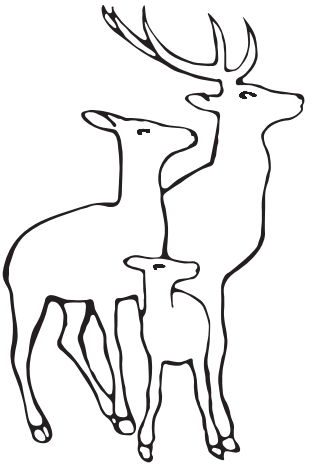
ISSN 0041-4301
www.turkishjournalpediatrics.org

THE TURKISH JOURNAL OF PEDIATRICS

ORIGINAL ARTICLES

volume 62
number 2
March-April 2020



THE TURKISH JOURNAL OF PEDIATRICS

www.turkishjournalpediatrics.org

Volume 62 ▪ Number 2
March-April 2020

ISSN: 0041-4301

THE TURKISH JOURNAL OF PEDIATRICS

ISSN 0041-4301
www.turkishjournalpediatrics.org

Cilt: 62 Sayı: 2, Mart-Nisan 2020

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

YAYININ TÜRÜ
Yerel süreli yayın

YAYIN ŞEKLİ
İki aylık İngilizce

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

BASIM TARİHİ: XX.XX.2020

ISSN 0041-4301
www.turkishjournalpediatrics.org

Vol: 62 Number: 2, March-April 2020

PRODUCTION MANAGER
Owner on behalf of the Publishers
Murat YURDAKÖK

ADMINISTRATOR
Enver HASANOĞLU

DOCUMENTATION and PUBLICATIONS
Ali DÜZOVA

PUBLISHED BY
Turkish National Pediatric Society,
Hacettepe University Institute Child Health and
The International Children's Center

EDITORIAL OFFICE
The Turkish Journal of Pediatrics
P.K. 36, Samanpazarı 06240 Ankara, Turkey
Faks: (312) 305 22 64

SUBSCRIPTION ADDRESS
The Turkish Journal of Pediatrics Editorial Office
Hacettepe University
İhsan Doğramacı Children's Hospital
06100 Ankara
Tel : (312) 305 26 76
Fax: 90 (312) 305 22 64

PUBLICATION TYPE
International peer-reviewed journal

PUBLICATION FREQUENCY and LANGUAGE
Bi-monthly ▪ English

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

PRINT DATE: XX.XX.2020

THE TURKISH JOURNAL OF PEDIATRICS

The Turkish Journal of Pediatrics is joint publication of the Turkish National Pediatric Society, Hacettepe University Institute of Child Health and The International Children's Center. The journal has been published since 1958 and has been available online since 2002. The Turkish Journal of Pediatrics is published 6 times a year. The journal does not have article processing charges or article submission charges.

The Turkish Journal of Pediatrics is a multidisciplinary, peer reviewed, open access journal that seeks to publish research to advance the field of Pediatrics. The Journal publishes original articles, case reports, review of the literature, short communications, clinicopathological exercises and letter to the editor in the field of pediatrics. Articles published in this journal are evaluated in an independent and unbiased, double blinded peer-reviewed fashion by an advisory committee.

This publication is indexed in BIOSIS Previews, CABI Abstracts (Helminthological Abstracts, Nutrition Abstracts and Reviews Series A, Protozoological Abstracts, Review of Medical and Veterinary Entomology), EMBASE/Excerpta Medica, EBSCOhost (Medline with Full Text), IBIDS (International Bibliographic Information on Dietary Supplements), ProQuest (Medline, Professional ProQuest Central, ProQuest Health and Medical Complete, ProQuest Medical Library, ProQuest Pharma Collection), Science Citation Index (SCI) Expanded, and Turkiye Citation Index.

CONTENTS

VOLUME: 62 NUMBER: 2 MARCH-APRIL 2020

ORIGINAL ARTICLES

CONTENTS

VOLUME: 62 NUMBER: 2 MARCH-APRIL 2020

CONTENTS

VOLUME: 62 NUMBER: 2 MARCH-APRIL 2020

The Turkish Journal of Pediatrics 2020; 62: 167-174
https://doi.org/10.24953/turkjped.2020.02.001

Original Article

Canakinumab in colchicine resistant familial Mediterranean fever and other pediatric rheumatic diseases

Mustafa Çakan, Şerife Gül Karadağ, Nuray Aktay Ayaz

Clinic of Pediatric Rheumatology, Kanuni Sultan Süleyman Research and Training Hospital, İstanbul, Turkey

ABSTRACT

Background and objectives. The aim of this observational retrospective cohort study was to demonstrate indications and response rates of the patients with pediatric rheumatic diseases that used canakinumab.

Method. The files of the patients that used canakinumab between December 2012 and July 2017 were reviewed. Canakinumab was used in 29 patients. Diagnosis of the patients were; colchicine resistant familial Mediterranean fever (crFMF) (19 cases), hyperimmunoglobulin D syndrome-mevalonate kinase deficiency (HIDS-MKD) (3 cases), cryopyrin-associated periodic syndrome (3 cases), systemic juvenile idiopathic arthritis (sJIA) (2 cases), idiopathic recurrent pericarditis (1 case) and pyoderma gangrenosum (1 case).

Results. Canakinumab was used for 21.8 ± 15.8 months (6-54 months). crFMF patients had a female predominance; 16 girls and 3 boys. Mean age at the first symptoms of FMF was 2.8 ± 2.2 years. Mean number of attacks per year before colchicine was 18.7 ± 6.9 (10-36), after colchicine was 8.2 ± 2.7 (6-12) and after biologic agent the number dropped to 0.1 ± 0.3 (0-1). Canakinumab led to resolution of attacks in 3 HIDS-MKD cases. Two familial cold autoinflammatory syndrome patients were using canakinumab for 13 months with total remission. Chronic infantile neurological cutaneous articular syndrome patient did not show dramatic response to standard doses of IL-1 blockers and remission was achieved with high doses of canakinumab. Canakinumab led to the resolution of all systemic and articular manifestations in one sJIA case but the other sJIA case developed polyarticular joint involvement under canakinumab treatment. A severe pyoderma gangrenosum patient that failed dapson and anakinra, also failed canakinumab treatment that was used for 9 months. We have successfully treated a case of idiopathic recurrent pericarditis with canakinumab. Canakinumab was discontinued due to inefficacy only in two cases.

Conclusion. Overall efficacy of canakinumab was 93.1% in this study. No major adverse event was observed under canakinumab treatment. Canakinumab seems to be effective and safe in children with rheumatic diseases.

Key words: autoinflammatory diseases, canakinumab, colchicine resistant familial Mediterranean fever.

Autoinflammatory diseases (AIDs) are a group of disorders characterized by recurrent attacks of fever, and systemic inflammation mainly mediated by the cells of the innate immune system.[1] The world of autoinflammation is expanding rapidly with new diseases described every year since the first description of autoinflammation in 1999.[2] Monogenic periodic fever syndromes (MPFS), including familial Mediterranean fever (FMF), hyperimmunoglobulin D syndrome-mevalonate kinase deficiency (HIDS-MKD), cryopyrin-associated periodic syndrome (CAPS) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS) comprise the most well defined group of AIDs and are characterized by attacks of fever, rash, polyserositis and systemic inflammation. FMF is the most common form of MPFS.[1-3]

The signs and symptoms in MPFS are due to overproduction of interleukin 1 (IL-1). Pyrin and NRLP3 mutations induce IL-1β release from myeloid cells by activating the inflammasome

✉ Mustafa Çakan
mustafacakan@hotmail.com

Received 20th October 2018, revised 12th February 2019, 19th April 2019, accepted 3rd July 2019.

that regulates caspase-1 activity. Description of the role of the inflammasome and IL-1 in the pathogenesis of many AIDs have led to the use of more targeted treatment options.[4,5] Colchicine is the mainstay therapy in FMF and most of the patients show dramatic response to it.[6] However, nearly all of the patients with other MPFS do not respond to colchicine and IL-1 blockage is now regarded as the recommended treatment modality in these disorders. Also, around 5-10% of FMF patients are nonresponsive to colchicine and there are many reports on beneficial effects of IL-1 blockers in colchicine resistant FMF (crFMF) patients.[6-8] Canakinumab is a human monoclonal anti-IL-1β antibody that neutralizes the activity of IL-1β by binding to it. There are also two other IL-1 blockers; anakinra (a recombinant IL-1 receptor antagonist) and rilonacept (receptor fusion protein acting as IL-1 decoy receptor).[9,10]

The aim of this observational retrospective cohort study was to demonstrate diagnosis, demographic features, indications and response rates of the patients with pediatric rheumatic diseases that used canakinumab.

Material and Methods

This study was conducted in the pediatric rheumatology clinic of Kanuni Sultan Süleyman Research and Training Hospital. The files of the patients that canakinumab have been used between December 2012 and January 2017 were reviewed. Diagnosis, laboratory parameters, indications, duration of the treatment and response of the patients to the canakinumab were recorded. To be included into the study; the patient had to have either clinical and/or molecular diagnosis for that rheumatic disease and had to be using canakinumab for at least 6 months. All FMF patients fulfilled diagnostic criteria of Turkish pediatric FMF criteria.[11] Also FMF, HIDS-MKD and CAPS patients fulfilled 'The Eurofever clinical diagnostic/ classification criteria for autoinflammatory periodic fevers' criteria set.[12] In crFMF patients, FMF exacerbation was defined as having fever and abdominal and/or chest pain for at least 12 hours or more. Colchicine resistance was defined as having ≥3 attacks for 6 consecutive months despite maximal colchicine dose. Initial colchicine dose was arranged according to age and started as 0.5 mg/day for children <5 years of age, 1 mg/day in two divided doses for children between 5 and 10 years of age and 1.5 mg/day in three divided doses for children >10 years of age. Colchicine dose was increased 0.25 mg every 3-6 months in partial or non-responders as 2 mg/day being the maximum daily dose. Canakinumab was started at a dose of 2 mg/kg in all indications and, if needed, the dose was gradually increased (2 mg/kg per each increment) every 1 or 2 months until all signs and symptoms of that disease are controlled. Colchicine treatment was continued in all crFMF patients. The study was performed according to the tenets of Declaration of Helsinki and was approved by the local ethics committee (ethics approval number: KAEK/2018.3.27). Informed consent was taken from the legal guardians of the children.

Clinical and demographic characteristics were summarized by mean and standard deviation for continuous variables and count and percent for categorical variables. Statistical analyses were performed using the SPSS software package for Windows (version 22.0; SPSS, Chicago, IL, USA).

Results

Canakinumab was used in 29 patients during 4.5 years. The cohort consisted of 19 crFMF, 3 HIDS-MKD, 3 CAPS [2 familial cold autoinflammatory syndrome (FCAS) and 1 chronic infantile neurological cutaneous articular syndrome (CINCA)], 2 systemic juvenile idiopathic arthritis (sJIA), 1 idiopathic recurrent pericarditis and 1 pyoderma gangrenosum case. Mean duration of follow-up was 5.1 ± 3.7 years. Canakinumab was used for 21.8 ± 15.8 months (6-54 months). Overall beneficial clinical response to canakinumab, based on the expert opinion, was seen in

93.1% of the patients and canakinumab was discontinued only in 3 patients. These were one crFMF patient due to control of the symptoms, one sJIA and one pyoderma gangrenosum case due to persistence of the clinical features.

In crFMF patients, the most common reason for use of biologics was resistance to colchicine. In 7 of the patients, in addition to colchicine resistance, presence of subclinical inflammation was another indication for biologic use and one patient had renal amyloidosis. Table I shows demographic and clinical features of the crFMF patients. The crFMF patients had a female predominance; 16 girls and 3 boys. Mean age at the first symptoms of FMF was 2.8 ± 2.2 years (3 months - 7 years) and mean age at diagnosis of FMF and use of colchicine was 5.3 ± 3.1 years (3 months - 11 years). Fifteen patients had M694V homozygous mutation, one patient had M694V/R761H compound heterozygous mutation and in 3 of the cases we could not have demonstrated a pathogenic mutation in the *MEFV* and other monogenic periodic fever syndrome genes. In 14 of 19 crFMF patients, anakinra was used before canakinumab for 6.0 ± 2.9 months (3 - 12 months). The duration between the start of colchicine and interleukin-1 blocker was 4.3 ± 2.6 years (1 – 9 years). Mean number of attacks per year before colchicine was 18.7 ± 6.9 (10 - 36), after colchicine it was 8.2 ± 2.7 (6 – 12) and after biologics the number dropped to 0.1 ± 0.3 (0 – 1) attacks per year. The most common reason for switching anakinra to canakinumab was painful daily injections. The mean dose of canakinumab was 4.0 ± 0.9 mg/kg (3-6 mg/kg). At the time of enrollment, in 11 patients canakinumab was being used every month and in 8 patients every 3-4 months.

Clinical and demographic features of patients with HIDS-MKD and CAPS are shown in Table II. Canakinumab was used in three HIDS-MKD cases. Two of them were brothers and both had homozygous V377I mutation in the *MVK* gene, and the other patient had heterozygous V377I mutation. Initially anakinra was used for 7 months and canakinumab was being used for 10 months with resolution of the attacks. Two FCAS patients were using canakinumab for 13 months with total disappearance of rash and fever attacks. CINCA patient did not show dramatic response to standard doses of both IL-1 blockers (anakinra and canakinumab) with partial control of the acute phase reactants (APRs). The dose of canakinumab was increased to 12 mg/kg/month that made the control of APRs and clinical features. In one of the sJIA patients canakinumab was used for 27 months with good response. But other sJIA case initially followed a polycyclic course and for 28 months canakinumab was used with a good response but later turned into polyarticular course under canakinumab treatment. A severe pyoderma gangrenosum patient that failed dapson and anakinra also failed canakinumab that was used for 9 months. We have successfully treated a case of idiopathic recurrent pericarditis with canakinumab. No major adverse event was observed under canakinumab treatment. One patient had impetigo and five patients had upper respiratory tract infections. All canakinumab injections were well tolerated with no injection site reactions.

Discussion

The world of autoinflammation has seen breakthrough developments in the recent years with the description of new monogenic diseases. Better understanding of the pathophysiology of the diseases led to the discovery of more targeted, more selected therapies, namely cytokine blockers.[2,13]

Familial Mediterranean fever is the prototype of autoinflammatory diseases and caused by gain of function mutations in the *MEFV* gene. Mutated pyrin protein leads to over activity of inflammasomes and production of abnormal amounts of inflammatory cytokine IL-1β.[1,2,7] Colchicine is the standard treatment option in FMF and prevents both the attacks and decreases the risk of amyloidosis. Less than 5% of FMF patients do not show any improvement with colchicine. These patients are called as crFMF but there is no internationally accepted

Table I. Demographic and clinical features of the patients with colchicine resistant familial Mediterranean fever.

	Gender	MEFV mutation	Age at onset of the symptoms (years)	Age at diagnosis (years)	Clinical features	Number of attacks before colchicine (per year)	Number of attacks after colchicine (per year)	Number of attacks after canakinumab (per year)	Duration of canakinumab treatment (months)
1	Female	M694V Homozygous	4	8	F,AP, CP, ELE	24	6	0	34
2	Female	M694V Homozygous	0.3	0.3	F,AP	24	12	0	28
3	Female	M694V Homozygous	2	4	F,AP,M	12	6	0*	9
4	Female	M694V Homozygous	2	4	F,AP,CP,A	24	6	1	50
5	Female	M694V Homozygous	1	4	F,AP,CP	36	6	0	42
6	Female	M694V Homozygous	4	9.5	F,AP,CP,A,ELE	10	6	0	32
7	Female	M694V Homozygous	5	11	F,AP,CP,A,ELE	12	6	1	32
8	Female	M694V Homozygous	0.5	2	F,AP,CP,M	24	6	1	50
9	Female	M694V Homozygous	0.5	4.5	F,AP,A	12	6	0	54
10	Female	M694V Homozygous	1	3	F,AP,A	12	6	0	54
11	Male	M694V Homozygous	2	8	F,AP,A,M,ELE	12	8	0	12
12	Male	M694V Homozygous	1	2.5	F,AP,M	18	9	0#	6
13	Female	M694V Homozygous	7	10	F,AP,A,ELE	18	12	0#	6
14	Female	M694V Homozygous	5	6	F,AP,A,M	18	12	0#	6
15	Male	M694V Homozygous	6	7	F,AP,M	10	6	0	23
16	Female	M694V Heterozygous R761H Heterozygous	5	8	F,AP,CP,M	24	8	0*	9
17	Female	Normal	5.5	6	F,AP,CP	18	12	0	22
18	Female	Normal	0.5	1	F,AP	24	12	0	12
19	Female	Normal	1.5	2.5	F,AP,CP,M	24	12	0*	9
Mean ± standard deviation			2.8 ± 2.2	5.3 ± 3.1		18.7 ± 6.9	8.2 ± 2.7	0.1 ± 0.3	25.7 ± 17.6

In these patients numbers of attacks are given for * 9 months and for # 6 months.
F: fever, AP: abdominal pain, CP: chest pain, ELE: erysipelas-like erythema, M: myalgia, A: arthralgia and/or arthritis.

Table II. Demographic and clinical features of the patients with HIDS-MKD and CAPS.

