniques for surgical closure of coronary fistulae have been used in this subset of patients. In a patient with favourable morphology, the fistula could be closed using simple ligation. However, it should be noted that this approach was not recommended. In four patients, fistulae were closed using cardiopulmonary bypass and cardioplegic arrest. In patients with coronary to RA fistulae, the communication was visualised after the onset of cardiopulmonary bypass but before cardioplegic arrest. This sequence was helpful for identifying the right atrial opening of the fistula. In another patient with a RCA-RV fistula and aneurysmal dilatation of the RCA, the orifice was sutured and closed primarily through the RCA, with identification of distal continuity and the orifice of the acute margin branch. Transesophageal echocardiography was used to confirm complete closure of the fistulae in most recent cases.

Early complications developed in two patients during the transcatheter procedure. At the beginning of the procedure, a 4mm x 3cm coil was embolized into the right ventricle and then into the left pulmonary artery. The procedure was continued by deploying a coil that was one size larger. Closure was successfully completed with using four coils. To remove the embolized coil which is in the left pulmonary artery, 5F sheat was replaced with 7F long sheat. With the help of microsnare catheter, the embolized coil was caught in the left pulmonary artery and was safely removed by taking into the long sheat. To close the CAF, five platinum coils were used in our second patient who has developed an early complication. The subsequent angiogram showed that the fistula was not completely closed and there was a significant amount of transition of contrast material. A flow-guided microcatheter (1.2 Fr Marathon catheter, Covidien, Mansfield, Massachusetts, USA) was navigated using a micro-guidewire (Mirage 8, Covidien) into the feeding artery as close as possible to the fistula. The microcatheter was washed with 5% dextrose. A 50% mixture of NBCA and Lipiodol (Guerbet LLC., Bloomington, Indiana, USA) was injected under digital subtraction angiography control. After correct penetration of the glue, the microcatheter was rapidly withdrawn. During this procedure, the electrocardiogram showed significant ST segment elevation. A control angiography was performed considering there may be myocardial infarction due to the reflux of the tissue adhesion material. The angiography demonstrated some degree of occlusion in the LAD and Cx branches with LAD edge irregularities, but the lumen of LAD and Cx were patent. Seven days after the procedure, coronary angiography was repeated and normal coronary flow patterns in the LAD and Cx were documented with previously-occluded branches and the persistence of LAD edge irregularities. Ejection and shortening fraction were measured using echocardiography as 52% and 24%, respectively, and echocardiography showed abnormalities in the apical and septal wall motion. This patient was diagnosed with myocardial infarction. He received streptokinase for three days, heparin for four days, and then acetylsalicylic acid (5 mg/kg/day) treatment for six months. During the last visit, echocardiographic examination showed normal cardiac function. Right bundle branch block (RBBB) was seen as an early surgical complication in one patient. RBBB due to surgical trauma improved spontaneously at the last follow-up. There were no late complications identified in treated patients. Medical records showed that 51 (91.1%) patients were followed regularly, while five patients (8.9%) were

lost during follow-up. No treatment-related deaths were observed. At the time of discharge, anticoagulant therapy was administered to only one patient who had a myocardial infarction. No residual shunting or recanalization was observed among treated patients in long-term follow-up.

Discussion

Coronary artery fistulae are usually congenital in nature, and represent approximately 0.2-0.4% of all congenital heart diseases.[3,4,9] It is reported that CAF commonly originates from the RCA, LAD or Cx and terminates in the right ventricle, right atrium and pulmonary artery.[1-4,9-11] The origin and termination sites for CAF in our patients were similar to those reported in the literature. Fistula-related complications tend to increase with age. Complication rates were reported to be 11% in patients younger than 20 years old, while these rates increased to 35% in patients older than 20 years old.[12,13]

Enlargement of the cardiac chamber, left ventricle hypertrophy, congestive heart failure, coronary artery dilation, dysrhythmia, angina pectoris, chronic myocardial ischaemia that causes myocardial dysfunction, papillary muscle dysfunction, valve regurgitation and cardiomyopathy are common complications reported in patients with CAF.[2-5,8] Our results were comparable to the literature, which showed that coronary artery dilation, coronary steal syndrome, enlargement in the heart chamber, and congestive heart failure were common complications that developed in our patients.

In the literature, associated lesions have been reported to be found in 20-60% of patients.[1-4,9] We described CHD in 42.9% of patients, which was in agreement with previous findings in the literature.

Echocardiography is a highly-useful, non-invasive imaging modality that can determine the extent of dilation in the heart chamber, the origin, size, and drainage sites of fistulae.[3-5,9,10,14] Angiography provides detailed information about fistulae such as size, course, origin and drainage sites, and it also may help illustrate stenosis and associated CHD.[3,4,15] Angiography may also be used in therapeutic intervention.[3,15] In addition to echocardiography and catheters, angiography was the main diagnostic tool used in our study. We also effectively employed transesophageal echocardiography to determine the origin site and the termination chamber of fistulae during surgical intervention.

In this study, CAF could not be confirmed in seven patients (12.5%) who were suspected to have fistulae based on echocardiographic images, because selective coronary angiography was not performed. It is difficult to predict how small fistulae will change over time; some may enlarge over time in some patients, while they may narrow and close spontaneously in others. In this study, CAF became larger over time among three patients; two of these CAF were occluded interventionally, while the other was closed surgically. We suggest that small fistulae do not require cardiac catheterisation if they are differentiated from abnormal origin of the coronary artery. However, they should be examined periodically by echocardiography because it is probable that they may enlarge and become symptomatic during the follow-up period.

It is well known that small CAF's can grow with age and may cause severe haemodynamic, thrombotic, or ischaemic complications during adulthood. Some authors reported that treatment of CAF in adulthood seems to be more difficult due to the increased tortuosity of the CAF.[16] Although it is controversial to treat asymptomatic patients with a small fistula, it is recommended to treat patients who have symptoms or large fistulae since complications may be observed frequently in these patients.[1-4,9] Since complications and late symptoms are likely in patients with significant shunting (Qp/Qs ratio>1.5), these patients should be treated soon after their diagnosis.[2,4,9,17] Improvement in fistula-related dilation of the coronary artery was reported in mid-long

term after treatment.[5] Early intervention is also important for myoproliferation, which helps restore normal calibration in the aneurysmal coronary segment. Many studies indicate that transcatheter intervention has a high success rate with less complications compared to surgery.[1-4,18-21] The major advantage of transcatheter closure is that the procedure is performed without cardiopulmonary bypass and median sternotomy, which prevents iatrogenic complications.[1-4] A shorter recovery time, reduced mortality rates, and lower cost are other advantages of this treatment. However, Collins et al.[22] showed that vessel tortuosity and the calibre of the lumen seem to limit delivery of the device.

A surgical approach for closure of CAF may be performed safely regardless of the patient's age or the size of the fistula.[23] Surgical closure is the treatment of choice when CAF is associated with congenital heart defects that also require surgery. Surgery offers an easier way to correct large aneurysms and proximal fistulae. The epicardial and endocardial approaches are two techniques that are used in surgical closure. The life expectancy of CAF patients is normal, with 9-19% recurrence after transcatheter treatment and 25% after surgical ligation.[10] The recurrence rate in published series varies significantly, from no incidence of recanalization in some studies, to 10-15% rate of reoccurrence in others.[1,24,25] Jama et al.[20] reported 30% of patients had residual shunting, but less than 1% of these patients needed reintervention. Transcatheter occlusion was the preferred method for treating CAF in our institution. Although the balloon occlusion test prolongs the procedure, it is valuable for identifying out the most distal functional branch of the CAF, but it cannot always be performed. In our clinic, fistulae were closed using the most distal part to prevent disrupting myocardial perfusion. There were no instances of thrombotic complications during the period of involution among the transcatheter-treated patients group, so none of the patients needed antithrombotic therapy. Surgery was another treatment option when transcatheter intervention failed, or when transcatheter device closure was unsuitable. Our success rate for transcatheter treatment of CAF was 83.3%.

Although anticoagulant recommendations are not available, anticoagulation after transcatheter closure is recommended in some studies.[1,26] In this study, anticoagulant agents were administered to one patient who experienced a myocardial infarction caused by tissue adhesive. However, the remainder of patients did not need anticoagulation therapy. We do not recommend routine anticoagulant therapy after a transcatheter procedure unless there is a certain indication.

The mean follow-up period of the treated group was 8.3 ± 4.4 years, and it was impressive that no patients had residual shunting or recanalization during this time. Late complications, such as thrombus formation and myocardial ischaemia, were not seen in this group. It is recommended that all patients with closed CAF be followed regularly, since residual shunting and recanalization continue to be potential dangers for them in the long-term.

The risk of complications related to transcatheter or surgical approaches is low. Dysrhythmia, changes in the ST-T segment, myocardial infarction, coronary artery spasm, fistula dissection and coil embolisation are some of the complications that may develop after treatment.[1,2,5,13,20,27] The mortality rate among patients with complications was reported to be 0-2%.[13,27] We reported two complications during the transcatheter procedure. One of them was coil embolisation, while another was myocardial infarction in the patient that received an injection of tissue adhesive. Based on this experience, we determined that tissue adhesive could translocated to and block the coronary artery, which may cause myocardial infarction. RBBB developed in one patient due to surgery and this improved spontaneously at the last follow-up. There was no procedure-related death, and no reinterventions required for residual shunting or recanalization in this study.

A retrospective study is a significant disadvantage in the collection and evaluation of data. The change of medical equipment according to technological developments during this period is a major limitation. Transcatheter closure of the fistulae is considered a limitation among a small number of patients. Considering we had data from 56 patients, this may be a factor to consider.

In conclusion, echocardiography is an adequate tool for monitoring small, asymptomatic and hemodynamically-insignificant fistulae. Large, symptomatic fistulae and fistulae that cause haemodynamic disturbance and dilation of the coronary artery should be treated. Transcatheter closure should be the treatment of choice for CAF since it offers a high success rate and a low rate of complications with a shortened hospital stay. In patients with fistulas that are not appropriate for transcatheter occlusion, surgery is an effective alternative.

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Original Article

Pigeon breeder's disease as a cause of hypersensitivity pneumonia in children

Tuğba Ramaslı Gürsoy[1], Zeynep Reyhan Onay[1], Tuğba Şişmanlar Eyüboğlu[2], Ayşe Tana Aslan[1], Öznur Boyunağa[3]

Departments of [1]Pediatric Pulmonology and [3]Radiology, Gazi University Faculty of Medicine, Ankara; [2]Department of Pediatric Pulmonology, Dr Sami Ulus Maternity and Children Research and Training Hospital, Ankara, Turkey.

ABSTRACT

Background and objectives. Hypersensitivity pneumonia is a complex condition due to exposure time, intensity, different clinical presentation, and treatment practices. We aimed to evaluate the patients that were diagnosed with hypersensitivity pneumonia (HSP) due to exposure to pigeons and a review of the literature for diagnosis and treatment of Pigeon Breeder's Disease (PBD) in children.

Method. Between the years of 2009-2018, patients who were diagnosed with HSP due to PBD were included in the study in a pediatric pulmonology department. Findings of our patients, treatments, and prognoses were compared with 17 articles in the literature about PBD in children.

Results. In a 9 year-period, 6 patients were diagnosed as HSP due to PBD. The mean age of the patients was 8.8 ± 5.4 years and the average duration of pigeon exposure was 60.1 ± 6.5 days. Precipitating antibodies were positive in 3 patients. In four cases, symptoms were resolved with only prevention of pigeon exposure. Two patients who had close contact with pigeons needed oxygen supplementation and steroid therapy.

Conclusion. Hypersensitivity pneumonia should be considered for the differential diagnosis of patients that present with respiratory distress, cough, fever, and weight loss. Prolonged exposure and close contact may worsen the clinical symptoms. In most cases, only exposure prevention is enough, while steroid therapy, oxygen support, and intensive care monitoring may be required in severe cases.

Key words: children, hypersensitivity pneumonia, interstitial lung disease, Pigeon Breeder's disease.

Hypersensitivity pneumonia (HSP), also called extrinsic allergic alveolitis, is a non-Ig E mediated immunologic disorder, which develops as a result of repeated inhalation of animal or plant antigens. This typically affects the respiratory tract and pulmonary parenchyma.[1] HSP was described as "farmer lung" in 1713 in cereal workers by Ramazzani.[2] The epidemiology of HSP is not definitively known.[3] Pigeon breeder's disease (6-20%) and farmer lung (1-19%) are the most common HSPs.[1,3]

In the pathogenesis of HSP, host sensitivity, immune system, and genetic factors play a role together with antigen exposure. After inhalation of environmental antigens, Th1-mediated type III and IV hypersensitivity reactions are observed in interstitium, alveoli, and the central and terminal airways.[4,5] Complaints such as cough, dyspnea, chest pain, fever, chills, sweating, weakness, myalgia, and headache can start hours or years after the antigen exposure.[5] Hypersensitivity pneumonias are defined in three forms as acute, subacute and chronic for clinical course. In acute form, symptoms such as fever, sweating, nausea, headache, muscle pain occur 2-9 hours after exposure and these

✉ Ayşe Tana Aslan
aysetugbapp@gmail.com

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symptoms regress within hours-days. Subacute form symptoms develop slowly in days-weeks, coughing and shortness of breath are prominent. Chronic form shows insidious onset in months, progressive cough and exertional dyspnea, fatigue and weight loss may be the dominant symptoms.[6]

Pigeon breeder's disease (PBD) is one of the most common causes of HSP and is the result of the inhalation of pigeon avian protein antigens.[6,7] Precipitating antibodies in the serum show exposure and sensitization to pigeon avian protein antigen.[8,9] In this study, we aimed to evaluate the children that were diagnosed as HSP due to PBD in a pediatric pulmonology department with a review on literature.

Material and Methods

Between the years of 2009-2018, all patients that were diagnosed as HSP due to PDB were included in the study in a tertiary pediatric pulmonology department. Clinical findings, laboratory and radiological data of patients were noted. Complaints, duration of complaints, contact history, physical examination, laboratory results, pulmonary function test (PFT), chest radiography and thorax CT findings, treatments, and prognosis were recorded. Acute phase reactants of all patients and arterial blood gas results with low oxygen saturation were evaluated. PFT was performed in patients older than 6 years. The presence of precipitating antibody against pigeon avian protein antigen was evaluated in all patients using Ouchterlony plaque method with gel precipitation technique. Thorax CT was performed in patients with auscultation findings on physical examination and infiltration on chest x-ray. The presence of ground-glass appearance, mosaic pattern and centrilobular nodules on thorax CT were found to be compatible with HSP.

All available studies about PBD in children from the literature were reviewed. Findings of our patients, treatments, and prognoses were compared to 17 articles in the literature about PBD in children.

Statistical analysis was performed using SPSS v.23.0 program (SPSS Inc., Chicago, IL, USA). The data was expressed as mean and standard deviation. The level of statistical significance was set at p-value of less than 0.05.

This report was performed using the principles of the Declaration of Helsinki and approval was granted by the Gazi University Hospital Ethics Committee (Date: 08/10/2018, Number: 740). Our study is a retrospective archival study. For this type of study formal consent is not required.

Results

In a 9 year-period, 6 patients were diagnosed as HSP due to PDB. The results of these 6 patients and 71 cases researched from 17 articles in the literature were evaluated.

Clinical features of cases with HSP

In our six patients, the mean age was 8.8 ± 5.4 years and the average duration of pigeon exposure was 60.1 ± 6.5 days. Four of them were female and two were male. All patients' fathers were breeding pigeons as a hobby or for economic reasons. Patients who bred pigeons at home and touched pigeons with their hands were evaluated as close contact. All results of the patients are summarized in Tables I and II. In the family of four patients, there were other patients that were followed up with the diagnosis of HSP due to PBD. All patients admitted with a cough and three patients had a high fever and shortness of breath. The mean duration of complaints was 28.6 ± 4.3 days. All cases were evaluated as subacute form. One of the patients was followed up with remission of lymphoma for 6 years and the other five patients had no chronic disease before these complaints. Tachycardia and low oxygen saturation were detected in case 4 and 5. These patients had widespread crackles and weight loss (4% and 5.5%) in the physical examination, case 4 had pulmonary hypertension, and none of them

Table I. Clinical features of 6 cases with HSP due to pigeon.

	Case 1	Case 2	Case 3	Case 4*	Case 5*#	Case 6
Age (year)	2.5	2.3	9	10.5	14	15
Type of exposure	Her father and uncle were breeding pigeons at home.	Her father and uncle were breeding pigeons at home.	Her father and uncle were breeding pigeons at home.	Her father was breeding pigeons at home.	His father was breeding pigeons near his home.	His father and uncle were breeding pigeons at home.
Duration of symptoms (days)	30	30	30	20	32	30
Form of hypersensitivity pneumonia	Subacute	Subacute	Subacute	Subacute	Subacute	Subacute
Symptoms	Cough	Cough	Cough, shortness of breath and high fever.	Cough, shortness of breath and high fever.	Cough, shortness of breath and high fever.	Cough
Physical examination	Normal	Normal	Normal	Tachycardia, low saturation, widespread crackles and weight loss	Tachycardia, low saturation, widespread crackles and weight loss	Normal

* These cases were contacted closely to pigeons (handling pigeons).
* # This patient was followed up with a diagnosis of remission of lymphoma for 6 years.

had clubbing. The patient was diagnosed with pulmonary hypertension by echocardiography. In cases 4 and 5, respectively, respiratory rates were 48 and 42 per minute, oxygen saturation were 66 and 82%. Case 4 and 5 that admitted with the findings of severe respiratory distress had longer and more contact with the pigeons than the other patients ($p > 0.05$).

Laboratory results, PFT, chest x-ray and thorax CT findings, treatments and prognosis of cases

All patients' mean erythrocyte sedimentation rate was 58 mm/h and it was high in all patients. The arterial blood gases of cases 4 and 5 with respiratory distress were normal. Three patients had precipitating antibody positivity and two patients could not perform PFT. The PFT was normal in 2 patients and two patients had restrictive pattern in PFT. Patients with a restrictive pattern in PFT results, respectively, FEV_1 71 and 68%; FVC 69 and 62%; FEV_1/FVC 102 and 109% were detected. Bronchodilator reversibility was not performed. Four of our patients had normal chest x-ray and two of them had diffuse interstitial infiltration on chest x-ray. The thorax CT was performed in only two patients and bilateral symmetric ground-glass appearance and multiple centrilobular nodules were detected. Other causes such as virus, bacteria including atypical bacteria, vasculitis, immunodeficiency, allergy, rheumatologic and other interstitial lung diseases were excluded. All patients were prevented from contact with the pigeons. Families took the pigeons away from their homes. Families were informed that their homes and clothes should remove the pigeon droppings and other products. Case 4 and 5 were hospitalized for approximately two weeks. Oxygen supplementation and inhaled corticosteroid therapy were given to two patients that were followed-up tachypnea and desaturation that had close contact with pigeons. Despite receiving inhaled corticosteroids, their clinical status did not improve and systemic steroid treatment was started. Pulmonary hypertension regressed in

Table II. Laboratory results, PFT, chest X-ray and thorax CT findings, treatments and prognosis of cases.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Serum precipitating antibodies	-	-	+	+	-	+
Pulmonary function test	-	-	Normal	Restrictive pattern	Restrictive pattern	Normal
Chest x-ray and thorax CT findings	Normal	Normal	Normal	Bilateral symmetric ground-glass appearance and multiple centrilobular nodules	Bilateral symmetric ground-glass appearance and multiple centrilobular nodules	Normal
Treatments	Allergen avoidance	Allergen avoidance	Allergen avoidance	Allergen avoidance Oxygen supplementation Systemic steroids	Allergen avoidance Oxygen supplementation Systemic steroids	Allergen avoidance
Prognosis	Complaints regressed at the first week follow-up	Complaints regressed at the first week follow-up	Complaints regressed at the first week follow-up	Progressive clinical and radiological improvement	Progressive clinical and radiological improvement	Complaints regressed at the first week follow-up

control echocardiography without additional treatment in case 4. In four patients, symptoms were resolved with only prevention of pigeon exposure. Case 4 and 5 showed improvement in chest x-rays and PFTs at follow-up.

Discussion

Hypersensitivity pneumonia is a complex condition rather than a single disease due to exposure time, intensity, different clinical presentation, and treatment practices.[27,28] Diagnostic criteria for the diagnosis of HSP have been developed, but none of these criteria have been confirmed. The disease is usually diagnosed by suspicion of contact history and complaints.[28,29] In differential diagnosis of hypersensitivity pneumonia, viral or bacterial pneumonias, vasculitis, immunodeficiencies, asthma, allergic, rheumatic diseases and other interstitial lung diseases should be investigated and excluded.[2,4] In cases where laboratory tests and radiological imaging do not benefit, the exposure history may be helpful in the diagnosis.

Pigeon breeder's disease is the most common HSP in children[21] and it was first described in 1967 by Stiehm et al.[10] In this study, we evaluated contact histories, physical examination findings, laboratory results, treatments and prognoses of our cases in the light of the literature. Our six patients were reviewed with 71 patients in 17 articles. Case series reporting the PBD are shown in Table III.[10-26] The literature on PBD was reviewed in detail: The results of 17 articles about pigeon breeder's disease in children and 71 patients were evaluated. The mean age of these patients was 10.1 ± 0.7 years. The contact frequency of 32 patients was unknown, while 20 of them had close contacts. The mean duration of complaints was 1.1 ± 1.8 years. One of these patients was asymptomatic and the other patients had complaints such as cough, shortness of breath, weight loss, fever, and growth retardation. In these studies, physical examination of 6 patients was normal and the physical examination findings of 32 patients were not given. Other patients had physical

examination findings such as bilateral crackles, respiratory distress, cyanosis, and clubbing. PFT could not be performed on 15 patients due to age or clinical status, and it was found normal in one case. Obstructive pattern was present in 5 patients, restrictive pattern in 35 patients, and both obstructive and restrictive in 15 patients. Only one of these patients had normal chest x-ray, while interstitial pneumonitis and reticulonodular changes were observed in other cases. In 52 patients, diffuse ground glass appearance and reticulonodular changes were detected on thorax CT. In 64 patients, the precipitating antibody positivity was shown. Bronchoalveolar lavage was performed in 36 patients, it was normal in one patient, it was inconclusive in another patient, and the other patients had lymphocytosis. Lung biopsy was performed in 11 patients and it was found compatible with HSP. Allergen avoidance was applied to all cases except 5 cases. Steroid treatment was given to 59 patients systemically and 5 patients as inhaled.

Breeding pigeons at home and handling pigeons have been considered as close contact. In our patients, two patients with more severe clinical findings (tachypnea, desaturation, respiratory distress, and pulmonary hypertension etc.) were found to be in close contact for a longer time with pigeons. As in our patients, it was observed in the literature that 20 of the patients were in closer contact with the pigeons and their clinical findings were heavier than the others.[10-12,14-16] In acute cases, cough, shortness of breath, and fever were observed.[13,19,25] In chronic cases, it was observed that findings such as weight loss and clubbing were added to these findings.[12,21,24] It is stated that PFTs in PBD could be obstructive, restrictive, or both patterns.[10,12-15,17,19-26] In our two patients, restrictive findings were found in the patients' PFTs.

Chest x-ray is normal in 30% of acute form cases. In our patients, it was observed that patients that have normal chest x-ray have normal physical examination findings. In the literature, only one of the 6 patients with normal physical examination findings had normal chest x-ray.[10,12,17,18,20] Bilateral diffuse homogeneous / heterogeneous areas of opacity and micronodular appearance in the middle and lower zones may occur. In HRCT, ground glass pattern, centrilobular nodules, mosaic pattern, emphysema and honeycombing appearance can be detected. In our patients, as in the literature, bilateral symmetric ground-glass appearance and multiple centrilobular nodules were detected in the thorax CT of two patients with severe respiratory distress.[15-18,20-26]

In the literature, although precipitating antibody positivity was not shown in seven of the 71 patients, antibody positivity was detected only in three of our cases. Physical examination and chest x-ray findings were normal in two cases with antibody positivity in our study. It was observed that in a case with severe respiratory distress findings, precipitating antibody was negative. Similarly, in the literature, although 7 patients had severe respiratory distress, the antibody was negative.[18,21,22,26] The main reason for this is that tests cannot show some of the responsible antigens. Pigeon breeder's disease was investigated in terms of the antibody, but no valuable information was found about treatment and prognosis.

In the literature, 34 of 36 patients' bronchoalveolar lavage samples had lymphocytosis consistent with HSP.[14,15,18,21,22,25] It was observed that the diagnosis of HSP due to pigeons was made according to contact history, clinical findings, chest x-ray, thorax CT and bronchoalveolar lavage results in patients that were negative for precipitating antibodies. Literature showed that lung biopsy was performed in 11 patients.[10,12,18,21,24] Lung biopsy can be performed in patients that cannot be diagnosed with clinical, physical examination, laboratory, and radiological findings. In our study, bronchoscopy was not performed in two patients that had severe respiratory distress because of the allergen avoidance and steroid treatment were required to be given rapidly. Parents of other four patients did not give permission for bronchoscopy probably because their clinical findings were mild. Lacasse et

Table III. Case series reporting the pbreeder's disease in the literature.[11-27]

Authors/ Year	n	Average age (years)	Type of exposure	Duration of symptoms	Symptoms	Physical examination
Stiehm et al (1967)	5	12.2	Case 1: He was breeding 2 pigeons at home for 1 month. Case 2: His father was breeding pigeons at home. Case 3: He visited the pigeon coop. Case 4: He spent an average of 6 hours per day with pigeons for 3 years. Case 5: He raised pigeons for a hobby for 3 years	4 months, 2 years, Acute onset, 1 year, 6 hours	Cough, shortness of breath, weight loss, fever	Bilateral crackles (4/5), hepatosplenomegaly -Normal (1/5)
Chandra et al (1972)	3	13	Patient's father was breeding pigeons at home.	6 weeks, 3 weeks, 5 weeks	Cough, shortness of breath, weight loss	Crackles and respiratory distress
Keith et al (1981)	4	15	The family moved to a home where pigeons were kept.	5 years*	Growth failure, chronic cough, clubbing, progressive dyspnea, Asymptomatic (1/4)	Moderate clubbing, bilateral crackles, -Normal (1/4)
Tsai et al (1998)	1	7	For the past two years, the pigeons had been living on the same floor as the patient's bedroom.	2 months	Dyspnea, anorexia, fever, nonproductive cough	Bilateral basilar crackles
Grech et al (2000)	5	10.8	Case 1: He was breeding pigeons at home Case 2: His father was breeding pigeons at home. Case 3: His father was breeding pigeons at home Case 4: His family were breeding pigeons at home Case 5: He visited the pigeon coop.	1 month, -, -, 5 months, 10 months	Cough, shortness of breath	Bilateral crackles, wheezing
F. Ratjen et al (2002)	9	10.7	-	3-8 weeks*	Fatigue, cough and dyspnea	-
Yalçın et al (2003)	1	5.5	Her father was breeding pigeons at home for many years.	8 months	Progressive cough, dyspnea, fever, lack of appetite	Clubbing, bilateral crackles
Nacar et al (2004)	5	11.4	Case 1: Her family were breeding pigeons at home for 6 months Case 2: His parents were breeding pigeons at home. Case 3: There was a family history of contact with neighbours' pigeons. Case 4: His father was breeding pigeons at home for two months. Case 5: Her family were breeding pigeons at home	3 months, 7 years, 2 days, 1 month, 3 weeks	Fever, weight loss, cough, shortness of breath, weakness	Crackles and respiratory distress, retractions -Normal (1/5)
Ettlin et al (2005)	3	5.5	Case 1: 2 weeks holiday on a farm Case 2: Holiday on a farm Case 3: Living next to a pigeon house	1.5-2.5 months*	Dyspnea, weight loss, fatigue, fever and mild signs of respiratory distress	Bilateral crackles -Normal (1/3)
Nacar et al (2005)	1	13	She was in contact with pigeons	1 month	Cough, shortness of breath, fever	Crackles and respiratory distress

* average duration of symptoms

Table III. Continued.