Diagnosis	Gene analysis	Gender	Age at the onset	Age at the diagnosis	Clinical features	Duration of canakinumab treatment
HIDS-MKD	V377I Homozygous	Male	1 year	5.5 years	Fever, abdominal pain, diarrhea, lymphadenopathy, oral ulcers, splenomegaly	9 months
HIDS-MKD	V377I Homozygous	Male	2 years	9 years	Fever, abdominal pain, arthralgia, oral ulcers, painful lymphadenitis	9 months
HIDS-MKD	V377I Heterozygous	Male	1 year	2 years	Fever, abdominal pain, rash, diarrhea, vomiting, aphthous stomatitis	12 months
CAPS (CINCA)	V198M Heterozygous	Female	Neonatal	1.5 years	Premature delivery, fever, rash, arthropathy, developmental delay	17 months
CAPS (FCAS)	Q703K Heterozygous	Male	7 years	8 years	Fever, rash, myalgia, conjunctivitis	15 months
CAPS (FCAS)	V198M Heterozygous	Female	8 years	9 years	Fever, rash, arthralgia, myalgia, conjunctivitis	11 months

HIDS-MKD: hyperimmunoglobulin D syndrome-mevalonate kinase deficiency, CAPS: cryopyrin-associated periodic syndrome, CINCA: chronic infantile neurological cutaneous articular syndrome, FCAS: familial cold autoinflammatory syndrome.

consensus definition for colchicine resistance.[6] The most widely used one was defined by Hentgen et al.[14] as having more than 6 typical FMF attacks per year or more than 3 typical attacks over a 4-6 month period. In cases where attacks are incomplete, then increases in at least 2 of 3 acute phase reactants (C-reactive protein, erythrocyte sedimentation rate, serum amyloid A) between attacks would identify the patient as colchicine resistant. The most important issue before calling an FMF patient as colchicine resistant is the compliance of the patient.[6,8] Compliance was reassured in our institution by daily pill counting that was made by the parents. We did not have any colchicine intolerant patient in the cohort that may be a problem in 2-5% of FMF patients.[8]

It is known that FMF patients with exon 10 mutations have the most severe phenotype, especially the ones having M694V homozygous mutations. These patients tend to have initial symptoms of FMF at young ages, have more frequent attacks and need higher doses of colchicine. Nearly 25-30% of FMF patients have only one mutation in the *MEFV* gene and a small number of FMF patients do not exhibit any known pathogenic mutation in the *MEFV* gene.[1,5,8] In this study, 15 patients (78.9%) had M694V homozygous mutation and the mean age at the first symptoms of FMF was 2.8 years, supporting the notion that FMF patients with homozygous exon 10 mutations have a severe course. One patient had compound heterozygous mutations in exon 10 (M694V/R761H) and in 3 cases we were unable to demonstrate any pathogenic mutation in the *MEFV* and other monogenic periodic fever syndrome genes, namely *MVK, TNFRSF1A*, and *NLRP3*. We have classified them under crFMF group, because these patients fulfilled criteria sets for FMF (both Eurofever and Turkish pediatric FMF criteria) and had classical symptoms of FMF without any peculiar signs and symptoms suggestive of another AID and had not a complete, but partial response to colchicine and had a dramatic response to IL-1 blockers.[11,12]

Elucidation of the role of IL-1 in the pathogenesis of FMF and other autoinflammatory diseases led to the discovery of IL-1 blockers. Anakinra (Kineret®) is a short-acting recombinant IL-1 receptor antagonist and blocks IL-1α and IL-1β

binding to the IL-1 receptor, and administered as daily subcutaneous injections.[10] There are many case reports and case series on beneficial effects of anakinra in crFMF patients.[15] Ben-Zvi et al.[16] conducted a double-blind randomized placebo-controlled trial in 25 colchicine resistant adult FMF patients over 4 months of period. The attack rate, the primary outcome, was significantly less among the anakinra group versus placebo (1.7 ± 1.7 versus 3.5 ± 1,9 attacks per patient month, respectively, p= 0.037). Başaran et al.[17] presented 8 cases of colchicine resistant pediatric FMF patients that all showed complete remission under anakinra treatment. The most problematic issue with anakinra is daily painful injections which is more problematic in children. So, in many of the manuscripts written on FMF and IL-1 blockers, anakinra was switched to canakinumab.[17,18] We have used anakinra before canakinumab in 14 of 19 crFMF patients with a mean duration of 6 months and have seen that anakinra was effective in all patients in both controlling the frequency of attacks and subclinical inflammation. Painful daily injections were the reason in all cases for switching to canakinumab.

Canakinumab (Ilaris®) is a recombinant fully humanized selective anti-IL-1β monoclonal antibody and binds to serum IL-1β and neutralizes its activity by blocking the interaction with IL-1 receptors. It is administered subcutaneously every one or two months depending on the indication and has approval for CAPS and sJIA but not for crFMF in Europe and North America.[1,9,10] There are two open-label phase II studies looking for efficacy and safety of canakinumab in crFMF patients. Both studies have shown that canakinumab was effective in reducing the frequency of FMF attacks and maintaining low levels of APRs with no unexpected adverse events.[7,19] Kara Eroglu et al.[18] presented 9 cases of pediatric crFMF patients and all responded well to canakinumab. Başaran et al.[17] presented 4 children with crFMF in whom canakinumab was used. In all of them anakinra was used initially. Three cases showed sustained remission under canakinumab but in one case canakinumab was switched back to anakinra after 3 months due to clinical and laboratory worsening. Recently published international CLUSTER study (The phase 3 canakinumab pivotal umbrella study in three hereditary periodic fevers) evaluated canakinumab efficacy in crFMF, HIDS-MKD and TRAPS patients and it was shown that at week 16, 61% of patients with crFMF had complete response to canakinumab while this number was 6% on placebo group.[3] We have used canakinumab in 19 crFMF patients with a mean duration of 25.7 months, and in 6 of them canakinumab was used for less than a year. We have seen that canakinumab was effective both in short and long term in all cases in both controlling the attacks and APRs. There is no consensus definition for optimal dose interval and duration of canakinumab treatment in crFMF patients.[9] We have used canakinumab every month for 6 months at the beginning of the treatment and then every 2-4 months depending on the severity of the previous FMF attacks and have observed that decreasing the intervals of injections did not lead to reemergence of the FMF attacks in the majority of the patients. Only 3 patients had mild FMF attacks once per year without necessitating increasing the frequency of injections.

The other two autoinflammatory diseases that canakinumab was used in this cohort were HIDS-MKD and CAPS. Life-long IL-1 inhibition is indicated in patients with CAPS. In HIDS-MKD patients with frequent attacks and/or subclinical inflammation between attacks, maintenance therapy with IL-1 blockade or etanercept is recommended.[20,21] Two FCAS cases showed dramatic response to standard doses of canakinumab (2 mg/kg/2 months) with total resolution of rash and constitutional symptoms. But CINCA patient did not show the same response to both anakinra and canakinumab. The symptoms and APRs were controlled only with very high dose of canakinumab (12 mg/kg/month). Kuemmerle-Deschner et al.[22] reported that real-life effectiveness of canakinumab in daily clinical practice of CAPS was lower (53%

with standart dose and 72% after dose increase) than reported in a placebo-controlled, double-blinded trial, in which complete remission was achieved in 97% of patients. Russo et al.[23] used canakinumab in 10 CAPS (8 Muckle-Wells syndrome and 2 CINCA) patients. Canakinumab was used for a median of 21 months and clinical remission was obtained in all patients. Sixty percent of the patients needed dose adjustments and CINCA cases required higher doses of canakinumab than those with Muckle-Wells syndrome. HIDS-MKD patients are treated depending on the frequency of attacks. TNF-alpha inhibitors and IL-1 blockers (both anakinra and canakinumab) have been used with variable success. Case reports and case series have shown that 80% of HIDS-MKD patients improved using anakinra continuously or during attacks only.[24] The study of Galeotti et al.[25] demonstrated that in 11 patients with HIDS-MKD, continuous IL-1 blocking was associated with complete remission in 4 cases and partial remission in seven. They have stated that IL-1 blockade brings substantial benefit to HIDS-MKD patients. We initially used anakinra and later canakinumab in 3 HIDS-MKD cases and all showed dramatic resolution of attacks and normalization of APRs under IL-1 blocking regimens, confirming a key role of IL-1 in HIDS-MKD pathogenesis.

Systemic JIA is characterized by fever, rash, arthritis, serositis, lymphadenopathy and hepatosplenomegaly. Early use of biologics (anakinra, canakinumab or tocilizumab) is recommended in patients with severe disease course or impending macrophage activation syndrome.[26] The study of Ruperto et al.[27] reported that 76% of 190 sJIA patients attained ACR Pedi-90 response with canakinumab at the end of the withdrawal phase. We have used canakinumab in 2 sJIA patients. Both cases had a severe course complicated with macrophage activation syndrome. One case followed a polycyclic course and canakinumab resulted in resolution of sJIA flares. But the other case initially followed a polycyclic course and later turned into polyarticular course under canakinumab treatment.

This is one of the manuscripts that include high number of crFMF patients on canakinumab with relatively long duration of follow-up time. Canakinumab was used in 29 children and only in 2 cases discontinued due to inefficacy. Overall efficacy of canakinumab was 93.1% in this study. In conclusion, canakinumab seems to be effective, safe and well tolerated in children with rheumatic diseases. In patients with crFMF, monthly canakinumab injections may be decreased to every other month after the completion of initial six months.

REFERENCES

1. Barron KS, Kastner DL. Periodic fever syndromes and other inherited autoinflammatory diseases. In: Petty RE, Laxer RM, Lindsey CB, Wedderburn LR (eds). Textbook of Pediatric Rheumatology (7th ed) Philadelphia: Elsevier, 2016: 609-626.
2. Manthiram K, Zhou Q, Aksentijevich I, Kastner DL. The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. Nat Immunol 2017; 18: 832-842.
3. De Benedetti F, Gattorno M, Anton J, et al. Canakinumab for the treatment of autoinflammatory recurrent fever syndromes. N Engl J Med 2018; 378: 1908-1919.
4. Oda H, Kastner DL. Genomics, biology, and human illness: advances in the monogenic autoinflammatory diseases. Rheum Dis Clin North Am 2017; 43: 327-345.
5. Omenetti A, Carta S, Delfino L, Martini A, Gattorno M, Rubartelli A. Increased NLRP3-dependent interleukin 1β secretion in patients with familial Mediterranean fever: correlation with MEFV genotype. Ann Rheum Dis 2014; 73: 462-469.
6. Ozen S, Kone-Paut I, Gül A. Colchicine resistance and intolerance in familial Mediterranean fever: definition, causes, and alternative treatments. Semin Arthritis Rheum 2017; 47: 115-120.
7. Gül A, Ozdogan H, Erer B, et al. Efficacy and safety of canakinumab in adolescents and adults with colchicine-resistant familial Mediterranean fever. Arthritis Res Ther 2015; 17: 243.
8. Özen S, Batu ED, Demir S. Familial Mediterranean fever: recent developments in pathogenesis and new recommendations for management. Front Immunol 2017; 8: 253.
9. Haviv R, Hashkes PJ. Canakinumab investigated for treating familial Mediterranean fever. Expert Opin Biol Ther 2016; 16: 1425-1434.

10. Grattagliano I, Bonfrate L, Ruggiero V, Scaccianoce G, Palasciano G, Portincasa P. Novel therapeutics for the treatment of familial Mediterranean fever: from colchicine to biologics. Clin Pharmacol Ther 2014; 95: 89-97.

11. Yalçınkaya F, Özen S, Özçakar ZB, et al. A new set criteria for diagnosis of familial Mediterranean fever in childhood. Rheumatology (Oxford) 2009; 48: 395-398.

12. Federici S, Sormani MP, Ozen S, et al; Paediatric Rheumatology International Trials Organisation (PRINTO) and Eurofever Project Evidence-based provisional clinical classification criteria for autoinflammatory periodic fevers. Ann Rheum Dis 2015; 74: 799-805.

13. Jesus AA, Goldbach-Mansky R. IL-1 blockade in autoinflammatory syndromes. Annu Rev Med 2014; 65: 223-244.

14. Hentgen V, Grateau G, Kone-Paut I, et al. Evidence-based recommendations for the practical management of familial Mediterranean fever. Semin Arthritis Rheum 2013; 43: 387-391.

15. Van der Hilst JCH, Moutschen M, Messiaen PE, Lauwerys BR, Vanderschueren S. Efficacy of anti-IL-1 treatment in familial Mediterranean fever: a systematic review of the literature. Biologics 2016; 10: 75-80.

16. Ben-Zvi I, Kukuy O, Giat E, et al. Anakinra for colchicine-resistant familial Mediterranean fever: a randomized, double-blind, placebo-controlled trial. Arthritis Rheumatol 2017; 69: 854-862.

17. Başaran Ö, Uncu N, Çelikel BA, Taktak A, Gür G, Çakar N. Interleukin-1 targeting treatment in familial Mediterranean fever: an experience of pediatric patients. Mod Rheumatol 2015; 25: 621-624.

18. Kara Eroğlu F, Beşbaş N, Topaloğlu R, Ozen S. Treatment of colchicine-resistant Familial Mediterranean fever in children and adolescents. Rheumatol Int 2015; 35: 1733-1737.

19. Brik R, Butbul-Aviel Y, Lubin S, et al. Canakinumab for the treatment of children with colchicine-resistant familial Mediterranean fever: a 6-month open-label, single-arm pilot study. Arthritis Rheumatol 2014; 66: 3241-3243.

20. ter Haar NM, Oswald M, Jeyaratnam J, et al. Recommendations for the management of autoinflammatory diseases. Ann Rheum Dis 2015; 74: 1636-1644.

21. Çakan M, Aktay-Ayaz N, Keskindemirci G, Karadağ ŞG. Two cases of periodic fever syndrome with coexistent mevalonate kinase and Mediterranean fever gene mutations. Turk J Pediatr 2017; 59: 467-470.

22. Kuemmerle-Deschner JB, Hofer F, Endres T, et al. Real-life effectiveness of canakinumab in cryoprin-associated periodic syndrome. Rheumatology (Oxford) 2016; 55: 689-696.

23. Russo RAG, Melo-Gomes S, Lachmann HL, et al. Efficacy and safety of canakinumab therapy in paediatric patients with cryopyrin-associated periodic syndrome: a single-centre, real-world experience. Rheumatology (Oxford) 2014; 53: 665-670.

24. Moll M, Kuemmerle-Deschner JB. Inflammasome and cytokine blocking strategies in autoinflammatory disorders. Clin Immunol 2013; 147: 242-275

25. Galeotti C, Meinzer U, Quartier P, et al. Efficacy of interleukin-1-targeting drugs in mevalonate kinase deficiency. Rheumatology (Oxford) 2012; 51: 1855-1859.

26. Benedetti FD, Schneider R. Systemic juvenile idiopathic arthritis. In: Petty RE, Laxer RM, Lindsey CB, Wedderburn LR (eds). Textbook of Pediatric Rheumatology (7th ed) Philadelphia: Elsevier, 2016: 205-216.

27. Ruperto N, Brunner HI, Quartier P, et al; for the PRINTO and PRCSG. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. N Eng J Med 2012; 367: 2396-2406.

The Turkish Journal of Pediatrics 2020; 62: 175-181
https://doi.org/10.24953/turkjped.2020.02.002

Original Article

Sacroiliitis associated with familial Mediterranean fever in childhood: a case series and review of literature

Deniz Gezgin Yıldırım[1], Hatice Kibriya Fidan[2], Sevim Gönen[3], Oğuz Söylemezoğlu[1]

[1]Divisions of Pediatric Rheumatology and [2]Pediatric Nephrology, [3]Department of Pediatrics, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background and objectives. Familial Mediterranean fever (FMF) is an autosomal-recessive auto-inflammatory disorder characterized by recurrent episodes of fever with serositis. Sacroiliitis associated with FMF is very rare, especially in children. We aimed to describe the demographic, clinical, laboratory features, and treatment responses of pediatric patients with FMF -related sacroiliitis.

Methods. The study consisted of seven pediatric patients younger than 16 years, diagnosed with sacroiliitis associated with FMF between 2010 and 2017. Medical records of patients were retrospectively evaluated. Sacroiliitis was diagnosed based on magnetic resonance imaging. We also reviewed previous studies of FMF related sacroiliitis.

Results. Five of the seven patients (male:female ratio of 5:2) had a *M694V* (homozygous) mutation, one patient had a *M694V* (heterozygous) mutation, and one patient had a *V726A* (heterozygous) mutation. All patients were *HLA-B27* negative. One of the cases achieved remission with colchicine plus non-steroidal anti-inflammatory drug treatment, and one patient's symptoms were managed by the addition of sulfasalazine. Four patients responded to etanercept treatment, and one patient's symptoms were suppressed with canakinumab.

Conclusion. Sacroiliitis can be seen in pediatric FMF patients suffering with inflammatory back pain. This manifestation generally occurs in FMF patients who have *M694V* mutation. Etanercept could likely show a beneficial effect in patients who are resistant to disease modifying anti-rheumatic drugs and non-steroidal anti-inflammatory drugs. In addition, canakinumab treatment should be considered as a successful alternative therapy in this rare group of patients.

Key words: childhood, familial Mediterranean fever, sacroiliitis, treatment.

Familial Mediterranean fever (FMF) is an autosomal-recessive autoinflammatory disorder characterized by recurrent episodes of fever with serositis. Untreated patients may develop AA type amyloidosis which results in renal impairment and associated morbidity and mortality.[1] In terms of the ethnicity spectrum, FMF is found in Armenian, Turkish, Jewish and Arabic people in Mediterranean regions.[2] Mediterranean fever gene (*MEFV*) mutation located on chromosome 16p13.3, encodes a 781–amino acid length protein denoted as pyrin or marenostrin, leads to the hyperactivity of inflammasome which in turn increases IL-1β, and causes severe inflammation.[3]

Monoarthritis of the large joints of the lower extremities with a self-limiting course is one of the most common features of the musculoskeletal involvement in FMF.[4] In addition to peripheral arthritis, some FMF patients with chronic arthritis fulfil the diagnostic criteria of spondyloarthritis (SPA) such as the presence of

✉ Deniz Gezgin Yıldırım
gezgindeniz@gmail.com

Received 18th February 2019, revised 19th July 2019, accepted 17th September 2019.

This manuscript was presented as a poster presentation in abstract form at the International Society of Systemic Auto-Inflammatory Diseases (ISSAID) Congress in 4-7 May 2017, Turkish Rebuplic of Northern Cyprus.

sacroiliitis, enthesitis, inflammatory back pain; although they are *HLA-B27* negative.[5] Lehman et al.[6] described the first case report of *HLA-B27* negative FMF-related sacroiliitis in 1978.

Sacroiliitis associated with FMF is very rare, especially in children. In this study, we aimed to describe the demographic, clinical, laboratory features, and treatment responses of pediatric patients with FMF-related sacroiliitis.

Material and Methods

The retrospective study consisted of seven pediatric patients younger than 16 years, diagnosed with sacroiliitis associated with FMF between 2010-2017 in our department. All the patients were evaluated clinically according to Tel Hashomer criteria.[2] The diagnosis of FMF was confirmed genetically using a panel of common *MEFV* mutations; *A744S, F479L, I720M, K695R, M680I, M694I, M694V, P369S, R761H* and *V726A*. *MEFV* gene was evaluated using direct sequencing of the Polymerase Chain Reaction (PCR) amplified fragments. *HLAB27* antigen was performed by flow cytometry method. Clinical findings, direct radiography and magnetic resonance imaging (MRI) technique were used to confirm sacroiliitis. The patients were followed up at 3-months intervals. At follow-ups, the patients underwent a physical examination, and systemic arthritic activity was evaluated using Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores.[7] Acute phase reactants, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), were also monitored.

Patients' characteristics, such as age, sex, age at onset of FMF, age at onset of sacroiliitis, treatments (disease modifying anti-rheumatic drugs [DMARDs] and biologics) disease duration, MRI of sacroiliitis, existence of peripheral arthritis, BASDAI scores and acute phases (at onset and last visit), were recorded.

Clinical-radiological-laboratory responses were evaluated according to the response of therapies: resolution of all sacroiliitis symptoms with decreasing acute phases was accepted as a complete response, while reduced of sacroiliitis symptoms without full recovery was a partial response.

We also reviewed previous studies of patients with FMF, complicated by sacroiliitis. A search of PubMed for studies from inception to April 2017 was conducted using 'familial Mediterranean fever' and 'sacroiliitis' as keywords. Studies that discussed the treatment of FMF- related sacroiliitis were included. Studies of *HLA-B27* positive spondyloarthropaties (SPAs) and coexistence of FMF with SPAs were excluded.

The study protocol was approved by the Institutional Ethics Committee. Informed consent was obtained from all individual participants included in the study. All of the analyses were entered as variables into a database and analysed using SPSS, version 23. Descriptive statistics were evaluated.

Results

Among 392 pediatric FMF patients, 7 out of them (1.7%) had sacroiliitis associated with FMF. Demographic, clinical and laboratory features of patients are described in Table I. Two of the seven patients were females (28%). One patient had *V726A* (heterozygous), five patients had a M694V (homozygous) mutation and one patient had a *M694V* (heterozygous) mutation. All the patients were *HLA-B27* negative, rheumatoid factor negative and anti-nuclear antibodies negative. There was no amyloidosis. All patients had restricted movement in Schober's test and suffered from inflammatory back pain in the physical examination, supporting a diagnosis of sacroiliitis. Direct radiography and MRI findings were positive for sacroiliitis. FMF was diagnosed at a median of 5 (2-9) years, and sacroiliitis detected at median of 8 (7-15) years. The median duration between FMF diagnosis and development of sacroiliitis was 5 (0-12) years.

All the patients received treatment with colchicine and naproxen, a non-steroidal anti-

Table I. Patients characteristics, treatment features and response indicators.

Cases	1	2	3	4	5	**6**	**7**
Age (years)	14	9	14	16	11	9	16
Gender	male	male	male	female	male	male	female
FMF onset age(years)	5	6	3	9	2	8	3
Sacroiliitis onset age (years)	7	8	13	15	7	8	15
Peripheral arthritis/enthesitis	-	knee	ankle	ankle	ankle		
FMF diagnosis to sacroiliitis (months)	24	24	120	72	60	0	144
Sacroiliitis (unilateral/bilateral)	Bilateral	Unilateral	Bilateral	Bilateral	Bilateral	Unilateral	Bilateral
NSAIDs	+	+	+	+	+	+	+
SSZ	+	+	+	+	+	-	+
MTX	-	+	-	-	+	-	+
Anti-IL1	+	-	-	-	-	-	-
Anti-TNF	-	+	-	+	+	-	+
MEFV mutation	*V726A* (heterozygous)	*M694V* (homozygous)	*M694V* (heterozygous)	*M694V* (homozygous)	*M694V* (homozygous)	*M694V* (homozygous)	*M694V* (homozygous)
DMARDs duration (months)	40	17	12	12	30	-	12
Biologic duration (months)	44	16	-	1	30	-	1
BASDAI onset of sacroiliitis	4.9	3.7	2.9	2.7	3	3.2	5.5
BASDAI last visit	1.9	1.1	1.3	1.5	1	1.1	4.4
ESR onset sacroiliitis	42	37	12	33	48	36	39
ESR last visit	5	8	11	14	20	10	29
CRP onset sacroiliitis	36	20.1	1.5	15.2	18.3	17.5	22
CRP last visit	3	4.4	1	3	2.3	2	8

FMF: familial Mediterranean fever, BASDAI: bath ankylosing spondylitis disease activity index, NSAIDs: non-steroidal anti-inflammatory drugs, DMARDs: disease modifying anti-rheumatic drugs, MEFV: Mediterranean fever, SSZ: sulfasalazine, MTX: methotrexate, ESR: erythrocyte sedimentation rate (0-20 mm/h), CRP: C-reactive protein (0-5 mg/L).

inflammatory drug (NSAID). The colchicine dose was gradually increased to a maximum dose of 2 mg/day, followed by treatment NSAIDs and DMARDs, such as sulfasalazine (SSZ) and methotrexate (MTX). One patient whose clinical course included resistant fever attacks was managed with anti-IL-1 therapy. Four patients were treated with etanercept (ETC) after failure of NSAID and DMARD therapy. The clinical status of all patients treated with anti-TNF improved within 1 month of treatment commencement. The clinical course of only one patient was managed using colchicine and NSAIDs. Six patients received DMARD therapy. At the 12-month follow-up, only one patient, (i.e. the patient with the M694V [heterozygous] mutation) showed a therapeutic response to DMARDs.

The duration of DMARD treatment ranged from 12 to 40 months, whereas the duration of biological treatment varied from 1 to 44 months. Four (57.1%) patients had peripheral arthritis and enthesitis, including knee and/or ankle arthritis. The median BASDAI score at treatment onset was 3.2 (2.7-5.5) and the median score at the final follow-up was 1.3 (1.0- 4.4).

Acute phase reactants decreased by medical therapies. At the onset of sacroiliitis, the median ESR rate was 37 (12-48) mm/h and the median CRP was 18,3 (1.5-36) mg/L. At the last visit, the median ESR rate had decreased to 11 (5-29) mm/h, and the median CRP had decreased to 3 (1-8) mg/L.

Discussion

HLA-B27 negative FMF-related sacroiliitis is a rare disorder, with limited studies on the topic in a subgroup of pediatric patients. According to the literature, almost all reports of FMF-related sacroiliitis comprise adult patient series. Thus, the present pediatric case series is important.

Distinguishing FMF-related sacroiliitis from FMF coexisting with SPA is difficult, as there are no standardized definitions. Sönmez et al.[8] described that FMF patients with sacroiliitis had higher acute phases, less common *HLA-B27* positivity, enthesitis and vertebral involvement than in patients with SPA. Additionally, they showed that M694V mutation was the most common *MEFV* mutation among FMF patients with sacroiliitis. In the present series, all patients had restricted Schober test and increased acute phase reactants. There were no *HLA-B27* positivity. 6 of 7 patients (86%) had M694V mutation. 4 out of 7 patients (57%) had peripheral arthritis and enthesitis. Additionally, we did not reveal any vertebral involvement in our cases.

In a study of 157 adult FMF patients, Akar et al.[9] reported that only 15 (7.5%) patients had *HLA-B27* negative SPAs. They also found that the *M694V* mutation was common in this group. Their study suggested that factors other than *HLA-B27* played a role in the coexistence of FMF and SPA.

The primary treatment for FMF is colchicine, which effectively suppresses the frequency of attacks and prevents the development of amyloidosis.[10] Colchicine is well tolerated by pediatric patients.[11] In recent years, biological agents have been used as an alternative treatment for colchicine-resistant patients. Anti-IL 1 treatment, including anakinra and canakinumab, suppresses inflammation caused by IL-1 activation.[12] Anti Tumour Necrosis Factor (TNF) treatment, such as ETC, infliximab or adalimumab, is also effective in controlling FMF attacks in patients with chronic arthritis or sacroiliitis.[13]

We prepared a mini literature review regarding to sacroiliitis accompanied with FMF in Table II and III. Lehman et al.[6] described the first case report of *HLA-B27* negative FMF-related sacroiliitis in 1978, noting that the patient showed a partial response to treatment with colchicine and a NSAID. Majeed et al.[4] reported the use of colchicine plus NSAID therapy in the treatment of a 14-year-old patient with FMF-related sacroiliitis and achieved a partial response. Langevitz et al.[14] reported a large study of 3000 FMF patients. The primary

Table II. Literature review of demographic data to HLA-B27 negative FMF related sacroiliitis.

Author	Number of patients	Age at dx	MEFV mutation
Lehman et al.[6]	2	10	NA
Majeed et al.[4]	1	14	NA
Langevitz et al.[14]	11	25-51	NA
Eifan et al.[23]	1	11	M694V/M694V
Demirag et al.[15]	1	22	M694V/M694V
Borman et al.[16]	2	18-29	M694V/M694V
Erten et al.[17]	3	33-48	M694V/M694V, M694V/M680I
Bilgen et al.[18]	8	25-42	M694V/M694V, M694V/M680I, E148Q/E148Q
Erten et al.[24]	1	18	M694V/M694V
Sahin et al.[21]	1	45	A744S/E148Q
Estublier et al.[19]	1	39	M694I/M694I
Varan et al.[20]	1	39	V726A/ -
Ugan et al.[22]	1	22	M694V/M694V

FMF: familial Mediterranean fever, NA: not avialable.

Table III. Literature review of treatment approach to HLA-B27 negative FMF related sacroiliitis.

Author	Anti TNF	Anti IL-1	Agents prior biologics	Median follow-up time	Clinical effects
Lehman et al.[6]	-	-	Colchicine, NSAID	NA	PR
Majeed et al.[4]	-	-	Colchicine, NSAID	10 months	PR
Langevitz et al.[14]	-	-	Colchicine, NSAID, SSZ, MTX	NA	PR, PD
Eifan et al.[23]	-	-	Colchicine, NSAID	24 months	CR
Demirag et al.[15]	-	-	Colchicine, NSAID, Gold (im)	8 months	PR
Borman et al.[16]	-	-	Colchicine, NSAID, SSZ	NA	PR
Erten et al.[17]	-	-	Colchicine, SSZ	NA	CR
Bilgen et al.[18]	INF, ADA, ETC	-	Colchicine, NSAID, SSZ, MTX	NA	PR, CR
Erten et al.[24]	INF, ETC	-	Colchicine	NA	CR
Sahin et al.[21]	ETC	-	NSAID, pred, SSZ, LFN, MTX, HCQ	96 months	PR
Estublier et al.[19]	ADA, ETC	ANA	Colchicine, NSAID, SSZ, Pred	144 months	CR
Varan et al.[20]	ADA, ETC	ANA	Colchicine, NSAID, SSZ	51 months	PR
Ugan et al.[22]	-	-	Colchicine, NSAID, SSZ, Pred	1 month	CR

FMF: familial Mediterranean fever, NA: not avialable, INF: infliximab, ADA: adalimumab, ETC: etanercept, ANA: anakinra, NSAID: non-steroidal anti-inflammatory drug, Pred: prednisolon, SSZ: sulfasalazine, MTX: methotrexate, LFN: leflunomide, AZA: azatiopurin, PR: partial response, PD: progressive disease, CR: complete response.

objective of their study was to determine the association between FMF and seronegative SPA, coexisting with FMF and ankylosing spondylitis (AS) or FMF-related sacroiliitis. They detected *HLA-B27* negative sacroiliitis in only 11 (0.4%) patients, nine of whom were males. All patients had inflammatory back pain. Six patients had enthesitis, and seven patients suffered from heel pain. Peripheral arthritis was monoarticular in five patients and oligoarticular in the other patients. Treatment consisted of MTX, SSZ, NSAIDs and colchicine. The outcome was favorable in eight patients, but disease progression occurred in three patients. In addition, they emphasized that in these patients who had FMF-related sacroiliitis, none of them had any radiologic vertebral changes. Demirag et al.[15] reported a partial response of a

22-year-old patient who had sacroiliitis related to FMF, to intramuscular gold therapy. Borman et al.[16] and Erten et al.[17] presented adult cases that showed favorable responses to colchicine plus SSZ. Bilgen et al.[18] examined the response of colchicine-resistant adult patients with FMF-associated sacroiliitis to anti-TNF therapy. In their study, eight patients had a M694V mutation (homozygous) and compound heterozygous *M680I/M694V* mutation. One patient had a *E148Q* (homozygous) mutation. The duration and frequency of attacks decreased in two patients, and a complete response was obtained in five patients.[18] In recent years also anti IL-1 treatments have been used effectively in this small group of patients. Estublier et al.[19] and Varan et al.[20] reported two adult cases of FMF-related sacroiliitis resistant to anti TNF therapy. Both patients showed favorable responses to anakinra treatment, demonstrating that this drug may be useful in the treatment of FMF-related sacroiliitis.

As noted above, *HLA-B27* negative FMF-related sacroiliitis cases treated with anti-TNF or anti-IL-1 therapy are very rare. There are no reports of canakinumab treatment of pediatric and adult patients in FMF related sacroiliitis. Herein, we described seven cases of FMF-related sacroiliitis, five with a *M694V* (homozygous) mutation and one with a heterozygous mutation of the same gene. Four of 7 patients improved with etanercept therapy. The patient who had *V726A* (heterozygous) mutation, was successfully controlled with canakinumab.

In conclusion, we suggested that in FMF patients who are suffering from inflammatory back pain with increased acute phases in laboratory work-up, should be investigated for sacroiliitis with MRI. This manifestation generally occurs in FMF patients who have *M694V* mutation. Firstly, NSAIDs and DMARDs could be prefer for the treatment of sacroiliitis. When there is insufficient response to these therapies, biological agents (anti TNF and anti IL-1) could be useful in this rare group patients.

Acknowledgement

Authors thank the parents of the patients for participation in this study.

REFERENCES

1. Onen F. Familial Mediterranean fever. Rheumatol Int 2006; 26: 489-496.
2. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. Am J Med 1967; 43: 227-253.
3. Bernot A, Clepet C, Dasilva C, et al; The French FMF Consortium. A candidate gene for familial Mediterranean fever. Nat Genet 1997; 17: 25-31.
4. Majeed HA, Rawashdeh M. The clinical patterns of arthritis in children with familial Mediterranean fever. QJM 1997; 90: 37-43.
5. Balaban B, Yasar E, Ozgul A, Dincer K, Kalyon TA. Sacroiliitis in familial Mediterranean fever and seronegative spondyloarthropathy: importance of differential diagnosis. Rheumatol Int 2005; 25: 641-644.
6. Lehman TJ, Hanson V, Kornreich H, Peters RS, Schwabe AD. HLA-B27-negative sacroiliitis: a manifestation of familial Mediterranean fever in childhood. Pediatrics 1978; 61: 423-426.
7. Zochling J, Braun J. Assessment of ankylosing spondylitis. Clin Exp Rheumatol 2005; 23(Suppl 39): S133-S141.
8. Sönmez HE, Batu ED, Demir S, Bilginer Y, Özen S. Comparison of patients with familial Mediterranean fever accompanied with sacroiliitis and patients with juvenile spondyloarthropathy. Clin Exp Rheumatol 2017; 35(Suppl 108): 124-127.
9. Akar S, Soysal O, Balci A, et al. High prevalence of spondyloarthritis and ankylosing spondylitis among familial Mediterranean fever patients and their first-degree relatives: further evidence for the connection. Arthritis Res Ther 2013; 15: R21.
10. Zemer D, Revach M, Pras M, et al. A controlled trial of colchicine in preventing attacks of familial mediterranean fever. N Engl J Med 1974; 291: 932-934.
11. Padeh S, Gerstein M, Berkun Y. Colchicine is a safe drug in children with familial Mediterranean fever. J Pediatr 2012; 161: 1142-1146.
12. Meinzer U, Quartier P, Alexandra JF, Hentgen V, Retornaz F, Koné-Paut I. Interleukin-1 targeting drugs in familial Mediterranean fever: a case series and a review of the literature. Semin Arthritis Rheum 2001; 41: 265-271.

13. Ozgocmen S, Ozçakar L, Ardicoglu O, Kocakoc E, Kaya A, Kiris A. Familial Mediterranean fever responds well to infliximab: single case experience. Clin Rheumatol 2006; 25: 83-87.

14. Langevitz P, Livneh A, Zemer D, Shemer J, Pras M. Seronegative spondyloarthropathy in familial Mediterranean fever. Semin Arthritis Rheum 1997; 27: 67-72.

15. Demirag MD, Ozturk MA, Goker B, Haznedaroglu S. Intramuscular gold for the treatment of seronegative spondyloarthropathy associated with familial Mediterranean fever. Rheumatol Int 2008; 29: 77-79.

16. Borman P, Gökoğlu F, Taşbaş O, Yilmaz M, Yorgancioğlu ZR. Familial Mediterranean fever-related spondyloarthropathy. Singapore Med J 2009; 50: e116-e119.

17. Erten S, Erzurum C, Altunoglu A. Three family members with familial Mediterranean fever carrying the M694V mutation showed different clinical presentations. Intern Med 2012; 51: 1765-1768.

18. Bilgen SA, Kilic L, Akdogan A, et al. Effects of anti-tumor necrosis factor agents for familial mediterranean fever patients with chronic arthritis and/or sacroiliitis who were resistant to colchicine treatment. J Clin Rheumatol 2011; 17: 358-362.

19. Estublier C, Stankovic Stojanovic K, Bergerot JF, Broussolle C, Sève P. Myositis in a patient with familial Mediterranean fever and spondyloarthritis successfully treated with anakinra. Joint Bone Spine 2013; 80: 645-649.

20. Varan O, Kucuk H, Tufan A. Anakinra for the treatment of familial Mediterranean fever-associated spondyloarthritis. Scand J Rheumatol 2016; 45: 252-253.

21. Sahin A, Yetişgin A, Sahin M. Rheumatoid arthritis and familial mediterranean fever or sacroiliitis accompanied by FMF. Case Rep Rheumatol 2013; 2013: 636713.

22. Ugan Y, Doğru A, Şencan H, Şahin MF, Tunç ŞE. Sacroiliitis and polyarteritis nodosa in a patient with familial mediterranean fever. Case Rep Med 2016; 2016: 5134546.

23. Eifan AO, Ozdemir C, Aydogan M, Gocmen I, Bahceciler NN, Barlan IB. Incomplete attack and protracted sacroiliitis: an unusual manifestation of FMF in a child. Eur J Pediatr 2007; 166: 383-384.

24. Erten S, Erten SF, Altunoglu A. Successful treatment with anti-tumor necrosis factor (anti-TNF)-alpha of proteinuria in a patient with familial mediterranean fever (FMF) resistant to colchicine: anti-TNF drugs and FMF. Rheumatol Int 2012; 32: 1095-1097.

The Turkish Journal of Pediatrics 2020; 62: 182-190
https://doi.org/10.24953/turkjped.2020.02.003

Original Article

Bone marrow involvement in pediatric malignancies: a comparison study of Positron emission tomography-computed tomography and bone marrow biopsy

Özlem Tezol[1], Fatih Sağcan[1], Pınar Pelin Özcan[2], Elvan Çağlar Çıtak[3]

Departments of [1]Pediatrics, [2]Nuclear Medicine and [3]Pediatric Oncology, Mersin University Faculty of Medicine, Mersin, Turkey.

ABSTRACT

Background and objectives. The comparison of Positron emission tomography- computed tomography (PET-CT) and bone marrow biopsy (BMB) modalities in detecting bone marrow disease is an up to date research topic. In this study, we aimed to compare the results of PET-CT and BMB procedures in detecting bone marrow involvement in pediatric malignancies.

Method. At the time of diagnosis, PET-CT imaging and BMB performed patients' data were evaluated, retrospectively. Malign diagnoses were Hodgkin's lymphoma in 23 (30.7%), non-Hodgkin's lymphoma in 20 (26.7%), neuroblastoma in 11 (14.7%), Ewing sarcoma in 10 (13.7%), Langerhans cell histiocytosis in 6 (8%), and rhabdomyosarcoma in 5 (6.6%) patients.