Chest x-ray findings	Pulmonary function tests	Serum precipitating antibodies	Thorax CT findings	BAL	Treatment	Improvement time
Diffuse intersitial pneumonitis (4/5) -Normal (1/5)	Obstructive pattern (1/4)	5/5	-	-	Allergen avoidance (5/5) Corticosteroid therapy (1/5)	Progressive clinical and radiological improvement
Miliary mottling throughout both lung fields	Normal (1/3)	+, +, +	-	-	Allergen avoidance (3/3), corticosteroid therapy (2/3)	Progressive clinical and radiological improvement
Bilateral interstitial Markings most prominent in the bases	Restrictive pattern	+	-	-	Allergen avoidance (4/4), corticosteroid therapy (4/4)	Complaints regressed in the follow-up
Widespread nodular interstitial pattern	Obstructive and restrictive pattern	+	_	_	Allergen avoidance	1 month
Reticulonodularshadowing	Restrictive pattern	+	-	Lymphocytosis+ (1/5)	Allergen avoidance (5/5), Corticosteroid therapy (5/5)	Complaints regressed in the follow-up
Diffuse reticular-nodular changes	Obstructive and restrictive pattern	+	Diffuse reticular-nodular changes	Lymphocytosis +, CD4/CD8 ratio is the normal range (9/9)	Allergen avoidance (9/9), corticosteroid therapy (9/9)	Progressive clinical and radiological improvement
Bilateral peribronchial thickening	-	+	Bilateral diffuse ground-glass appearance, disseminated centrilobuler densities and air entrapments in the lungs	-	Allergen avoidance, corticosteroid therapy	Progressive clinical and radiological improvement
Widespread nodular infiltrates, patchy infiltration	Obstructive and restrictive pattern (2/5)	+, +, +, +, +	Bilateral micronodular infiltrate	-	Allergen avoidance (5/5) (1/5 only allergen avoidance), Systemic steroids (3/5), Inhaled steroids (2/5)	Complaints regressed in the follow-up
Bilateral micronodular infiltrate	-	+, -,+	Diffuse noduler and patchy infiltration	Lymphocytosis+, decreased/ reduced/normal CD4/CD8 ratios	Allergen avoidance (3/3), treatment with oral prednisone (1-2 mg/kg/ day) (3/3), Inhaled steroids (3/3)	9 months, 6 months, 1 year
Nodular and patchy infiltrative appearance in both lungs	Obstructive and restrictive pattern	+	-	-	Allergen avoidance Systemic steroids Inhaled steroids	Complaints regressed in the follow-up (5 months)

Table III. Continued.

Authors/ Year	n	Age	Type of exposure	Duration of symptoms	Symptoms	Physical examination
Ozmen et al (2013)	4	8.5	Case 1: His father was breeding pigeons at home. Case 2: Pigeons had been bred at home for a long time. Case 3: They had been breeding pigeons at home for 7 years. Case 4: She was in contact with pigeons for the last two months	6 months, 2 weeks, 7 years, 2 months	Cough, wheezing, fever and exercise-induced cough, dyspnea, sputum production	-Crepitant crackles (2/4) - Normal (2/4)
Griese et al (2013)	23	9.8 ± 3 years	-	1.3 ± 1 months*	Chronic cough, dyspnea, cyanosis, clubbing, weight loss	-
Cardoso et al (2014)	3	11	Living in a rural area, patients had contact with pigeons and canaries.	5 months*	Dyspnea, fever, nonproductive cough	Crackles and respiratory distress
Bahçeci-Erdem S, et al (2015)	1	9	He lived above an Office in which birds and bird manure were merchandised.	4 months	Cough, dyspnea, chest pain	Crackles and respiratory distress
Tsangla, et al (2015)	1	12	They were breeding around 60 pigeons at home	3 years	Dry cough, dyspnea and weight loss	Cachexic, dyspneic, tachycardia, tachypnea, use of accessory muscles for respiration, pectus excavatum and bilateral basilar crackles
Woicka-Kolejwa et al (2017)	1	11	Several dozen years ago, the boy's grandfather had bred 400 pigeons in the attic of the house where the boy lived.	1,5 years	Persistent coughing and shortness of breath	Bilateral basilar crackles
Esenboga et al (2017)	1	16	Patient was a pigeon fancier and had close contact for 5 years (Patient was follow up as chronic granulomatous disease)	Since he had close contact with pigeons	Chronic cough and dyspnea	Crackles and respiratory distress

* average duration of symptoms

Table III. Continued.

Chest x-ray findings	Pulmonary function tests	Serum precipitating antibodies	Thorax CT findings	BAL	Treatment	Improvement time
Normal-paracardiac-perihilar involvement	Restrictive pattern (3/4)	+,+,+	Mosaic perfusion, ground-glass pattern and centrilobular micronodules	-	Allergen avoidance (4/4), inhaled fluticasonepropionate treatment (4/4)	Complaints regressed in the follow-up
Hilar lymph nodes, linear opacities, reticular opacities, nodular opacities, cystic opacities, bronchiectasis, ground glass pattern, increased attenuation consolidation	Restrictive pattern (22/23)	+ (21 of 23)	Hilar lymph nodes linear opacities reticular opacities nodular opacities bronchiectasis ground glass pattern, increased attenuation	Done in 17 children lymphocytosis +, CD4/CD8 ratio is elevated.	Allergen avoidance (18 of 23) Systemic steroids (20 of 23) Inhaled steroids (11 of 23)	17 healthy 5 improved 1 worse
Bilateral diffuse perihilar interstitial infiltrate	Obstructive pattern	_	Parenchymal thickening in both lungs, small nodules with ill-defined borders, and ground-glass changes	Lymphocytosis +, CD4/CD8 ratio is decreased	Allergen avoidance (3/3) Systemic steroids (2/3) Inhaled steroids (3/3)	Progressive clinical and radiological improvement
Patchy noduler infiltration	Obstructive pattern	+	Ground glass areas in both lungs	-	Allergen avoidance Systemic steroids	Progressive clinical and radiological improvement
Bilateral ground glass pattern	Restrictive pattern	+	Diffuse mosaic pattern and multiple ill-defined centrilobular nodular lesions in both upper lobes and interstitial thickening in the apical segment of left lower lobe.	Inconclusive	Allergen avoidance Oxygen supplementation Corticosteroid therapy Inhaled budesonide	Complaints regressed in the follow-up
Bad aeration and parenchymal– interstitial lesions with atypical changes in the hilar	Obstructive and restrictive pattern	+	Interlobular nodules in both the lungs, ground glass pattern	Reduced level of macrophages (57%) and increased percentages of neutrophils (31%) and eosinophils (6%) with 6% of lymphocytes.	Allergen avoidance, methylprednisolone 2 mg/kg/day up to a maximum of 60 mg/day	Improved and auscultatory changes resolved.
Pathcy ground glass appearance with fine	Obstructive and restrictive pattern	-	Patchy, vaguely centrilobular ground-glass opacification with air trapping areas, interlobular septal thickening and subpleural bullae	Normal	Allergen avoidance, Oxygen supplementation	Complaints regressed in the follow-up

al.[6] showed that appropriate cases could be diagnosed as HSP with history, clinical, physical examination findings and simple laboratory tests without the need for invasive procedures such as bronchoalveolar lavage and biopsy.

The most important factor in treatment is the removal of the antigen. Systemic or inhaled corticosteroid therapy has been used in cases with respiratory insufficiency. In the acute form, prognosis has usually been good and often the symptoms are reduced with the prevention of exposure. Fibrosis development determines prognosis in subacute and chronic forms.[27-30]

In our patients, four patients were treated only with pigeon exposure prevention. In the literature, allergen avoidance was not performed in 5 patients for social reasons and 7 patients were treated only by allergen avoidance.[10,11,13] It was observed that these patients were admitted with findings such as cough and fever without respiratory complaints and that thorax CT was not required because of the mild clinical and physical examination findings. Systemic steroids were used in the treatment of patients with respiratory failure, desaturation, ground glass appearance, and reticulonodular changes on thorax CT. In the literature, 5 patients were treated only with inhaled steroids and 59 patients with systemic steroids. It was shown that patients with severe clinical, physical examination, and thorax CT findings needed steroid treatment. In all cases in the literature, as in our patients, it was observed that the clinical findings regressed in the follow-up with avoidance of allergen, inhaled, or systemic steroid therapy.[10-26] In the literature, a patient with chronic granulomatous disease was given steroid treatment as well as hydroxychloroquine treatment.[26]

In conclusion, HSP should be considered in the differential diagnosis of patients that present with respiratory distress, cough, fever, and weight loss. Detailed contact history should be questioned. Close and long-term pigeon contact can lead to severe clinical findings. Serum precipitating antibodies may not be present in every patient and do not give information about treatment and prognosis. Radiological diagnosis may be helpful for diagnosis. Patients with mild clinical and radiological findings may be treated with pigeon exposure prevention, while steroid treatment, oxygen support, and intensive care follow-up may be necessary in severe cases.

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Original Article

Bee product efficacy in children with upper respiratory tract infections

Yılmaz Seçilmiş[1], Sibel Silici[2]

[1]*Division of Pediatric Emergency, Department of Pediatrics, Erciyes University Faculty of Medicine, Kayseri;* [2]*Department of Agricultural Biotechnology, Erciyes University Faculty of Agriculture, Kayseri, Turkey.*

ABSTRACT

Background and objectives. The most common infectious disease in children is acute upper respiratory tract infection (URTI). Many drugs, especially antitussive drugs, are used for symptomatic treatment. Bee products (propolis, royal jelly, and honey) have antiviral, antibacterial, and antioxidant properties, and they have synergistic effects with antibiotics. The aim of this study was to evaluate the effectiveness of a mixture of bee products in URTI in children.

Methods. The patients were divided into four groups consisting of two bacterial groups receiving either antibiotics or antibiotics + bee products and two viral groups treated with either placebo or bee products. Disease severity and improvement duration were assessed by the Canadian Acute Respiratory Illness and Flu Scale (CARIFS) Score.

Results. One hundred and four patients (59 male, 56.7%; 45 female, 43.3%) aged between 5–12 years were included in the study. Fifty patients (48%) were evaluated for bacterial infections and 54 (52%) for viral infections. Patients with viral infection receiving a mixture product showed earlier improvement, compared to placebo group. CARIFS scores were significantly lower in antibiotic + mixture group on day-2 and day-4, compared to antibiotic alone group ($p < 0.05$). None of the patients developed any reactions or side effects to the mixture product.

Conclusions. Bee products are effective in symptomatic treatment of upper respiratory tract infections. Bee products can be considered as a good treatment option because the available drugs already used for symptomatic treatment are not cost effective and can also have serious side effects in children.

Key words: honey, propolis, royal jelly, upper respiratory tract infection.

The most common infectious disease in children is acute upper respiratory tract infection (URTI).[1] Uncomplicated infections typically cause runny nose and obstruction, fever, throat pain, and cough.[2] URTIs can be of either viral or bacterial origin, whose incidence markedly increase in winter months, threatening public health.[2,3] Many drugs, especially antitussive drugs, are widely used for symptomatic treatment.[4] Numerous adverse effects have been reported in children under 6 years using antitussive, decongestant, and antihistamine drugs.[5-8]

The Canadian Acute Respiratory Illness and Flu Scale (CARIFS) Score was established for the first time in a study involving 220 children in 2000, in order to evaluate the severity and duration of the infection objectively. There are 18 different questions related to URTI symptoms on the scale, as families will be classified according to its seriousness.[9] Honey has antibacterial and antiviral effects and is recommended by the World Health Organization (WHO) for symptomatic treatment, primarily for cough in URTIs.[10-12] Propolis and royal jelly are bee-derived products. As a positive effect, they reduce bacterial motility and synergistically

✉ Yılmaz Seçilmiş
yildosec@hotmail.com

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increase antioxidant activity with their honey component.[13,14] There are studies in children using honey or propolis together or separately for URTIs.[15-18]

The aim of this study was to determine the effectiveness of a mixture of bee products (honey, royal jelly, propolis), as they are inexpensive (the price of the product is about one dollar), reliable, and have been used for many years for both symptoms and infection progression in URTI-infected children. To the best of our knowledge, it is the first study in which three different products were used, so that viral and bacterial infections could be evaluated separately.

Material and Methods

The study was performed prospectively. A randomized, double-blind, placebo-controlled trial procedure was used, which included two hundred patients aged 5-12 years who were diagnosed with viral and bacterial tonsillopharyngitis at Erciyes University Mustafa Eraslan-Fevzi Mercan Children's Hospital and had complaints such as fever, sore throat and a cough, during the period from 1 October 2015 to 29 February 2016. Group selection was done completely randomly by computer assignment. The computer program randomly decided a patient's allocation into one of the groups.

Fifty patients were included in four groups of volunteers of similar age range and gender. The placebo group (control) received starch and brown sugar in equal amounts with a mixture of bee products, which was prepared by a commercial company. Ninety-six patients who did not return the case form, who did not attend the control examination, who missed the case form, who did not use appropriate treatment were excluded from the study. Of the remaining 104 patients, 59 (56.7%) were male and 45 (43.3%) were female. Eleven (11.4%) patients were 5 years old, 8 (8.3%) were 6 years old, 14 (14.6%) were 7 years old, 16 (16.6%) were 8 years old, 14 (14.6%) were 10 years old, 12 (12.5%) were 11 years old, and 11 (11.4%) were 12 years old. The products given to all patients were numbered and given in boxes of ten. For this reason, neither the doctor nor patient knew what product was used. Informed consent was received from all families.

Clinical findings and follow-up

At the time of admission, a detailed medical and allergy history of each patient was taken; it included school absenteeism, history of allergy, the number of illnesses per year, smoking at home, parents' school degrees and ages, and the number of siblings. We recorded the physical examination findings (including height and weight) and filled out the case form.

We delineated the clinical findings of each case, with CARIFS score tables (for 10 days) given to each set of parents who were included in the study. In this scoring, parents were asked to fill out these forms by asking 18 questions that included presence of a sore throat, muscular pain, fever, cough, nasal congestion or runny nose, headache, poor appetite, insomnia, irritable, unwellness, tiredness, excessive crying, need for extra care, clingy, vomiting, uninterested, lack of willingness to sleep and unamenable to play. When the complaints began, we also asked whether the child received any medication before treatment, and if any side effects occurred during that time. The doctor was seen on the first day and the fever was recorded. The answers to each question (no problem=0, little problem=1, medium problem=2, and large problem=3) were considered.

Laboratory tests

Five ml blood samples were taken from all subjects: C-reactive protein (CRP), a complete blood count (CBC), and erythrocyte sedimentation rate (ESR) were studied. The tests were carried out at Erciyes University Mustafa Gündoğdu Central Laboratory.

Bacterial and viral analyses

A throat culture for diagnostic purposes was taken from each patient. Sheep bloody agar was used for growing beta hemolytic streptococcus group A, as it is frequently encountered as the etiology of URTIs. Nasal swab polymerase chain reaction (PCR) and Epstein-Barr viral capsid antigen immunoglobulin M were used to detect a viral etiology. Patients with cryptic tonsillitis, leukocytosis, and elevated CRP (plus combining these three findings) were evaluated, as bacterial infections were included in the study. Patients without bacterial infections who had normal throat cultures were excluded from the evaluation. Patients were initially divided into two groups, i.e., those with both viral and bacterial infection.

In the group of bacterial infections, half the patients took antibiotics and the other half took antibiotics and a mixture of the products. Half of the viral infection group was given a placebo; the remaining half took a mixture product. A total of 104 patients were divided into four groups.

We used paracetamol at a dose of 10 mg/kg for 6 hours or ibuprofen at a dose of 5 mg/kg for 8 hours when the body temperature exceeded 38°C while medical treatment was applied in the groups.

The mixture products were consumed once a day for 10 days (20 g/day for children under 30 kg, 40 g/day for children over 30 kg). A jar mixture product consisted of 25 ml. The mixture was 96.7% honey, 3% royal jelly, and 0.03% propolis. The maximal moisture content of the honey was determined to be 20%, the maximum free acidity was 50 mEq/kg, the hydroxymethyl furfural (HMF) value was a maximum of 40 mg/kg, the fructose and glucose content was a minimum of 60%, and the sucrose content was a maximum of 5%. Antibiotics and pesticides were analyzed for honey, royal jelly, and propolis to ensure they did not exert any residual risk. The research lasted 10 days, with phone calls made to parents every three days.

The study was approved by the Ethics Committee of Erciyes University (07.03.2014, No. 2014/140).

Statistical analysis

SPSS for Windows, v. 21.0 was used for statistical analysis. Normal distribution of numerical variables was evaluated by Shapiro-Wilk normality test and Q-Q graphs. Descriptive statistics were given as number of units (n), percentage (%), mean ± standard deviation, mean ± standard error, median (25^{th} -75^{th} percentiles). Comparisons between the groups were performed with one-way analysis of variance (ANOVA) analysis for the normal distribution of maternal age, fever, hemoglobin, hematocrit, and platelet count variables. If there was a difference in the ANOVA results, Tukey HSD test was used as a post hoc test. Comparisons between the groups were performed with Kruskal-Wallis analysis for the non-normal distribution of the number of siblings, school absenteeism, number of illnesses per year, body weight, laboratory parameters such as CBC, CRP, anti-streptolysin O (ASO) titer and ESR. The Dunn-Bonferroni test was used as a post hoc test in case of a difference between the Kruskal-Wallis analysis. Two-way analysis of variance was used for repeated measurements of general linear models in comparison of time scores of the groups. Bonferroni test was used as a multiple comparison test. A p value <0.05 was considered statistically significant.

Results

Two hundred patients were included in the study, but 104 score tables were evaluated (52%), including fifty-nine males (56.73%) and 45 females (43.27%). Those who were admitted to Erciyes University Mustafa Eraslan-Fevzi Mercan Children's Hospital between 1 October 2015 and 29 February 2016 were included in the study. Complaints identified in patients diagnosed with URTIs were fever, cough, sore throat, vomiting and nasal obstruction.

The analysis of the answers about the secondary factors revealed that 78.1% of patients' parents did not work and 34.7% of them were primary school graduates. In addition, 77.1% of patients were found to have no allergies, but 53.5% were around smokers at home. Patients treated with the mixture product had no reaction or side effects. CBC and CRP values were measured to give an etiological evaluation to patients in the study. Laboratory values on the first day of admission to the hospital are given in Table I. There was no statistically significant difference between the groups in terms of number of siblings, school absenteeism, number of URTIs for one-year, maternal age, fever, and body weight ($p > 0.05$; data are not shown). CRP, WBCs, and neutrophil values were found to be higher in patients with a bacterial infection ($p < 0.05$); platelet count was significantly higher in patients with viral-based URTIs ($p < 0.05$).

The scoring table for 10 days is presented in Table II. Measurement results of the general linear model were statistically significant ($p < 0.05$). Clinical severity and complaints at the beginning of treatment period were similar in all four groups ($p > 0.05$). In the following days, however, in bacterial infection groups, a statistically significant decrease was found in the clinical score on the 2nd and 4th days in the group that received the mixture product and antibiotic, compared to antibiotic alone ($p < 0.05$). In the viral infection groups, while there was no statistically significant difference in the clinical score at the beginning of the treatment, statistically significant differences were found between the patients who received placebo and mixture products from the 4th day until the last day of the treatment ($p < 0.05$).

Discussion

In this study, we included children between the ages of 5 to 12 years, who presented to our pediatric emergency department with URTIs. The mixture product group showed rapid improvement in their symptoms according to CARIFS scoring, compared to the groups not using it. This finding was found to be more significant with mixture-product users with viral infections.

In the viral infection group, the symptoms of the URTI continued at a similar level for 10 days while using the placebo product, as there was no satisfactory improvement for parents and patients. There are studies reporting the effectiveness of honey, royal jelly, and propolis individually for symptomatic improvement of URTIs.[18,27,28]

In 2007, Paul et al.[15] reported a significantly better treatment with honey versus treatment with placebo among 100 children with

Table I. Laboratory parameters according to infection and treatment groups.

Laboratory parameters	Infection and treatment groups					
	Bacterial infection			Viral infection		
	Antibiotic + Mixture (n=23)	Antibiotic (n=27)	p-1	Mixture (n=25)	Placebo (n=29)	p-2
Hemoglobin, g/dl	12.94 ± 1.3	12.91 ± 0.9	0.954	13.04 ± 0.8	12.81 ± 0.7	0.401
White blood cell, $x10^3/mm^3$	11.4 (5.9-16)	14.4 (6.9- 16.2)	0.809	7.2 (4.6-10)	8.01 (5.1-11.6)	0.961
Neutrophil, $x10^3/mm^3$	7.3 (4.5-12.4)	10.8 (4.1-12.6)	0.919	4.1 (2.1-6.7)	4.32 (3.08-7.53)	0.889
Lymphocyte, $x10^3/mm^3$	1.79 (1.42-2.41)	2.04 (1.34-2.99)	0.227	2.0 (1.38-2.74)	1.94 (1.42-3.09)	0.842
Platelet, $x10^3/mm^3$	300 ± 105	299 ± 66	0.973	250 ± 93	231 ± 54	0.490
C-reactive protein, g/L	25.8 (11.1-56.2)	20.9 (11-56.8)	0.995	3.2 (3.27-12.6)	10.7 (3.48-37.7)	0.232
Sedimentation, mm/hour	12 (7-20.7)	14.5 (5.2-34.2)	0.511	15 (8-28.5)	14.5 (8-22.5)	0.576

Data are presented as mean ± standard deviation or median (25^{th} percentile - 75^{th} percentile) as appropriate.
p-1: comparison between "antibiotic + mixture" and "antibiotic" groups in bacterial infection
p-2: comparison between "mixture" and "placebo" groups in viral infection

Table II. The Canadian Acute Respiratory Illness and Flu Scale (CARIFS) Score according to infection and treatment groups for 10 days.

Days	Infection and treatment groups					
	Bacterial infection			Viral infection		
	Antibiotic + Mixture (n= 23)	Antibiotic (n= 27)	p-1	Mixture (n= 25)	Placebo (n= 29)	p-2
1	21.2 ± 2.8	26.5 ± 2.62	0.176	17.3 ± 2.0	22.4 ± 2.9	0.152
2	16.0 ± 2.5	25.9 ± 3.2	0.017*	13.2 ± 2.1	19.7 ± 2.9	0.071
3	11.9 ± 2.2	18.8 ± 2.7	0.053	10.1 ± 2.1	16.1 ± 2.9	0.093
4	10.1 ± 2.1	17.0 ± 2.4	0.035*	6.2 ± 1.2	15.9 ± 2.6	0.001*
5	10.7 ± 2.7	13.8 ± 2.3	0.399	5.0 ± 1.2	12.4 ± 2.3	0.005*
6	7.3 ± 2.1	12.5 ± 2.3	0.088	5.2 ± 1.3	11.5 ± 2.4	0.020*
7	6.2 ± 1.7	9.5 ± 1.8	0.195	4.5 ± 1.1	12.4 ± 2.6	0.005*
8	6.6 ± 1.8	7.6 ± 1.7	0.678	4.1 ± 1.0	12.3 ± 2.7	0.004*
9	5.1 ± 1.7	5.7 ± 1.3	0.791	2.8 ± 0.9	10.5 ± 2.7	0.005*
10	3.5 ± 1.0	4.3 ± 1.2	0.600	1.8 ± 0.7[a]	9.9 ± 2.6	0.003*

Data are presented as mean ± standard error.
p-1: comparison between "antibiotic + mixture" and "antibiotic" groups in bacterial infection
p-2: comparison between "mixture" and "placebo" groups in viral infection
*: p <0.05

URTIs, showing significant improvement of the symptoms of cough, although similar results were attained as compared with dextromethorphan.

Oduwole et al.[19], in a study on honey use in URTIs dated 2014, reported that honey was effective against cough compared to placebo treatment and had a similar efficacy with dextromethorphan. In our study, three bee products were used as a mixture: when compared with the placebo and bacterial groups using antibiotics, not only the cough, but all symptoms (i.e. CARIFS score) improved in a significantly shorter period. Yuksel et al.[20], as indicated by a review published in 2016, found that a protective mixture of royal jelly, honey, and propolis in specific ratios was also very effective, with antibacterial, antiviral, and anti-inflammatory properties.[15] In 2004, Cohen et al.[21] conducted a placebo-controlled double-blind study with 430 patients aged 1-5 years. They used propolis and echinacea as a mixture product and at a 12-week follow-up, a significant decrease in URTI frequency and symptoms was found. In the same study, it was reported that the duration of fever, the use of antibiotics and antipyretics, and recurrent visits to the doctor were significantly reduced.[21] In our study, using all three bee products, we obtained effective results in the bacterial infection and viral infection groups. These findings support the study of Cohen et al.[21] in terms of antibacterial and antiviral activity.

El-Shouny et al.[22] found accelerated healing in 2-5 days in forty-one pediatric patients with URTIs, due to beta-hemolytic streptococcus throat infections when using propolis. Krol et al.[23] and Speciale et al.[24] found that in vitro studies showed synergistic effects between propolis and antibiotics.

Mirzoeva et al.[25] in a similar study in 1997, found combined effects with propolis and antibiotics, especially penicillin G and ampicillin for URTIs. At the same time, they also found that synergistic efficacy increased with increasing doses of propolis.

In our study, the rate of infection recovery was found to be higher in the group receiving antibiotics and mixture products than those that received antibiotics alone. The results found in our study were similar to the studies of Krol[23] and Mirzoeva.[25]

This study has some limitations. Initially, 200 patients were planned to be included in the study, but it was completed with 104 patients due to patient-related and technical issues.

Despite known adverse effects of antibiotics for treatment of URTIs, there are drugs like dextromethorphan, which are used to treat coughs (although they are no more effective than placebo); they are known to have numerous side effects and a higher cost, so patients decide to use herbal and bee products.[26]

This study has shown the effectiveness of a mixture of bee products for URTIs in children. These functional products are not only inexpensive, but have a number of beneficial effects. For this reason, we think that a mixture of bee products will be useful as an alternative treatment in URTI treatment in children.

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Case Report

A rare cause of secondary hemophagocytic lymphohistiocytosis: systemic loxoscelism

Tuğba Erat[1], Aysun Yahşi[1], Cem Çanakçı[2], Aybike Korkmaz[2], Ceyda Karahan[2], Talia İleri[3], Savaş Serel[4], Huban Atilla[5], Nihal Kundakçı[6], Halil Özdemir[1], Erdal İnce[1], Ergin Çiftçi[1]

Divisions of [1]Pediatric Infectious Diseases, [3]Pediatric Hematology and Oncology, [2]Department of Pediatrcis, Departments of [4]Plastic Reconstructive and Aesthetic Surgery, [5]Ophthalmology and [6]Dermatology, Ankara University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Loxoscelism is caused by the bite of a specific spider type called the Loxosceles genus. In Turkey, most cases are seen after *L. rufescens* bites. Clinical manifestation of the bites ranges from local cutaneous reaction to severe ulcerative necrosis. Systemic loxoscelism may also occur.

Case. Herein, we report a previously healthy five-year-old male patient who developed a secondary hemophagocytic lymphohistiocytosis after a presumed brown spider bite. He was treated with dexamethasone. Within the following 14 days, hemophagocytic syndrome resolved. Local hyperbaric oxygen therapy was applied to the necrotic areas.

Conclusion. Secondary hemophagocytic lymphohistiocytosis may develop after systemic loxoscelism. In the presence of persistent fever, hepatosplenomegaly and laboratory findings this clinical entity should be kept in mind.

Key words: hemophagocytic lymphohistiocytosis, necrotic arachnidism, systemic loxoscelism, violin spiders.

Loxoscelism is caused by the bites of Loxosceles genus spider, generally known as brown spiders or violin spiders. They have a worldwide distribution, predominantly found in the hot climate and tropical areas. Loxosceles recluse is the best known Loxosceles spider in the world, especially in the United States. *L. rufescens* is common around entire Europe and Mediterranean countries. In Turkey, most of the cases are caused by *L. rufescens*.[1]

The clinical condition caused by Loxosceles spider bite is called loxoscelism. In cutaneous loxoscelism, local skin reaction develops at the bite site and varies from mild and local to severe ulcerative necrosis with eschar formation. The ulcerative necrosis may develop due to the enzymes secreted by the spiders' venom, including hyaluronidase, esterase, alkaline phosphatase, and sphingomyelinase D. Severe systemic reactions such as fever, chills, nausea, arthralgia, myalgia, hemolysis, coagulopathy, and organ dysfunction rarely develop. These clinical manifestations are called systemic loxoscelism and is mostly encountered in children.[2-4]

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome characterized by severe inflammation caused by pathologic immune activation and is nearly always fatal if left untreated. HLH is classified as primary and secondary. Primary HLH, an inherited form of hemophagocytic lymphohistiocytosis syndrome, is a heterogeneous autosomal recessive disorder and found to be more

✉ Tuğba Erat
tugbacancan84@hotmail.com

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prevalent with parental consanguinity. Secondary HLH usually refers to patients who have no known underlying genetic cause of HLH. In secondary HLH, pathologic immune activation is often precipitated by an infection, toxins, rheumatologic disorder, or malignancy. The guidelines of HLH was published by Henter et al.[5] in 2004 and diagnostic criteria of HLH include fever >38.5°C, splenomegaly, bicytopenia, hypertriglyceridemia and/or hypofibrinogenemia, and hemophagocytosis. In HLH-2004, the following three additional criteria were introduced; low/absent NK-cell-activity, hyperferritinemia, and high- soluble interleukin-2-receptor levels. Altogether five of these eight criteria must be fulfilled unless family history or molecular diagnosis is consistent with HLH.[5,6] In Turkey, HLH associated with toxins of loxoscelism has not been reported. We reported a previously healthy five years old male patient who developed secondary HLH from a presumed brown spider bite. Informed consent was obtained from the parents.

Case Report

A previously healthy five years old boy living in the Zonguldak area of the Northern region of Turkey had a red spot, like an insect bite, just above the left eyebrow, which was noticed by his parents (Fig. 1). Three days after the red spot was seen, the erythema expanded, and edema occurred on the left eyebrow and eyelid. He was hospitalized at a local hospital's dermatology service on the second week (Fig. 2). Ampicillin-sulbactam, amikacin, vancomycin, ceftriaxone, metronidazole, methylprednisolone, and pheniramine treatments were initiated. He received the treatment for five days; however, erythema and edema continued to expand. Originating from the first red spots localization, skin necrosis developed above-left eyebrow. He was referred to the city's university hospital after five days. Systemic penicillin, metronidazole, clindamycin, gentamicin, streptomycin, ciprofloxacin, fluconazole, methylprednisolone, pheniramine, ibuprofen, and local therapy, including moxifloxacin eye

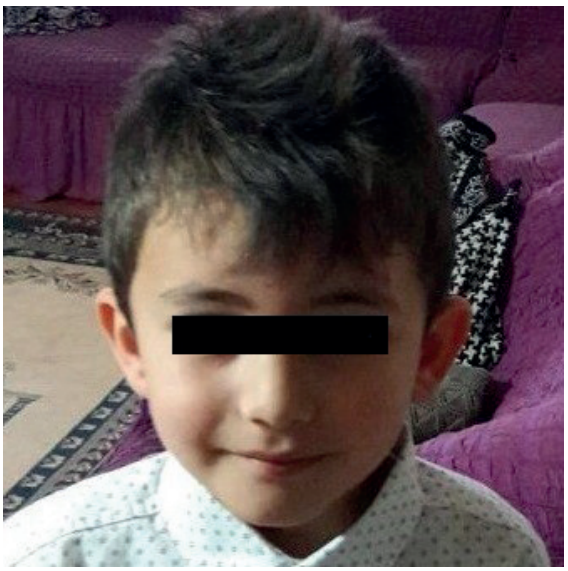

Fig. 1. Spider bite lesion above the left eyebrow (photo taken by the family).

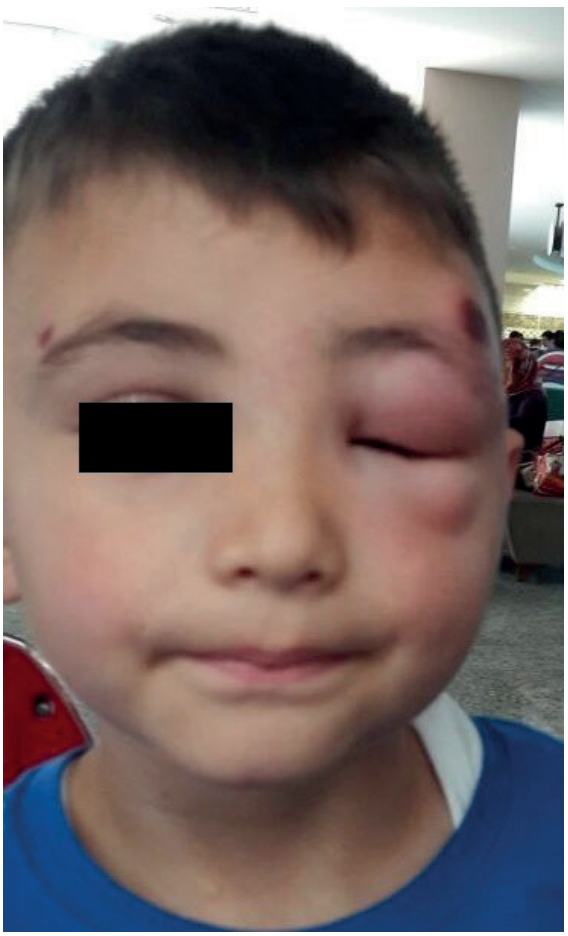

Fig. 2. Left eyebrow lesion (photo taken by the family before admission to regional hospital).

drop, bacitracin, and neomycin pomade, were initiated. A biopsy was obtained from the necrotic area, and gram positive cocci and yeast cell presence was reported. Tests were negative for tuberculosis and tularemia. In spite of the treatment, erythema, edema and necrosis expanded, and fever developed. After fourteen days, the patient was referred to our hospital with a diagnosis of treatment-resistant preseptal cellulitis.

Upon admission, the patient had an acute onset of malaise, nausea, and fever. The patient's vital signs were normal, except for a body temperature of 38.5°C. On clinical examination, the patient had bilateral severe eyelid edema that was more prominent on the left side, a superficial necrotic crust at above-left eyebrow (about 6 cm in diameter), erythema of bilateral eyelids and left side of the face and neck (Fig. 3). Ophthalmological examination revealed 0.7/0.7 visual acuity for both eyes, with normal anterior and fundus findings as well as regular motions with normal pupillary reflexes. Palpable noninflamed lymph nodes were present in left submandibular and cervical areas. Liver and spleen were two centimeters palpable under arcus costalis. Initial laboratory evaluation revealed high ALT and AST, and slightly low white-cell count and platelet count (Table I). Computer tomography scan revealed; periorbital, facial, and submental diffuse subcutaneous edema that was more prominent on the left side. The inflammation was considered as preseptal cellulitis (orbital cellulitis was excluded). The left parotid gland was also under the effect of inflammation. Left cervical, submandibular, and supraclavicular lymph nodes were palpable. Acute thrombosis was present at the left superficial temporal vein. When the medical history of the patient was revised in detail, the parents remembered that they found a little brown spider at home, and mentioned that the patient had a red spot like an insect bite approximately one centimeter above the left eyebrow. Based on the history and presenting symptoms, the etiology of his disease was consistent with a brown spider bite causing systemic loxoscelism.

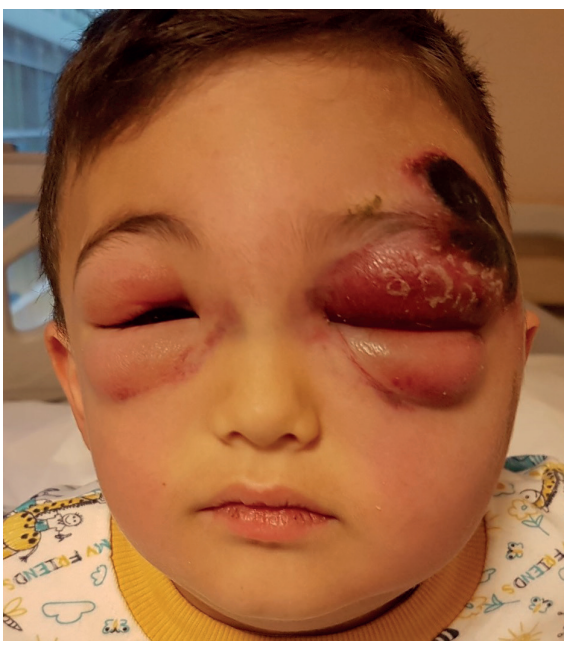

Fig. 3. Necrotic lesion on the left eyebrow and diffuse eyelid and facial edema (photo taken during our referral in our hospital).

The patient received a variety of antibiotics, including vancomycin, meropenem, and liposomal amphotericin B. On the 10th day, liposomal amphotericin B stopped because of resistant hypokalemia, and micafungin was initiated. The patient received 2 g/kg IVIG treatment. Enoxaparin was started for acute venous thrombosis prophylaxis, and dapsone was initiated for possible loxoscelism. Local hyperbaric oxygen therapy (HBOT) was applied for necrotic areas. Our patient received 30 days of HBOT. The patient was consulted with the pediatric immunology department, and the immunologic tests were normal. Drug reaction and ecthyma gangrenosum were excluded.

After three days, the patient had a fever, hepatosplenomegaly, deepened pancytopenia, elevated triglyceride, elevated ferritin, and low fibrinogen levels (Table I). HLH was considered, and bone marrow biopsy was performed and the result was consistent with hemophagocytic syndrome (Fig. 4). Dexamethasone 10 mg/m^2 was intravenously initiated. Within the

Table I. Laboratory data of the patient.

Variable	On admission	8th day of admission	30th day of admission	Reference range*
White-cell count (per mm^3)	4570	1600	9000	5000-13500
Hemoglobin (g/dl)	12.1	7.3	8.7	11.5-15
Hematocrit (%)	34.3	21.3	26.8	34-45
Differential count (%)				
Neutrophils	55.6			35-65
Lymphocytes	37.7			30-55
Monocytes	4.5			2-9
Platelet count (per mm3)	106.000	76.000	324.000	150.000-450.000
Erythrocyte sedimentation rate (mm/hr)	8			0-20
C-reactive protein (mg/L)	0.1	0.1		<5.0
Sodium (mEq/L)	137	135		136-145
Potassium (mEq/L)	4.5	3.2		3.5-5.1
Calcium (mg/dl)	8.2	8.5		8.7-10.4
Creatinine (mg/dl)	0.27	0.32		0.32-0.6
Aspartate aminotransferase (U/L)	163			<34
Alanine aminotransferase (U/L)	208			10-49
Ferritin (ng/ml)		6856	32	22-322
Triglyceride (mg/dl)		516	165	<150
Fibrinogen (g/L)		1.42	1.66	

*Reference values are affected by many variables. The ranges used at Ankara University Hospitals are adjusted for patient who are not pregnant and do not have medical conditions that could affect the results. And ranges are not age-adjusted. They may therefore not be appropriate for all patients.

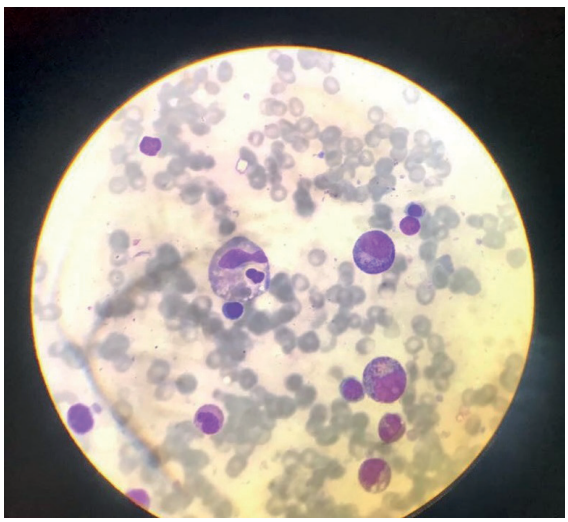

Fig. 4. A bone marrow aspirate demonstrated hemophagocytosis.

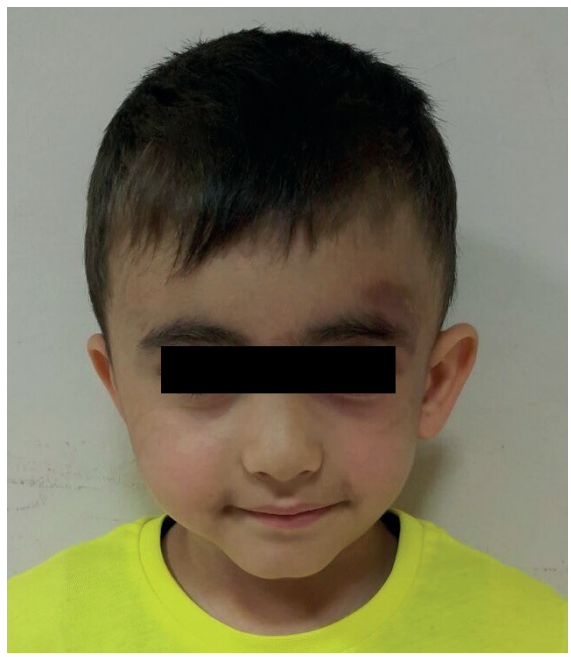

Fig. 5. Regression of necrotic lesion on the left eyebrow and facial edema (photo taken during follow-up).

following 14 days, hemophagocytic syndrome findings gradually resolved. His facial lesions showed improvement for the first of two weeks, with a decrease in erythema and edema. Necrotic crust expanded through the left eyelid. The patient picked off the necrotic crust, and fresh tissue came into view. He received vancomycin for 14 days, meropenem for 28 days, liposomal amphotericin B for eight days, micafungin for six days. At follow-up admissions, his facial lesions improved (Fig. 5). Abdominal ultrasound and temporal artery doppler ultrasound were performed, and both reported regular findings.

Discussion

The diagnosis of a spider bite is typically based on patients' history and epidemiological findings, clinical signs and symptoms, and morphological appearance of the cutaneous lesion. The patient was referred to our hospital with the diagnosis of treatment-resistant preseptal cellulitis. However, on clinical examination, a local cutaneous reaction was observed with severe ulcerative necrosis and eschar formation at the bite site. Clinical signs, systemic symptoms, and appearance of the cutaneous lesion supported the diagnosis of loxoscelism. Similar cases with dermonecrosis were previously reported in the literature.[7,10]

The venom triggers a complex inflammatory response that causes an ischemic-reperfusion injury and enhances dermonecrosis. The pathophysiology of the ischemic reperfusion injury includes adherence of neutrophils to microvascular endothelium and subsequent basal membrane and interstitial tissue penetration. These neutrophils produce reactive oxygen metabolites that cause tissue cytotoxicity. Because of the microvascular injury, hyperbaric oxygen therapy (HBOT) should be considered.[11,12] Hadanny et al.[11] reported the effect of HBOT on non-healing wounds caused by brown spider bites. Our patient received 30 days of HBOT.

Presence of fever, pancytopenia, hepatosplenomegaly, hypertriglyceridemia, hyperferritinemia raised the concerns of HLH. Bone marrow biopsy was performed and lead us to the confirmation of HLH diagnosis. Hemophagocytic syndrome findings resolved with dexamethasone therapy. The patient had no underlying genetic disorder suggesting primary HLH. Age of the patient, absence of consanguinity between parents and family history of HLH, early recovery of findings only by steroid, and treatment of the underlying disease support the diagnosis of secondary HLH. To the best of our knowledge the case presented here is the first secondary HLH associated with loxoscelism in Turkey. The pathologic process affecting patients with HLH is the uncontrolled production of cytokines creating an abnormal accumulation and dysregulation of cytotoxic T cells, NK cells, and macrophages. Interferon-gamma, IL-6, IL-10, IL-12, and soluble IL-2 receptors are the key cytokines found in patients with HLH.[5,6,13,14] The underlying pathogenesis affecting patients with systemic loxoscelism is not fully understood, but the venom of loxosceles spider contains several enzymes. Sphingomyelinase D, a pro-inflammatory protein stimulating the activation of IL-6, IL-8, IL-10, is the main component of the venom.[6,15] Therefore, the HLH clinic can also occur in systemic loxoscelism. Initial standard therapy for HLH includes dexamethasone and etoposide. In our patient, hemophagocytic syndrome resolved with dexamethasone.

In conclusion, the case presented here is the first case of secondary HLH associated with loxoscelism in Turkey. Clinicians must be aware of the possibility of HLH after systemic loxoscelism. Further investigation is needed to clarify the relationship between HLH and loxoscelism.

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Case Report

A neglected cause of recurrent rhabdomyolysis, *LPIN1* gene defect: a rare case from Turkey

Sevgi Topal[1], Melis Demir Köse[2], Hasan Ağın[1], Ferhat Sarı[3], Mustafa Çolak[1], Gülhan Atakul[1], Utku Karaarslan[1], Rana İşgüder[4]

Departments of [1]Pediatric Intensive Care Unit, [2]Pediatric Metabolism and Nutrition and [4]Department of Child Health and Diseases, University of Health Sciences, Dr. Behcet Uz Children's Diseases and Surgery Training and Research Hospital, İzmir; [3]Department of Pediatric Intensive Care Unit, Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, Turkey.

ABSTRACT

Background. Rhabdomyolysis; can occur due to toxic, infectious, metabolic, and genetic causes. Severe rhabdomyolysis may progress to several clinical manifestations such as cardiac arrest and may pose a risk of mortality if it is not treated timely.

Case. In this article, we presented a 26-month-old patient who was admitted with an acute rhabdomyolysis attack and a venovenous hemodiafiltration (CVVHDF) was initiated on the 5th hour of hospitalization. Creatine kinase (CK) levels of the patient continued to increase (max: 943 452 IU/L) until the 5th day of treatment and hereafter began to decrease. As the common causes of rhabdomyolysis were excluded and the CK levels were the highest values reported in the literature, although, *LPIN1* deficiency was the most suspected diagnosis, to facilitate the diagnostic procedures a whole-exome sequencing was performed. A homozygous [c.1696G>C p.(Asp566His)] mutation was detected on *LPIN1* gene. This variant has not been described previously, however, when examined with programs such as SIFT and Mutation taster, it has been considered as pathogenic.

Conclusion. In the pediatric age group, especially in infants presenting with severe rhabdomyolysis, *LPIN1* deficiency should also be considered; as early diagnosis and appropriate treatment may reduce mortality.

Key words: creatine kinase, rhabdomyolysis, *LPIN1* deficiency.

Rhabdomyolysis can occur both due to metabolic and genetic diseases and also acquired causes like trauma, exercise, drugs, and toxins. Severe rhabdomyolysis may progress to several clinical manifestations such as cardiac arrest and may pose a risk of mortality if it is not treated timely.[1]

Most of the metabolic myopathies characterized by recurrent myoglobinuria triggered by fever and exercise are composed of glycogen storage diseases, fatty acid oxidation defects, carnitine metabolism disorders, and myopathic mitochondrial cytopathies. All of those reasons cause an energy shortage in the muscles. Differential diagnosis is very important in distinguishing metabolic myopathies. Particularly, muscle myophosphorylase activity and glycogen accumulation may provide specific diagnostic information for muscle glycogenosis. Additionally, staining and quantitative enzymatic analyzes for oxidative phosphorylation chain complexes may provide insight into single or combined oxidative phosphorylation chain deficiencies. However, the diagnosis of muscle biopsy in this highly genetic heterogeneous group is very limited. In pediatric patients presenting with rhabdomyolysis, when a specific diagnosis could not be made by first step metabolic analyses, electromyography, muscle biopsy (after the acute phase), and mitochondrial DNA

✉ Sevgi Topal
sevgi_topal86@hotmail.com

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analysis may be performed. Despite all these investigations, the cause of rhabdomyolysis may not be found in at least half of the cases. Mutations of the *LPIN1* gene have been identified as the cause of severe recurrent rhabdomyolysis in pediatric patients. *LPIN1* gene defects have an autosomal recessive hereditary model. Biallelic mutations cause severe, recurrent rhabdomyolysis attacks with high-mortality rates, although there are only a few cases reported in the literature when compared to other metabolic myopathies. The pathophysiology of Lipin 1 deficiency is not well known and the prognosis is poor.[2,3] Lipin 1 is found most abundant in the adipocytes and skeletal muscle, and it acts as a transcriptional coactivator by interacting with transcription factors that regulate the expression of genes involved in energy pathways.[4,5] It is thought that rhabdomyolysis which develops in *LPIN1* deficiency may be caused by disruptions in all these functions, but further studies are needed to clarify the pathogenesis.

In this article, we describe a patient with acute rhabdomyolysis, who to the best of our knowledge had the highest creatine kinase (CK) level reported in the literature. She was diagnosed with *LPIN1* deficiency, with a homozygous missense mutation which was not previously described in the literature. With an effective and immediate intensive care treatment and CVVHDF, we successfully got over this severe rhabdomyolysis attack.

Case Report

A twenty-six months old girl admitted to the emergency department with complaints of loss of appetite for 2-3 days and somnolence on the last day. She also had a fever. Dark color of the urine was first observed in the emergency department (Fig. 1) before high levels of serum CK: 10 529 IU/L (reference range: 29-168), AST (aspartate aminotransferase): 229 IU/L (reference range: 5-40), and ALT (alanine aminotransferase): 53 IU/L (reference range: 7-40) were determined. CK level was increased to 40 850 IU/L at the 4th hour of admission. The patient was transferred to the pediatric intensive care unit with the diagnosis of rhabdomyolysis, when a rapid rise in muscle enzymes together with a change of consciousness was observed. However, her renal function and blood gas analysis were normal.

Prenatal and natal history was unremarkable. The patient did not have similar symptoms before and her neuromotor development was appropriate for the age. Her parents were first degree cousins, she had a healthy sister and there was no family history of rhabdomyolysis. On physical examination, the Glasgow Coma Score was 8, blood pressure was 98/58 (65) mmHg, and heart rate was 160/min. There was no pseudohypertrophy and muscle weakness. Systemic examination was normal except for the change in consciousness, nystagmus and dark color of the urine. Laboratory evaluation revealed; BUN: 20 mg/dl, creatinine: 0.6 mg/dl, uric acid: 6.5 mg/dl, Na: 138 mmol/L, K: 3.8 mmol/L. Electrocardiogram (ECG) and echocardiography which were performed to exclude cardiac pathologies and rhythm disorders put forward normal results. Urine ketone was positive. Toxic causes were excluded by a detailed history and with toxicological studies. Blood and urine culture tests and

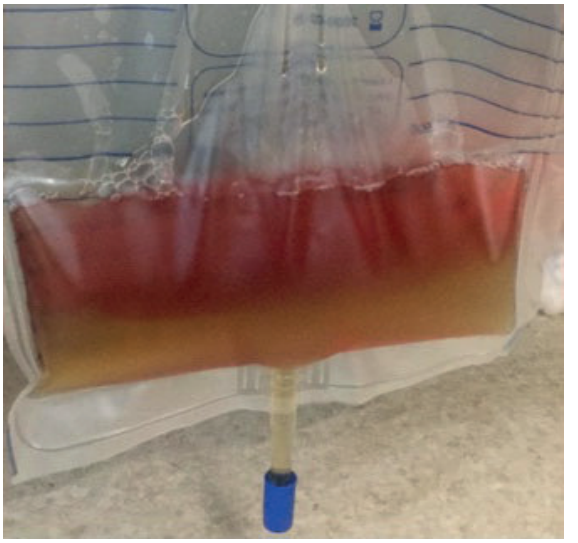

Fig. 1. Change in the urine color of the patient within 4 hours.

viral serology for infectious agents were also negative.

For initial treatment; 3000 ccs/m^2 intravenous fluid, urine alkalinization, and diuretic were given. Since the patient's consciousness was gradually worsened, she was intubated. Serum (reference range: 0-65), and urine myoglobin levels (normal<5 ng/ml) were measured over than 5000 ng/ml. CK was 170 000 IU/L at the 5th hour of admission, and continuous venovenous haemodiafiltration (CVVHDF) was started. Creatine kinase values continued to increase in the first 4 days of hemodiafiltration (The peak CK: 943 452 IU/L, AST: 9599 IU/L, ALT: 2616 IU/L; at the 5th day of CVVHDF), and then they began to decrease. Hemodiafiltration was discontinued on the 9th day of hospitalization, following a reduction of CK level to 26 467 IU/L (Fig. 2). On the 12th day of hospitalization, she was extubated. Serum glucose, ammonia, lactate, carnitine-acyl carnitine profile, homocysteine, plasma amino acids, and urine organic acid analysis, which were taken in the critical period were normal. The blood ketone level was within the normal limits. In this step differential diagnosis is very important, although the *LPIN1* gene defect was considered in the foreground. As inherited causes of rhabdomyolysis are a large group of diseases, it is time-consuming to evaluate all of them. So whole-exome sequencing was performed and a homozygous [c.1696G> C p. (Asp566His)] mutation of the *LPIN1* gene was detected. This variant has not been described previously, but when evaluated with programs such as SIFT and Mutation taster, it has been considered as pathogenic. Mother, father, and sister are tested with Sanger's sequencing. The mother, father, and sister were heterozygous for this mutation (Fig. 3). Eleven months after the first attack of rhabdomyolysis, the patient is still healthy.

We gave information and took informed consent from the parents of the patient.

Discussion

Metabolic myopathies characterized by recurrent myoglobinuria and triggered by catabolic processes like fever and exercise are glycogen storage diseases, fatty acid oxidation defects, carnitine metabolism disorders, and mitochondrial pathologies. Differential diagnosis is very important in distinguishing metabolic myopathies which are a crowded group from the other acquired conditions or neurological causes. Basic metabolic examinations are guiding in the first stage of diagnosis, especially in fatty acid oxidation

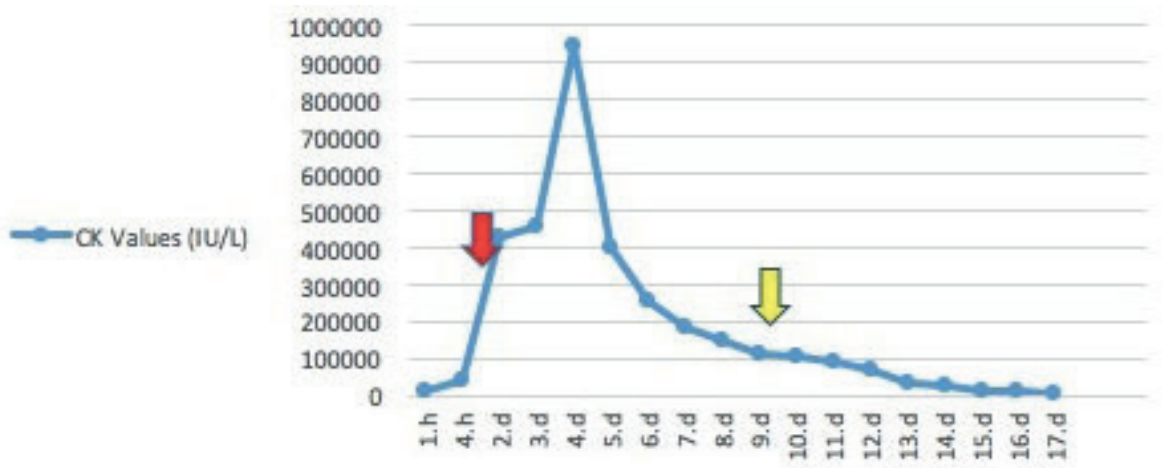

Fig. 2. Change of creatine kinase (CK) values of the patient in course of time (h: hour, d: day).
> Hemodiafiltration initiation time
> Hemodiafiltration termination time

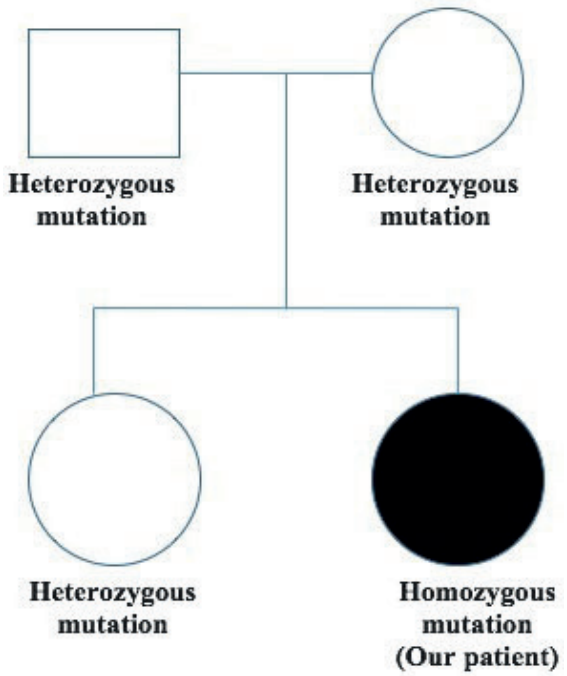

Fig. 3. The pedigree of the [c.1696G>C p. (Asp566His)] mutation on *LPIN1* gene.

defects, if critical samples were taken in time. Since our patient's plasma ammonia level was normal, urine ketone was positive and blood ketone was normal; fatty acid oxidation defects were excluded. Due to the normal detection of carnitine levels and the acylcarnitine profile, another frequent cause of CPTII deficiency was also excluded. Some of the mitochondrial myopathies that have an important role in the differential diagnosis are characterized with neuromotor retardation and myopathic findings.[2] However, our patient did not have any neurological abnormalities and plasma lactate/pyruvate levels were normal. Then after, with very high CK levels *LPIN1* gene defect was our preliminary diagnosis.[3,4] Muscle biopsy is an option if any abnormal finding is detected in the first step of metabolic examinations. However, the diagnostic value of muscle biopsy in this highly heterogeneous genetic disease group is limited and can be done 1 or 2 months after the acute phase. Molecular tests are increasingly being used to explore the genetic cause of rhabdomyolysis.[6] Like our case, in severe rhabdomyolysis without a known neurological disease and normal metabolic investigation results which are ruling out fatty oxidation defects, mitochondrial pathologies, and glycogen storage diseases, the diagnosis is based on molecular analysis.

LPIN1 gene defect is a common cause of childhood serious rhabdomyolysis, which accounts for more than half of the cases in infancy.[2,3] The first attack of rhabdomyolysis usually occurs during a febrile illness like in our case. The lipin 1 protein plays a critical role in adipocyte differentiation and lipid metabolism.[2,7] However, further studies are needed to determine which changes in these pathways bring out the symptoms.