Results. Bone marrow involvement was detected in 39 (52%) of 75 patients. Bone marrow involvement was identified by both PET-CT and BMB in 18 (46.1%) patients, by only PET-CT in 12 (30.7%) patients, by only BMB in 9 (23%) patients. The sensitivity of PET-CT was 66%, specificity was 75%, positive predictive value was 60%, and negative predictive value was 80%. Sensitivity, spesificity, positive and negative predictive values of PET-CT were different in before mentioned malignancy groups.

Conclusion. PET-CT may not have high sensitivity and specificity to identify bone marrow involvement for each type of cancer. The approach of using bone marrow biopsy and PET-CT as complementary modalities seems reliable.

Key words: bone marrow involvement, pediatric malignancies, PET-CT.

Cancer a major cause of mortality, is rare in childhood. Bone marrow involvement (BMI) is a sign of advanced disease and poor prognosis in childhood malignancies. In the pediatric population, at the time of diagnosis, Hodgkin's lymphoma (HL) has BMI in 4-14% of patients, and stage-IV non-Hodgkin's lymphoma (NHL) frequency is 8.4-25.4% in recent studies.[1-3] The frequency of BMI in newly-diagnosed pediatric patients with Ewing sarcoma was reported to be 8.5%, with neuroblastoma it was reported to be 54.5%.[4,5] Bone marrow biopsy (BMB) is a procedure for diagnosis, staging, identification of prognostic risk factors, treatment monitoring in pediatric malignancies. BMB is accepted as a "gold standart" modality in identifying bone marrow involvement.

Positron emission tomography-computed tomography (PET-CT) is the most frequently used imaging method in staging of malign diseases in adult patients. It provides information about the anatomic structures and metabolic activities of tumors, and visualizes the whole body including bone marrow. BMB is an invasive and painful procedure with disadvantages such as general anesthesia need in young children or sedation need in older children, false negative results due to sampling failure, failure in detection of focal involvement.[6]

✉ Özlem Tezol
ozlemtezol@hotmail.com

Received 21st January 2019, revised 15th June 2019, 27th July 2019, accepted 29th July 2019.

By means of high fluor-18 fluorodeoxyglucose (18F-FDG) avidity of the bone marrow, imaging of the entire marrow, and detecting focal involvements are known as the superiorities of PET-CT.[7] In case of incompatibility between different imaging procedures, PET-CT imaging has the highest diagnostic accuracy in 90% of cases, it changes the stage of disease in 61% of cases, and it changes the clinical approch in 24% of patients.[8] There is emerging evidence to suggest that FDG PET-CT has an established role in staging pediatric malignancies, and BMB can be safely excluded in patients with normal bone marrow on PET-CT.[9] For instance, Zapata et al.[6] reported no false negative BMI results with PET-CT in pediatric solid tumors, and Hassan et al.[7] reported a high sensitivity, specificity and negative predictive value for BMI on PET-CT in pediatric HL, likewise Chen et al.[15] reported a sensitivity and specificity greater than 90% in pediatric NHL.

The comparison of PET-CT and BMB modalities in detecting bone marrow disease is a research topic that remains update. In this study, we aimed to compare the results of PET-CT and BMB modalities in detecting BMI in pediatric malignancies that are known to metastasize to the bone marrow, and to investigate the sensitivity and specificity of PET-CT.

Material and Methods

Patients' data diagnosed with Hodgkin's lymphoma, non-Hodgkin's lymphoma, Ewing sarcoma, neuroblastoma, rhabdomyosarcoma and langerhans cell histiocytosis (LCH) between June 2008 and June 2018 were evaluated, retrospectively. At the time of diagnosis and before the initiation of systemic therapy, PET-CT imaging and BMB performed patients' data were included. Demographic and clinical characteristics were collected as data.

Following 4-6 hours fasting, PET-CT imagings of patients were performed who were hidrated with dextrose free fluid. One hour after 18F-FDG injection the entire body was scaned in 3D mode (Siemens Medical Solusions, Erlangen, Germany and GE Discovery PETCT 610, US). Imaging results were evaluated by a nuclear medicine specialist who did not know the results of BMBs. PET-CT results of patients with FDG uptake similar to adjacent soft tissues in bone marrow were accepted as negative. Results with FDG uptake equal with primary tumor or more than adjacent tissues in bone marrow were accepted as positive. Diffuse or multifocal FDG uptake patterns were noted.

Bone marrow biopsies were performed by the same pediatric oncologist from the standard iliac crest region before the PET-CT scan. Bilateral sampling from iliac crests were performed. All biopsy samples were adequate, and examined histopathologically and immunohistochemically in pathology laboratory. The pathologist performed CD1a/S100 staining in LCH and choromogranin, synaptophysin, neuron spesific enolase, CD99, desmin, vimentin stainings in small-blue-round-cell tumors. Leukocyte common antigen, CD20, CD79a, CD3, CD5, CD15, bcl2, bcl-6, CD30, CD10, CD30, terminal deoxynucleotidyl transferase stainings were performed in lymphomas.

PET-CT result was accepted as true negative if there was not metastasis in bone marrow biopsy, PET-CT result was accepted as true positive if there was metastasis in bone marrow biopsy. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the PET-CT modality were calculated. The study protocol was approved by the local ethics committee (MEU 2018/315).

Results

Over a period of ten years, we evaluated 75 patients in whom both BMB and PET-CT procedures were performed. Malign diagnoses were Hodgkin's lymphoma (HL) in 23 (30.7%), non-Hodgkin's lymphoma (NHL) in 20 (26.7%), neuroblastoma in 11 (14.7%), Ewing sarcoma in 10 (13.7%), langerhans cell histiocytosis (LCH)

in 6 (8%), and rhabdomyosarcoma in 5 (6.6%) patients. Mean (SD) age was 13.0 (4.1) years in HL, 10.6 (4.4) years in NHL, 2.3 (1.6) years in neuroblastoma, 10.5 (4.2) years in Ewing sarcoma, 6.9 (4.8) years in LCH, 9.0 (3.8) years in rhabdomyosarcoma group. Percentage of male patients was 52.1% in HL, 65% in NHL, 72.7% in neuroblastoma, 60% in Ewing sarcoma, 50% in LCH, 60% in rhabdomyosarcoma group. BMI was detected in 39 (52%) patients. The malignancy group with the highest frequency of bone marrow involvement was non-Hodgkin's lymphoma (16 of 20 patients, 80%). Diagnoses and BMI frequencies are shown in Table I.

BMI was identified by both PET-CT and BMB in 18 (46.1%) patients, by only PET-CT in 12 (30.7%) patients, by only BMB in 9 (23%) patients. The examples for these groups of patients were given in Figures 1, 2 and 3. For the whole group, the sensitivity of PET-CT was 66%, specificity was 75%, positive predictive value was 60% and negative predictive value was 80% (Table II). The sensitivity values of PET-CT for HL, NHL, neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, LCH groups were 100, 50, 75, 50, 100, 100%, respectively. The specificity values of PET-CT for HL, NHL, neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, LCH groups were 73, 50, 100, 83, 100, 60%, respectively. The positive predictive values of PET-CT for HL, NHL, neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, LCH groups were 44, 60, 100, 66.6, 100, 33.3%, respectively. The negative predictive values of PET-CT for HL, NHL, neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, LCH groups were 100, 40, 87.5, 71.4, 100, 100%, respectively.

Table I. Diagnoses and bone marrow infiltration frequencies.

Diagnosis	BM infiltration
Hodgkin's lymphoma	9 (39.1%)
Non-Hodgkin's lymphoma	16 (80.0%)
Neuroblastoma	4 (36.3%)
Ewing sarcoma	5 (50.0%)
Langerhans cell histiocytosis	3 (50.0%)
Rhabdomyosarcoma	2 (40.0%)

BM: bone marrow.

Table II. Comparison of BMB results & PET-CT results.

PET-CT results	BMB results	
	BMB positive	BMB negative
Positive	18	12
Negative	9	36

BMB: bone marrow biopsy, PET-CT: positron emission tomography-computed tomography.

PET-CT had false negative results in 9 patients. Six of these PET negative BMB positive patients were diagnosed with NHL, and there were generalised hypermetabolic lympadenopathies on their PET-CT scans (SUVmax range = 4.52 – 28.59). Two of these PET negative BMB positive patients were diagnosed with Ewing sarcoma, and there were tumoral uptakes on their PET-CT scans (SUVmax values =6.87 and 4.79). One of these PET negative and BMB positive patients was diagnosed with neuroblastoma, and there was tumoral uptake on his PET-CT scan (SUVmax = 3.73).

PET-CT detected abnormal up-take in different bone marrow regions defined as multifocal uptake pattern in 12 BMB negative patients. Increased multifocal F-FDG uptake in 11 patients was in several bone marrow regions except iliac crests (cranium, rib, sternum, humerus, tibia, femur, sacrum). One patient diagnosed with HL had abnormal F-FDG uptake in left iliac crest and negative BMB result.

Clinical course of BMB negative patients was good as anticipated, these patients had higher one-year survival rates. The distribution of procedures identifying bone marrow involvement according to the diagnosis and one-year survival rates are shown in Table III.

Discussion

Early diagnosis and disease screening have great importance for pediatric oncology. For the determination of the treatment protocol, BMI is assessed by BMB, as the gold standard method.

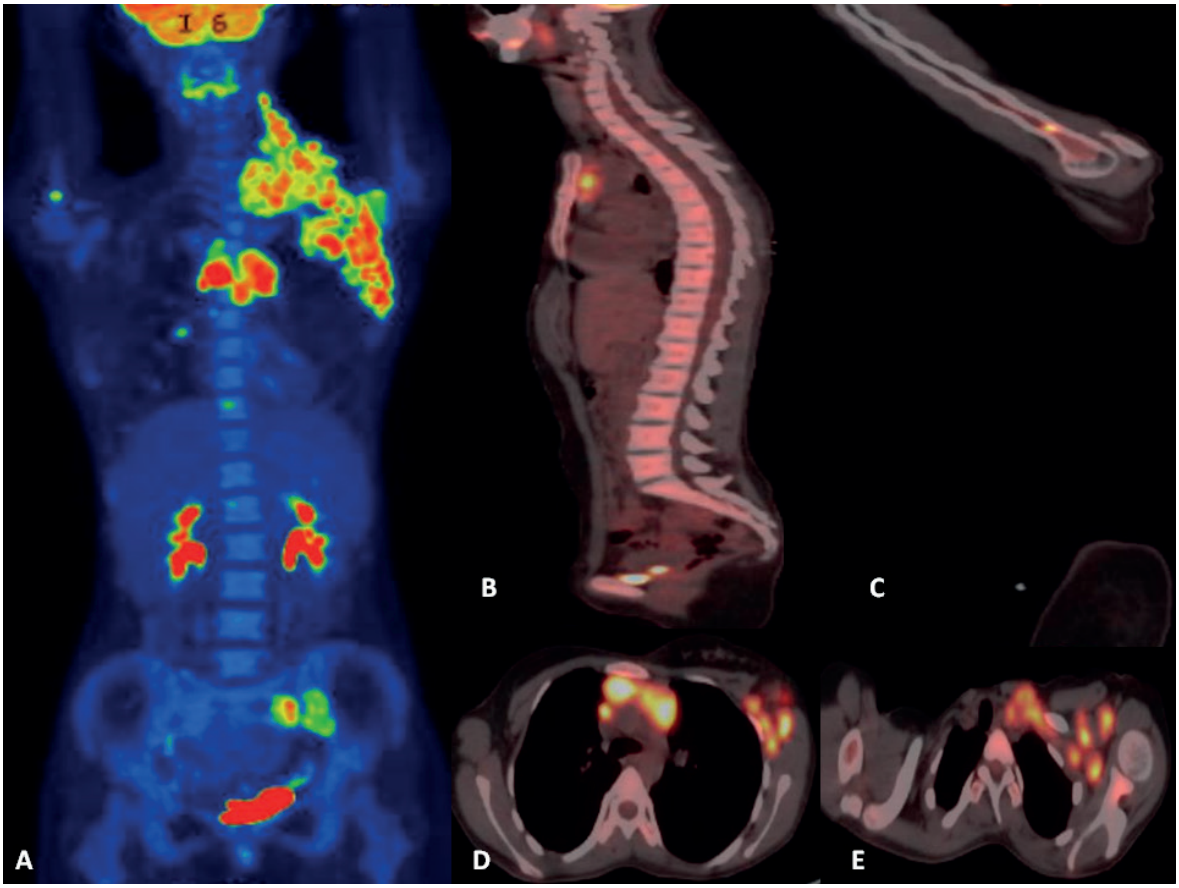


Fig. 1. FDG PET-CT images of a 15 year old female patient with Hodgkin Lymphoma diagnosis. Maximum intensity projection (MIP) image (A), sagittal (B, C) and axial (D,E) fusion images demonstrate multipl hypermetabolic lymphadenopaties and bone-bone marrow diffuse and multifocal FDG uptake compatible with lymphoma diagnosis. Bone marrow biopsy was negative for this patient.

PET-CT has found a wide and reliable area of use in terms of diagnosis, accurate staging, and evaluation of treatment response in pediatric malignancies. In pediatric tumor staging, the sensitivity values of PET and PET-CT for all cancers were reported to be 90-97% and 99-100%, respectively.[10-12] In the current study, PET-CT showed 66% sensitivity and 75% specificity for demonstrating BMI.

Most pediatric studies investigated BMI by PET-CT in patients with lymphoma. PET-CT shows all the lesions detected by BMB, and it is able to differentiate diffuse uniform involvement due to anemia or reactive changes and multifocal bone involvement due to lymphoma.[8] In a study conducted with 54 patients with a lymphoma diagnosis (31 HL, 24 NHL), the specificity and positive predictive values were reported to be 100% for both PET-CT and BMB, and the sensitivity and negative predictive values were 92% and 54%, respectively for PET-CT, and 98% and 87%, respectively for BMB.[13] Our study showed lower results in whole lymphoma group for PET-CT in detecting BMI compared with BMB (62% versus 52% sensitivity, 66% versus 75% specificity).

In a study of 784 pediatric patients diagnosed with Hodgkin's lymphoma, 13.3% of patients had BMI. The sensitivity, specificity, positive and negative predictive values of PET-CT were found to be 93.6, 94, 53, and 99.4%, respectively, and this method was reported to be more sensitive than BMB for detecting BMI. The authors recommended that BMB should only be

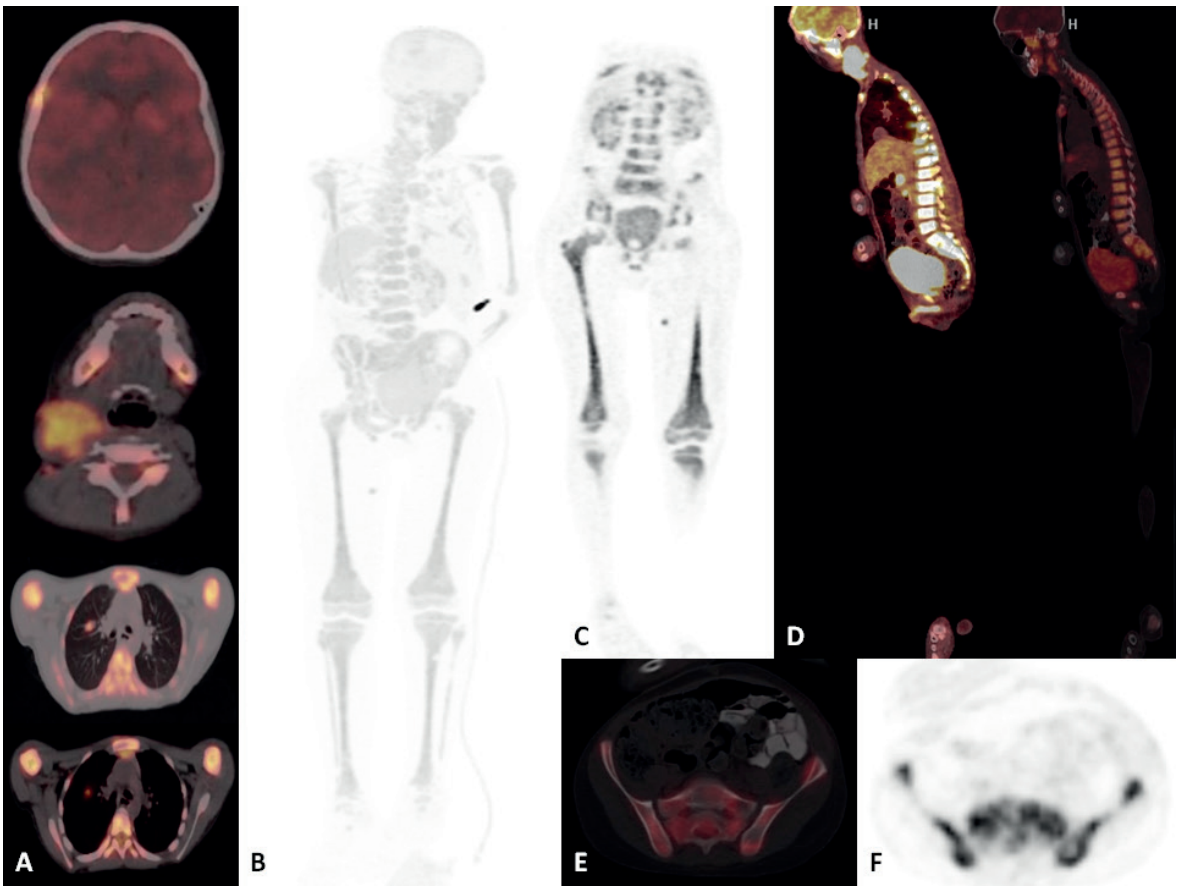


Fig. 2. FDG PET-CT images of a 12 year old male patient with non-Hodgkin's lymphoma diagnosis. Axial fusion (A), Maximum intensity projection (MIP) image (B), coronal PET (C), sagittal fusion (D), axial fusion (E) and axial PET (F) images demonstrate multipl hypermetabolic lymphadenopaties, soft tissue lesions and bone-bone marrow diffuse and multifocal FDG uptake compatible with lymphoma diagnosis. Bone marrow biopsy was positive for this patient.

performed in patients with normal BM activity or increased diffuse activity that suggests reactive bone marrow.[7] In another pediatric Hodgkin's lymphoma study, PET-CT did not give any false negative result compared to the reference methods BMB, CT and magnetic resonance imaging. Furthermore, the specificity and negative predictive value of PET-CT were reported as 100%. Due to the typical multifocal BMI of HL, the authors noted that the sensitivity of BMB from the iliac crest was low.[14] For the patients diagnosed with Hodgkin's lymphoma in the current study, the sensitivity and negative predictive value of PET-CT for detecting BMI were both 100%, and its specificity and positive predictive values were 73% and 44%, respectively. So we can suggest that PET-CT could idendify bone marrow metastatic HL patients as accurately as BMB.