Acute renal failure and cardiac arrhythmia are the most critical complications of rhabdomyolysis and are usually associated with high mortality if the patients have not renal replace-ment therapy in time.[3,8] In our case, no abnormal ECG findings were observed. In one case reported by Bergouniouxet al.[9], ECG had shown diffuse symmetrical high-amplitude T waves with no other changes. No specific ECG findings have been reported in other arti-cles.[2,3,9]

Although there is not a specific treatment, early suspicion of *LPIN1* deficiency will lead to a better prognosis and avoid unnecessary invasive procedures such as muscle biopsy.[10,11] Michot et al.[3]; reported that from 29 infantile cases with rhabdomyolysis, whose etiology could not be determined and all metabolic myopathies were excluded, 59% of them had *LPIN1* gene mutations. Our case also had a homozygous missense mutation on the *LPIN1* gene, which was not described.

In this article; we present an infantile case of severe rhabdomyolysis, which was triggered by a febrile illness, to share a rare disease of *LPIN1* deficiency. Also, to the best of our knowledge, this case has the highest CK levels reported in the literature with *LPIN1* deficiency and also the first case with *LPIN1* deficiency described from Turkey.

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Case Report

Different clinical presentation in a patient with two novel pathogenic variants of the *FBXL4* gene

Engin Köse[1], Melis Köse[2], Selvinaz Edizer[3], Zeynep Akışın[4], Zehra Burcu Yılmaz[5], Ahmet Şahin[5], Ferah Genel[6]

[1]Division of Pediatric Metabolism and Nutrition, Department of Pediatrics, Ankara University Faculty of Medicine, Ankara; [2]Division of Pediatric Metabolism and Nutrition, Department of Pediatrics, Katip Çelebi University Faculty of Medicine, İzmir; Divisions of [3]Pediatric Neurology and [6]Allergy and Immunology, [5]Department of Pediatrics, [4]Nutrition and Dietetics, Dr. Behçet Uz Children Research and Training Hospital, İzmir, Turkey.

ABSTRACT

Background. The recently described *FBXL4*-related encephalomyopathic mitochondrial DNA depletion syndrome 13 (MTDPS13) manifests with severe encephalopathy, early-onset lactic acidosis, hypotonia, developmental delay and feeding difficulty. Less than 100 cases with *FBXL4*-related MTDPS13 and 47 pathogenic mutations in the *FBXL4* gene have been identified thus far. Here, we describe a patient diagnosed with MTDPS13 with two novel variants of the *FBXL4* gene.

Case. A 51-day-old male was admitted with the complaint of bloody stool. His physical examination revealed facial dysmorphic features, developmental delay and truncal hypotonia with lack of head control. Laboratory investigations showed anemia, neutropenia, metabolic acidosis with hyperlactatemia, elevated fumaric acid, 2-ketoglutaric acid in urine and elevated alanine level in plasma which were consistent with mitochondrial dysfunction. Brain magnetic resonance imaging (MRI) showed large ventricles, thin corpus callosum and poor myelination. Drug-resistant epilepsy developed during the clinical follow-up. Ketogenic diet was initiated for intractable epilepsy; which was then interrupted due to severe metabolic acidosis. Compound heterozygous pathogenic variants were detected in the *FBXL4* gene [p.Gly258* (c.772G>T, Exon 5)/p.Trp354Ser (c.1061G>C, Exon 6)] with whole-exome sequencing.

Conclusion. We detected two novel variants of the *FBXL4* gene. To the best of our knowledge, this is the first case in the literature that presented with gastrointestinal bleeding as an encephalomyopathic form of mitochondrial DNA depletion syndromes and for whom ketogenic diet was initiated due to intractable epilepsy, which was not reported in previous cases.

Key words: FBXL4, MTDPS13, novel variants.

The *FBXL4* (F-box and leucine-rich repeat-containing protein) related encephalopathy referred to as encephalomyopathic mitochondrial DNA depletion syndrome 13 (MTDPS13) was first described in 2013.[1] The *FBXL4* mutation has pathogenetic effects on mitochondrial respiratory chain enzyme activities, disturbs the dynamic mitochondrial network and causes loss of mitochondrial membrane potential as well as mtDNA depletion.[1] Most patients with *FBXL4*-related MTDPS13 present with severe encephalopathy, early-onset lactic acidosis, hypotonia, developmental delay and feeding difficulty.[2-4] Cardiac, ophthalmological and genitourinary manifestations, seizure and hyperammonemia are reported as the less common features of the condition.[4]

To date, 94 patients with *FBXL4*-related MTDPS13 have been reported from different countries. Forty-seven pathogenic mutations in *FBXL4* have been identified, and no genotype-phenotype correlation has been determined.[5]

✉ Engin Köse
enginkose85@hotmail.com

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Here, we describe the 95th patient with *FBXL4*-related MTDBS13 who presented with intestinal dysmotility, hypotonia, lactic acidosis and refractory epilepsy and had two novel pathogenic variants of the *FBXL4* gene. To the best of our knowledge, this is the second patient with *FBXL4*-related MTDBS13 for whom ketogenic diet treatment was provided.

Case Report

A 51-day-old male was admitted to emergency department with the complaint of bloody stool. He was born to nonconsanguineous parents after a full-term uneventful pregnancy. His birth weight, height and head circumference were 2680 g (-1.6 SD), 50.5 cm (0.23 SD) and 35.5 cm (0.43 SD), respectively. In postnatal history, treatment due to a diagnosis of pneumonia for one month was reported by his parents. He had been exclusively breast-fed until the age of one month and his mother then proceeded feeding the infant with commercial formula. There was no family history of genetic disease or congenital abnormalities. The patient had a 3-year-old healthy brother.

During the physical examination, facial dysmorphic features such as micrognathia, low-set ear, bossing forehead and long philtrum were noted (Fig. 1). His weight was 4000 g (-1.53 SD), height was 55 cm (-0.61SD) and head circumference was 39.5 cm (0.26 SD). Developmental delay and truncal hypotonia with lack of head control were observed. While abdominal distension was detected, there was no hepatomegaly or splenomegaly. Ophthalmological evaluation revealed optic atrophy. Hearing impairment was not detected. Other systemic examinations were unremarkable.

Laboratory investigations showed anemia (hemoglobin 7.6 mg/dl), neutropenia ($0.8/10^3$/uL), metabolic acidosis (pH: 7.22, HCO_3: 14.5 mmol/L, pCO_2: 20 mmHg) with hyperlactatemia (101 mmol/L, RR: <2.2). Renal function tests and transaminase levels were within normal range. Serum ammonia level was normal. Metabolic screening revealed elevated fumaric acid (45 μmol/L; RR: 0 - 28) and 2-ketoglutaric acid (2743 μmol/L; RR: 0 - 631) in urine, and elevated alanine (580 μmol/L; RR: 139 - 474) level in plasma which were consistent with mitochondrial dysfunction. Acylcarnitine profile was nonspecific. For the evaluation of etiology of gastrointestinal tract bleeding, specific Ig E levels and skin prick test were assessed. No evidence was revealed for IgE mediated food allergies.

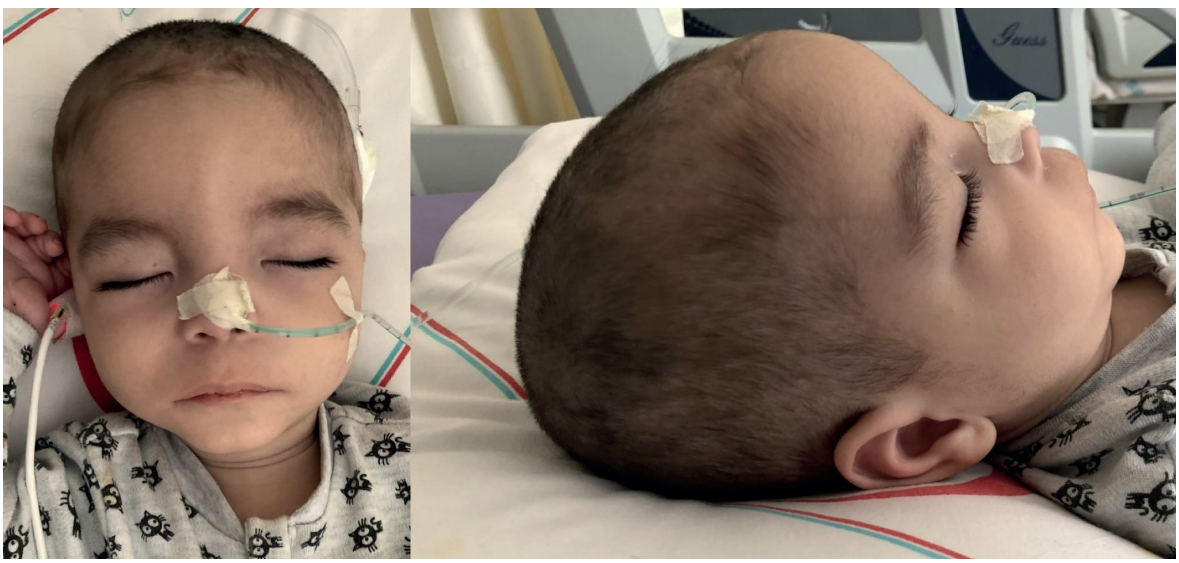

Fig. 1. Facial dysmorphic features of the patient.

Abdominal sonography, echocardiogram, electrocardiogram and electroencephalogram (EEG) were normal. Brain magnetic resonance imaging (MRI) showed large ventricles and thin corpus callosum (Fig. 2a). Lactate peak was detected in brain MR spectroscopy (MRS) (Fig. 2b). Abdominal X-ray (posterior-anterior view) revealed dilated bowel (Fig. 2c). Clinical state of patient did not allow us to preform colonoscopy.

Metabolic acidosis and hyperlactatemia were treated with sodium bicarbonate and dichloroacetic acid. Coenzyme Q10, riboflavin, thiamine, biotin treatments were initiated for the differential diagnosis of mitochondrial disease. Nasogastric tube feeding was required due to hypotonia, dysphagia and feeding difficulties. Treatment with domperidone and ranitidine was initiated upon the findings of gastrointestinal dysmotility. Elimination diet with amino-acid based infant formula was initiated. However, episodic lower GI bleedings were observed and non-IgE mediated food allergies were ruled out. During his follow-up with these treatments, compound heterozygous pathogenic variants were detected in the *FBXL4* gene [p.Gly258* (c.772G>T, Exon 5) / p.Trp354Ser (c.1061G>C, Exon 6)] with whole-exome sequencing. Genetic investigation was performed for his parents and sibling (Fig. 2d).

After 7 months, when he was 9 months old, the patient presented with feeding difficulty and fever. Pneumonia was diagnosed. Nystagmus, roving eyes and jerks of left arm were noted during the clinical examination, and EEG showed slow, irregular background activity and asynchronous sharp waves activity consistent with myoclonic epileptic encephalopathy (Fig. 2e). Brain MRI revealed large ventricles in addition to cerebral and cerebellar atrophy (Fig. 2f). Treatment with phenobarbital, levetiracetam and vigabatrin was initiated due to intractable epilepsy and ketogenic diet was introduced. However, while blood ketone level reached 5 mmol/L on the fifth day of the ketogenic diet, metabolic acidosis and hyperlactatemia occurred. Ketogenic diet was therefore discontinued. Finally, epileptic activity was treated with phenobarbital, levetiracetam, vigabatrin and topiramate medications.

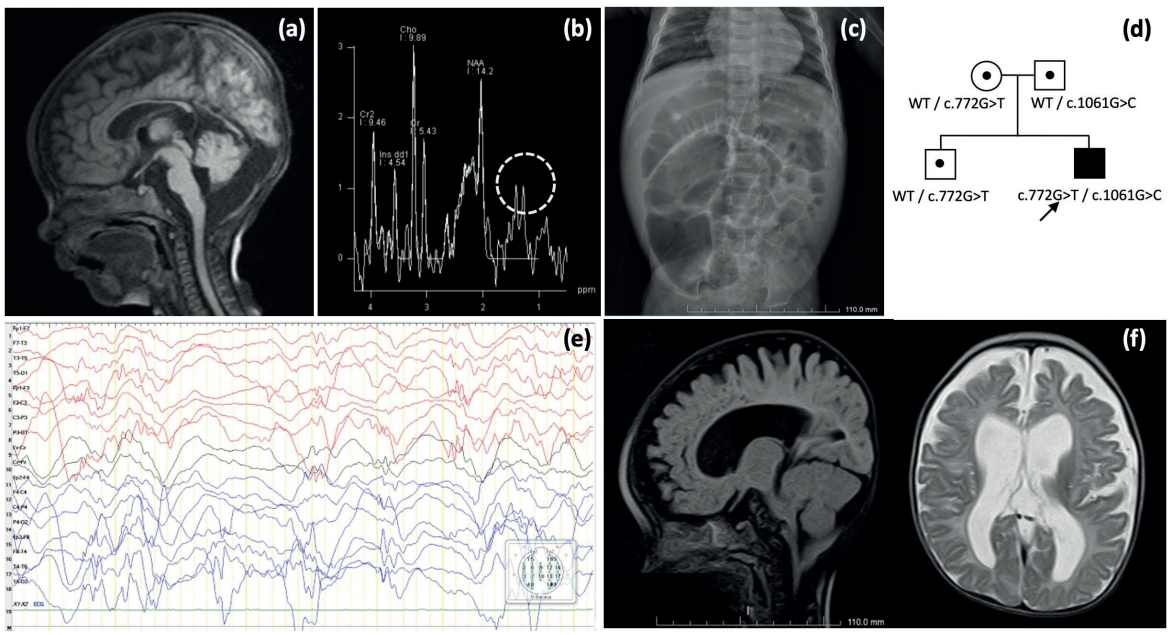

Fig. 2. (a) Thin corpus callosum in MRI. **(b)** Lactate pick on MRS. **(c)** Dilated intestinal loops. **(d)** Pedigree of the patient's family. **(e)** EEG findings of patient. **(f)** Enlarged ventricles, cerebral and cerebellar atrophy in MRI.

At his most recent admission at 11 months of age, the patient was followed up with supportive treatments for developmental delay, seizures and eye involvement by a multidisciplinary team including specialists from pediatric metabolism, neurology, ophthalmology, nutrition and developmental pediatrics departments. Written informed consent was recieved from the family.

Discussion

To the best of our knowledge, our patient is the 95th case of *FBXL4*-related MTDPS13 in the literature.[4-7] We have identified two novel pathogenic variants in the FBXL-4 gene. While patients with *FBXL4*-related MTDPS13 presented with hypotonia, lactic acidosis and development delay in previous reports, our patient presented with dysmotility and lower gastrointestinal bleeding as an encephalomyopathic form of mitochondrial DNA depletion syndromes. Fukuyama et al.[8] reported a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) which presented with transient non-occlusive small intestine ischemia that eventually triggered mucosal necrosis. They suggest that the presence of a large number of abnormal mitochondria in MELAS is closely linked to mucosal necrosis of the small intestine. Our patient was admitted with gastrointestinal bleeding as previously reported in literature. However, he didn't need any operation or intestinal resection and gastrointestinal bleeding spontaneously resolved. These findings were not compatible with intestinal ischemia. Furthermore, we did not perform endoscopy for the evaluation of gastrointestinal bleeding.

Typical manifestations of *FBXL4*-related MTDPS13, i.e. lactic acidosis, neurological involvement, developmental delay, feeding difficulties were also observed in our patient. However, microcephaly and hyperammonemia, which were observed in almost half of previous cases, were not noted in the patient presented herein.[4] Cardiac and genitourinary (hypospadias, cryptorchidism) manifestations, renal tubular acidosis and hearing impairment -which are less frequent- were also not detected.[2] We observed clinical findings such as epilepsy and nystagmus during the clinical follow-up which were not detected at the time of first admission. These findings suggest that long-term follow-up is required to better understand the clinical progress of the condition as well as the genotype-phenotype correlation.

Epilepsy is reported in 28% of affected patients, mainly in the form of generalized tonic-clonic seizures. Drug-resistant epilepsy has not been reported in any of the cases presented thus far.[5] To date, ketogenic diet was initiated in a patient with *FBXL4*-related MTDPS13 due to differential diagnosis of pyruvate dehydrogenase complex deficiency which was then discontinued owing to elevated alanine aminotransferase and aspartate aminotransferase levels.[9] In our case, we initiated ketogenic diet due to intractable epilepsy; however, severe metabolic acidosis led us to interrupt the diet.

Abnormalities in brain MRI have been observed in almost all individuals with *FBXL4*-related MTDPS13. White matter abnormalities, cerebral atrophy and lactate peak in MRS are the most common findings in neuroimaging. Thin corpus callosum and cerebellar atrophy are detected in 20% of patients.[2,4,10] Consistent with previous reports, in our case we determined enlarged ventricles due to cerebral atrophy together with thin corpus callosum and lactate peak in MRS. Cerebellar atrophy was noted during the clinical course.

Currently, there is no treatment for *FBXL4*-related MTDPS13, and supportive treatment is essential. Administration of vitamins and antioxidants have limited benefits, similar to other mitochondrial disorders.[2] In our experience, we have not detected any positive effects of the mitochondrial cocktail.

We identified two novel pathogenic variants, a stop-gain variant [p.Gly258* (c.772G>T, Exon 5)] located in the F-box region and a missense

variant [p.Trp354Ser (c.1061G>C, Exon 6)] located in leucin-rich repeats region of *FBXL4*. Although no genotype-phenotype correlation has been determined, El-Hattab et al.[4] reported that patients with biallelic missense variants or compound heterozygous mutations of missense and null variants have longer survival compared to patients with biallelic null variants.

Huemer et al.[10] and Gai et al.[11] reported that mutations in the *FBXL4* are associated with decreased respiratory chain complex activity in their functional studies on mitochondrial membrane potential. They also showed severe mtDNA depletion in patients with *FBXL4* gene mutations. On the other hand, Barøy et al.[9] found normal respiratory chain complex activity. It is important to assess both genetic findings and functional tests to better understand the disease and the effects of variants. However, we were not able to evaluate these parameters in our patient.

Our case suggests that patients with *FBXL4*-related MTDPS13 may present with gastrointestinal bleeding. This is the first report that shows intractable epilepsy in *FBXL4*-related MTDPS13 and failure of ketogenic diet due to metabolic acidosis. Functional tests are required to reveal the unknowns of this disease.

Acknowledgments

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Case Report

Two patients with glutaric aciduria type 3: a novel mutation and brain magnetic resonance imaging findings

Sevil Dorum[1], Cengiz Havalı[2], Özlem Görükmez[3], Orhan Görükmez[3]

Divisions of [1]Metabolism Unit and [2]Pediatric Neurology, Department of Pediatrics, [3]Department of Genetics, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey.

ABSTRACT

Background. Glutaric Aciduria Type 3 (GA-3) is a rare metabolic disease which is inherited autosomal recessively and characterized by isolated glutaric acid excretion. To date, a limited number of cases have been reported in the literature. We present two patients with GA3 who were diagnosed with the isolated increased level of glutaric acid in urine.

Case. Glutaric aciduria type 1 and type 2 were excluded by genetic analysis and other laboratory and clinical findings. One of our patients had a homozygous mutation p.Arg322Trp (c.964C> T) of SUGCT (NM_001193311) gene. To the best of our knowledge this mutation has not been reported in the literature previously. Symmetrical periventricular and deep cerebral white matter abnormalities were detected on his brain magnetic resonance imaging (MRI).

Conclusion. We present two patients with GA-3 and a novel mutation in the SUGCT gene. Our findings expand the spectrum of causative mutations and clinical findings in GA-3.

Key words: brain magnetic resonance imaging, glutaric aciduria type 3, novel mutation, SUGCT gene.

Glutaric aciduria type 3 (GA-3) is a rare metabolic disorder caused by variants of the SUGCT (C7orf10) gene. It is characterized by the reduced transformation of free glutaric acid to glutaryl-coA due to succinate-hydroxymethylglutarate-CoA-transferase enzyme deficiency. This deficiency leads to isolated glutaric acid accumulation or excretion.[1,2]

While other types of glutaric acidemia have been known as mitochondrial disease, it has been accepted that GA-3 is caused by a peroxisomal dysfunction. In contrast to, glutaric aciduria type types 1 (GA-1) and 2 (GA-2), GA-3 is less known and rarely occurs. Some authors have suggested that GA-3 is not a disease.[3] There is limited knowledge regarding the disease that is composed of a several case reports in the literature. Additionally, nearly half of patients with GA3 that have been reported in the literature were asymptomatic and were identified through the newborn screening programme.[1-6]

In this report, two patients with GA3 who had distinct genotypes and phenotypes have been presented. Furthermore, one of the patients had brain magnetic resonance imaging (MRI) findings we believe were previously undefined.

Case 1

An 11-year-old female patient was admitted for sensorineural hearing loss and intellectual disability. She had a seizure at the age of 1 year. Her parents are from a consanguineous marriage (Fig. 1A). She had been diagnosed with global developmental delay and hearing loss at the age of 2 years. She had intellectual and speech impairments. Systemic physical examination was otherwise normal. Plasma

✉ Sevil Dorum
sevildorum@gmail.com

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acylcarnitine, and amino acid profiles were unremarkable. Increased glutaric acid levels were noticed in urine organic acid profiles (108 mg/g creatinine). In the genetic analysis of SUGCT (NM_001193311) gene, Arg108 (c.322C>T) mutation was homozygous (Fig. 1B). Riboflavin (vitamin B2) treatment was started at 200 mg/day. No laboratory and clinical changes were observed with riboflavin (vitamin B2) treatment.

Clinical exome sequencing and Sanger sequencing were applied for both of the patients. Genomic DNAs were extracted from peripheral venous blood using the QIAamp® DNA Mini Kit (QIAGEN, Ankara, Turkey). The Clinical Exome Solution (SOPHiA GENETICS, Switzerland) was used for exome enrichment. All procedures were carried out according to the manufacturer's protocols. It is a capture-based target enrichment kit and covers 4,900 genes with known inherited disease causing mutations.

Next generation sequencing showed homozygous nonsense mutation, c.322C>T, p.Arg108* in the *SUGCT* (NM_001193311) gene, and homozygous missense mutation, c.250C>T, p.Arg84Trp in *TMIE* (NM_147196) gene and these mutations were confirmed by sanger sequencing in this family (Fig. 1B).

Case 2

A 13-month old male, was admitted due to neuromotor developmental delay and tremor. He was born at 40 weeks gestational ages with a birth weight of 2200 grams. He was the third child of healthy consanguineous Syrian parents. He had two healthy siblings. His family history and antenatal, perinatal and postnatal histories were unremarkable. He had seizures in clusters at four months of age for three days in the course of acute gastroenteritis. Brain MRI and electroencephalography were normal during that period. The patient sat with support at the age of 7 months, and he did not achieve the ability to sit without support at the age of 1 year. At the age of 13 months, subacute regression in his motor abilities occurred. On examination, his weight, height and head circumference were <3rd percentile. He had axial hypotonia and bilaterally clonus and spasticity. The patients' ability to sit with support regressed. Blood gas, blood ketone level, dry blood acylcarnitine and

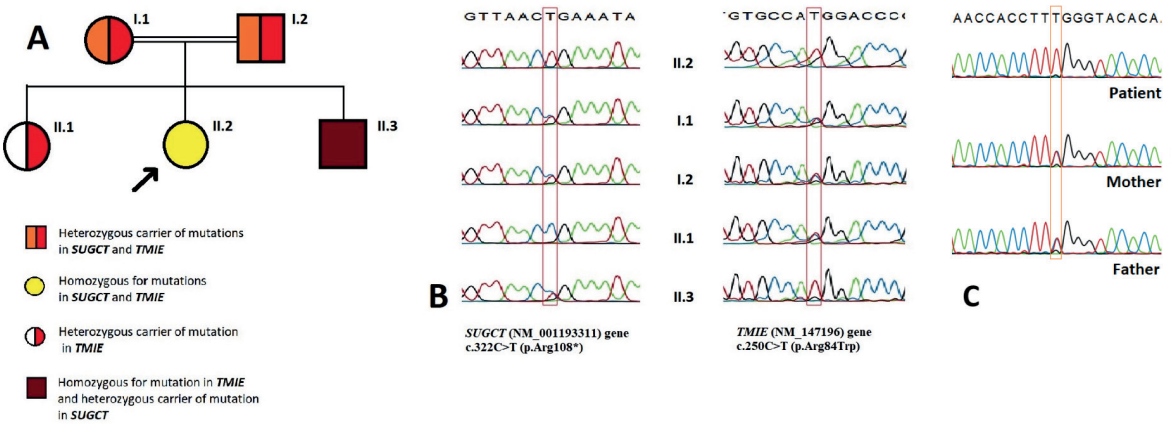

Fig. 1. Pedigree of the family showing the identified variants and mutation analysis

[A]. Shown is a consanguineous family segregating Autosomal Recessive Deafness 6 (DFNB6) and Glutaric Aciduria III (GA III). The arrow indicates the proband.

[B]. Electropherograms of Sanger's sequencing analysis of the family. Germline mutations in *SUGCT* and *TMIE* genes (indicated by red frames).

[C]. Result of DNA sequencing of the second family. A novel homozygous germline mutation, c.964C>T, p.Arg322Trp in *SUGCT* gene of the case 2 (indicated by red frame).

blood amino acid profile were normal. Auditory Brainstem Response test was normal. There was an increase in the level of glutaric acid in urine organic acids to 26.4 mg/g creatinine (N<5 mg/g creatinine). Molecular analysis of the *SUGCT* (NM_001193311) gene showed the homozygous variant of p.Arg322Trp (c.964C> T), not previously reported in the literature (Fig. 1C).

In this case, we detected a homozygous missense mutation, c.964C>T, p.Arg322Trp in the *SUGCT* (NM_001193311) gene, which was confirmed by sanger sequencing as mentioned before. This variant has not been previously reported in the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php) and in 1000 Genomes Project. Silico analysis programs (MutationTaster; disease causing, PolyPhen2; probably damaging, and SIFT; damaging) showed that this change could be the cause of the disease. We also showed the same mutation as heterozygous in the parents (Fig. 1C).

On the brain MRI, there were extensive, symmetrical periventricular and deep cerebral white matter abnormalities with sparing of the U fibers of the cerebral hemispheres. The internal capsule, corticospinal tracts in the brain stem and the cerebellar white matter were normal (Fig. 2). Carnitine (50 mg /kg/ day), and riboflavin (100 mg/day) treatments were started. Lorazepam and physical therapy were initiated for spasticity. During follow-up, the patients' weight increased. His spasticity resolved. Motor development and mobility of patient moderately ameliorated.

Informed consent was received from both of family.

Discussion

In this article, two patients with GA-3 who were diagnosed with urine organic acids and genetic tests were presented. GA-3 remains less described and known in comparison with other types of glutaric aciduria. In the literature only a few cases have been reported to date and most cases were asymptomatic and were revealed by a screening-programme. The remaining symptomatic patients with GA-3 presented with different clinical and laboratory findings such as diarrhea, vomiting, cyclic vomiting attacks, metabolic acidosis, ketosis, and hypoglycemia.[1,2,6]

Our first case was evaluated with intellectual disability and sensorineural hearing loss. In this case, sensorineural hearing loss was thought to be associated with a concomitant additional mutation in the *TMIE* gene. Brain MRI could not be performed because of the cochlear implant. The patient was also started on riboflavin treatment.

Our second case had neuromotor delay and hypotonia, similar to the cases previously described in the literature.[7] On brain MRI of a few patients previously reported in the literature, nonspecific white matter changes were described.[1-3,6] On brain MRI of our patient, there were extensive, symmetrical periventricular and deep cerebral white matter abnormalities with sparing of the U fibers of the cerebral hemispheres. These findings have not previously been reported to be associated with GA3 in the literature.