In pediatric patients with a NHL diagnosis, Chen et al.[15] reported the sensitivity and specificity of PET-CT as 95% and 98%, respectively based on multifocal accumulations that were not detected by BMB but visualized by PET-CT. In the same study, the sensitivity and specificity of BMB was 56% and 100%, respectively. However, the authors commented that the role of PET-CT was dependent on the type of lymphoma.[15] In the current study, for the patients with NHL, the PET-CT sensitivity and specificity were 50%, and its positive and negative predictive values were 60% and 40%, respectively. These results

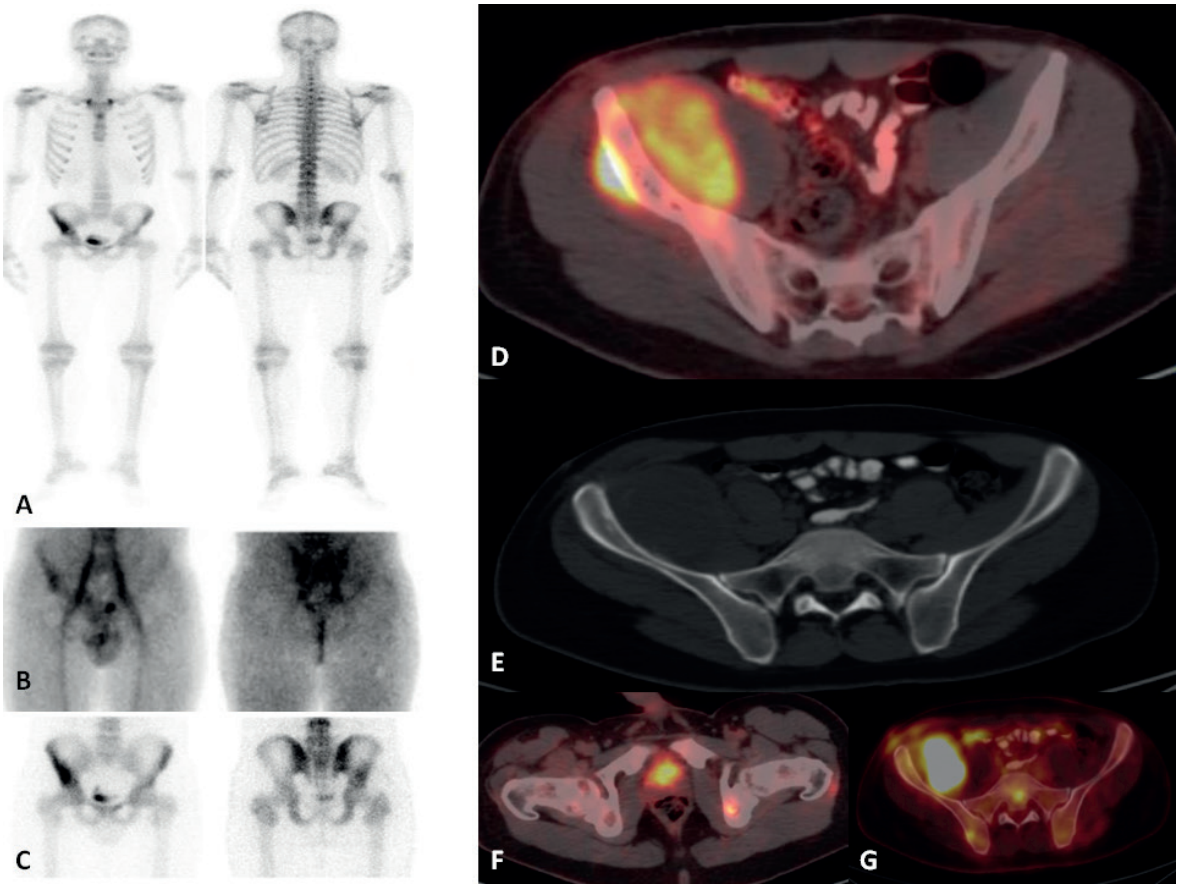


Fig. 3. Images of a 17 year old male patient with Ewing Sarcoma diagnosis. Whole body bone scintigraphy (A), blood pool (B) and statik (C) images demonstrates radioactivity uptake on right iliak crest. FDG PET-CT images of the same patient illustrate both right iliac crest mass and multipl bone-bone marrow FDG uptake on images of axial fusion (D), axial CT (E) and axial fusion pelvic (F,G) images. Bone marrow biopsy was negative for this patient.

support the approach to perform BMB on NHL patients at the time of diagnosis and use PET-CT imaging as a complementary method.

In pediatric solid tumors, the number of studies that evaluated BMI using PET-CT is less than those undertaken for lymphomas. In a study conducted with 34 patients with a lymphoma diagnosis accompanied by a solid tumor (7 rhabdomyosarcomas, 7 Ewing sarcomas, and 20 neuroblastomas), Zapata et al.[6] detected no false negative results with PET-CT.

18F-FDG-PET-CT is increasingly used in the diagnosis, staging and prognosis prediction of pediatric neuroblastoma. Li et al.[16] evaluated 26 patients with a neuroblastoma diagnosis and observed BMI in 16 patients, of whom 10 (62.5%) had bone marrow lesions and 6 (37.5%) had diffuse FDG uptake. Taking a positive BMB as the reference standard, the sensitivity, specificity and accuracy of PET-CT in detecting BMI were found to be 100, 50 and 80.7%, respectively. In addition, PET-CT was able to demonstrate multifocal involvement in 2 patients with a negative result in the iliac crest BMB.[16] In the current study, the PET-CT specificity and positive predictive value were 100%, and sensitivity and negative predictive value were 75% and 87.5%, respectively, so we can suggest that PET-CT could idendify non-bone marrow metastatic neuroblastoma patients as accurately as BMB.

Table III. The distribution of procedures identifying bone marrow involvement according to the primary diagnosis and one-year survival rates.

Diagnosis	BMI status			
	PET-CT + / BMB +	PET-CT + / BMB -	PET-CT - / BMB +	PET-CT - / BMB -
HL	4	5	0	14
Survival rate (%)	100	80	-	100
NHL	6	4	6	4
Survival rate (%)	50	75	50	100
Neuroblastoma	3	0	1	7
Survival rate (%)	33	-	100	100
Ewing sarcoma	2	1	2	5
Survival rate (%)	0	0	0	60
LCH	1	2	0	3
Survival rate (%)	100	100	-	100
Rhabdomyosarcoma	2	0	0	3
Survival rate (%)	0	-	-	33

BMB: bone marrow biopsy, BMI: bone marrow involvement, PET-CT: positron emission tomography-computed tomography, HL: Hodgkin's lymphoma, NHL: non-Hodgkin's lymphoma, LCH: Langerhans cell histiocytosis.

Ewing sarcoma is the second most common malignant bone tumor. In recent years, PET-CT has been used for imaging, staging and monitoring treatment response of this tumor.[17] Kasalak et al.[18] retrospectively evaluated 38 BMB samples (bilateral in 18 and unilateral in 2 patients) and PET-CT scans belonging to 20 patients with the diagnosis of Ewing sarcoma in terms of BMI. The PET-CT and BMB findings were similar in 36 posterior iliac cristas. According to the patient-based evaluation, the findings from both methods were similar in 19 patients. Considering these results, the authors suggested that in Ewing sarcoma patients, the routine BMB procedure should be reconsidered when it is possible to perform PET-CT during the evaluation of BMI at the time of diagnosis.[18] In the current study, in patients with Ewing sarcoma, the PET-CT specificity was 83%, sensitivity was 50%, and positive and negative predictive values were 66.6% and 71.4%, respectively. In Ewing sarcoma, metastatic status is the most important factor for a risk-adaptive treatment approach. Based on our results, we consider that for accurate staging, BMB should be performed at the time of diagnosis, and PET-CT can be used as a supporting method.

The most common soft tissue sarcoma in childhood is rhabdomyosarcoma. With the widespread use of PET-CT imaging, BMB procedure is now frequently employed in the staging of rhabdomyosarcoma.[17] Federico et al.[19] reviewed 30 pediatric cases with rhabdomyosarcoma retrospectively and detected BMI in 4 patients (13%) by using BMB, of whom 2 also had a positive PET-CT result, and suggested that PET-CT could be used routinely in staging rhabdomyosarcoma. However, in their literature review, Norman et al.[20] reported that the results of Federico et al.[19] were not clear, and the sensitivity of PET-CT was limited. In the current study, the specificity, sensitivity, positive and negative predictive values of PET-CT for revealing BMI were found to be 100%, so we can suggest that PET-CT could detect BMI as accurately as BMB in rhabdomyosarcoma patients.

LCH is a reactive proliferative disease of unknown etiology, characterized by the proliferation of langerhans cells. BMI is associated with high risk and low survival, and indicates multisystemic LCH and poor prognosis. In the literature, the BMI rates were reported as 26.3% and 33.3%.[21,22] In the current study, BMI was presented at a rate of

44.4%, and the PET-CT sensitivity, specificity, negative and positive predictive values for demonstrating BMI were 100%, 60%, 100%, and 33.3%, respectively. So we can suggest that PET-CT could idendify bone marrow metastatic LCH patients as accurately as BMB. In order not to diagnose multisystemic LCH based on false positive results, we consider that it is not appropriate to evaluate BMI using the PET-CT method alone.

Limitations of our study are small sample size, the retrospective nature, heterogeneity, only the iliac crest region preference for BMB, and not evaluating the results of other imaging methods. We also know that the involvements we regard as bone marrow metastasis by PET-CT may be paraneoplastic activity. For this reason, the detection of BMI should be investigated by different modalities, and associations between different modality results and survival data should be studied with larger samples prospectively.

In conclusion, the diagnosis should be considered when choosing a procedure to investigate bone marrow involvement in pediatric malignancies. PET-CT may not have high sensitivity and specificity to identify BMI for each type of cancer. The approach of using bilateral BMB and PET-CT as complementary modalities seems reliable.

REFERENCES

1. Paulino AC, Margolin J, Dreyer Z, Bin S Teh, Stephen Chiang. Impact of PET-CT on involved field radiotherapy design for pediatric Hodgkin lymphoma. Pediatr Blood Cancer 2012; 58: 860-864.
2. Faizan M, Anwar S, Khan S. Demographics and outome in paediatric non-Hodgkin lymphoma: single centre experience at The Children Hospital Lahore, Pakistan. J Coll Physicians Surg Pak 2018; 28: 48-51.
3. Budiongo AN, Ngiyulu RM, Lebwaze BM, et al. Pediatric non-Hodgkin lymphomas: first report from Central Africa. Pediatr Hematol Oncol 2015; 32: 239-249.
4. Khan SJ, Ishaq I, Saeed H, Fayyaz MB, Baqari SAS, Mohammad Wali R. Bone marrow involvement in metastatic pediatric ewing sarcoma. J CollPhysicians Surg Pak 2017; 27: 502-504.
5. Rastogi P, Naseem S, Varma N, et al. Bone marrow involvement in neuroblastoma: a study of hemato-morphological features. Indian J Hematol Blood Transfus 2015; 31: 57-60.
6. Zapata CP, Cuglievan B, Zapata CM, et al. PET/CT versus bone marrow biopsy in the initial evaluation of bone marrow infiltration in various pediatric malignancies. Pediatr Blood Cancer 2018; 65: e26814.
7. Hassan A, Siddique M, Bashir H, et al. ^{18}F-FDG PET-CT imaging versus bone marrow biopsy in pediatric Hodgkin's lymphoma: a quantitative assessment of marrow uptake and novel insights into clinical implications of marrow involvement. Eur J Nucl Med Mol Imaging 2017; 44: 1198-1206.
8. Ozcan Kara P. Pediatrik lenfomalarda PET/BT görüntüleme. Turkiye Klinikleri J Nucl Med-Special Topics 2017; 3: 93-99.
9. Chambers G, Frood R, Patel C, Scarsbrook A. ^{18}F-FDG PET-CT in paediatric oncology: established and emerging applications. Br J Radiol 2019; 91: 20180584.
10. Tatsumi M, Miller JH, Wahl RL. 18F-FDG PET/CT in evaluating non-CNS pediatric malignancies. J Nucl Med 2007; 48: 1923-1931.
11. Odalovi´c S, Sobic-Saranovic D, Pavlovic S, et al. Preliminary experience with 18f-fluoro-deoxy-glucose positron emission tomography/computed tomography in pediatric oncology patients. Acta Chir lugosl 2011; 58: 67-73.
12. Wegner EA, Barrington SF, Kingston JE, et al. The impact of PET scanning on management of paediatric oncology patients. Eur J Nucl Med Mol Imaging 2005; 32: 23-30.
13. Cheng G, Chen W, Chamroonrat W, Drew A Torigian, Hongming Zhuang, Abass Alavi.Biopsy versus FDG PET/CT in the initial evaluation of bone marrow involvement in pediatric lymphoma patients. Eur J Nucl Med Mol Imaging 2011; 38: 1469-1476.
14. Purz S, Mauz-Korholz C, Korholz D, et al. [^{18}F] fluorodeoxyglucose positron emission tomography for detection of bone marrow involvement in children and adolescents with Hodgkin's lymphoma. J Clin Oncol 2011; 29: 3523-3528.
15. Chen S, Wang S, He K, Ma C , Fu H , Wang H. PET/CT predicts bone marrow involvement in paediatric non-Hodgkin lymphoma and may preclude the need for bone marrow biopsy in selected patients. Eur Radiol 2018; 28: 2942-2950.

16. Li C, Zhang J, Chen S, et al. Prognostic value of metabolic indices and bone marrow uptake pattern on preoperative 18F–FDG PET/CT in pediatric patients with neuroblastoma. Eur J Nucl Med Mol Imaging 2018; 45: 306-315.

17. Harrison DJ, Parisi MT, Shulkin BL. The role of ^{18}F-FDG-PET/CT in pediatric sarcoma. Semin Nucl Med 2017; 47: 229-241.

18. Kasalak O, Glaudemans AWJM, Overbosch J, Jutte PC , Kwee TC . Can FDG-PET/CTreplace blind bone marrow biopsy of the posterior iliac crest in Ewing sarcoma? Skeletal Radiol 2018; 47: 363-367.

19. Federico SM, Spunt SL, Krasin MJ, et al. Comparison of PET-CT and conventional imaging in staging pediatric rhabdomyosarcoma. Pediatr Blood Cancer 2013; 60: 1128-1134.

20. Norman G, Fayter D, Lewis-Light K, et al. An emerging evidence base for PET-CT in the management of childhood rhabdomyosarcoma: systematic review. BMJ Open 2015; 5: e006030.

21. Kumar M, Singh Sachdeva MU, Naseem S, et al. Bone marrow infiltration in Langerhan's cell histiocytosis - An unusual but important determinant for staging and treatment. Int J Hematol Oncol Stem Cell Res 2015; 9: 193-197.

22. Goyal A, Rani S, Singh T, Choudhury P, Dubey A. Childhood histiocytoses: a review of twenty two cases. Indian Pediatr 1998; 35: 151-156.

The Turkish Journal of Pediatrics 2020; 62: 191-198
https://doi.org/10.24953/turkjped.2020.02.004

Original Article

Performance of SNAPPE-II score in neonatal sepsis: an experience from a tertiary care center

Moumita Samanta[1], Chiranjib Biswas[2], Nishith Kumar Pal[3], Soma Sarkar[4], Avranil Goswami[4], Avijit Hazra[5], Arijit Bhowmik[2], Tapas Kumar Sabui[6]

Departments of [1]Pediatrics and [3]Microbiology, Nil Ratan Sircar Medical College & Hospital (NRSMC&H) Medical College, Kolkata; Departments of [2]Pediatrics and [4]Microbiology, Medical College and Hospital, Kolkata; [5]Department of Pharmacology, Institute of Postgraduate Medical Education and Research, Kolkata; [6]Department of Pediatrics, RGKAR Medical College and Hospital, Kolkata, India.

ABSTRACT

Background and objectives. The Score for Neonatal Acute Physiology II with Perinatal Extension (SNAPPE-II) is a vital tool for prognostication in newborns. The study was conducted with the hypothesis that the performance of the SNAPPE-II score might be affected by the presence of sepsis in newborns admitted with possible early onset septicemia and whether score performance varies between culture positive and culture negative sepsis.

Methods. The prospective observational study was conducted over a period of 1 year (January 2014 to January 2015) in neonates presenting with clinical suspicion of sepsis to the Sick Newborn Care Unit (SNCU) of a tertiary care hospital in Eastern India.

Results. SNAPPE-II score cut-off of ≥20 offered the highest sensitivity of 74.5% with specificity 48.3%, PPV 27.6% and NPV 87.7%. Comparison of mortality proportions between the two subgroups defined by this cut-off returned p= 0.005 with OR 3.47 (95% 1.40 to 8.64). No significant association was found between SNAPPE-II score and blood culture results; mean scores for culture positive (25.16 ± 15.6) and negative groups (24.49 ± 15.6) were comparable (p= 0.920).

Conclusions. At a cut-off value of ≥20 in presence of sepsis, SNAPPE-II score offers acceptable indices to predict mortality outcome. Prediction of outcome by SNAPPE-II score is not affected by positive or negative blood culture sepsis.

Key words: neonatal sepsis, SNAPPE score, blood culture.

Neonatal sepsis continues to be a major cause of neonatal morbidity and mortality worldwide.[1-4] Globally, neonatal sepsis is responsible for 4 million deaths annually and 99% of them occur in developing countries.[5] Very often, the signs of infections may be absent or subtle or hard to detect and fatal septicemia may occur with little warning.[6] Hence timely diagnosis of neonatal sepsis is critical.

Over the years blood culture has been considered the gold standard for diagnosis of neonatal septicemia. However, it is time consuming and to circumvent this drawback various prognostication scores or tools have been developed based on risk factors, acute physiological changes and biochemical parameters. These strategies help predict onset of acute sickness in newborns and help monitoring while on therapy. Management begins at the earliest suspicion of sepsis and newborns are subjected to level II or level III care based on the severity of illness scores. The Score for Neonatal Acute Physiology (SNAP) II with Perinatal Extension (SNAPPE-II) by Richardson et al.[7,8] and modified over the years, comprises of physiology-based indicators which are measured within the first 12 hours of admission.[9]

✉ Tapas Kumar Sabui
tsabui@gmail.com

Received 25th February 2019, accepted 8th April 2019.

In our study we explored the association between SNAPPE-II score and mortality in neonatal sepsis of our referral hospital setting. Neonates with sepsis usually have more physiological instability which may affect the scores. To test this hypothesis we compared scores of newborns with sepsis (clinical or culture proven) to those without sepsis. Previous studies have shown significantly higher mortality rates in blood culture positive than culture negative neonates.[10] Thus, we further decided to test the score performance in blood culture positive versus negative neonates. This could have implications in clinical practice in assessment of score and its predictability in the presence of sepsis.

Henceforth to explore these possibilities, we conducted this study with the hypothesis that the performance of the SNAPPE-II score might be affected by the presence of sepsis in newborns admitted with possible early onset septicemia and whether score performance varies between culture positive and culture negative sepsis.

Material and Methods

A prospective observational study was conducted over a period of 1 year (January 2014 to January 2015) in neonates presenting with clinical suspicion of sepsis to the Sick Newborn Care Unit (SNCU) of a tertiary care hospital in Eastern India. The study was approved by the Institutional Ethics Committee for clinical research on 2 January 2014 (MC/Kol/IEC/227/1-2014).

Cases were recruited on alternate days of the week, excluding the weekends, through purposive sampling. Parental written informed consent was obtained after selection and before enrolment of subjects. The inclusion criteria were neonates with features suggestive of neonatal sepsis like respiratory distress (excluding hyaline membrane disease), labored breathing, cyanosis, apnea in a previously healthy baby, temperature instability, lethargy, refusal to suck, abdominal distension, feed intolerance, subtle seizure / vacant stare and suspected necrotizing enterocolitis (NEC). Appropriate investigations were done. Neonates with congenital infection, congenital metabolic disease, prior antibiotic use, and unavailable blood culture or sepsis screen reports were excluded.

The severity of illness of each infant was systematically assessed for the first 12-hour period after enrolment. The time of enrolment once the neonate was diagnosed to have sepsis was considered as '0' hour. The worst value for each scoring parameter in the 12-hour period was taken for determining the total SNAPPE-II score. Data on the perinatal extension parameters were collected from perinatal assessment records. The SNAPPE-II score was thereafter calculated by the resident doctor trained in neonatology and subsequently verified by a senior investigator. Other variables recorded were sex of the baby, place and type of delivery, postnatal age at admission and intrauterine growth restriction. This information were collected from labor theatre records in case of inborn and discharge summary in case of outborn babies. The subjects were closely monitored every day till discharge or death. A structured case report form was used for capturing data.

Sepsis screen (which includes total leukocyte count, absolute neutrophil count, immature/total neutrophil, micro-ESR and C-reactive protein)[1] and blood culture (using BacT/ALERT 3D 60 automated blood culture system) were performed for all neonates recruited. Neonates with two or more sepsis screen parameters positive were considered to have screen positive sepsis. Positive blood cultures were further analyzed for species identification and antimicrobial susceptibility testing using VITEK® 2 instrument (of Compact Biomerieux, Inc. Durham, North Carolina, USA). In neonates with clinical features suggestive of meningitis, or culture proven sepsis, cerebrospinal fluid (CSF) examination was performed. The cohort for analysis was subdivided into blood culture positive (definitive sepsis) and blood culture negative (includes clinical sepsis where sepsis

screen is negative and probable sepsis where screen is positive) groups.

Statistical analysis: Data were analyzed with SPSS statistics software version 17 (Illinois, Chicago: SPSS Inc., 2008). Numerical variables were compared between subgroups by Student's independent samples t-test while Fisher's exact test was employed for comparison of independent proportions. For these comparisons, p value less than 0.05 was considered to be statistically significant. Ninety five percent confidence interval (95% CI) values and Odds ratio (OR) have been presented where deemed relevant. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio positive (LR+) and likelihood ratio negative (LR–) were calculated to evaluate performance of different cut-offs of the SNAPPE-II score in predicting mortality. A receiver operating characteristics (ROC) curve analysis was also done to discriminate the best performing cut-off of SNAPPE-II score to predict outcome.

Results

Of the 246 neonates enrolled, 21 were excluded (2 had glucose-6-phosphate dehydrogenase deficiency, 1 galactosemia, 5 congenital infections, and rest 13 received antibiotics before enrolment) leaving an analysis cohort of 225 neonates, of whom 124 (55.1%) were males. The mean gestational maturity of the whole cohort was 35.1 ± 2.5 weeks (range 28-40 weeks), with 96 (42.7%) neonates being term (≥37 weeks gestational maturity). The characteristics of whole group are shown in Table I.

Aerobic blood culture was done for each enrolled neonate at the onset of suspicion of sepsis. Blood culture results (n=225) showed growth of organism in 97 neonates. However, 8 growths (6 being coagulase negative *Staphylococcus*, 1 *Micrococcus* and 1 *Candida*) were considered as contaminants in the background of non-corroborative clinical features and favorable outcome in all without antibiotics. Hence, the total number of neonates with growth of pathogenic organism in blood culture (culture positive) was 89 (39.6%); while the rest 136 neonates (60.4%) were culture negative. Blood culture growth was dominated by gram negative organism in 75 (84.3%) and gram positive in the rest. *Klebsiella* species was by far the most common organism identified (54%); followed by *Pseudomonas* (13.5%), *E. coli* (10.1%) and *Acinetobacter* (6.7%). *Staphylococcus aureus* (13.79%) and *Enterococcus* (2.2%) were the gram positive organisms isolated.

Respiratory distress was the most common presenting symptom (69.8%); with 43.9% of these being culture positive (p= 0.041 chi-square; OR 1.9, 95% CI 1.02-3.46). Feed intolerance seen in 5.3% neonates was also significantly more in the culture positive group than in the culture negative group (66.7% vs. 33.3% respectively; p= 0.048 Pearson chi-square; OR 3.3, 95% CI 0.95-11.17) Table II. The mean gestational maturity of newborns who survived were modestly but significantly different from that for the expired neonates (35.4 ± 2.3 weeks vs. 34.1 ± 2.8 weeks respectively; p= 0.002; 95% CI of the difference 0.48 to 2.07). Mean birth weight in survivors was also significantly more than for expired neonates (2276.3 ± 730.1 g vs. 1808.5 ± 659.6 g respectively, p <0.001; 95% CI of difference 236.4 to 699.3 g). SGA neonates were significantly more prone to death than their appropriate for gestational age counterparts (32.4% vs. 15.6% respectively; p= 0.004; OR 2.60; 95% CI 1.34 to 5.03). However, survival rates in blood culture positive and negative groups were not statistically significant (74.16% vs. 82.35% respectively; p= 0.139). The mean age in hours of life at SNAPPE-II measurement was 31.10 ± 24.15 (median 27 hours, 95% CI 27.93-34.27).

Total mortality observed in our study was 47/225 (20.9%; 95% CI 15.6 to 26.2%). Comparison of SNAPPE-II scores revealed higher SNAPPE-II score in neonates who expired (range 5 – 73, mean 30.4 ± 15.7, median 28) than in those who survived (range 0 – 65, mean 23.7 ± 15.3, median 22.0) and this was significantly different

Table I. Characteristics of infants according to sepsis groups (N=225).

Characteristics	Sepsis groups: True sepsis (Culture proven, n=89)	Sepsis groups: Suspected sepsis (Culture negative, n=136)	P value
Male, n (%)	48 (53.9)	76 (55.9)	0.775
Gestational age, n (%)			
≥ 37 weeks	29 (32.6)	66 (48.5)	0.55
32 to 36 weeks	53 (59.5)	61 (44.9)	
< 32 weeks	7 (7.9)	9 (6.6)	
Birth weight, n (%)			
≥ 2.5 kg	33 (37.1)	53 (38.9)	0.689
1.5 to < 2.5 kg	41 (46.1)	56 (41.2)	
< 1.5 kg	15 (16.8)	27 (19.8)	
Birth weight, n (%)			
> 999 g	89 (100)	135 (99.3)	0.713
750 – 999 g	0	1 (0.7)	
< 750 g	0	0	
Small for gestational age, n (%)	17 (19.1)	26 (19.1)	0.014
APGAR score at 5 minutes, n (%)			
≥ 7	58 (65.2)	74 (54.4)	0.002
< 7	31 (34.8)	62 (45.6)	
Lowest mean arterial pressure, n (%)			
> 29 mmHg	83 (93.3)	134 (98.5)	0.923
20 – 29 mmHg	5 (5.6)	2 (1.5)	
< 20 mmHg	1 (1.1)	0	
Lowest temperature, n (%)			
> 35.6°C	39 (43.8)	61 (44.9)	0.601
35 – 35.6°C	21 (23.6)	33 (24.2)	
< 35°C	29 (32.6)	42 (30.9)	
PaO_2/FiO_2, n (%)			
> 2.49	34 (38.2)	64 (47.1)	0.576
1.0 – 2.49	36 (40.4)	49 (36.0)	
0.3 – 0.99	19 (21.2)	23 (16.9)	
< 0.3	-	-	
Lowest pH, n (%)			
> 7.19	83 (93.6)	132 (97.1)	0.984
7.10 – 7.19	6 (6.7)	4 (2.9)	
< 7.10	0	0	
Seizure, n (%)	42 (47.2)	51 (37.5)	0.025
Urine output, n (%)			
> 0.9 ml/kg/h	69 (77.5)	104 (76.5)	0.920
0.1 – 0.9 ml/kg/h	20 (22.5)	32 (23.5)	
< 0.1 ml/kg/h	0	0	
SNAPPE II Score	25.16 ± 15.6	24.49 ± 15.6	0.936

PaO_2/FiO_2 is ratio of arterial oxygen partial pressure and fractional inspired oxygen. SNAPPE score is expressed in mean ± SD

Table II. Blood culture results according to clinical features (N=225).

Clinical features	Total*	Blood Culture		P value	Odd's ratio (95% CI)
		Culture positive**	Culture negative**		
Respiratory distress	157 (69.8)	69 (43.9)	88 (56.1)	0.041	1.88 (1.02-3.46)
Lethargy, refusal to feed	71 (31.6)	28 (39.4)	43 (60.6)	0.980	-
Apnea	23 (10.2)	11 (47.8)	12 (52.2)	0.392	-
Feed intolerance	12 (5.3)	8 (66.7)	4 (33.3)	0.048	3.3 (0.95-11.17)
Abdominal distension	11 (4.9)	4 (36.4)	7 (63.6)	0.824	-
Suspected NEC	6 (2.7)	3 (50.0)	3 (50.0)	0.596	-
Subtle seizure	4 (1.8)	2 (50.0)	2 (50.0)	0.666	-
Temp. instability	3 (1.3)	1 (33.3)	2 (66.7)	0.824	-

*: results are presented as n (%); column percentile
**: results are presented as n (%); row percentile
NEC: necrotizing enterocolitis

(p=0.008; 95% CI of difference 1.76 to 11.69). In general there was progressive rise in mortality with increasing SNAPPE-II score. This trend was statistically significant (p= 0.004) by chi-square for trend analysis. Table III indicates the performance of various SNAPPE-II cut-offs in predicting mortality in terms of standard diagnostic indices. The cut-off of ≥20 offered the highest sensitivity of 74.5% with specificity 48.3%, PPV 27.6% and NPV 87.7%. Comparison of mortality proportions between the two subgroups defined by this cut-off returned an OR of 3.47 (95% CI 1.40 to 8.64; p= 0.005).

No significant association was found between SNAPPE-II score and blood culture results (25.16 ± 15.6 in culture positive vs. 24.49 ± 15.6 in culture negative group; p= 0.920). Performance of SNAPPE-II score with cut-off ≥20 was further assessed in different gestational age subgroups and it was found that score performed best in 32-36 weeks gestational age group in predicting the outcome irrespective of blood culture results. SNAPPE-II scores compared between survivors and fatal cases in various gestational maturity categories, showed a statistically significant difference for the 32-36 week gestational age band in both blood culture positive and blood culture negative cases (Table IV) but not for other bands. When ROC curve analysis was done for different subgroups to assess discriminatory power of SNAPPE-II score to predict mortality outcome, the area under curve (AUC) was reasonable only for 32-36 weeks gestational maturity group (AUC 0.705) (Fig. 1).

Discussion

Results of the whole cohort revealed significant association between mortality in neonatal sepsis and increasing SNAPPE-II score (p= 0.004). Similar results have been reported by other authors.[11-14] RA et al.[11] reported that SNAPPE II has the best performance in predicting first

Table III. Performance of SNAPPE-II score in predicting mortality at various cut-offs.

SNAPPE-II score cut-offs	≥20	≥30	≥35	≥40	≥50	≥60
Sensitivity	74.5%	42.5%	31.9%	25.5%	12.8%	4.2%
Specificity	48.3%	64%	74.7%	87%	95.5%	97.7%
Positive predictive value	27.6%	23.8%	25%	34.3%	42.8%	33.3%
Negative predictive value	87.7%	80.8%	81%	81.6%	80.6%	79.4%
Likelihood ratio positive	1.44	1.2	1.26	1.98	2.84	1.89
Likelihood ratio negative	0.53	0.9	0.91	0.85	0.91	0.98

Table IV. SNAPPE II score in gestational maturity categories stratified by blood culture results.

Gestational age	Outcome	SNAPPE-II score	P value
Blood culture positive sepsis (n=89)			
< 32 weeks (n=18)	Survived (n=7)	31.0 ± 21.42	0.762
	Died (n=11)	34.0 ± 19.29	
32 to 36 weeks (n=42)	Survived (n=32)	27.2 ± 18.86	0.025
	Died (n=10)	43.1 ± 18.84	
≥ 37 weeks (n=29)	Survived (n=27)	30.9 ± 17.57	0.699
	Died (n=2)	36.0 ± 21.21	
Blood culture negative sepsis (n=136)			
< 32 weeks (n=22)	Survived (n=17)	32.8 ± 18.12	0.241
	Died (n=5)	43.6 ± 14.98	
32 to 36 weeks (n=47)	Survived (n=38)	29.4 ± 17.69	0.026
	Died (n=9)	44.2 ± 16.37	
≥ 37 weeks (n=67)	Survived (n=57)	27.5 ± 17.97	0.650
	Died (n=10)	30.3 ± 16.94	

P value in the last column is for comparison between survivors and fatal cases.

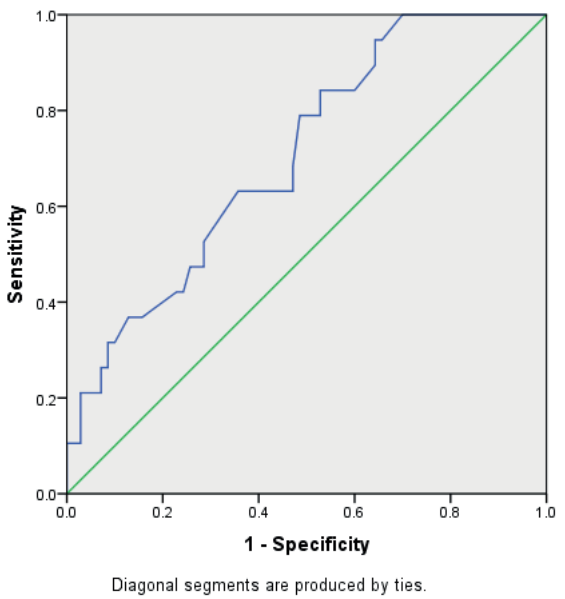


Fig. 1. Receiver operating curve for SNAPPE-II Score to predict mortality in neonates at gestational age of 32 to 36 weeks.

6-days mortality. However, the cut-off point of SNAPPE II scoring system to predict mortality has been found to differ in various studies. While RA et al.[11] suggested a cut-off point 30 we found a cut-off point of 20 for maximum sensitivity of mortality prediction with fairly acceptable specificity. Jain et al.[15] observed that, as the score increased to 40 and above probabilities of mortality increased and it was maximum with score of 80 and above.

Ramirez et al.[13] found that SNAPPE-II score showed higher values for newborns who died compared to survivors, with analysis of the ROC curve showing an area under the curve for SNAPPE II of 0.77 (CI 95% 0.69–0.86).

On analysis of SNAPPE-II score among the demographic subgroups of the entire cohort, we found that performance of SNAPPE-II score in predicting mortality was statistically significant only in 32-36 weeks gestational maturity group. SNAPPE-II did not seem to predict outcome well in those with gestational age <32 weeks or >36 weeks. Possibly, prematurity and birth asphyxia are the confounding factors that have overshadowing effect on the outcome in this regard. This could also be an incidental finding and needs further studies to prove its reproducibility.

Positivity of blood culture did not seem to alter the performance of SNAPPE-II score. In other words SNAPPE-II score predicted outcome independent of blood culture results. Moreover no significant difference of mean score in the

two groups was found (p= 0.920). This is in contrary to the hypothesis that newborns with true sepsis might have greater SNAPPE score. The mortality rate in our study was 20.1% which is less than that reported in various studies from India, (37% to 47%) among the early onset sepsis cases.[16,17] This could possibly be due to a secular trend or due to changes in obstetric and neonatal care practices. No significant difference in death between blood culture positive (25.8%) and blood culture negative (17.6%) neonates were found in our study. This finding is unusual as previous studies have reported significantly higher mortality rates in culture positive than culture negative neonates.[18] Vinay et al.[19] also had a similar finding of overwhelmingly higher death rates in culture positive (83.6%) than in culture negative (16.4%) cases.

One of the most common causes of neonatal mortality in our country is septicemia and delayed referral to higher centers. Currently established country level guidelines for early referral include mostly the serious complications like recurrent seizures, shock, hypoglycemia etc.[20] Our study analysis shows that SNAPPEII scores does perform well in predicting outcome but are not useful in differentiating true sepsis from suspected (or clinical sepsis). The existing country level guidelines for early referral are still good enough.

Like any other study our study also has its share of limitations. Firstly sample size was smaller and skewed resulting in difficult subgroup analyses. Late onset sepsis was not included to avoid various confounding variables involved in transport of these newborns. Healthy newborns had they been included as matched control group would have helped to compare SNAPPE-II score between the septic (both suspected and true) and non-septic healthy groups.

In conclusion we can say that, notwithstanding the limitations, at a cut-off value of ≥20 in presence of sepsis, SNAPPE-II score offers acceptable indices to predict mortality outcome. Prediction of outcome by SNAPPE-II score is not affected by presence or absence of true sepsis. SNAPPE II score of ≥20 could be a possible cut-off for early referral of sick newborns to higher centers, particularly between 32-36 weeks of gestational age.

Acknowledgement

We thank Prasenjit Saha, Lab Technician, Deepanwita Pal, Lab Techinician and Arunava Bhattacharyya from Medical College and Hospital, Kolkata for their logistic help. We would like to thank National Health Mission for funding this project.

REFERENCES

1. Puopolo KM. Bacterial and fungal infections. In: Cloherty JP, Eichenwald EC, Stark AR (eds). Manual of Neonatal Care (6th ed). Philadelphia: Lippincott Williams & Wilkins, 2008: 274-300.
2. Sharma M, Yadav A, Yadav S, Goel N, Chaudhary U. Microbial profile of septicemia in children. Indian J Pract Doct 2008; 5: 5-8.
3. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics 2002; 110(2 Pt 1): 285-291.
4. Bang AT, Bang RA, Baitule SB, Reddy MH, Deshmukh MD. Effect of home-based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet 1999; 354: 1955-1961.
5. Darmstadt GL, Saha SK, Choi Y, et al; and the Bangladesh Projahnmo-2 (Mirzapur) Study Group. Population-based incidence and etiology of community-acquired neonatal bacteremia in Mirzapur, Bangladesh: an observational study. J Infect Dis 2009; 200: 906-915.
6. Shirazi H, Riaz S, Tahir R. Role of the hematological profile in early diagnosis of neonatal sepsis. Ann Pak Inst Med Sci 2010; 6: 152-156.
7. Richardson DK, Gray JE, McCormick MC, Workman K, Goldman DA. Score for neonatal acute physiology: a physiology severity index for neonatal intensive care. Pediatrics 1993; 91: 617-623.
8. Richardson DK, Corcoran JD, Escobar GJ,Lee M SK. SNAP-II and SNAPPE-II: Simplified newborn illness severity and mortality risk scores. J Pediatr 2001; 138: 92-100.