GA-1 and GA-2 that are present with increased glutaric acid in urine have relatively different clinical findings and distinctive patterns of involvement on brain MRI. GA-1 has a highly variable clinical manifestation. It frequently occurs with acute encephalitis-like encephalopathy in early childhood. It is often precipitated with gastroenteritis, febrile illness, and immunization. The typical neurological findings include dystonia, axial hypotonia, spasticity, akinetic-rigid parkinsonism.[8] Enlarged cerebrospinal fluid spaces anterior and temporal areas, subdural collections, white matter involvement and cerebral atrophy, are included MRI findings in GA-1.[9] Also, T2 hyperintensity of caudate and lentiform nuclei is frequently encountered on MRI. Corpus callosum, globus pallidus, dentate nuclei, and

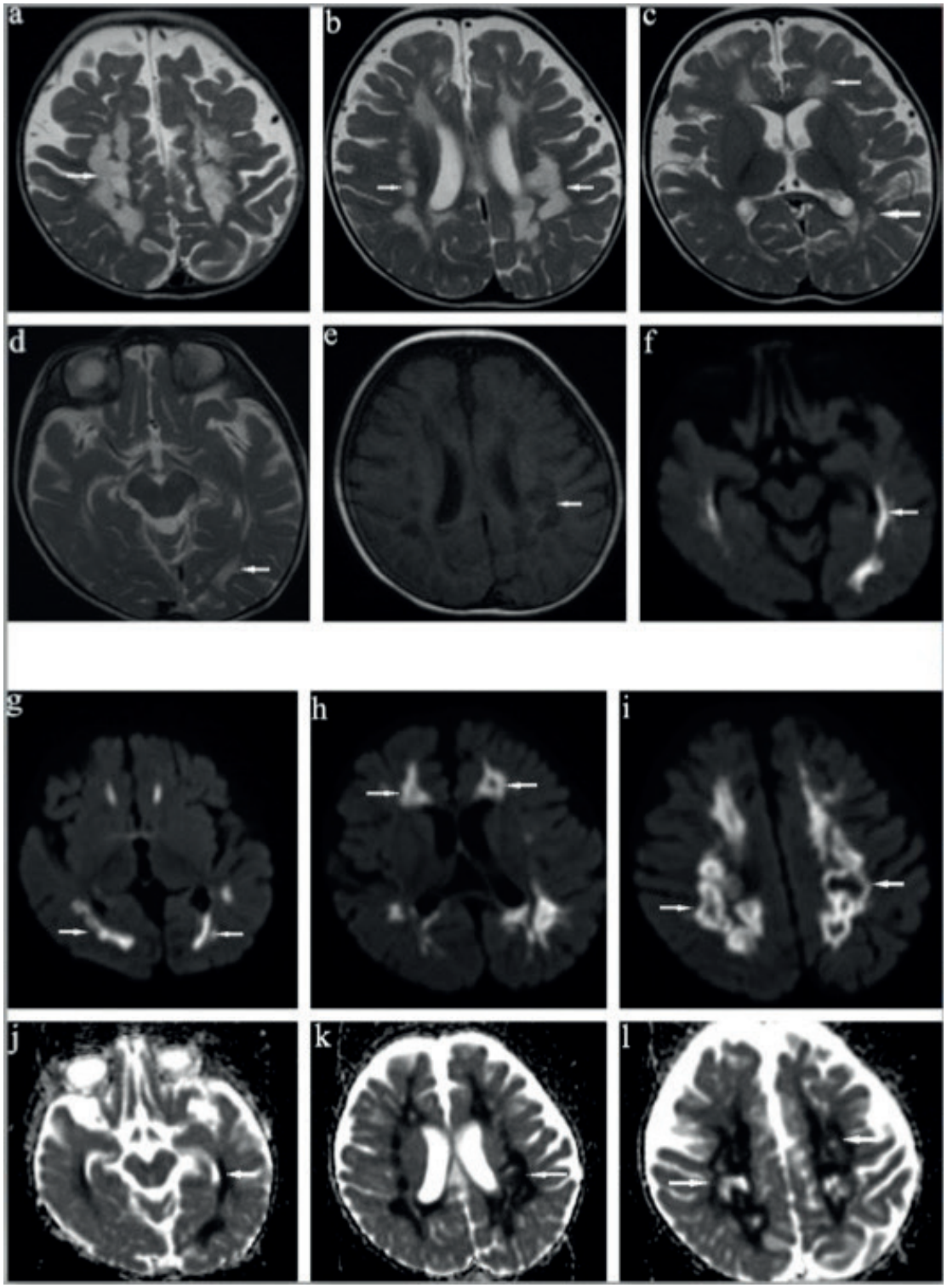

Fig. 2. There are extensive, symmetrical periventricular and deep cerebral white matter abnormalities with sparing of the U fibers of the cerebral hemispheres. The internal capsule, corticospinal tracts in the brain stem and the cerebellar white matter are normal (a,b,c,d). The T1-weighted images reveal that the involvement has an abnormally low signal intensity and cystic nature (e). Also, there are extensive signal abnormalities that refer to abnormal restricted diffusion in the diffusion-weighted imaging (f, g, h, i, j, k, l).

substantia nigra may also participate in brain involvement in patients with GA-1.[10]

GA-2 appears with recurrent vomiting, lethargy, hypoglycemia, and metabolic acidosis. Lipid-storage myopathy is found of muscle involvement. Three distinct forms of GA-2 are described. Type I and Type II are neonatal-onset form and associated with non-ketotic hypoglycemia, and metabolic acidosis. Type 1 also includes congenital anomalies. Type III is the late-onset form and moderately clinical form.[11] Reported MRI findings include, leukodystrophy, recurrent episodes of CNS demyelination and bilaterally symmetrical T2 hyperintensities in the globus pallidus.[12,13]

In addition to GA-1 and GA-2, a group of mitochondrial disease should be considered in the differential diagnosis with patients who have symmetrical periventricular and deep cerebral white matter abnormalities on the brain MRI. The pattern of involvement, dysmyelinated areas of white matter, basal ganglia and cortical structures participation are noteworthy in the differential diagnosis. Complex I and II deficiency may lead to isolated symmetrical periventricular cystic involvement. Determinations of plasma and CSF lactate and pyruvate levels, blood gas analysis, analysis of urinary organic acids are essential hallmark tests.[14] There was no mutation in the NDUFS4, SDHA, SDHD and SDHAF1 genes in the clinical exome analysis.

As recommended in the literature, we started therapy with carnitine, riboflavin.[1,2] We have observed weight gain and an improvement in motor abilities in the case 2.

GA-1 and GA-2 were excluded by genetic analysis in our patients who had high levels of glutaric acid in urine. Genetic analysis revealed homozygous variants in SUGCT (NM_001193311) genes. The homozygous variant p.Arg322Trp (c.964C> T) detected in the second patient was previously unreported.

In conclusion we present two patients with GA3 and a novel mutation in the SUGCT gene. Our findings expand the spectrum of causative mutations and clinical findings in GA III.

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Case Report

A partial response to abatacept in a patient with steroid resistant focal segmental glomerulosclerosis

Eren Soyaltın[1], Belde Kasap Demir[1,2], Caner Alparslan[1], Seçil Arslansoyu Çamlar[1], Demet Alaygut[1], Önder Yavaşcan[3], Fatma Mutlubaş[1]

[1]Department of Pediatric Nephrology, University of Health Sciences, Tepecik Training and Research Hospital, [2]Department of Pediatric Nephrology, Katip Çelebi University Faculty of Medicine, İzmir; [3]Department of Pediatric Nephrology, İstanbul Medipol University Faculty of Medicine, İstanbul Turkey.

ABSTRACT

Background. Herein we present our experience with abatacept in a patient diagnosed with primary focal segmental glomerulosclerosis (FSGS) and resistant to steroid and other immunosuppressives.

Case. A 17-year-old girl was diagnosed with idiopathic nephrotic syndrome (NS) at the age of 8 years. Kidney biopsy was performed when she did not respond to 6-weeks of steroid (2mg/kg) therapy followed by three doses of pulse methylprednisolone (PMP) and considered as steroid resistant NS. The biopsy revealed focal segmental glomerulosclerosis (FSGS) and cyclophosphamide was added to the steroid treatment but the patient had no response. The genetic analysis revealed G34G/A318A compound homozygous synonym aminoacid variation in NPHS2 gene, thus all immunosuppressive regimes were stopped and she was put on supportive treatment. Throughout this period, she had nephrotic range of proteinuria, however serum albumin levels were >3g/dl. At the end of two years, the patient had NS with severe edema and hypoalbuminemia. When the genetic analysis was interpreted again, it was found to be consistent with a polymorphism rather than a mutation. Following 3 doses of PMP, oral steroid treatment was resumed and cyclosporine (CsA) was added to the treatment at the fifth year of follow up. However, she was unresponsive to CsA at the end of the first year as well as mycophenolate mofetil used for 12 months and rituximab used for 6 months, respectively. Then abatacept was instituted and proteinuria decreased below 1 gr/day and serum albumin levels increased to 3 g/dl at the end of 6 doses. Serum albumin levels remained stable in the following 7 months.

Conclusion. Partial remission including the decrease in proteinuria and increase in albumin levels achieved in our patient encourages the usage of abatacept in patients who do not respond to multiple immunosuppressive therapies.

Key words: focal segmental glomerulosclerosis, abatacept, nephrotic syndrome.

Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in childhood.[1,2] It is classified according to the response to steroid treatment as steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS).[3] Although most children are diagnosed with SSNS, 10-20% of patients have no response to steroids and are diagnosed with SRNS. These patients have a greater risk for end-stage renal failure when compared to patients with SSNS.[4] The etiology of SRNS is also unknown in children; however podocytopathies are considered to be responsible for both minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). The circulating factors derived from immune dysregulation may also cause podocyte injury as well as genetic structural defect.[5,6]

Steroid resistance is defined as a failure to response to 4 weeks of daily therapy and high-dose steroid pulses.[1,7] Following failure of standard steroid therapy, second-line

✉ Eren Soyaltın
erensoyaltin@hotmail.com

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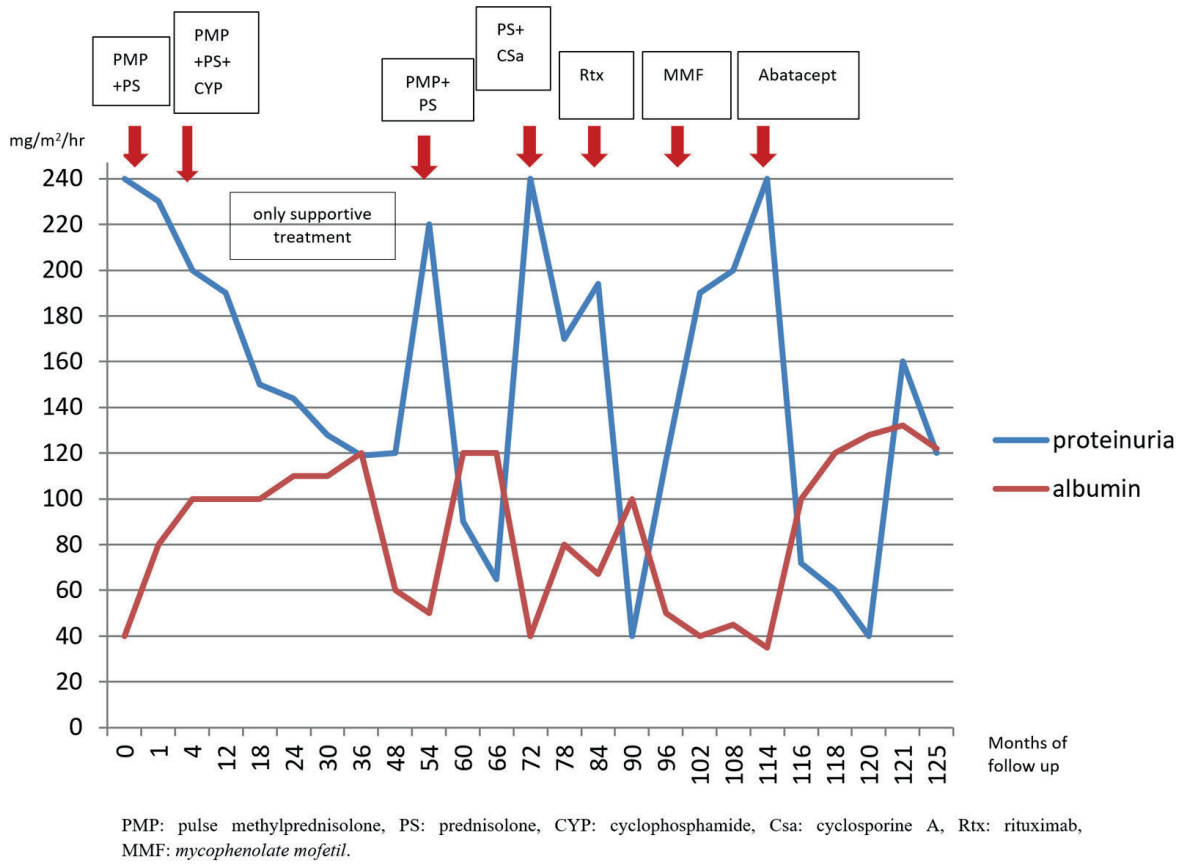
immunosuppressive agents like calcineurin inhibitors, cyclophosphamide (CYC), mycophenolate mofetil (MMF), Rituximab (RTX); can be initiated in combination with corticosteroids for remission.[3,7] A new biological agent abatacept, a cytotoxic T-lymphocyte-associated antigen 4-immunoglobulin fusion protein [CTLA-4-Ig] is available in recent studies.[5,8] Herein we present our experience with Abatacept in a patient diagnosed with primary FSGS and resistant to steroid and second-line immunosuppressive agents.

Case Report

A 17-year-old girl, diagnosed with INS at the age of 8 years, had been treated with oral steroids (2mg/kg/day) as initial treatment. When she did not respond to 6-weeks of oral steroid therapy followed by three doses of daily pulse methylprednisolone (PMP), she was considered as SRNS and a kidney biopsy was performed, which confirmed FSGS as the underlying pathology. Cyclophosphamide (1 mg/kg/day orally) was added to the steroid treatment. The genetic analysis revealed G34G/A318A compound homozygous synonym amino acid variation in NPHS-2 gene. Then, immunosuppressive therapies were discontinued; angiotensin converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), fish oil and statin treatment were initiated. Throughout this two-year period, she had nephrotic range of proteinuria, however serum albumin levels were >3g/dl. At the end of two years, the patient had NS with severe edema and hypoalbuminemia. When the genetic analysis was interpreted again, it was found to be consistent with a polymorphism rather than a mutation. Further genetic analysis revealed no mutation in NPHS-1, NPHS-3, NPHS6, TRPC-6, WT1, LAMB2, DGKE, ARHGDIA, COQ2, COQ6, CD2AP, ACTN4, CRB2, INF2, PAX2, MYO1E, APOL1 and ADCK-4 genes either. Following 3 doses of daily PMP, oral steroid treatment was resumed. In the follow-up, partial remission (50% decrease in proteinuria and serum albumin >3 g/dl) was achieved and cyclosporine (CsA) (5 mg/kg/day) was added to the treatment. When she experienced relapses in the first year of CsA treatment, RTX (375 mg/m^2/dose) was added to her therapy and CD19 levels were measured weekly. Second dose of RTX was given one month after the first dose due to increased CD19 levels (>1%). The administration of second dose of RTX was associated with a dramatic fall in urinary protein excretion (120 mg/m^2/hour to 40.5 mg/m^2/hour). At the sixth month of therapy, a third dose of RTX was given due to increased levels of CD19; but this time she did not respond to therapy well. Then, steroid and CsA therapies were stopped by tapering doses. Supportive treatments including ACEI and ARB were sustained and MMF (500 mg/m^2/dose at 2 doses) was initiated. However, there was no response to MMF treatment. She had severe proteinuria with albumin levels between 2 and 2.5 g/dl causing edema. At the end of 1.5 years, MMF therapy was discontinued and abatacept was initiated. She received six doses of abatacept (750 mg/dose) in 4 months as three doses over a month, then at monthly intervals. After administration of third dose of abatacept; the patient achieved partial remission and albumin levels increased above 3 g/dl (Fig. 1). As planned, 6 doses of abatacept were applied without any adverse effects and the proteinuria level decreased to 40 mg/m^2/hour after the last dose. One month after the last dose, the patient had 160 mg/m^2/hour of proteinuria, however albumin levels were over 3 g/dl and she had no edema anymore. At her last visit 7 months after the last dose abatacept, 24-hour proteinuria was 120 mg/m^2/hour, but serum albumin was 3.1 g/dl, serum creatinine was 0.6 mg/dl and the glomerular filtration rate was 142 mL/min per 1.73 m^2. There was no decrease in mean glomerular filtration rate values compared to pretreatment period (131.5 mL/min per 1.73 m^2).

An informed consent was received from the family to report the patient.



PMP: pulse methylprednisolone, PS: prednisolone, CYP: cyclophosphamide, Csa: cyclosporine A, Rtx: rituximab, MMF: *mycophenolate mofetil*.

Fig. 1. The proteinuria, albumin levels and the treatments of the patient in follow up period.

Discussion

FSGS is a histological variant of INS and is considered as a podocytopathy. In recent studies, the expression of *de novo CD80* in podocytes has been reported during relapse period and it is suggested to be a causal role for podocyte injury and proteinuria by disrupting the binding of talin to ß1-integrin.[3,5,9] *CTLA-4* is a *CD80 inhibitor* and is expressed in human podocytes.[5,10] Abatacept is a cytotoxic T-lymphocyte-associated antigen 4-immunoglobulin fusion protein and a costimulatory *B7-1 (CD80) inhibitor*.[5,8,11] Yu et al.[8] reported partial or complete remission with abatacept in one patient with glucocorticoid-resistant primary FSGS and four patients with RTX-resistant recurrent FSGS after kidney transplantation (KTx). *B7-1* staining of podocytes was shown in the biopsy specimens of whole patients and it has been shown to be a predictive factor for responding to abatacept therapy. Alachkar et al.[12] shared data with respect to five patients with FSGS after KTx who did not have a response to plasmapheresis and RTX. The patients did not have any positive therapeutic response to abatacept treatment; despite positive podocyte B7-1 expression in their kidney-biopsy specimens. Delvillle et al.[13] reported a prospective study of 9 patients with recurrent FSGS after KTx, with no remission after abatacept therapy in contrast with the recent report by Yu et al.[8] B7-1 expression was not detected in podocytes of biopsies performed at the time of recurrence. Following this study, Delvielle et al.[13] tested B7–1 in other proteinuric diseases of native kidneys including primary and secondary forms of FSGS, diabetic nephropathy, membranous nephropathy,

ANCA vasculitis, and lupus nephritis and B7–1 was not detectable in podocytes in any of these patients. As a result, they did not support the use of B7-1 immunostaining as a biomarker to treat patients with abatacept. Novelli et al.[14] analyzed B7-1 protein expression in the kidney biopsies of 31 patients diagnosed with MCD and FSGS and they could not show B7-1 expression on podocytes in biopsy specimens. They suggest that the anti-proteinuric effect of abatacept determined in previous studies may be a result of immune cell inhibiting rather than a direct effect on podocytes.

The necessity of B7-1 staining in biopsy preparations before initiation of treatment still contains a suspicion and its relationship with disease activation and treatment has not been clearly demonstrated yet. In our case, we thought that we would start abatacept treatment despite any evidence for B7-1 staining due to this uncertainty. In addition, B7-1 immunstaining was not available in our pathology clinic.

Jayaraman et al.[15] reported abatacept experience in a 62 year-old man with steroid and RTX resistant FSGS, who failed to respond to therapy. In addition, Garin et al.[5] have reported progressive fall in urinary protein excretion after abatacept administration in a patient with MCD and high urinary CD80 excretion, in contrast to patients diagnosed with FSGS (one patient with primary FSGS and three patients with recurrent FSGS after KTx) and no CD80 excretion. However, they reported that proteinuria increased on day 9 in their patient with MCD, who responded to abatacept. In our patient, proteinuria decreased to non-nephrotic ranges and albumin levels increased following the third dose of abatacept; but as stated by Garin et al.[5], proteinuria increased rapidly 1 month after the last dose of abatacept.

In our report, there appears to be a similarity between the response of Abatacept and RTX. Partial remission was achieved after RTX treatment and once after pulse steroid administration too. However, with these treatments, the response was not long-lasting, was not achieved at repeated doses and required additional immunosuppressive treatments. There are different protocols of RTX applied in NS but there is still no certain protocol yet. One of these protocols is to administer the drug, once weekly for four weeks.[16] We prefered to follow the patients with serum CD19 levels considering the possible side effects of RTX and repeat the doses when CD19 levels increase above 1%. Although we received a partial response after the second dose, we did not see any benefit after the last dose. Although partial remission was achieved only once with pulse steroid treatment, relapse was observed rapidly on the follow-up period. On the other hand, we did not prefer repeated applications due to side effects such as cataract and impaired glucose tolerance.

In conclusion, although the hope for abatacept failed with regard to the studies carried out after the promising publications of Yu et al.[8] and Alackhar et al.[12] because of the negative consequences of the anti-proteinuric effect of the drug, partial remission including the decrease in proteinuria and increase in albumin levels achieved in our patient encourages the usage of abatacept in patients who do not respond to multiple immunosuppressive therapies and decreased quality of life due to persistent edema and recurrent hospitalizations. As there is no consensus in terms of duration, dosages, safety and efficiency of abatacept yet, further studies to clarify these issues are needed.

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Case Report

Terlipressin in the treatment of neonatal refractory hypotension caused by septic shock: a case report

Quanyao Chen, Zhiyi Huang, Yao Chen

Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, Fujian, China.

ABSTRACT

Background. While the clinical benefits of terlipressin (TP) have been reported in adults and children with refractory hypotension, data in neonates are limited.

Case. Herein, we report a case of off-label rescue TP therapy in a neonate with septic shock and persistent hypotension. The patient's blood pressure was normalized, and tissue perfusion improved without serious adverse reactions. However, genetic testing revealed mitochondrial gene defects in the patient, and the parents subsequently elected to stop treatment after 25 doses of TP (20 μg/kg/min every 4 h for 100 h).

Conclusion. While TP treatment appeared to help control hypotension and may prolong the survival time, there are no conclusive data regarding the safety and efficacy of TP in neonates.

Key words: hemorrhagic shock, hypotension, rescue therapy, Terlipressin, vasoconstriction.

Refractory hypotension in neonates is defined as hypotension with signs of inadequate perfusion despite volume expansion and administration of inotropic agents and/or corticosteroids.[1] This condition is associated with severe intraventricular hemorrhage, a high mortality rate of about 50%, hearing loss, and adverse neurodevelopmental outcomes.[2]

Terlipressin (TP), a long-acting analog of vasopressin (VP), is indicated for treating hemorrhage from esophageal varices and hepatorenal syndrome. Several randomized and retrospective studies in adults and children (> 1-month-old) have shown its clinical benefits in the treatment of refractory hypotension.[3-5] However, TP as a rescue treatment for neonatal hypotension is considered off-label use. We report our experience with the use of TP in a case of neonatal refractory hypotension resulting from septic shock.

✉ Quanyao Chen
chenquanyao1@gmail.com

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Case Report

A boy was born via normal vaginal delivery at 37 weeks and 6 days, with a birth weight of 3,700 g and an Apgar score of 10 at 1, 5, and 10 min. The neonate was transferred to our neonatal intensive care unit owing to the appearance of signs of facial cyanosis 4 h after birth. After tracheal intubation and sputum suction, we found bloody sputum. Chest radiograph suggested pulmonary hemorrhage. His oxygen saturation was 85-90% and pulmonary artery pressure (PAP) was 55 mmHg. Treatment was initiated using high-frequency oscillatory ventilation combined with nitric oxide inhalation. Then the blood pressure was 75/43 mmHg with a mean arterial pressure (MAP) of 53 mmHg, the knees and elbows were cold, the femoral arteries were weak, the complexion was pale, the capillary refill time (CRT) was 3 seconds, and the shock score was 5 points. Dopamine and dobutamine infusions were administered to improve his microcirculation. Twenty-two hours after admission, the blood pressure had dropped to 40/32 (Mean Arterial Pressure (MAP): 35) mmHg despite dopamine treatment of 8.8 μg/kg/min. Thus, dopamine

was replaced with epinephrine, which was gradually increased to a dose of 0.66 µg/kg/min. In addition, hydrocortisone was administered at a dose of 10 mg four times daily. Under this treatment regime, the neonate's MAP increased to 50 mmHg, with mild fluctuations.

Routine blood testing revealed a white blood cell count of 16.08×10^9/L and a C-reactive protein level of 51.5 mg/L. His prothrombin and activated partial thromboplastin times were extended by >1.5-fold. Chest radiography revealed pneumonia, and the newborn was diagnosed with septic shock. Fifty-one hours after admission, he developed hypotension with a blood pressure of 49/32 (MAP: 38) mmHg. His hypotension did not respond to high-dose inotropic drug treatment (epinephrine at 0.82 µg/kg/min and milrinone at 0.5 µg/kg/min) and transfusion therapy, and his oliguria did not respond to furosemide treatment. Thus, TP rescue therapy (20 µg/kg/min every 4 h) was started after obtaining approval from the parents. About 15 minutes after the first dose of TP treatment, the blood pressure increased to 52/43 (MAP 46) mmHg. After the second dose of TP, the values for MAP (maintained at 50-55 mmHg), blood oxygen saturation (90-93%), blood pH (7.12-7.25), lactate levels (15.26-14.33 mmol/L), base excess in the extracellular fluid compartment (-20.9 to -1.73 mmol/L), and urine output (0.3-4.7 mL/kg·h) also improved. Therefore, we decreased the positive inotropic drug support by discontinuing the milrinone treatment. The epinephrine treatment was gradually discontinued starting 72 h after

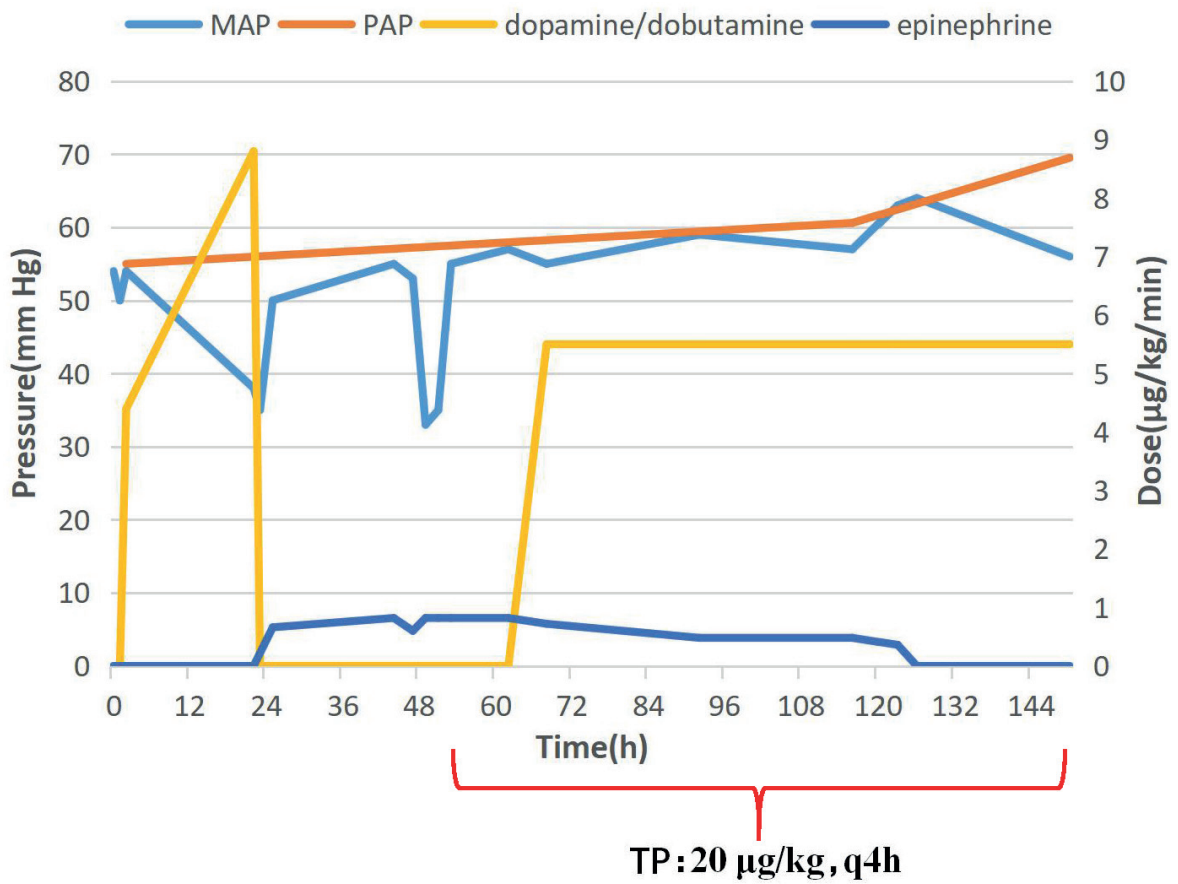

Fig. 1. Male neonate with refractory hypotension: blood pressure values and medications administered during the entire hospitalization process.

MAP: mean arterial pressure, PAP: pulmonary artery pressure, TP: terlipressin.

the first dose of TP. MAP was maintained at approximately 64 mmHg. Blood pressure values and medication administered during the hospitalization process have been given in Figure 1. However, genetic testing revealed mitochondrial gene defects four days after the first dose of TP, and the parents subsequently elected to stop the TP treatment. The newborn died shortly afterward.

Informed consent was obtained from the parents of the patient for the publication of this report.

Discussion

The hypothalamus produces vasopressin (VP), a powerful vasoconstrictor, used to treat cardiac arrest, advanced hemorrhagic shock, and hypotension caused by orthostasis or hemodialysis. In addition to its antidiuretic effect, which is mediated by the V_2 receptor, VP can mediate vasoconstriction of vascular smooth muscle by binding to the V_1 receptor. The TP molecule, a synthetic analog of the VP molecule, has a higher selectivity for the vascular V_1 receptor and consequently a stronger vasoactive and weaker antidiuretic effects than VP. Its pharmacokinetics involve the continuous conversion of the TP prodrug to a vasoactive lysine-VP by endopeptidase, providing a long-lasting and dose-dependent effect. The half-life of TP is 6 h (2-10 h of drug action) while that of VP is 6 min (with 30-60 min of drug action). This difference allows TP to be administered at greater intervals without the rebound hypotension that is usually observed after stopping a VP infusion.[6] However, the relatively long half-life requires adjustment of the TP dose in clinical practice, which may also be a disadvantage.