9. Harsha SS, Archana BR. SNAPPE- II (Score for Neonatal Acute Physiology with Perinatal Extension-II) in predicting mortality and morbidity in NICU. J Clin Diagn Res 2015; 9: SC10-SC12.

10. Gebrehiwot A, Lakew W, Moges F, et al. Predictors of positive blood culture and death among neonates with suspected neonatal sepsis in Gondar University Hospital, Northwest Ethiopia. Euro J Exp Bio 2012; 2: 2212-2218.

11. RA M, Etika R, Harianto A, Indarso F, Damanik SM. The use of score for neonatal acute physiology perinatal extention II (SNAPPE II) in predicting neonatal outcome in neonatal intensive care unit. Paediatr Indones 2005; 45: 241-245.

12. Thimoty J, Hilmanto D, Yuniati T. Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE II) as the predictor of neonatal mortality hospitalized in neonatal intensive care unit. Paediatr Indones 2009; 49: 155-159.

13. Ramirez MNM, Godoy LE, Barrientos EA. SNAP II and SNAPPE II as Predictors of Neonatal Mortality in a Pediatric Intensive Care Unit: Does Postnatal Age Play a Role? Int J Pediatr 2014; 2014: 298198.

14. Reid S, Bajuk B, Lui K, Sullivan EA; NSW and ACT Neonatal Intensive Care Units Audit Group, PSN. Comparing CRIB-II and SNAPPE-II as mortality predictors for very preterm infants. J Paediatr Child Health 2015; 51: 524-528.

15. Jain S, Bansal A. SNAPPE II score for predicting mortality in a level II neonatal intensive care unit. Dicle Med J 2009; 36: 333-335.

16. Anwer SK, Mustafa S. Rapid identification of neonatal sepsis. J Pak Med Assoc 2000; 50: 94-98.

17. Chacko B, Sohi I. Early onset neonatal sepsis. Indian J Pediatr 2005; 72: 23-26.

18. Sriram R. Correlation of blood culture results with the sepsis score and the sepsis screen in the diagnosis of neonatal septicemia. Int J Biol Med Res 2011; 2: 360-368.

19. Vinay BS, Girish GN, Adhikari S, Hugara S. Evaluation of septic screen as a diagnostic tool for neonatal sepsis in a tertiary hospital at Mysore. Sch J App Med Sci 2015; 3(2G): 1005-1010.

20. Facility Based Newborn Care Operational Guide. Guidelines for Planning and Implementation. Ministry of Health and Family Welfare, Government of India, 2011.

The Turkish Journal of Pediatrics 2020; 62: 199-207
https://doi.org/10.24953/turkjped.2020.02.005

Original Article

Osteoarticular involvement of brucellosis in pediatric patients: clinical and laboratory characteristics

Dilek Yılmaz Çiftdoğan[1,2], Selda Aslan[3]

[1]*Department of Pediatric Infectious Diseases, İzmir Katip Çelebi University, İzmir;* [2]*Department of Pediatric Infectious Diseases, Tepecik Training and Research Hospital, İzmir;* [3]*Department of Infectious Diseases, Gaziantep Children State Hospital, Gaziantep, Turkey.*

ABSTRACT

Background and objectives. The aim of this study was to evaluate the clinical and laboratory characteristics of osteoarticular involvement in children with brucellosis.

Methods. A total of 202 pediatric brucellosis patients were evaluated from April 2012 to August 2013.

Results. Among the 202 patients, 53 (26.2%) had osteoarticular involvement. In patients with osteoarticular involvement, the mean values of estimated sedimentation rate (ESR) and C-reactive protein (C-RP) were significantly higher than in patients without osteoarticular involvement ($p = 0.001$, $p = 0.01$, respectively). The majority of patients with osteoarticular involvement ($n = 48$, 90.6%) had a higher standard tube agglutination (STA) test titer (≥ 1/640) than patients without osteoarticular involvement ($n = 69$, 46.3%) ($p = 0.001$). The most commonly found osteoarticular involvement was peripheral arthritis. The second most commonly found osteoarticular involvement was sacroiliitis ($n = 5$, 9.4%). Three patients (5.7%) had spondylitis. Only one patient (1.9%) had osteomyelitis.

Conclusions. Osteoarticular involvement was detected in nearly one of every four childhood brucellosis patients in our study. Brucellosis should be considered as a pre-diagnosis in children with osteoarticular complaints, especially in regions where the disease is endemic.

Key words: Brucellosis, osteoarticular, pediatric.

Brucellosis is a serious public health issue throughout the world; it is of special concern in the Mediterranean region which includes Turkey.[1-3] Turkey's geographic situation is a risk factor for many infectious diseases.[4] The yearly incidence rate of this zoonotic disease in Turkey is 23 per 100,000 people.[3] However, the true incidence rate of brucellosis is not known because underreporting of the disease is believed to be common. Despite being endemic in Turkey, brucellosis remains underdiagnosed due to non-specific clinical manifestations and laboratory parameters.

Brucellosis is a multisystem disease which may present with a broad spectrum of clinical manifestations. It may mimic other infectious and non-infectious conditions. Fever, sweats, malaise, lethargy, anorexia, and joint pain are the most common complaints in childhood brucellosis.[5] Osteoarticular involvement has been extensively reported in adults and is also the predominant manifestation of brucellosis in children.[5-7] Osteoarticular involvement in brucellosis was first reported by Kennedy in 1904.[8] There are several reports of osteoarticular involvement in brucellosis in adults and children from various regions.[2,5-12] However, the prevalence and characteristics of osteoarticular involvement in brucellosis are influenced

✉ Dilek Yılmaz Çiftdoğan
drdilekyilmaz@hotmail.com

Received 18th December 2018, revised 1st June 2019, 29th June 2019, accepted 11th November 2019.

This study was presented at the 53rd Turkish Pediatr Congress, 14-18 May 2017, Girne, Turkish Rebuplic of Northern Cyprus.

by many factors: regional differences, environmental factors, differences in hosts, and the age of the patients.[9-12]

The aim of this study was to evaluate the demographic, clinical, and laboratory characteristics of osteoarticular involvement in children with brucellosis.

Material and Methods

A total of 202 pediatric patients who had been diagnosed with brucellosis at the Gaziantep State Children Hospital's Department of Pediatric Infectious Diseases were evaluated from April 2012 to August 2013. Among the 202 patients, 53 (26.2%) had some degree of osteoarticular involvement.

The diagnosis of brucellosis was based on the Centers for Disease Control and Prevention's definition, which includes the presence of clinical signs and symptoms with evidence of *Brucella* spp. invasion in a positive culture or a single high titer against *Brucella* spp. of ≥ 1:160.[13] Patients who had positive Rose Bengal test results were further tested by the standard tube agglutination test (STA). Seropositivity was defined as an STA titer of 1/160 or more. During the study period, Coombs test and 2-mercaptoethanol agglutination test could not be performed routinely in the laboratory.

Based on the duration of disease, patients were classified as having acute brucellosis (< 3 months), subacute brucellosis (3-12 months), or chronic brucellosis (> 12 months). Any history of drinking unpasteurized milk, consumption of unpasteurized dairy products and handling animals or animal excretions were recorded. Blood, bone marrow aspirates and synovial fluid samples were not routinely cultured for *Brucella* spp. Demographic data, clinical manifestations, and outcomes were evaluated. Complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (C-RP), and blood chemistry profile were analyzed.

Peripheral arthritis was defined as pain and the presence of at least two other signs of inflammation of the affected joint, including swelling, restriction of joint movement, heat, and redness. Hip arthritis was defined as pain and a positive flexion-abduction-external rotation (FABER) or Stinchfield test with the presence of the abnormality shown on radiologic examination. Clinically, sacroiliitis was diagnosed as inflammatory pain and functional disability associated with a positive FABER test. The patients with back pain and progressive stiffness of the spine were evaluated for spondylitis. Radiography, computed tomography (CT), or magnetic resonance imaging (MRI) was performed on all patients with clinical signs that suggested sacroiliitis, spondylitis, osteomyelitis, or hip involvement.

All patients were treated with various antimicrobial combinations. The antimicrobial combinations consisted of oral rifampin, oral doxycycline in children ≥ 8 years old, oral trimethoprim/sulfamethoxazole, intramuscular streptomycin, and intramuscular/intravenous gentamicin. Therapeutic failure was defined as the persistence of symptoms or clinical signs during the antimicrobial combination treatment. Relapse was defined as a recurrence or exacerbation of symptoms and the presence of clinical signs after completion of the antimicrobial combination treatment.

The study was approved by the local ethics committee (no.55-02/05/2013). Informed consent was obtained from all the individual's parents.

Data analysis was performed using the IBM SPSS Statistics ver. 19.0 (IBM Corporation, Armonk, NY, USA) software. We expressed continuous variables as the mean ± standard deviation (S.D.) with the median, using a *Chi-square* test to compare proportions. The Fisher's exact test was used for expected frequencies below five in the contingency tables. A one-way analysis of variance (ANOVA) test was used for multiple comparisons. P values < 0.05 were considered statistically significant.

Results

In this study, 202 children with brucellosis were analyzed. Among these patients, 123 (60.9%) were male, and 79 (39.1%) were female. The rate of male patients was significantly higher than female patients ($p < 0.05$). The age distribution of the patients was 1-18 years (mean age 7.37 ± 4.14 years). A majority of 188 (93.1%) patients were in the acute stages of brucellosis, 11 (5.5%) had subacute brucellosis, and three (1.4%) had chronic brucellosis. Among these 202 brucellosis patients, 53 (26.2%) had osteoarticular involvement, and their mean age was 10.3 ± 4.06 years. However, the mean age of patients without osteoarticular involvement was lower than in patients with osteoarticular involvement (6.34 ± 3.61 years, $p < 0.0001$). Gender distribution rates of patients with and without osteoarticular involvement were similar. While there was no correlation between gender and osteoarticular involvement ($p > 0.05$), a significant correlation was found between age and osteoarticular involvement ($r = 0.43$, $p = 0.001$). All of the patients with chronic brucellosis ($n = 3$) had osteoarticular involvement. Differences in the distribution of disease classifications based on the duration of disease between patients with and without osteoarticular involvement were statistically significant ($p = 0.006$).

Eight patients with osteoarticular involvement (15.1%) had a history of handling animals or animal excretions. Seventeen patients without osteoarticular involvement (11.4%) had a history of handling animals or animal excretions. None of patients with and without osteoarticular involvement admitted to drinking unpasteurized milk. However, 28 patients with osteoarticular involvement (52.8%) and 71 patients with osteoarticular involvement (47.6%) had a history of ingesting unpasteurized dairy products. There were no significant differences in history of handling animals or animal excretions and a history of ingesting unpasteurized dairy products between patients with and without osteoarticular involvement (p = 0.48, p = 0.51; respectively).

The specific demographic and clinical characteristics of patients with and without osteoarticular involvement are shown in Table I. The most frequent patient symptoms were fever, malaise, arthralgia, and anorexia. The frequency of fever, malaise, arthralgia, and myalgia were higher in patients with osteoarticular involvement (overall, $p < 0.05$). The frequency of hepatomegaly, splenomegaly, and lymphadenopathy were similar in patients with and without osteoarticular involvement (overall, > 0.05). Laboratory characteristics and distribution of STA titers of patients with and without osteoarticular involvement are shown in Table II. In patients with osteoarticular involvement, the mean values of ESR and C-RP were significantly higher than in patients without osteoarticular involvement ($p = 0.001$, $p = 0.01$, respectively). The frequency of leukocytosis was higher in patients with osteoarticular involvement ($p = 0.002$). However, the frequencies of leukopenia, thrombocytopenia, and anemia were not significantly different between the patients with and without osteoarticular involvement (overall, $p > 0.05$).

Among all patients, STA titers ranged from 1/160 to > 1/1280 (Table II). The differences of the distribution of the STA titers between patients with and without osteoarticular involvement were statistically significant ($p = 0.001$). The majority of patients with osteoarticular involvement ($n = 48$, 90.6%) had a higher STA titer (≥ 1/640) than patients without osteoarticular involvement ($n = 69$, 46.3%), and this difference was statistically significant ($p = 0.001$).

After the treatment, in 4 patients with ongoing disease symptoms, 2-mercaptoethanol agglutination tests were performed at external laboratory centers. Only one patient's test result was positive. The Coombs test were performed on only 5 patients who had relapsing brucellosis or prolonged active infection. In the study, there were no patients who were negative with Wright test but positive with Coombs test.

Table I. Demographic and clinical characteristics of patients with and without osteoarticular involvement.

	Patients with osteoarticular involvement (n=53)	Patients without osteoarticular involvement (n=149)	*p*-value
Age*	10.3 ± 4.06	6.31 ± 3.56	0.001
Gender** (*n*, %)			
Male	36 (67.9)	87 (58.4)	0.25
Female	17 (32.1)	62 (41.6)	
Clinical type**			
Acute	42 (95)	143 (96)	0.006
Sub-acute	5 (9.4)	6 (4)	
Chronic	3 (5.7)	-	
Fever**	48 (90.6)	108 (72.5)	0.007
Malaise**	47 (88.7)	96 (64.4)	0.001
Anorexia**	40 (75.5)	104 (69.8)	0.434
Myalgia**	63 (45.3)	18 (28.6)	0.025
Weight loss**	19 (35.8)	42 (28.2)	0.29
Abdominal pain**	17 (32.1)	55 (36.9)	0.529
Dyspepsia**	18 (34)	47 (31.5)	0.747
Headache**	15 (28.4)	36 (24.2)	0.552
Night sweats**	5 (9.4)	15 (10.1)	0.89
Hepatomegaly**	8 (15.1)	28 (18.8)	0.54
Splenomegaly**	5 (9.4)	14 (9.4)	0.994
*Lymphadenopathy***	3 (5.7)	13 (8.7)	0.479

*Mean ± *SD.*, **n, %

Table II. Laboratory characteristics and distribution of STA titers of patients with and without osteoarticular involvement.

	Patients with osteoarticular involvement	Patients without osteoarticular involvement	*p*-value
*ESR (mm/h)**	36.3 ± 14.2	18.6 ± 12.6	0.001
*C-reactive protein (mg/dl)**	2.34 ± 1.72	1.75 ± 1.46	0.02
*Leukopenia***	6 (11.3)	21 (14.1)	0.611
*Leukocytosis***	13 (24.5)	12 (8.1)	0.002
*Thrombocytopenia***	5 (9.4)	14 (9.4)	0.994
STA titer**			
1/160	8 (5.8)	5 (7.9)	
1/320	13 (9.3)	12 (19.1)	
1/640	50 (35.9)	21 (33.3)	0.001
1/1280	49 (35.2)	16 (25.4)	
> 1/1280	19 (13.8)	9 (14.3)	
STA titer**			
< 1/640	21 (15.1)	46 (73.1)	0.001
≥ 1/640	118 (84.9)	17 (26.9)	

*Mean ± *SD.*, **n, % STA: standard tube agglutination test

Only 11 patient blood, bone marrow aspirates or synovial fluid samples were cultured for Brucella spp. Overall, 4 of 12 (33.3%) blood cultures obtained from eight patients were positive. Also, 2 bone marrow aspirates cultures from two patients and 1 synovial fluid samples culture from one patient were positive. In our study, no subtype was determined.

The frequency of the osteoarticular involvement of patients is shown in Table III. The most commonly found osteoarticular involvement in our patients was peripheral arthritis (n = 44, 83%). The majority of the peripheral arthritis (n = 42, 95.4%) was monoarticular. Only two patients with peripheral arthritis had pauciarticular joint involvement (one patient had bilateral hip involvement, and one patient had bilateral knee involvement). The most commonly affected peripheral joints were the hip (n = 21, 4.7%) and knee (n = 15, 34.1%). Other less commonly affected joints included the ankle (n = 3, 6.8%), wrist (n = 2, 4.5%, shoulder (n = 1, 2.3%), elbow (n = 1, 2.3%), and interphalangeal joints (n = 1, 2.3%).

Table III. Distribution of sites of osteoarticular involvement of patients.

Osteoarticular involvement	*n* (%)
Peripheral arthritis	44 (83)
Knee	21 (47.7)
Hip	15 (34.1)
Ankle	3 (6.8)
Wrist	2 (4.5)
Shoulder	1 (2.3)
Elbow	1 (2.3)
Interphalangeal joints	1 (2.3)
Sacroiliitis	5 (9.4)
Spondylitis	3 (5.7)
Osteomyelitis	1 (1.9)

The second most commonly found osteoarticular involvement in our patients was sacroiliitis (n = 5, 9.4%), which was unilateral in the majority of the patients (n = 4). In only one patient was the sacroiliitis bilateral. Three patients with osteoarticular involvement (5.7%) had spondylitis, and all these had lumbar region involvement. Only one patient (1.9%) had Brucella osteomyelitis of the right proximal tibia.

The mean age of patients with peripheral arthritis was lower than that of patients with sacroiliitis and spondylitis (9.7 ± 3.5 years, 13.4 ± 5.3 years, respectively, p = 0.01). Among the patients with peripheral arthritis, the mean age of patients with knee arthritis was lower than that of other patients (8.23 ± 3.7 years, 11.7 ± 3.6 years, respectively, p = 0.02). Also, the mean age of patients with hip arthritis was higher than that of patients with other joint involvement; however, this difference was not statistically significant (11.4 ± 2.8 years, 9.89 ± 4.4 years, respectively, p = 0.13). Gender distribution rates for the various types of osteoarticular involvement were similar (p = 0.12). All of the patients with chronic disease (n = 3) had osteoarticular involvement. Among these patients, two had sacroiliitis, and one had spondylitis.