Patients with septic shock have low VP levels and particularly a sensitive pressor response. Thus, single, intermittent, or continuous doses of TP can provide most patients with adequate blood pressure levels for >5 h, with a decreasing need for positive inotropic support after the first dose of TP.[5] Yildizdas et al.[7] studied 58 children with septic shock and refractory hypotension (randomized enrolment for TP treatment) and found that TP significantly increased MAP and survival time, but did not improve the mortality outcomes. Furthermore, a retrospective study of 14 children with septic shock revealed significant improvements in respiratory and hemodynamic parameters after TP treatment, with a rapid increase and maintenance of adequate blood pressure levels.[3] A prospective study of children also indicated that continuous infusion of TP was effective in improving and maintaining blood pressure.[5] Thus, there appears to be a role for TP treatment in childhood cases of hypotension.

Although these results suggest that TP treatment may also help improve the clinical symptoms and prognosis of neonatal refractory hypotension, there are limited high-quality data regarding this indication.[8] Matok et al.[9] were the first to report TP as rescue therapy in an 8-day-old neonate with septic shock and hypotension who did not respond to dopamine, milrinone, and epinephrine treatment. In that case, the newborn experienced a rapid improvement in both blood pressure and tissue perfusion after starting TP treatment, without any significant side effects. In another study in refractory hypotension in newborns and infants, TP appeared to help correct serum pH and lactate levels, promote the recovery of cerebral vascular tone, improve blood oxygen saturation, and reduce or eliminate the need for catecholamines, which may alleviate the adverse reactions caused by long-term high-dose catecholamine treatment.[10] In our case, the newborn experienced improvements in his clinical, physiological, and biochemical parameters after starting TP treatment, including normalization of cardiac preload and peripheral resistance, improvement of blood oxygen saturation and blood gas parameters, increased urine output, and rapid elevation of MAP from 38 to 55 mmHg, with consecutive maintenance of this level. The limited improvement in lactate levels may have been associated with a mitochondrial metabolic disorder.

The reported TP doses for neonates range from 7 μg/kg twice daily to 20 μg/kg every 4 h.[9,11,12] Some studies have indicated that TP treatment provides the greatest benefit when the norepinephrine requirements are between 0.8 and 2.5 μg/kg/min.[3,13,14] Our patient received TP treatment for a 100-h period divided into 25 doses of 20 μg/kg after the adrenaline dose was increased to 0.82 μg/kg/min. Thus, given the pharmacokinetics of TP, the dosing interval might be extended, and the dose might be decreased to reduce the incidence of adverse reactions, depending on the clinical efficacy and positive inotropic support in an individual case. Nonetheless, there are no conclusive data regarding whether TP administration before the occurrence of catecholamine resistance can improve outcomes in neonates, and additional research is needed to determine the optimal dose, dosing interval, and duration of TP treatment. However, Radicioni et al.[11] reported that TP may be associated with the over-contraction of coronary vascular in neonates (weighing 3,800 g and born at 40 weeks). Thus, in cases with high-dose catecholamine administration, it may be prudent to consider a small starting dose of TP.

The V_2 receptor mediates vasodilation in some blood vessels, and animal experiments have indicated that TP treatment may result in pulmonary vasodilation.[15] This is slightly different from our experience, as our patient experienced a continuous increase in PAP after starting TP treatment. We suspect that this might have been related to a down-regulation of nitric oxide. Furthermore, TP treatment may induce hyponatremia in children with postoperative catecholamine-resistant hypotension,[10,12] possibly via its effect on V_2 receptors.[16] In contrast, our patient had high sodium levels during treatment, which may be related to renal dysfunction and the high selectivity of TP for V_1 receptors, as well as the inhibition of V_2 receptor-mediated antidiuretic effects.

In conclusion, in our case of sepsis-induced neonatal refractory hypotension, TP rescue treatment improved the hypotension. The promising findings in our case require further investigation in future studies to examine their safety and efficacy. Given the risk of adverse reactions, we cannot recommend the replacement of catecholamines with TP as a first-line treatment. However, children who are considered for TP treatment are already in a critical state and have a high mortality rate. Thus, we recommend carefully weighing the pros and cons. If TP is administered, care should be taken to avoid excessive vasoconstriction through monitoring of the patient's heart, lung, kidney function, electrolytes, and hemodynamic status.

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We would like to thank the patient's parents for allowing us to publish the details of this case.

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Case Report

Giant ethmoidal mucocele leading to proptosis and hypertelorism in a pediatric patient with cystic fibrosis: a case report

Orhan Tunç, Burhanettin Gönüldaş, İsmail Aytaç, Semih Mumbuç, Muzaffer Kanlıkama

Department of Otorhinolaryngology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey.

ABSTRACT

Background. Chronic sinusitis and its complications are common in patients with cystic fibrosis. Mucoceles are one of these complications and can have life-threatening consequences if left untreated.

Case. We present the case of a giant ethmoid mucocele leading to proptosis and hypertelorism in a 5-year-old child with cystic fibrosis.

Conclusion. Chronic sinusitis and its complications are common in patients with CF. Mucoceles are a rare complication of sinusitis that can be treated surgically. As seen in this case if left untreated mucoceles can lead to orbital pathologies such as proptosis, hypertelorism. To the best of our knowledge, we report the first case report of giant ethmoidal mucocele leading to proptosis and hypertelorism in a patient with cystic fibrosis

Key words: cystic fibrosis, proptosis, mucocele, chronic sinusitis, endoscopic sinus surgery.

The prevalence of sinonasal pathology in patients with Cystic Fibrosis (CF) is extremely high. Due to the defect in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, stasis of viscous mucous and impaired mucociliary transport are believed to contribute to the development of sinonasal pathology.[1] In general, CF patients exhibit both, chronic inflammation of the lower respiratory tract as well as chronic rhinosinusitis (CRS). Almost 100% of CF patients exhibit morphological changes in computed tomography (CT) of the nose and paranasal sinuses. Furthermore, it was shown, that the prevalence of rhinosinusitis is up to 63% and the prevalence of nasal polyps is 50% in adult CF patients. Only 7.1% of CF patients are free from inflammatory changes in sinonasal histology.[2] Nasal polyps can be seen in up to 86% of children with CF, but only in 6% of those under 6 years of age. Those with the ΔF508 mutation are also more prone to have polypoidal manifestation.[3] Mucoceles are a well-known complication of sinusitis in adults but they very rarely occur in the pediatric age. Mucoceles are secreting cysts lined with upper respiratory epithelium which can grow in the paranasal sinuses with a slow concentric expansion. They are benign, but can enlarge by accumulation of secretions, and may displace and destroy or erode the surrounding bone with local, orbital or even intracranial complications.[4]

Herein we present a 5-year-old patient with bilateral giant ethmoidal mucoceles causing proptosis and hypertelorism.

Case Report

A 5-year-old boy with CF was referred from the department of pediatrics to the otolaryngology department for nasal obstruction. He was the seventh child of a consanguineous family,

✉ Orhan Tunç
orhantunc@gantep.edu.tr

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and his two brothers had previously died for unknown reasons. The patient was followed-up for recurrent lung infection at 5 months of age and ΔF508 mutations were detected and CF was diagnosed. The patient's complaints were long-standing nasal congestion, runny nose, and snoring.

A standard ears nose throat (ENT) examination was performed and an endoscopic evaluation of the nose was performed. There was intense mucoid secretion in the nasal cavity and both inferior and middle turbinates were highly edematous. Nasal passage was completely closed by the inferior and middle turbinates. The patient had proptosis and hypertelorism in both eyes. Paranasal sinus tomography showed giant ethmoidal mucoceles causing propitosis and hypertelorism by pushing the orbita laterally (Fig. 1).

Functional endoscopic sinus surgery (FESS) was performed using a 0^0 view, 2.7 mm rigid endoscope to remove the masses and decompress the sinuses. The mucocele, which pushes the middle turbinate medially, was drained and the yellow-green colored viscous discharge was drained through the mucocele (Fig. 2). Although anterior and posterior ethmoidal cells did not complete their development, they were completely opened. Sphenoid sinus was not fully developed and surgery was not performed on the sphenoid sinus. Maxillary sinus ostium was enlarged. The wall of the cyst was completely removed and the mucosa on the lamina paprisea was preserved. Samples from mucocele and the discharge were sent for microbiology and histological assessments. Pseudomonas aeruginosa was observed in microbiological investigations. Although it was too early two months after surgery, there was a mild improvement in propitosis and

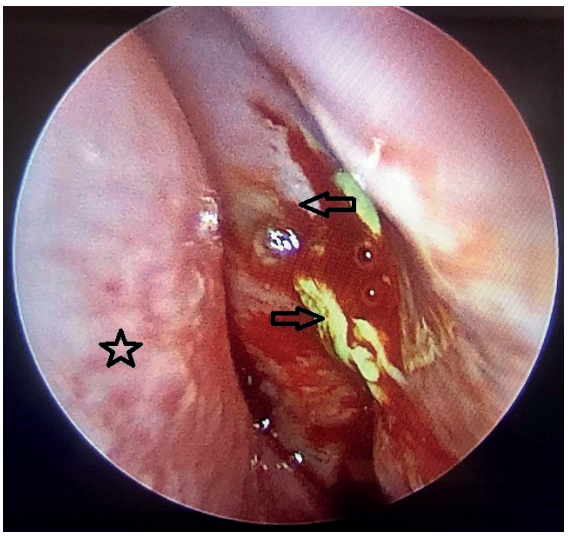

Fig. 2. Surgical image of left nasal cavity, (Black star; nasal septum, Right arrow; yellow green discharge, Left arrow; anterior wall of mucocele).

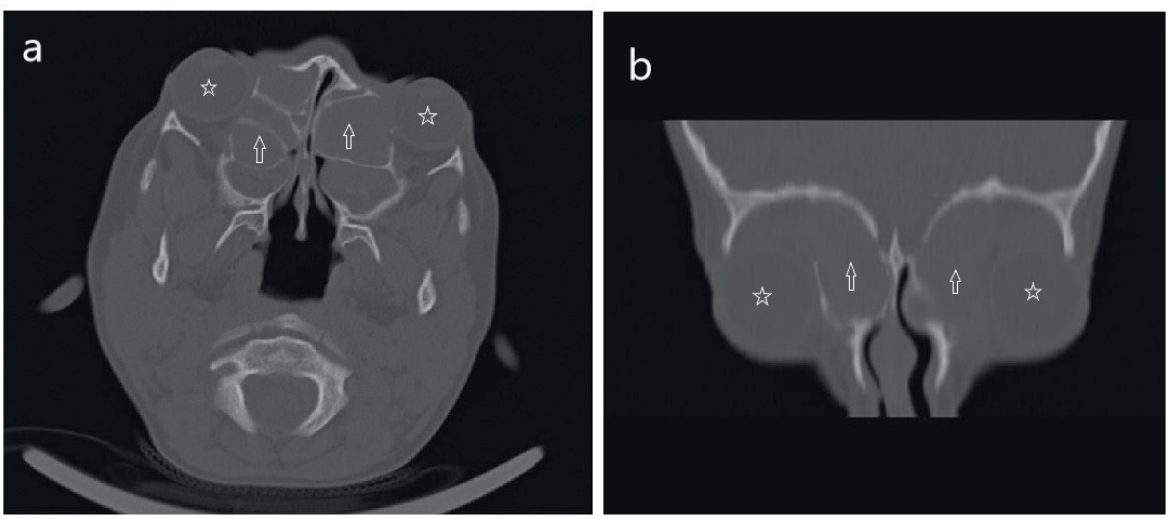

Fig. 1. (a) axial section of paranasal tomography, (b) coronal section of paranasal tomography (White stars; Approximate central alignment of the eye, White arrows; giant ethmoidal mucoceles).

heipertelorism. The parents of the patient were informed about the operation and informed consent form was obtained so that it could be written as a case report.

Discussion

Infectious rhinosinusitis is a common disease in children; secondary bacterial infection or CRS are common complications, particularly in children affected by CF, who generally harbour a large number of bacteria in sputum and nasal secretions.[4] Sinus mucoceles are common complications of CRS in adults but are rare in the pediatric population, and no prevalence data currently exist for children with CF. Although an association is known between the two conditions, mucoceles are rarely the presenting feature of the underlying disease. Frontal sinus is most commonly affected by mucoceles and is involved in approximately two-thirds of cases, the ethmoids are the next most common site, and the maxillary and sphenoidal sinuses are less likely to be involved.[5] Paranasal mucoceles leading to erosion of the surrounding bone walls was more frequent than expected. As the mucoceles expand, they distort local anatomy and apply pressure to surrounding structures. In patients with CF, mucoceles may grow too large and cause orbital symptoms such as proptosis and hypertelorism.[4]

Pathogenesis of mucoceles is still not well understood in CF. Many factors may be involved including the degree of viscosity of the CF mucus, decreased mucociliary clearance due to low mucus hidratation or loss of ciliated cells or the presence of bacterially induced factors. Additionally, some anatomical predisposing factors limiting the drainage of ostium meatal complex may play a pathogenetic role and it may be suggested that in cases with a diagnosis during the initial months of life, the formation of the mucocele had already began in intrauterine life.[4,5]

The pathophysiology for nasal polyp formation in CF is different from the non-CF population. Histologically, nasal polyps in CF consist of dilated mucous glandular structures, lack of eosinophils and a higher volume of neutrophils, mast cells and plasma cells. Microscopically, persistent bacteria colonization is the reason for a prolonged inflammatory response in the sinuses of patients with CF rather than the common viral vector. Pseudomonas aeruginosa is the commonest occupant, with a 95% prevalence rate.[3]

Current treatment involves FESS for drainage and marsupialisation of the mucocele. Topical steroid application is able to decrease the polyp size, but has limited effect on nasal symptoms due to the neutrophilic drive in CF nasal polyps. It seems performing FESS early in a child does not have any detrimental effect on the craniofacial development as first thought. Reviews have also shown a good safety profile for FESS in patients with CF. FESS relieves nasal symptoms, decreases hospitalization and assists with Pseudomonas aeruginosa eradication.[3]

Chronic sinusitis and its complications are common in patients with CF. Mucoceles are a rare complication of sinusitis that can be treated surgically. Untreated mucoceles can lead to orbital pathologies such as proptosis, hypertelorism.

To the best of our knowledge, we report the first case report of giant ethmoidal mucocele leading to proptosis and hypertelorism in a patient with CF. Our recommendation to clinicians is to closely monitor patients with CF on nasal pathologies and to prevent possible complications.

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Case Report

Two-year old girl with glial choristoma presented in a thyroglossal duct cyst

Oğuzhan Şal[1], Mehmet Ali Özen[2], Önder Peker[3], Egemen Eroğlu[4]

[2]Department of Pediatric Surgery, [1]Koç University School of Medicine, İstanbul; Departments of [3]Pathology and [4]Pediatric Surgery, American Hospital, Vehbi Koç Foundation, İstanbul, Turkey.

ABSTRACT

Background. Neuroglial choristomas are rare entities that are composed of differentiated neuroectodermal cells presenting where they do not belong.

Case. Here in this paper, we represent a two-year old patient with a very rare presentation of neuroglial choristoma which manifested itself within a thyroglossal duct cyst. In this paper we will also discuss pathogenesis, clinical manifestation, differential diagnosis and management of the neuroglial choristomas.

Conclusion. In conclusion we believe this unique case may aid in understanding the pathophysiology, differential diagnosis, and management of this rare congenital anomaly.

Key words: thyroglossal duct, neuroglial, choristoma, sistrunk.

Neuroglial choristomas are rarely seen in pediatric patients; they are usually located within the head and neck region. Unlike other cystic neuroectodermal pathologies such as meningoencephaloceles, they are not connected to the subarachnoid space and they do not have direct connection to central nervous system.[1] Furthermore they are not associated with any other neoplasia.

In this paper we present a case in which a two-year-old girl presents with a hard, firm swelling in the anterior midline neck region which has progressively enlarged.

Case Report

A two-year old girl presented with a mass located at the anterior midline neck. Caregivers stated that they noticed the mass since birth; however, it had progressively enlarged during the last 3 months. Physical examination revealed a round, firm, painless mass, 4x4 cm diameter in size (Fig. 1). Differential diagnosis was made in terms of other congenital anomalies or malignancies. Tumor biomarkers were within normal ranges. Complicated thyroglossal cyst was reported on preoperative ultrasonography and magnetic resonance imaging (MRI) (Fig. 2). The patient underwent Sistrunk operation which included resection of the specimen and hyoid bone section over it (Fig. 3). Post-operative outcomes were unremarkable. Histopathologic examination with Hematoxylin-Eosin (H&E) and Glial Fibrillary Acidic Protein (GFAP) revealed a heterotopic brain tissue which was proportional with the size of the intermingled thyroglossal cyst tissue without basal membrane invasion and angiogenesis (Figs 4, and 5). Neuroglial tissue was ruled as compatible with choristoma. The patient did not require further treatment. Informed consent from the patient's legal guardians were given to publish the findings of the case.

✉ Oğuzhan Şal
osal13@ku.edu.tr

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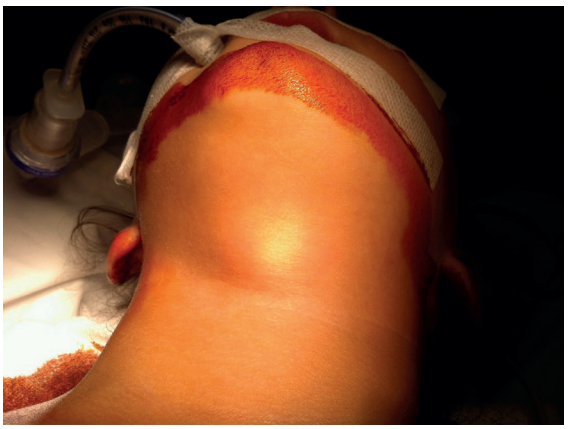

Fig. 1. Patient's preoperative image.

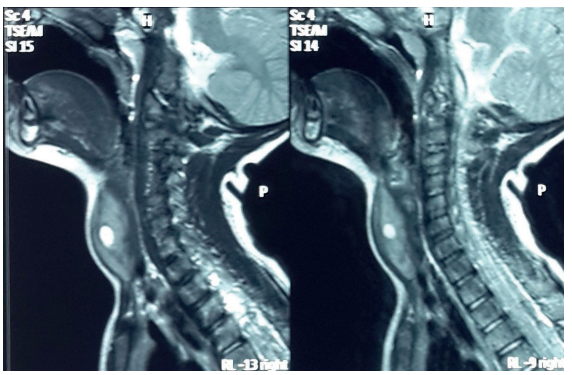

Fig. 2. Magnetic resonance imaging section of the neck.

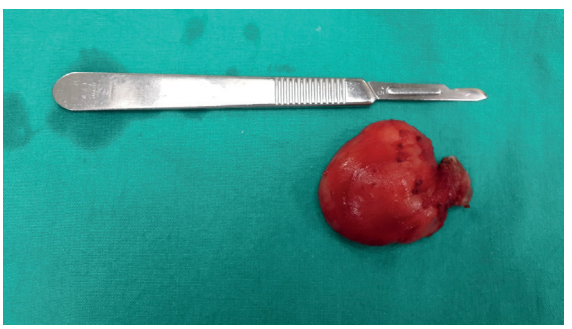

Fig. 3. Macroscopic appearance of the mass.

Discussion

Neuroglial choriostomas (NC) are heterotopic brain tissue, which can involve various locations, including the brain, nose, nasopharynx, and oropharynx.[2]

Pathogenesis of neuroglial chorsitomas are not clear, there are various theories trying to acknowledge the pathogenesis. One of the various theories suggests that neuroglial heterotopias are vestigial or absorbed components of encephaloceles which had formed before.[2] Another one proposes that these lesions are caused by displacement of neural crest cells within the region before termination of neural cell migration and further differentiation into heterotopic tissues which would represent themselves later on.[3] Furthermore development, separation and the tract that had been followed by the cranial nerves can be the etiology behind the neuroglial choristomas within head and neck region.[4] For our case one of the theories mentioned in the literature can enlighten us about the pathogenesis of this case. During invagination of the previously thickened endodermal thyroglossal duct, the nerve tissue innervating the mucosa of the posterior one-third of the tongue is entrapped within this invagination and formed the glial tissue within the duct cyst.[5] Although many theories have been constructed concerning the pathogenesis of neuroglial choristoma; according to our opinion the last one could be the most related theory which acknowledges the anatomical region and pathogenesis of our case. However, it is still a mystery to us why neuroglial choristoma was formed within a thyroglossal duct cyst.

The lesion in this case did not manifest any neoplastic behavior such as stromal and basal membrane invasion, metastasis and angiogenesis through the mass. These histopathologic features of the mass rules out any midline cervical neoplasms. Furthermore, the anatomic site and the size of the lesions accounted for the possible symptoms. Cervical midline choristomas may cause swelling, feeding difficulties, dyspnea, dysphagia, and mechanical obstruction of the airway.

In this case, differential diagnoses of an anterior neck mass include developmental lesions such as thyroglossal duct cyst, ranula, branchial cleft cyst, hygroma, teratoma, neuroglial choristoma, neoplastic lesions such as thyroid, parathyroid and lymphoproliferative neoplasms, reactive lymphadenitis secondary to infections and autoimmune diseases.[6,7] Differentiation between

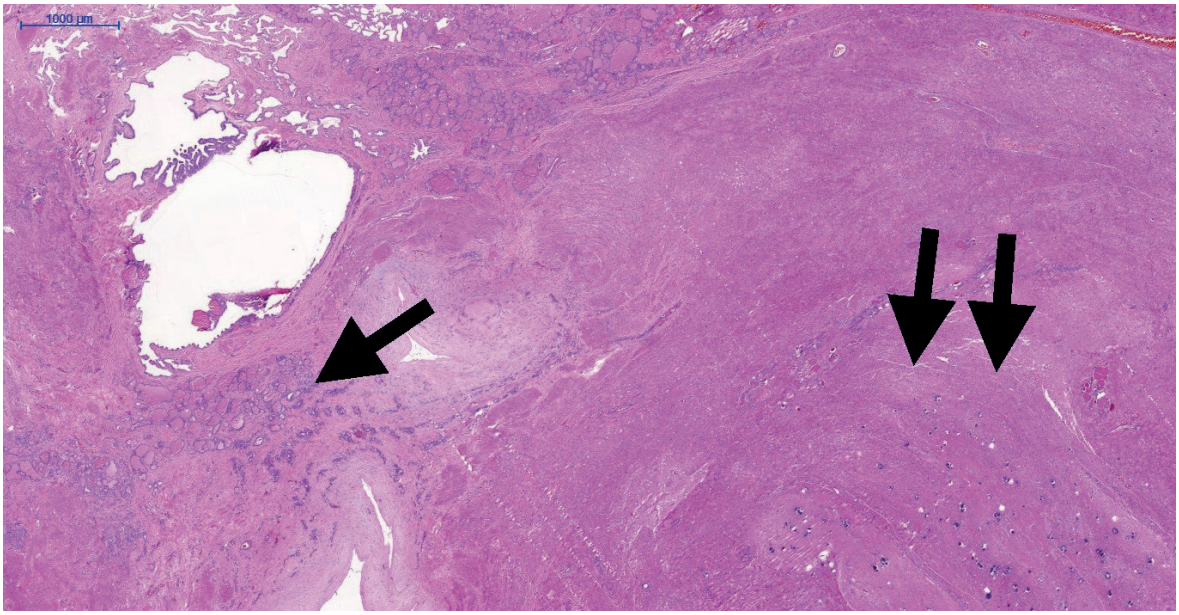

Fig. 4. Histopathologic examination of the mass confirmed the neuroglial choriostoma. Cyctic thyroid tissue (single arrow) and mature glial tissue with psammomas (double arrow). (HEx40).

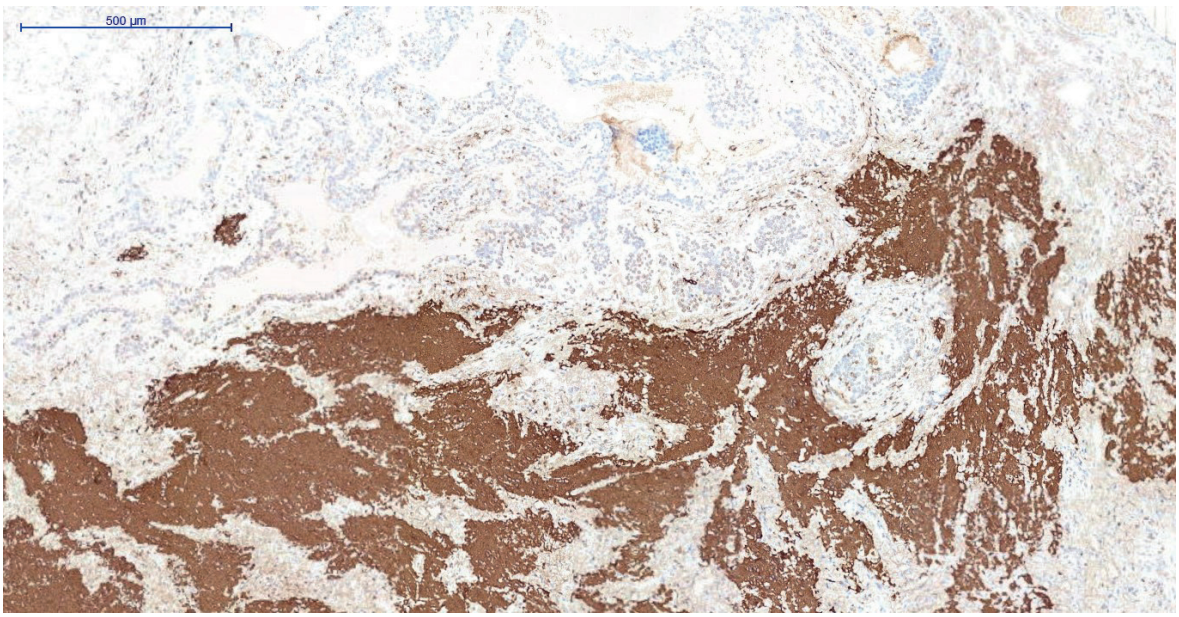

Fig. 5. Neuroglial tissue with Glial Fibrillary Acidic Protein (GFAPx40).

these entities can be done via radiological imaging such as ultrasonography and MRI.[8] However, MRI is superior to USG by its ability to demonstrate anatomy of the mass in more detail.[9] Ideally a combination of both MRI and USG imaging studies will provide much better comprehension of anatomy and pathology.

In addition to neuroglial choristomas, teratomas also have a solid part beside a cystic part.[10] Furthermore, these solid parts are visible in MRI.[11] When they are set side by side with brachial cleft cysts; neuroglial choristomas do not have cyst walls as thick as cystic hygromas; and they are not confined to nasopharynx

and oropharynx like cystic hygromas.[12] Furthermore, a cystic hygroma has an enhancing T2 signal due to its composition. In contrast to ranula; neuroglial choristoma do not have any discoloration that can be seen from outside; also the etiology of ranula is different from a neuroglial choristoma.[13] Ranulas are caused by blocked drainage of saliva and epithelial debris.[13] However, the definitive diagnosis of an anterior cervical mass can be declared by histopathologic examination of the specimen.

The mainstay treatment of neuroglial choristoma is surgery; however, there are some key points that will allow the surgeon and the patient to obtain better outcomes. One of them is the identification of the anatomical landmarks in detail to see the upmost anatomical functionality and aesthetic outcomes. The second point is to distinguish between neuroglial choristoma and other neuroectoderm derived pathologies such as meningoencephalocele; this is a quite vital point because connection between subarachnoid space would change the operation and would definitely have an impact on the post-operative period. The third point is to observe enough to allow vital neurovascular structure to mature so that it is not harmed during surgery due to its close proximity to lesion; also the blood volume of the patient would be greater in case of any hemodynamic instability during the operation.

In conclusion both neuroglial choristomas and thyroglossal cysts are congenital disorders of the midline. However, to the best of our knowledge their association has not previously been documented. Although in this paper we tried to construct possible pathophysiological pathway regarding how a neuroglial choristoma forms; there are spots in pathophysiology and anatomy of this case that needs to be further clarified; this unique case may aid in understanding the pathophysiology, differential diagnosis, and management of this rare congenital anomaly.

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Case Report

Pacemaker lead-induced tricuspid stenosis treated with percutaneous valvotomy

Nilüfer Çetiner[1], Işıl Yıldırım Baştuhan[1], Tolga Özyiğit[2], Ferit Onur Mutluer[3], Alpay Çeliker[1]

Departments of [1]Pediatric Cardiology and [3]Cardiology Koç University Faculty of Medicine, İstanbul; [2]Department of Cardiology, Amerikan Hospital, İstanbul, Turkey.

ABSTRACT

Background. Tricuspid stenosis is an uncommon complication of ventricular pacemaker electrode implantation, with few cases reported in the literature.

Case. We present an 18-year-old male who developed severe tricuspid stenosis 15 years after endocardial VVI pacemaker implantation for complete AV block following a surgically repaired ventricular septal defect.

Conclusion. In this case we have shown that percutaneous balloon valvuloplasty was performed and successful in treating this complication.

Key words: congenital heart defect, pacemaker, percutaneous balloon valvuloplasty, tricuspid stenosis.

Tricuspid stenosis is a rare late complication of endocardial pacemaker implantation that occurs generally in middle-aged patients.[1] As there are limited reports of treatment and outcome of tricuspid stenosis in the literature, we present a young man with endocardial pacemaker-induced symptomatic tricuspid stenosis which was treated by percutaneous balloon valvuloplasty.

Case Report

An 18-year-old male presented to our clinic with exertional fatigue. His medical history was remarkable for VVI transvenous pacemaker implantation at 3 years of age because of a third-degree atrioventricular block that occurred after a surgically repaired ventricular septal defect at 9 months of age. The patient received a diagnosis of tricuspid valve (TV) stenosis associated with a pacemaker implantation at 11 years of age, which worsened during follow-up. The transvenous pacemaker was replaced with an epicardial pacemaker to solve the transvenous lead related problem. The distal part of the electrode could not be removed due to firm adherence to the TV apparatus. At current presentation, he had no complaints of fever or weight loss. Physical examination revealed a loud middiastolic murmur and a 2/6 pansystolic murmur on the tricuspid region. He had no hepatomegaly, lower limb edema and jugular venous distension. The blood chemistry findings were unremarkable. Echocardiography revealed right atrial dilatation with dilated hepatic veins and vena cava inferior. On transthoracic echocardiography the thickened septal leaflet of the TV was noted (Fig. 1). Doppler interrogation revealed peak and mean gradients of 17,6 and 10 mmHg, respectively, across the TV and color flow imaging showed moderate tricuspid regurgitation (Figs. 2 and 3), along with mild mitral regurgitation. Echocardiographic findings were suggestive

✉ Nilüfer Çetiner
nilufercetiner@hotmail.com

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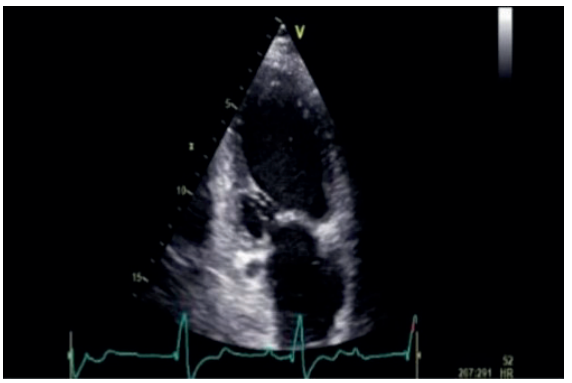

Fig. 1. Thickened septal leaflet of tricuspid valve.

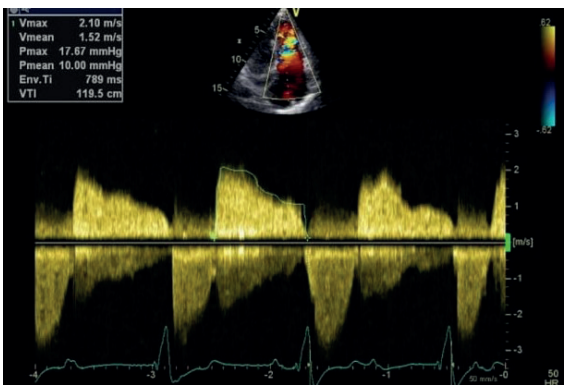

Fig. 2. Doppler Echocardiogram showing gradient across the tricuspid valve.

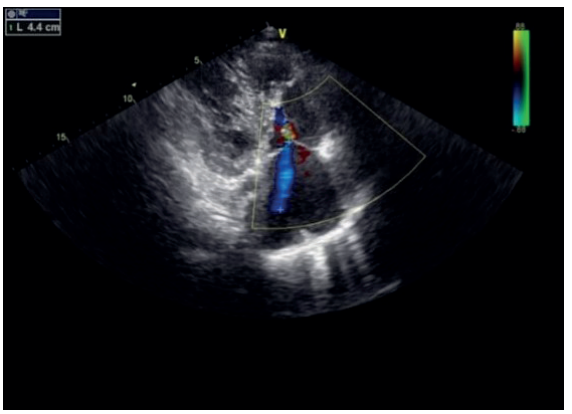

Fig. 3. Echocardiogram showing moderate tricuspid regurgitation.

of persistent and worsening TV stenosis. Percutaneous tricuspid valvuloplasty (PTV) was performed guided by 2D and 3D transesophageal echocardiography (TEE) under general anesthesia. TEE showed the stenotic annulus of TV (Fig. 4). The maximum and mean TV gradients on TEE were 13 and 7 mmHg, respectively. The TV area using planimetry and annulus were measured as 0,9 cm² and 34,5 mm, respectively. Cardiac catheterization showed an increased right atrium pressure (mean 10 mm Hg) and normal pulmonary artery and right ventricular pressures. The valve was dilated after four attempts of inflation with a 28-mm Inoue balloon (Figs. 5 and 6). After final inflation, a transvalvular maximum gradient of 6 mmHg and a mean gradient of 3 mm Hg were measured by echocardiography. On 3D TEE an enlarged tricuspid annulus was noted (Fig. 7). The TV area was measured as 1,9 cm² on 3D TEE. There was no increase in valve regurgitation. The patient was discharged after 24 hours without complications and remained asymptomatic, with a mean gradient across the TV of 3 mmHg at two years of follow-up. Consent for this case report was received from the family.

Discussion

Tricuspid stenosis is an uncommon complication of ventricular pacemaker implantation, with few cases reported in the literature and generally occurs in middle-aged patients. The usual mechanisms described are right ventricular inflow obstruction by TV vegetations (i.e. infective endocarditis), or multiple pacemaker leads and fibrosis secondary to mechanical trauma. The resulting endothelial damage triggers a series of local events, including chronic inflammation, fibrosis, calcification and valve stenosis.[1,2] The present patient was young and the most probable etiology of tricuspid stenosis was endothelial damage and fibrosis of the TV septal leaflet secondary to leaflet perforation by the lead.

Most patients are asymptomatic for a long time and present late with right heart failure.[3] The present case had exertional fatigue with echocardiographic findings of significant TV stenosis.

In most previous literature, the therapeutic management of the tricuspid stenosis revolves

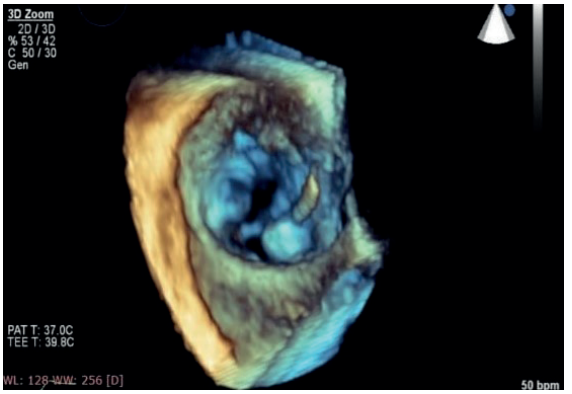

Fig. 4. TEE showing the stenotic annulus of tricuspid valve.

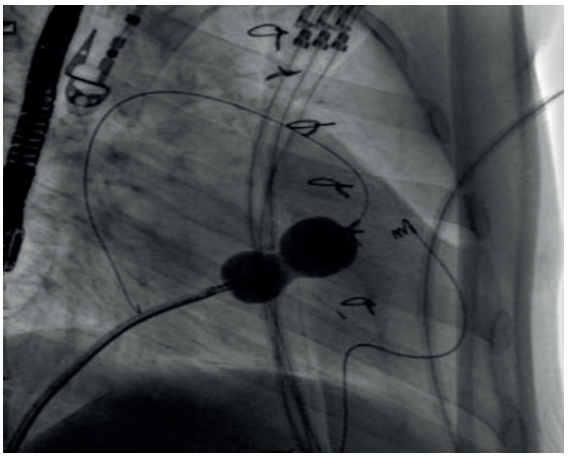

Fig. 5. Image of dilated balloon in the early period during valvuloplasty.

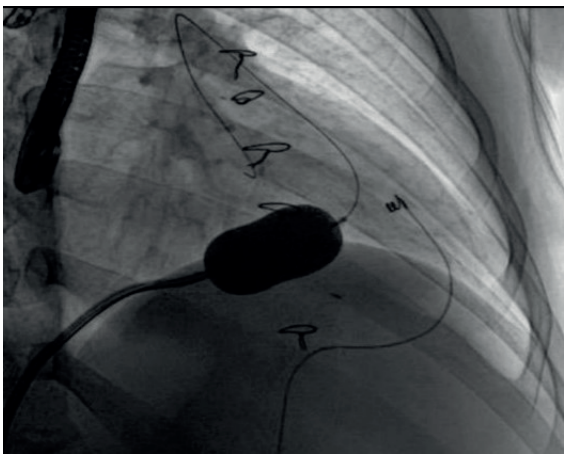

Fig. 6. Image of dilated balloon in the late period during valvuloplasty.

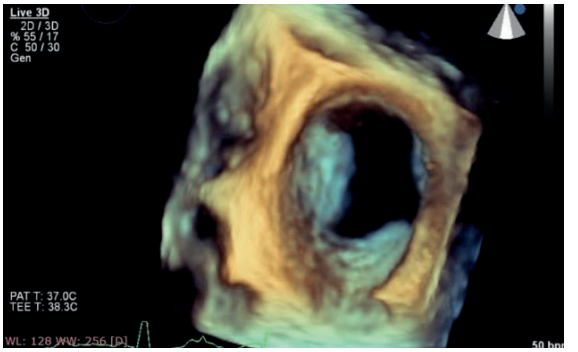

Fig. 7. 3D TEE showing the enlarged tricuspid annulus.

around decongestive therapy and surgical lead removal with TV replacement.[3] The reported management of these cases has included medical management in three (offer of surgery declined in two), surgical lead removal and TV replacement in two, and surgical lead removal followed by tricuspid valvuloplasty in one.[2]

Percutaneous management has also been lately reported, and shown to be safe and effective.[2,4-6] Limited data are available on long-term outcomes. A report of four patients with severe TS who were successfully treated with balloon valvotomy found that symptomatic and hemodynamic improvements persisted at six months follow-up.[2,4-6] In the present case the treatment was performed by successful balloon valvuloplasty. In view of the lasting reduction in gradient by balloon valvuloplasty, no further intervention has been necessary. After treatment the patient was asymptomatic and at two years of follow-up the mean gradient across the TV was 3 mmHg.

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Case Report

Extracorporeal shock wave lithotripsy in the management of a 14-year-old girl with chronic calcific pancreatitis

Özlem Boybeyi Türer[1], Tutku Soyer[1], Erkan Parlak[2], Hatice Nursun Özcan[3], Cenk Yücel Bilen[4], Feridun Cahit Tanyel[1]

Departments of [1]Pediatric Surgery, [2]Gastroenterology, [3]Radiology and [4]Urology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Chronic pancreatitis is very rare in childhood and causes chronic/relapsing abdominal pain, frequent hospitalizations, malnutrition, growth retardation, and stone formation in the main duct. Although pancreatic extracorporeal shock wave lithotripsy (P-ESWL) is commonly used in the treatment of pancreatic stones (PS) in adults, the use in children is still controversial. An adolescent girl with multiple PS is presented to discuss the use of ESWL as a treatment alternative in children with PS.

Case. A 14-year-old girl was admitted with abdominal pain and elevated pancreatic enzyme levels. Abdominal US showed irregularity and rough echogenicity in pancreas revealing pancreatitis. Multiple stones were seen in main pancreatic duct on Magnetic resonance cholangiopancreatography (MRCP). Endoscopic retrograde cholangiopancreatography (ERCP) was performed and dilated pancreatic duct, thickened pancreatic secretion were detected. Endoscopic sphincterotomy was performed. Endoscopic removal of stones could not be achieved since the largest stone was 17x8 mm. Pancreatic extracorporeal shock wave lithotripsy (P-ESWL) was performed using electromagnetic lithotripter under general anesthesia. Following ESWL, fragmentation of stones in the main duct was confirmed with ERCP. After 3 sessions of ESWL, no ESWL-related complication was observed. Pain relief was achieved. The patient is still under follow-up regarding endocrine and exocrine function of pancreas.

Conclusion. ESWL may be an effective and safe management option in pediatric PS which could not be removed by ERCP. The patients managed with ESWL should be followed-up for a long time regarding the endocrine and exocrine functions of the pancreas. As in management of adult pancreatitis, clinical guidelines are needed regarding the management of pediatric PS.

Key words: calcific pancreatitis, chronic pancreatitis, ESWL, pancreatic stone, children.

Chronic pancreatitis (CP) is rarely seen in children and causes extreme disruption not only in their health status but also in their education and development.[1] CP causes chronic and relapsing abdominal pain, frequent hospitalizations, malnutrition and growth retardation in children.[1] Approximately 50% of CP patients suffer from pancreatic ductal stones which increase intra-ductal pressure and cause severe abdominal pain.[1,2] In addition, children have increased cumulative risk of recurrence since their life expectancy is longer than adults. Therefore, treatment of choice should be minimal invasive as possible to ensure repetitive procedures could be done safely in children.

Although pancreatic stone (PS) management is considered as standard treatment in adults with pancreatic extracorporeal shock wave lithotripsy (P-ESWL), there is no consensus on management of PS in children. ESWL was first used for renal calculi by Chaussy et al.[3] in

✉ Özlem Boybeyi Türer
ozlemboy80@yahoo.com

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1980, and its use for pancreatic stones was by Sauerbrunch et al.[4] in 1987. Since then, it has become the first line therapy in PS management in adults as recommended by the European Society of Gastrointestinal Endoscopy (ESGE) guideline.[5] However, in the same guideline it was reported that endoscopic management should be the treatment of choice in children since effectiveness and safety of ESWL use in children was not yet verified.[5] Although a few studies have reported the use of P-ESWL in children in ESGE guideline, there is no standardized protocol or a guideline regarding use of P-ESWL in children. Herein, we reported a child with multiple PS who was treated with P-ESWL to discuss the management of PS in children.

Case Report

A 14-year-old girl was admitted to our department with abdominal pain starting from the epigastric region and surrounding her belly. Her physical examination revealed epigastric tenderness. The weight of the patient was 42 kg (10-25 percentiles) and height was 150 cm (5 percentiles). The laboratory work-up were normal except amylase and pancreatic amylase levels. The amylase level was 1621 U/L (N: 28-100 U/L) and pancreatic amylase level was 1216 U/L (N: 13-53 U/L). Abdominal ultrasonography (US) showed irregularity and rough echogenicity in the pancreas revealing pancreatitis. Therefore, she was hospitalized and medical treatment was initiated.

In her past medical history, she experienced three attacks of pancreatitis starting from 9 years of age. During the second episode of pancreatitis, endoscopic retrograde cholangiopancreatography (ERCP) was performed and partial divisum, dilated pancreatic duct, and thickened pancreatic secretions were detected. Endoscopic sphincterotomy was performed, but her complaints recurred. The blood lipid levels, serum chemistry, liver function tests, thyroid function tests and genetic analysis for cystic fibrosis were within normal limits.

At her present admission, multiple stones were seen in the main pancreatic duct on Magnetic resonance cholangiopancreatography (MRCP) (Fig. 1). Endoscopic removal of stones could not be achieved since the largest stone was 17x8 mm. Endoscopic sphincterotomy was performed again and our multidisciplinary council decided to perform a P-ESWL. P-ESWL was performed using a third-generation electromagnetic lithotripter (Siemens modularis variostar-Cplus, Erlangen, Germany) under general anesthesia. PS were targeted by lithotripter using fluoroscopy (Fig. 2). Not more than 5000 shocks with 3.5 microjoule per each shock were performed for each ESWL session (16 000 kV, 120 shock/min). The duration of each session was approximately 60 min. P-ESWL sessions were

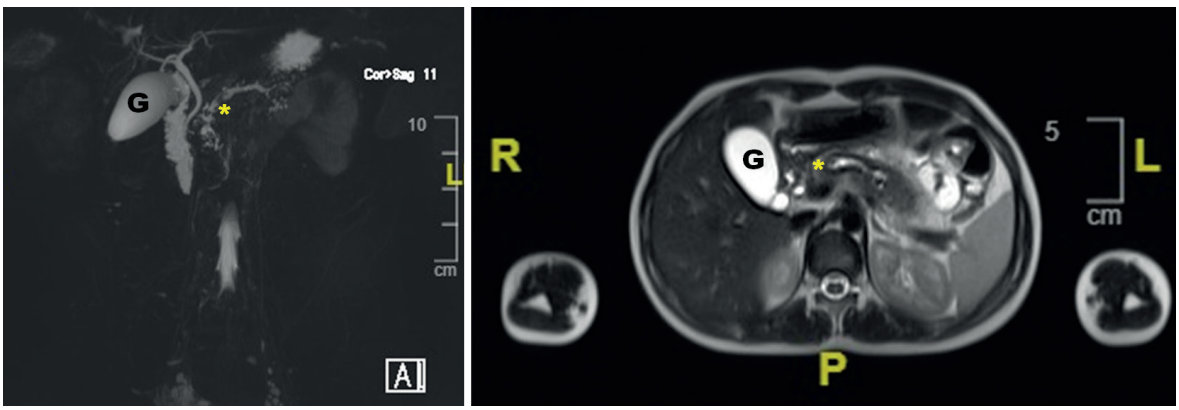

Fig. 1. The MRCP images showing dilated and tortoise main pancreatic duct with irregular borders and multiple stones within the main pancreatic duct (G: gallbladder, *: main pancreatic duct).

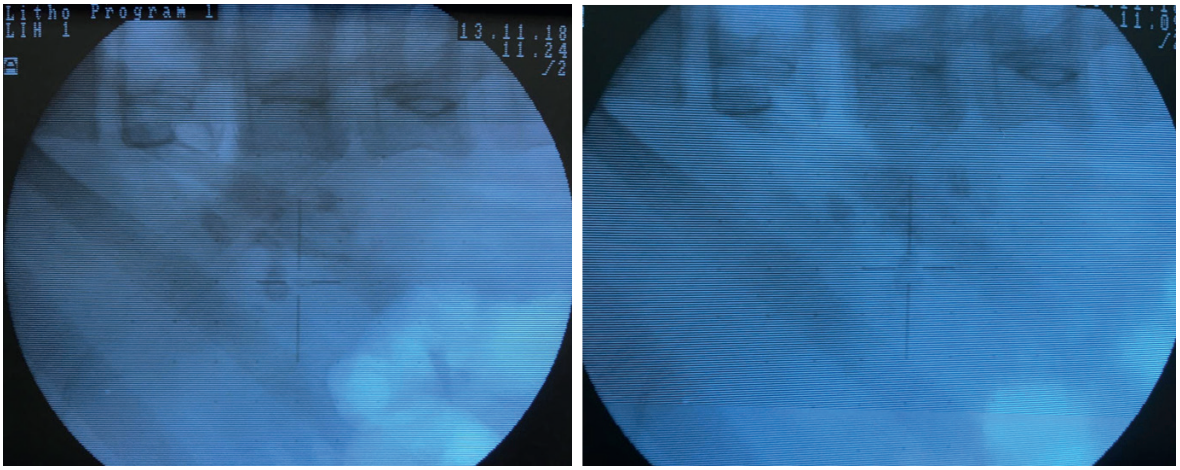

Fig. 2. Pancreatic stones were targeted by lithotripter using fluoroscopy showing a large stone at the middle of the target lines.

performed every other day, three times totally. Radiation protection was provided according to ESGE guidelines.[5] Following ESWL, ERCP was performed showing that the stones were fragmented and passage of contrast agent could be achieved into the main pancreatic duct. Since spontaneous clearance of stones was not seen, endoscopic wash of the pancreatic duct and re-sphincterotomy was performed. Additionally, 7Fr stent was inserted into the main pancreatic canal to prevent re-obstruction of the canal. After P-ESWL sessions, no ESWL-related complication was observed. Pain relief could be achieved with non-steroidal anti-inflammatory drugs in small doses.

Abdominal US was performed 1 month after the last ESWL session and revealed fragmented stones in pancreatic duct with a largest diameter of 7 mm. Therefore, we decided to repeat the P-ESWL sessions 3 months later. The pancreatic exocrine function profile was evaluated with testing for steatorrhea and fecal elastase test. Although steatorrhea in the stool was negative, fecal elastase level was lower than 15 mg/ml revealing pancreatic exocrine function deficiency. Therefore, pancreatic enzyme replacement 4 times/day (Creon®, Abbott Ltd., Istanbul, Turkey; 25000- 300 mg: 300 mg pancreatin + 18000 PEU amylase + 25000 PEU lipase + 1000 PEU protease) was ordered. Her vitamin D (Vit D) level was 5,4 μg/lt (severe deficiency) and Vit D replacement (50000 IU/15 ml, 6 drop/day) was ordered. The Vit D level increased to 20,19 μg/lt after replacement therapy. The pancreatic endocrine function was assessed with blood glucose level, 75-g oral glucose tolerance test and HbA1c level which were within normal limits. The patient is still under follow-up for 1 year regarding endocrine and exocrine function of pancreas. The current weight of the patient is 46 kg (10-25 percentiles) and height is 155 cm (10-25 percentiles) revealing a slow improvement. In addition, she still has a stent in the main pancreatic canal with fragmented and partially cleared stones. Since then, she has not experienced pancreatitis again. Informed consent was obtained from the parents of the patient that allows using the clinical data for scientific purposes.

Discussion

Pancreatic duct stones (PS) are seen in 50% of patients with chronic pancreatitis. The intra-ductal pressure increases in the presence of PS leading to ischemia and pain.[1,2] Since life expectancy of children is longer, life-long risk for PS recurrence is higher in children. The relapsing abdominal pain causes frequent admissions to emergency departments and longer hospital stays leading to increased school absences. Therefore, repeatable, safe

and effective methods are needed in the management of PS in children.

Although PS management is standardized in adults, there is no consensus on management of PS in children. The treatment alternatives of PS in children include surgical excision, endoscopic removal or fragmentation of stones by ESWL. Endoscopic stone removal is the first line treatment since it is less invasive than surgery. However, success rate of endoscopy is limited to 50% of all PS in children.[1,2] Additionally, the PS smaller than 3 mm and located at the head and body of the pancreas are considered as an indication for ERCP. The stones greater than 5 mm, multiple in number and distal location cannot be removed by ERCP. Therefore, fragmentation of such stones is needed to restore pancreatic juice flow and relieve pain.[2]

P-ESWL is reported to be safe, effective and minimal invasive in adults and recommended as first-line therapy combined with or without endoscopic removal of fragments.[5] The success in P-ESWL is defined as fragmentation of stones into pieces smaller than 3 mm or decreased stone density in radiological examinations.[5] A meta-analysis revealed that complete ductal clearance is achieved in 70% of cases and partial ductal clearance is achieved in 22% of cases.[6] According to the results in that meta-analysis, number of sessions varied according to the clinical response and ductal clearance, but better results were obtained when the number of sessions were more than three.[2,6] Additional endoscopic procedures such as removal of fragments, stent placement to main pancreatic duct and sphincterotomy are usually needed following ESWL.[5,6] In our case, partial ductal clearance, fragmentation of stones and pain relief were achieved after 3 sessions of ESWL. Endoscopic sphincterotomy was performed to increase flow of pancreatic juice; however, it was not possible to place a stent because of multiple fragments in the entire duct. Additional sessions were planned since the patient has residual stones in pancreatic duct.

The endocrine and exocrine function of the pancreas should be investigated in patients with chronic pancreatitis.[7] Although there is not enough data in the literature, ESWL seems to improve exocrine and endocrine pancreatic functions.[6,7] When we evaluated the endocrine and exocrine pancreatic function in this patient, we found decreased exocrine function with normal endocrine functions. However, this assessment was performed only after ESWL; we cannot comment on the baseline evaluations since we were the final referral center for this patient. Therefore, it will not be possible to suggest that ESWL either improves or alters the exocrine or endocrine pancreatic function for the present case. Thus, all clinicians should be aware of alterations in pancreatic functions in cases with chronic pancreatitis and follow-up the result of the management. On the contrary, Adamek et al.[8] stated that endoscopic management or ESWL do not affect the development of glandular insufficiency, since chronic pancreatitis causes progressive parenchymal destruction of pancreas. Therefore, future studies are needed to investigate the effect of management of chronic pancreatitis and PS to pancreatic functions in children.

Although P-ESWL was reported to be safe and effective in the management of PS, it may cause several complications including acute pancreatitis, bleeding, infection or perforation.[1] Wang et al.[1] reported that complication rates were 11% in children and 12% in adults and they found no statistically significant difference between pediatric and adult patients. The present case did not experience any complications after the P-ESWL sessions. Although future studies are needed to confirm these results, P-ESWL in children seems to have similar complication rates with adult patients and can be performed safely in childhood PS management.

In conclusion, ESWL is an effective and safe management option for pediatric PS that could not be removed by ERCP. The patients managed with ESWL should be followed-up for

a long time in regards to endocrine and exocrine functions of the pancreas. As in management of adult pancreatitis, clinical guidelines are needed regarding the management of pediatric PS.

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Case Report

Xanthogranulomatous pyelonephritis due to calculi in a 5-year-old girl

Esra Nagehan Akyol Önder[1], Mine Özkol[2], Nalan Neşe[3], Can Taneli[4], İpek Özünan[1]

Departments of [1]Pediatric Nephrology, [2]Radiology, [3]Pathology and [4]Pediatric Surgery, Celal Bayar University, Faculty of Medicine Manisa, Turkey.

ABSTRACT

Background. Xanthogranulomatous pyelonephritis is characterized by the inflammatory destruction of the renal parenchyma and intensive renal fibrosis. It is named because of its pathological appearance; that of its granulomatous inflammatory process with lipid-laden macrophages, which appear yellow, hence 'xantho' which is Greek for yellow. Xanthogranulomatous pyelonephritis is predominantly a disease of adults. In children it is diagnosed sporadically and is extremely rare in infants. The age of onset varies (21 days to 16 years), although 60-75% of cases have been diagnosed before 5 years of age. Recurrent urinary tract infections, obstructive nephropathy caused by renal calculus, malnutrition, abnormal lipid metabolism, altered immunologic response, lymphatic blockage, congenital urinary anomalies have been implicated in the etiology of xanthogranulomatous pyelonephritis in children.

Case. We report an unusual case of xanthogranulomatous pyelonephritis in a 5-year-old girl and discuss its clinical features, histopathological findings and treatment. In this article, we also emphasized the importance of diagnostic imaging in urinary tract infections which enabled us not to miss the underlying kidney stone disease.

Conclusion. Nephrolithiasis may lead to very serious conditions such as xanthogranulomatous pyelonephritis. This condition can be easily diagnosed by ultrasound, but if not detected, it can lead to complete loss of renal function as in the case.

Key words: Xantogranulomatous pyelonephritis, nephrolithiasis, kidney stones.

Xanthogranulomatous pyelonephritis (XPN) is an unusual and severely chronic inflammatory disease of the kidney, characterized by infiltration of the renal parenchyma with lipid laden macrophages.[1] It is rarely seen in childhood and the mechanism of XPN is poorly understood.[2] Recurrent urinary tract infections, obstructive nephropathy caused by renal calculus, malnutrition, abnormal lipid metabolism and altered immunologic response reported to predispose an individual to this rare renal parenchymal infection often mimicking neoplastic renal disorder.[3]

✉ Esra Nagehan Akyol Önder
esra.nagehan.7@hotmail.com

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Case Report

A five-year-old girl with complaints of flank pain, concomitant hyperpyrexia, loss of appetite for a month was referred to our clinic for further evaluation. She was treated with several courses of antibiotics for about a month in different centers. However, no diagnostic imaging was performed. Her history revealed that her development was uneventful. There was a positive family history of nephrolithiasis.

Her physical examination findings were as follows: weight, 15 kg (<3 percentiles); height,

110 cm (50-75 percentiles); heartbeat, 80/min; respiratory rate, 22/min; blood pressure, 90/50 mmHg (<90 p). A mass on the left side extending beyond the midline into the pelvis with dimensions of 8x10 cm was palpated. Costovertebral angle tenderness was not detected. Physical examination of other systems was unremarkable.

Her laboratory findings were as follows: white blood cell count 28420/mm3; platelets 752000/mm3. Peripheral blood smear findings revealed polymorphonuclear leucocytes (66%), toxic granulation, and hypochromia (18%). Acute phase reactants were also elevated: erythrocyte sedimentation rate: 120 mm/h, (0-15 mm/h), C-reactive protein: 15 mg/L (0-0,5 mg/dl) and procalcitonin: 0,34 ng/ml, (0-0,05 ng/ml). Biochemical values were within normal limits. Urine analysis showed: urine density, 1026; pH, 6,5; leukocytosis and intermittent haematuria. Urine culture was positive for Pseudomonas spp. and it was susceptible to ceftazidime.

On abdominal ultrasonography (USG), the left kidney was enlarged diffusely (111x61 mm). Five to six stones within the left pelvis renalis measuring 9 mm, and a few millimetric calculi within of the calyceal groups were detected. The pelvic-calyceal lumens appeared dilated and full of pus. There was inflammatory hyperechogenicity in perirenal adipose tissue. The right kidney was normal in size and echogenicity. Abdominal computed tomography (CT) was consistent with left renal multiple pelviceal stones, diffuse enlargement, foci of calcifications and hydro-pyonephrosis (Fig.1A). In a technetium 99m-dimercaptosuccinic acid renal scan a non-functional left kidney was revealed (Fig.1B).

Based on the clinical examination and imaging, the differential diagnosis included renal tuberculosis, Wilms tumor and XPN. Three early-morning urine samples sent for acid-fast stain were negative. Tuberculin skin test was negative. The patient had no concomitant clinical or radiographic findings suggestive of pulmonary involvement, thus we ruled out renal tuberculosis. We examine the patient for Wilms tumor associated anomalies, such as aniridia, hemihypertrophy, and genitourinary anomalies but we didn't find any signs related with these anomalies. Because of the suspected renal tumor, Doppler USG was performed for detecting tumor infiltration in the renal vein or inferior vena cava but no evidence was found. Additionally, chest radiograph and abdominal CT was negative for metastases. Although it is not easy to diagnose XPN preoperatively, based on the patient's typical clinical presentation of drug resistant urinary tract infection due to calculi and the combination of a nonfunctioning enlarged kidney, multiple central stones within the contracted renal pelvis, expansion of the calices, and inflammatory changes in the perinephric fat on the imaging features, a clinical diagnosis of XPN was made and a left radical nephrectomy was performed. No complication was observed during the operation and postoperative period. In the macroscopic examination of the specimen, left nephrectomy material was noted as having 11,5x7x6,5 cm dimensions and weighing 225 g. The outer layer of the specimen was multilobulated. There was a remarkable loss of renal parenchyma. All of the calyceal lumens were dilated and full of yellow-colored pus and multiple stones (Fig.1C). The sample tissues were fixed in 10% formalin and embedded in paraffin. Sections cut at 4 mm were stained with hematoxylin–eosin. Histopathological examination showed an acute and chronic inflammatory cell infiltrated with giant cells and lipid-laden macrophages (foam cells) on cortical areas (Fig. 1D). After surgery, she was feeling well and gained weight. Her acute phase reactants decreased to normal. She has been followed up at three-month intervals for nearly 1 year after surgery without complications. Informed consent was received from the family.

Discussion

Xanthogranulomatous pyelonephritis is an uncommon chronic obstructive pyelonephritis characterized by infiltration and fibrosis of

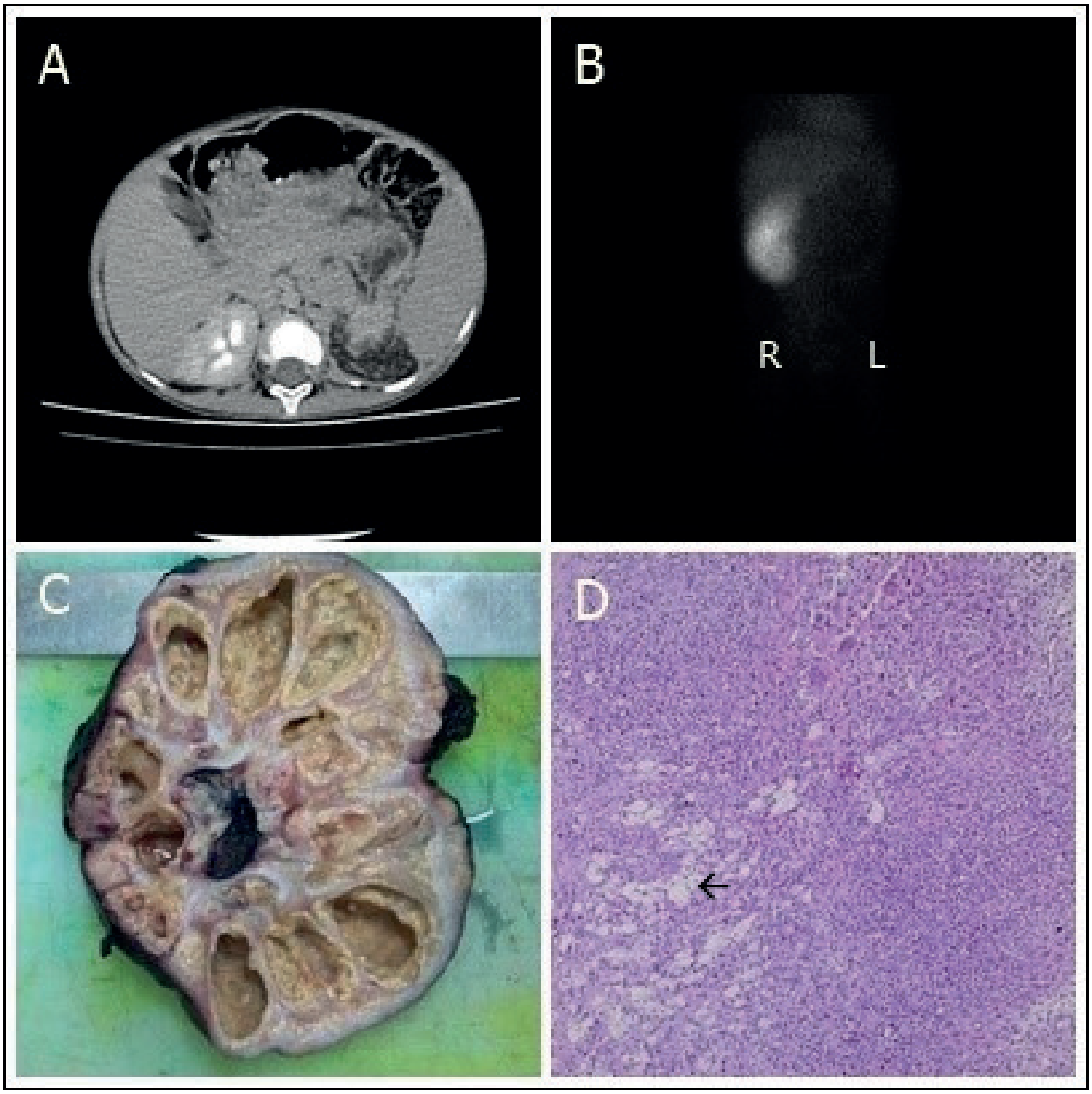

Fig. 1. (A) Computed tomography (CT) consistent with left renal multiple pelviceal stones, diffuse enlargement, foci of calcifications and hydro-pyonephrosis. (B) DMSA scan shows non-functional left kidney. (C) Macroscopic features of XPN: dilated calyces filled with pus and yellow tinge of fat laden macrophage layer is seen. (D) Histological features of XPN showing fibrosis, inflammatory infiltrate and the black arrow points fat laden macrophages.

the renal parenchyma composed of lipid laden macrophages, as well as a variety of inflammatory elements including, neutrophils ,lymphocytes, plasma cells, cholesterol clefts and multinucleated giant cells.[1,2,4] Recurrent urinary tract infections with E.coli, Proteus mirabilis, and rarely Pseudomonas species (54%), obstructive nephropathy caused by renal calculus (68%), malnutrition, abnormal lipid metabolism, altered immunologic response (5%), lymphatic blockage, congenital urinary anomalies (5%) have been reported to predispose an individual to this rare renal parenchymal infection often mimicking neoplastic renal disorder.[1,3] XPN has been termed "the great imitator" because the differential diagnosis

includes a large group of disease such as Wilms tumor, renal cell carcinoma, renal abscess, infected renal cystic disease, tuberculosis, malakoplakia, and transitional renal cell carcinoma. The preoperative diagnostic rates are higher in the pediatric population.[5]

Schlagenhaufer first described features of XPN in 1916.[1] Quinn et al.[6] reported the presence of multiple calculi in 26 out of 31 patients involving the left kidney. In the same study, affected kidneys of 23 cases were detected as being nonfunctional or inadequately functional (<10%).[6] Our patient had a non-functional left kidney with multiple calculi and her urine culture was positive for Pseudomonas spp.

Bingol-Koloğlu et al.[7] reported that XPN is predominantly a disease of adults however it may occur in all age groups with two peaks, one before the age of 10 and the other in the fourth to fifth decade. Only a third of all cases were seen in children and they found a male predominance (85%). However, there are some reports revealing that the diffuse form is seen equally in boys and girls while focal XPN is more common in girls.[8-10]

Histologically the disease is characterized by lipid laden foamy macrophages and acute and chronic renal inflammatory cells.

Treatment is always surgical excision of the damaged tissue (total or partial nephrectomy) depending on the extent of involvement and the prognosis after the surgery is excellent.

In conclusion, XPN is a rare condition in children, the symptoms are usually mild. It is a histopathological entity and preoperative diagnosis is challenging; thus it is difficult to diagnose. It should be kept in mind that fever and flank tenderness are the most common symptoms. The mainstay imaging technique is CT in clinically suspected patients. The definitive diagnosis of XPN is pathological. Nephrectomy is the curative treatment. In this article, we also emphasized the importance of diagnostic imaging in recurrent urinary tract infections. It is necessary to diagnose underlying kidney stone disease. Nephrolithiasis may lead to very serious conditions such as XPN. They can be easily diagnosed by ultrasound, but if not detected, this can lead to XPN and complete loss of renal function as in this case.

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We thank the parents of the patient described for allowing us to share her details.

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Case Report

Type 4 hypersensitivity development in a case due to mifamurtide

Meral Şimşek[1], Erman Ataş[2], Emin Ümit Bağrıaçık[3], Armağan Günal[4], Bülent Ünay[5]

Divisions of [2]Pediatric Hematology and Oncology and [5]Pediatric Neurology, [1]Department of Pediatrics, [4]Department of Pathology, University of Health Sciences, Gülhane Faculty of Medicine, Ankara; [3]Department of Immunology, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. This report aims to discuss the mechanism of pleural and pericardial effusion related to mifamurtide which is an immunological agent used as adjuvant chemotherapy in osteosarcoma.

Case. Mifamurtide (2 mg/m^2) and European and American Osteosarcoma Studies (EURAMOS) protocol were used together intravenously after complete surgical resection. No side effects occurred except for fever after the first dose. However, pleural, pericardial effusion, and splenic nodule formation began 11 months after discontinuation of mifamurtide treatment. Pleural biopsy revealed a type 4 hypersensitivity reaction. We treated the patient with 1,5 mg per day colchicine. Pericardial effusion attacks and nodules in the spleen disappeared. The patient had a mild pleural effusion attack which has not yet repeated.

Conclusion. Mifamurtide, which activates macrophages, can also activate immunity with a stand by effect and cause a hypersensitivity reaction.

Key words: child, osteosarcoma, mifamurtide, pleural effusion, pericardial effusion, hypersensitivity.

Osteosarcoma (OS) is the most common bone tumor in childhood. Considerable progress in survival has been obtained recently. Advances in chemotherapy, surgery, and immunotherapy have shown their influence in the treatment of OS. Mifamurtide is an immunological agent used in the treatment of OS.[1] It influences treatment at the level of micrometastasis. However, there are some side effects as well as positive contributions to treatment. Most of them are chills, fever, headache. Mifamurtide can affect all systems in the body. However, some of these side effects maybe life threatening.[2] Thus, we tried to reveal the pharmacodynamical mechanism of side effects in a patient who developed pleural and pericardial effusion attacks after mifamurtide.

✉ Meral Şimşek
meralulgersimsek@gmail.com

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Case Report

A 10-year-old male patient was admitted with left knee pain. Radiological examination of the left knee revealed a 9x5.5x4 cm mass. A biopsy was performed, and the pathology was consistent with high-grade OS. After a 10-week preoperative European and American Osteosarcoma Studies (EURAMOS) protocol, limb-sparing surgery was performed. Mifamurtide was added to the postoperative EURAMOS treatment. Treatment was discontinued in complete remission. Approximately 11 months after mifamurtide treatment, he was brought in with complaints of chest pain, back pain, and dyspnea. Thorax ultrasonography revealed a 5.5 cm pleural effusion that collapsed the lung in the left thorax. Echocardiography showed moderate pericardial effusion, which was approximately 15 mm in the anterior and posterior walls. It also included fibrin particles. Purified protein

derivative (PPD) was anergic. A chest tube was inserted. Vancomycin and ceftriaxone were started for complicated pneumonia. During the follow-up, vancomycin and meropenem treatments were started due to the fact that fever and acute phase elevation persisted. In the pleural fluid biochemical examination results were measured as glucose: 135 mg/dl, concurrent glycaemia: 114 mg/dl, protein: 5.7 mg/dl, serum protein: 4,82 mg/dl, LDH: 1674 U/L, serum LDH: 303U/L. Cytology revealed abundant neutrophils, leukocytes, and histiocytes. Pericardiocentesis was performed. Pericardial cytology also showed diffuse lymphocytes, plasma cells, and macrophages. In a pericardial biopsy, chronic inflammation and fibrosis tissue were detected. Cultures did not grow, and no Adenosine Deaminase Activity (ADA) activity was detected. Mycobacteria culture was negative. Pericardial effusion regressed to 11 mm in the second week of treatment. Abdominal ultrasound showed a slightly heterogeneous, hyperechoic, nonspecific area. It was barely distinguishable from the normal spleen parenchyma. On computer tomography (CT), a solid lesion with a diameter of 3 cm was confirmed, and fluorodeoxyglucose -positron emission tomography with computer tomography (FDG PET / CT) and bone scintigraphy showed no significant increase in metabolic activity. A biopsy was not performed. After this event, the patient was brought back with the same complaints five months later. Pleural effusion was seen on chest X-ray, and thorax ultrasonography was compatible with 25 mm pleural effusion. Echocardiography showed a size of 7 mm. Bilateral pericardial effusion revealed pericarditis. A chest tube was inserted. The pericardial window was opened. Vancomycin, ceftriaxone, and anti-inflammatory ibuprofen treatments were initiated. Clarithromycin was added when fever and acute phase elevation persisted. Exudate or transudate was not differentiated from the pleural fluid sample. Pleural biochemical examination revealed pH: 7.8, LDH: 200 U/L, serum LDH: 155 U/L, glucose: 160 mg/dl, serum glucose: 95 mg/dl, protein: 5.3 mg/dl, serum protein: 6.75 mg/dl. The pleural fluid culture was negative. Mycobacteria culture was negative, and no ADA activity was detected. The repeated PPD test was anergic. Cytological examination revealed no tumor cells. Pleural fluid smear showed abundant neutrophils, leukocytes, and histiocytes. ANA and Anti-ds DNA obtained for the etiology were negative. Urinary calcium, serum ACE level, and eye examination for sarcoidosis were normal. Genetically, FMF gene analysis was reported negative. The nodule in the spleen persisted with the same characteristics. There was no malignant involvement in repeated PET/CT. In a pericardial biopsy, chronic inflammation and fibrotic tissue were detected. Pleural biopsy revealed diffuse fibrotic tissue with focal mesothelial cell hyperplasia and congestion and no malignant involvement. The pleural biopsy specimen was stained with CD3 staining, and intense T cell foci were detected. CD19 stain revealed approximately 3-4 focal B lymphocyte foci in each area (Fig. 1). The patient was treated with colchicine 3x 0.5 mg and had a new pleural effusion attack five months after the treatment was started. However, he did not have a concomitant pericardial effusion and regressed with outpatient antibiotic therapy. Besides, abdominal ultrasonography performed during this period showed that the nodule in the spleen disappeared. It was observed that pleural and pericardial effusion attacks did not reoccur. The patient is still in complete remission.

Informed consent was received from the family for writing this case.

Discussion

Successful treatment of non-metastatic, metastatic, and recurrent OS has been obtained with multi-agent chemotherapy, and local control procedures such as amputation, limb salvage surgery with negative margins, surgical removal of lung/bone metastases.[3] Because of the low survival rate for those with metastatic disease, treatment opportunities before metastasis, that activate the immune

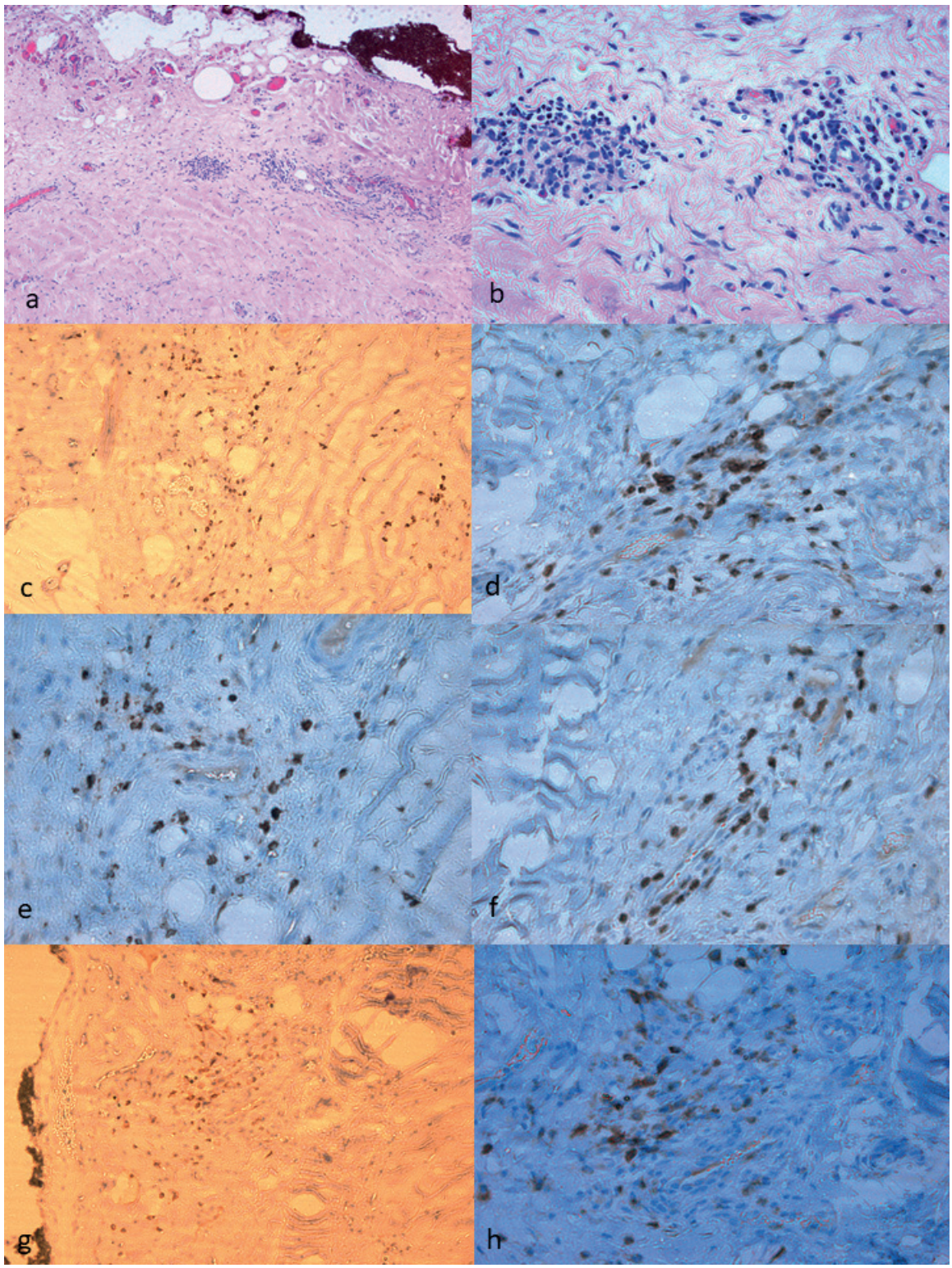

Fig. 1. (a) Intensive lymphocyte infiltration with HEx100 in pleural tissue biopsy specimen, (b) Focus of lymphocyte infiltration with HEx400, (c) T lymphocyte infiltration foci with IHC-CD3x100 in pleural tissue biopsy sample, (d, e, f) Intense infiltration of T lymphocytes with IHC-CD3x400, (g) B lymphocyte infiltration foci with IHC-CD19x100 in pleural tissue biopsy specimen, (h) B lymphocyte infiltration with IHC-CD19.

system, contributing to better outcomes, have been investigated. Infections associated with limb surgery have a positive effect on survival in humans with OS.[3] Higher absolute lymphocyte count recovery after chemotherapy also appears to be associated with improved survival.[3] In the phase 3 of the CCG/POG study where mifamurtide was begun after definitive surgery whether it was used or not, the addition of Liposomal-Muramyl TriPeptide-PhosphatidylEthanolamine (L-MTP-PE) made progress in event free survival (EFS) (p =0.08), and had a significant improvement in OS (78% vs. 70%; p= 0.03).[4] EURAMOS used interferon as immunotherapy in the good responders. The addition of interferon did not produce improvement of EFS.[5] Thus, we decided to use the EURAMOS protocol and add mifamurtide postoperatively in non-metastatic patients. To sum up, the addition of mifamurtide to treatment with chemotherapy may have some beneficial effects on metastatic or non-metastatic patients via eradication of residual micrometastases that are not eliminated by chemotherapy alone. Activated monocytes remain tumoricidal for up to 96 hours following mifamurtide infusion.[6] Mifamurtide does not accumulate during repeated administration.

When liposomes are applied systematically, they are accumulated by the phagocytic cells of the mononuclear phagocyte system, which is primarily in the liver, spleen, nasopharynx, thyroid, and lungs.[7] Muramyl dipeptide (MDP), the smallest component of immune potentiating activity from the cell wall of the Bacille Calmette-Guerin, stimulates innate immunity. The liposomal MTP-PE (L-MTP-PE) has similar immunostimulatory effects as natural MDP, with the additional advantage of a longer half-life in plasma and lower toxicity. The encapsulation of MTP-PE into liposomes has been shown in-vitro to enhance the activation of macrophages by 100-fold compared with free MDP.[8] Possible mechanism of mifamurtide can be muramyl dipeptide ligand to NOD2 receptors. In addition, lipoprotein on MTP-PE ligand to Toll-like receptors which are recognition receptors at the monocytes and macrophages is the other mechanism. Thus it forms a vesicle from the cell membrane and enters the cell. It activates the transcription factor NFκB via the intracellular domain MyD88 and activates protein synthesis by binding to the promoter region of DNA.[1] Normally, active monocytes turn to active macrophages, also called histiocytes in tissue. However, activated monocyte with mifamurtide in blood act like it's in tissue and works like a macrophage. In this stage, the release of cytokines, such as IL-1, IL-6, IL-8, IL-12, and TNF-alfa, IFN-gamma, called 'cytokine flu' occurs. Meanwhile, muramyl dipeptide epitopes bind to major histocompatibility complex (MHC) molecules and serve to the cell membrane. Antigen-presenting cell serves antigenic epitope to T and B lymphocytes in closest secondary lymph node follicles, as in the spleen follicles. Once T lymphocytes recognize MHC, they start to make permanent contact by adhesion molecules. For the first signal, ICAM-1 & LFA-1 adhesion molecules, a complex of "CD4 or CD8+T cell receptor (TCR)+CD4" and compatible MHC molecule reaction takes place. The second signal between CD28, and CD80/86 and the third signal provides differentiation and triggers the generation of Th1, Th2, Th17, and T regulatory. The active macrophages make a 'stand by' effect for overall immunoreactions. Within 96 hours, they react maximum, and then the impact of T cells reduces. Also, some of them last for years as T memory. MTP-PE shows adjuvant 'stand by' effect for T cells growth.

Activated macrophages move to that area via the chemokines, which are secreted by the antigen-presenting cell on tumoral tissue. Immune cells recognize the apoptotic signals like Phosphatidylserine (PtdSer), which translocated to the leaflet of tumor cells membrane with eat-me messages.[9] This is a macrophage-mediated tumoricidal effect. Similar to our study, some studies have reported that human macrophages after invitro activation with mifamurtide specifically recognize tumor cells and are not cytotoxic to healthy cells.[6,8] Similar to our research, one study reported

that anti-tumor activity is linked to both direct and indirect effects of macrophage activation, including contact-mediated tumor cytotoxicity and release of tumoricidal factor.[10]

The literature reports that mifamurtidine has some side effects, mostly potential infusion-related side effects.[2] Common side effects include fever, chills, headache nausea, tachycardia, vomiting, hypotension, hypothermia, dyspnea, constipation, and pain.[11] The severe side effects such as acute respiratory distress, hypoxia, malignant pleural effusion related to renal dysfunction, hepatic dysfunction, pyrexia, pericardial effusion (resolved in 1 week), respiratory distress, arrhythmia, peripheral sensory and centro- cerebellar neural involvement, skin disorders, ear disorders hearing-objective, febrile neutropenia, thrombocytopenia lead to discontinuation of drug.[8] However, there is limited data concerning the toxicity mechanism of mifamurtide in children with OS. In a phase 2 study, 12 patients aged 9-21 years with histologically proven OS with primary tumor resected that developed resectable metastases during or after adjuvant chemotherapy or those who initially presented with metastases that persisted despite chemotherapy were evaluated for side effects. Pleuritis and pericarditis were observed in 2 patients.[11] This status was similar to our case. In a study, 678 patients developed hypersensitivity reactions along with pleural and pericardial effusions, seizures, and muscle spasms.[12] Anderson et al.[3] also reported the side effects. They were generally Grade 1 or Grade 2 in 49% of cases. However, 2% of the patients had rare side effects potentially related to mifamurtide, such as pericardial and pleural effusions. Thus, we aimed to reveal the mechanism of pharmacodynamics and rare side effects of mifamurtide.

In our case, the patient had relapsing pericardial and pleural effusions which we believe may have occurred due to mifamurtide, since other etiological factors were negative. In the literature hypersensitivity reactions such as pleural and pericardial effusions, seizures, and muscle spasms, and severe hearing loss are mentioned.[12] Because of the lack of any survival benefit and the risk of serious adverse effects, the addition of mifamurtide to chemotherapy regimens is not advised. Similar to reports our patients had a severe side effect. Our patient's pathology of the pleural biopsy revealed so much focus on T cells and 3-4 focus in each area of B cells with diffuse fibrosis. It was compatible with type 4 hypersensitivity reactions. No macrophages were seen morphologically. Macrophage formation can be deformed. This may occur is the biopsy material is obtained a long time after the beginning of the inflammation. The absence of macrophages can also be attributed to the change of morphology of macrophages in the advanced stage of inflammation. Since the investigations for the etiology of this status were all normal. This clinical picture was thought to be due to the rare side effects of mifamurtide, an adjuvant immunological agent given to the patient. A type 4 hypersensitivity reaction was considered mainly. This hypersensitivity reaction may be caused by the inability to stop mifamurtide-induced immunoreactivity initiated by the 'stand by' effect. Primarily formed T memory and B memory cells may be effective in the formation of late-period type 4 hypersensitivity reactions. This autoimmune reaction may be due to the dose of the drug, route of administration, or the patient's immunity. Thus, MTP-PE can trigger hypersensitivity in some patients. The nodular lesion on spleen parenchyma may have also occured due to mifamurtide, but a biopsy was not performed for the splenic nodule. This exaggerated immune response was reduced by colchicine. Pericardial effusion attacks and nodules in the spleen also disappeared. However, like in our case the anti-inflammatory effect of drugs, such as ibuprofen may not prevent the progression of the attack, because non steroid anti-inflammatory drugs do not inhibit leucocytes, which are responsible for initiating inflammation. Besides, the effect on COX1>COX2 can be accountable for this.[13] Colchicine inhibits neutrophil activation and migration to the inflammation center. It also prevents microtubule, inflammasome,

and microtubule related chemotaxis, and phagocytosis.[14] TNF-α receptors on the surface of macrophages and endothelial cells are decreased by colchicines.[15] Although the patient had a mild pleural effusion attack, it has not yet repeated. This showed us that colchicine has a positive effect on inflammation.

In conclusion, mifamurtide is generally well-tolerated, with some side effects reported as either mild or moderate. However, it can cause hypersensitivity reactions. Future studies should focus more on the mechanism of the hypersensitivity reactions. Also, mifamurtide may be combined with PD-L1-Fc, which are regulation molecules for regulation of T cells. In addition, encapsulation with an inhibitory cytokine which TGF- β1 may increase transformation to T regulatory cells directly in order to reduce autoimmune reactions.

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NOTICE TO AUTHORS

Instruction for authors

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