The patients were treated with various antimicrobial combinations: oral rifampin, oral doxycycline in children ≥ 8 years old, oral trimethoprim/sulfamethoxazole, intramuscular streptomycin, and intramuscular/intravenous gentamicin. Twenty-seven patients (50.9%) with osteoarticular involvement received dual combination therapy (15 patients received trimethoprim-sulfamethoxazole and rifampicin, 12 patients received doxycycline and rifampicin) and 125 patients (83.9%) without osteoarticular involvement received dual combination therapy (72 patients received trimethoprim-sulfamethoxazole and rifampicin, 53 patients received doxycycline and rifampicin). Twenty-six patients (49.1%) with osteoarticular involvement received triple combination therapy (11 patients received trimethoprim-sulfamethoxazole and rifampicin and gentamicin or streptomycin, 15 patients received doxycycline and rifampicin and gentamicin or streptomycin) and 24 patients (16.1%) without osteoarticular involvement received triple combination therapy (14 patients

received trimethoprim-sulfamethoxazole and rifampicin and gentamicin or streptomycin, 10 patients received doxycycline and rifampicin and gentamicin or streptomycin). In the patients who had brucellosis without osteoarticular involvement, the triple combination therapy regime were used in complex cases of focal brucellosis such as neurobrucellosis, endocarditis or localized suppurative lesions and refractory diseases. In patients with osteoarticular involvement, the use of triple combination therapy was more common than patients without involvement (p= 0.001). In patients with osteoarticular involvement, the mean duration of treatment was 7.87 ± 2.98 weeks; this time interval was 7.05 ± 0.0.95 weeks in patients without osteoarticular involvement. This difference was statistically significant (p = 0.006). In patients with spondylitis, osteomyelitis, or therapeutic failure, the duration of treatment was from 12 up to 24 weeks. The frequency of treatment failure and relapse was not significantly different between patients with and without osteoarticular involvement (overall, $p > 0.05$) (Table IV).

Discussion

In this study, we found that osteoarticular involvement was present in 26.5% of childhood brucellosis cases. Studies have shown that the frequency of osteoarticular involvement in childhood brucellosis ranged from 6% to 75% in various regions of the world.[6,9,14-21] Brucellosis remains a significant public health problem, and studies from different districts showed that this rate ranges from 6.7% to 73.5% in Turkey, as was also found in our study.[7,22-25] In these studies, which were comprised of childhood and adult groups, osteoarticular involvement in brucellosis was found more frequently in adults than in children.[6,17] However, contrary to all these results, osteoarticular involvement in childhood was reported more in pediatric patients in one study from Turkey.[7] In a different study, osteoarticular involvement was found to be more likely in patients from 15 to 45 years of age.[10]

In some studies, osteoarticular involvement in childhood brucellosis was reported to be more common in males.[20,26,27] However, there are a few studies which reported it to be more common in females.[9,14] In our study, there was no gender difference between patients with or without osteoarticular involvement. A few studies reported that osteoarticular involvement increases with age and is especially low in infants.[12,20,26,27] Similarly to the results of these studies, patients with osteoarticular involvement were older than those without involvement in our study.

The routine screening of family members of index cases is a priority in endemic areas. However, in this study, family screening was not performed in all cases. Brucellosis is a multisystem disorder with unspecified clinical symptoms and findings. In our study, however, patients with osteoarticular involvement presented more frequently with fever, malaise, arthralgia, and myalgia than patients without involvement. Thus, the patients with osteoarticular involvement had more frequent complaints. Fever was the most frequent finding in other studies in patients with osteoarticular involvement.[9,12,20,26] Contradictory

Table IV. Duration of treatment and frequencies of therapeutic failure and relapse of patients with and without osteoarticular involvement.

	Patients with osteoarticular involvement	Patients without osteoarticular involvement	*p*-value
Duration of treatment (weeks)*	7.87 ± 2.98	7.05 ± 0.95	0.003
Therapeutic failure**	2 (3.8)	5 (3.4)	0.88
*Relapse***	5 (9.4)	11 (7.4)	0.63

*Mean ± *SD.*, ** n (%)

to other reported studies[9,12,20,26,28], there were no differences in the frequency of hepatomegaly and splenomegaly in brucellosis patients with osteoarticular involvement.

In our study, the most important abnormal laboratory findings were high-level ESR, C-RP, and leukocytosis. In the literature, high-level sedimentation rate and lymphocytosis were the most reported findings in childhood patients with osteoarticular involvement.[7,20,26,27] On the other hand, in some studies, leucopenia, leukocytosis, thrombocytopenia, high liver enzyme level, and a high C-RP were more frequently reported findings in patients with osteoarticular involvement.[9,12,14,20,27]

In previous studies, correlations were shown between a high standard Wright agglutination level and disease severity.[29,30] However, other studies reported that there were no significant differences between the standard Wright agglutination titers of patients with and without osteoarticular involvement.[31] In a different study, osteoarticular diagnosis involvement in brucellosis was verified in 98.7% of the patients via serologic tests, and three of the patients were reported as seronegative.[32] In our study, the patients with osteoarticular involvement showed a higher-level standard Wright agglutination titer than those patients without osteoarticular involvement.

Osteoarticular involvement in childhood brucellosis is mostly seen as peripheral arthritis, specifically monoarticular in character, as reported in most studies. The frequency of peripheral arthritis in these studies ranged from 6.4% to 90%.[6,9,12,14,20,22,26,33,34] The most involved joints were the hip, knee, and ankle.[9,10,14,18,20,33] In a few studies, the hip was the most frequently reported involved joint[9,10,19,28,33]; in others, the knee was the most often reported involved joint.[20,35] Contradictory to these studies, Gómez-Reino et al.[14] reported the most involved joint was the ankle. In the literature, minor joint involvement was rarely reported in brucellosis.[35] In our study, we found that the most osteoarticular involvement was peripheral arthritis in children with brucellosis; this supports the findings of other studies. The most involved joints were the hip and knee, respectively, and these were monoarticular in nature. The other affected joints were the ankle, wrist, shoulder, elbow, and interphalangeal joints.

Sacroiliitis was the most reported clinical form of osteoarticular involvement in adult type brucellosis.[5,10] In the literature, this form was reported at various frequencies, and it was generally unilateral in the pediatric age group with brucellosis.[9,10,14,26] Gómez-Reino et al.[14] reported 4.8%, Al-Eissa et al.[9] reported 8%, and Bosilkovski et al.[26] reported 6.8% in their studies. Contrary to this reported low frequency of sacroiliitis, Geyik et al.[10] reported that sacroiliitis was diagnosed in 48.7% of the child and 62.2% of the adult patients with brucellosis. In our study, sacroiliitis was diagnosed in 9.4% of the osteoarticular involvement patients with brucellosis, and this involvement was generally (80%) unilateral in form. On the other hand, all of the patients who had a sacroiliitis diagnosis were more than 11.5 years of age. Also, patients with sacroiliitis were older than patients with peripheral arthritis in accordance with findings in other studies.[9,26,27]

The other form of the osteoarticular involvement in brucellosis is spondylitis, and it generally increases in frequency in the fourth and fifth decades of adulthood.[10,36,37] In several studies, spondylitis was very low in frequency, or there was no report of it in the pediatric age group with brucellosis.[9,26,38] In our study, spondylitis was diagnosed in only three (5.7%) patients with osteoarticular involvement in brucellosis. Moreover, osteomyelitis has been a rarely reported form for such patients.[9,21,35,39] In several osteoarticular brucellosis studies, osteomyelitis was not detected.[7,10,14,26] In our study, only one patient was diagnosed with osteomyelitis.

Patients with spondylitis and sacroiliitis were older than patients with peripheral arthritis. The mean age of patients with knee involvement was significantly lower in peripheral arthritis

patients. Although the mean age of the patients with hip involvement was higher than other peripheral arthritis patients' mean age, this finding was not statistically significant. Also, osteoarticular involvement was frequently found in subacute and chronic brucellosis patients. Even sacroiliitis was detected in two and spondylitis was detected in one of the three chronic brucellosis patients.

Sequelae are very rare in osteoarticular brucellosis. Avascular necrosis of the femur head and functional disability of the hip joints were the most reported sequelae.[9,14,40] In our study, only one case of avascular necrosis of the femur head was detected.

The current study showed that osteoarticular involvement is a significant part of the multisystemic involvement in childhood brucellosis. In our study, osteoarticular involvement was detected in nearly one of every four childhood brucellosis patients. The most prominent form of the osteoarticular involvement was peripheral arthritis. The age of the patients with osteoarticular involvement and patients with sacroiliitis and spondylitis were older than patients with peripheral arthritis. Otherwise, the patients with osteoarticular involvement had more frequent complaints and higher ESR, C-RP, and STA levels. Additionally, brucellosis should be considered as a pre-diagnosis in children with osteoarticular complaints, especially in regions where the disease is endemic.

Acknowledgments

We are grateful to Efgan Gayyurhan and the children with brucellosis who volunteered to be part of this study.

REFERENCES

1. Pappas G, Papadimitriou P, Akriditis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis 2006; 6: 91-99.
2. Colmenero JD, Reguera JM, Martos F, et al. Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine (Baltimore) 1996; 75: 195-211.
3. Republic of Turkey, Ministry of Health, Brucellosis statistical data for 1970-2004.
4. Kılıç S, Ivanov IN, Durmaz R, et al. Multiple-locus variable-number tandem-repeat analysis genotyping of human Brucella isolates from Turkey. J Clin Microbiol 2011; 49: 3276-3283.
5. Young EJ. Brucella species (Brucellosis). In: Long SS, Pickering LK, Prober CG (eds). Principles and Practice of Pediatric Infectious Diseases. (4th ed). New York: Elsevier, 2012: 861-865.
6. Al Shaalan M, Memish ZA, Al Mahmoud S, et al. Brucellosis in children: clinical observations in 115 cases. Int J Infec Dis 2002; 6: 182-186.
7. Gür A, Geyik MF, Dikici B, et al. Complications of brucellosis in different age groups: a study of 283 cases in southeastern Anatolia of Turkey. Yonsei Med J 2003; 44: 33-44.
8. Kennedy JC. Notes on case of chronic synovitis, or bursitis, due to organism of Mediterranean fever. J Roy Army Med Corps 1904; 2: 178-180.
9. Al-Eissa YA, Kambal AM, Alrabeeah AA, Abdullah AM, al-Jurayyan NA, al-Jishi NM. Osteoarticular brucellosis in children. Ann Rheum Dis 1990; 49: 896-900.
10. Geyik MF, Gür A, Nas K, et al. Musculoskeletal involvement of brucellosis in different age groups: a study of 195 cases. Swiss Med Wkly 2002; 132: 98-105.
11. Dashti AS, Karimi A. Skeletal involvement of Brucella melitensis in children: a systematic review. Iran J Med Sci 2013; 38: 286-292.
12. Benjamin B, Annobil SH, Khan MR. Osteoarticular complications of childhood brucellosis: a study of 57 cases in Saudi Arabia. J Pediatr Orthop 1992; 12: 801-805.
13. Case definitions for infectious conditions under public health surveillance. Centers for Disease Control and Prevention. MMWR Recomm Rep 1997; 46: 1-55.
14. Gómez-Reino FJ, Mateo I, Fuertes A, Gómez-Reino JJ. Brucellar arthritis in children and its successful treatment with trimethoprim-sulphamethoxazole (co-trimoxazole). Ann Rheum Dis 1986; 45: 256-258.
15. Almuneef M, Memish ZA, Al Shaalan M, Al Banyan E, Al-Alola S, Balkhy HH. Brucella melitensis bacteremia in children: review of 62 cases. J Chemother 2003; 15: 76-80.

16. Mousa AR, Muhtaseb SA, Almudallal DS, Khodeir SM, Marafie AA. Osteoarticular complications of brucellosis: a study of 169 cases. Rev Infect Dis 1987; 9: 531-543.

17. Gotuzzo E, Seas C, Guerra JG, et al. Brucellar arthritis: a study of 39 Peruvian families. Ann Rheum Dis 1987; 46: 506-509.

18. Mantur BG, Akki AS, Mangalgi SS, Patil SV, Gobbur RH, Peerapur BV. Childhood brucellosis--a microbiological, epidemiological and clinical study. J Trop Pediatr 2004; 50: 153-157.

19. Roushan MRH, Ahmadi SAA, Gangi SMS, Janmohammadi N, Amiri MJS. Childhood brucellosis in Babol, Iran. Trop Doct 2005; 35: 229-231.

20. Zamani A, Kooraki S, Mohazab RA, et al. Epidemiological and clinical features of Brucella arthritis in 24 children. Ann Saudi Med 2011; 31: 270-273.

21. el-Desouki M. Skeletal brucellosis: assessment with bone scintigraphy. Radiology 1991; 181: 415-418.

22. Tanir G, Tufekci SB, Tuygun N. Presentation, complications, and treatment outcome of brucellosis in Turkish children. Pediatr Int 2009; 51: 114-119.

23. Parlak M, Akbayram S, Doğan M, et al. Clinical manifestations and laboratory findings of 496 children with brucellosis in Van, Turkey. Pediatr Int 2015; 57: 586-589.

24. Yoldas T, Tezer H, Ozkaya-Parlakay A, Saylı TR. Clinical and laboratory findings of 97 pediatric brucellosis patients in central Turkey. J Microbiol Immunol Infect 2015; 48: 446-449.

25. Çıraklı S, Karlı A, Şensoy G, Belet N, Yanık K, Çıraklı A. Evaluation of childhood brucellosis in the central Black Sea region. Turk J Pediatr 2015; 57: 123-128.

26. Bosilkovski M, Kirova-Urosevic V, Cekovska Z, et al. Osteoarticular involvement in childhood brucellosis: experience with 133 cases in an endemic region. Pediatr Infect Dis J 2013; 32: 815-819.

27. Alvarez de Bruego M, Gomez Reino FJ, Gomez Reino JJ. A long term study of 22 children with brucellar arthritis. Clin Exp Rheumatol 1990; 8: 609-612.

28. Lubani M, Sharda D, Helin I. Brucella arthritis in children. Infection 1986; 14: 233-236.

29. Issa H, Jamal M. Brucellosis in children in south Jordan. East Mediterr Health J 1999; 5: 895-902.

30. Benjamin B, Annobil SH. Childhood brucellosis in southwestern Saudi Arabia: a 5-year experience. J Trop Pediatr 1992; 38: 167-172.

31. Hashemi SH, Keramat F, Ranjbar M, Mamani M, Farzam A, Jamal-Omidi S. Osteoarticular complications of brucellosis in Hamedan, an endemic area in the west of Iran. Int J Infect Dis 2007; 11: 496-500.

32. Janmohammadi N, Roushan MRH. False negative serological tests may lead to misdiagnosis and mismanagement in osteoarticular brucellosis. Trop Doct 2009; 39: 88-90.

33. Galanakis E, Bourantas KL, Leveidiotou S, Lapatsanis PD. Childhood brucellosis in north-western Greece: a retrospective analysis. Eur J Pediatr 1996; 155: 1-6.

34. Al-Shamahy HA, Wright SG. A study of 235 cases of human brucellosis in Sana'a, Republic of Yemen. East Mediterr Health J 2001; 7: 238-246.

35. Shen MW. Diagnostic and therapeutic challenges of childhood brucellosis in a nonendemic country. Pediatrics 2008; 121: e1178-e1183.

36. Lifeso RM, Harder E, McCorkell SJ. Spinal brucellosis. J Bone Joint Surg Br 1985; 67: 345-351.

37. Nas K, Gür A, Kemaloğlu MS, et al. Management of spinal brucellosis and outcome of rehabilitation. Spinal Cord 2001; 39: 223-227.

38. al-Eissa YA, Kambal AM, al-Nasser MN, al-Habib SA, al-Fawaz IM, al-Zamil FA. Childhood brucellosis: a study of 102 cases. Pediatr Infect Dis J 1990; 9: 74-79.

39. Logan LK, Jacobs NM, McAuley JB, Weinstein RA, Anderson EJ. A multicenter retrospective study of childhood brucellosis in Chicago, Illinois from 1986 to 2008. Int J Infect Dis 2011; 15: e812-e817.

40. Benjamin B, Khan MR. Hip involvement in childhood brucellosis. J Bone Joint Surg Br 1994; 76: 544-547.

The Turkish Journal of Pediatrics 2020; 62: 208-214
https://doi.org/10.24953/turkjped.2020.02.006

Original Article

Increased potassium excretion in children with monosymptomatic nocturnal enuresis: could it be related to Kir 4.1- KCNJ10 gene polymorphism?

Ayşe Balat[1], Mesut Parlak[2], Sibel Oğuzkan Balcı[3], Bülent Göğebakan[3], Mithat Büyükçelik[4], Nilgün Çöl[5], Seval Kul[6], Keriman Tınaztepe[7]

[1]Department of Pediatric Nephrology, İstanbul Aydın University Faculty of Medicine, İstanbul; [2]Department of Pediatrics, Akdeniz University Faculty of Medicine, Antalya, Departments of [3]Medical Biology and Genetics, [4]Pediatric Nephrology, [5]Pediatrics and [6]Biostatistics, Gaziantep University Faculty of Medicine, Gaziantep; [7]Department of Pediatric Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background and objectives. There are controversial results in the literature regarding urinary electrolytes, especially potassium, in enuretic children. KCNJ10 channel protein, a member of the Kir 4.1 family is expressed in renal distal tubules and has an important function in renal ion transport. We investigated whether KCNJ10 gene polymorphisms are associated with clinical and laboratory findings of a group of Turkish children with monosymptomatic primary nocturnal enuresis (MNE).

Method. Ninety-seven MNE children and 100 healthy controls were tested for three single nucleotide polymorphisms (SNPs) in the KCNJ10 gene. The transversions in SNPs were G to A for intron 1(SNP1), G to A for exon 2 (SNP2), and T to C transition for promoter (SNP3). All SNPs were genotyped by PCR-restriction fragment length polymorphism.

Results. SNP3 in promoter of KCNJ10 gene showed strong association with MNE children for distribution of genotype and allele frequency, while SNP1 in intron 1 and SNP2 in exon 2 were noninformative.

The distribution of TT, TC, and CC genotypes for SNP3 was 66%, 26.8% and 7.2% respectively in MNE compared with 38%, 59% and 3% respectively in controls ($p < 0.0001$). In enuretic children, TT genotype was higher and there was an increased potassium excretion in children with TT genotype ($P < 0.05$).

Conclusion. We conclude that KCNJ10 gene promoter polymorphism may have a role on potassium excretion in Turkish MNE children. This is the first study in literature evaluating KCNJ10 gene polymorphism in this patient population. Future studies investigating the other SNPs, mutations or altered regulation of Kir4.1 in larger samples would help clarify the role (s) of KCNJ10 gene in enuresis.

Key words: KCNJ10 gene, Kir 4.1 family, monosymptomatic nocturnal enuresis, primary nocturnal enuresis, polymorphism.

Monosymptomatic nocturnal enuresis (MNE) is the involuntary urination during the night in the absence of an organic disease in a child over 5 years of age.[1] It is a common situation in children and accounts for about 85% of primary enuresis.[2] Although there are a lot of data concerning the epidemiology, pathophysiology, and management of MNE, etiology remains unclear. Recent researches indicate that it is mostly due to physiological reasons.[3] Diurnal pattern of urinary sodium (Na^+) and potassium (K^+) in normal children, with a marked reduction from daytime to night time, has not been found

✉ Ayşe Balat
aysebalat@hotmail.com

Received 16th January 2019, revised 29th May 2019, accepted 3rd July 2019.

This study presented partially in 14th Congress of the International Pediatric Nephrology Association, 31 August- 4 September 2007, Budapest, Hungary.

in enuretics having polyuria, natriuresis, and kaliuresis, despite normal levels of plasma atrial natriuretic peptide.[4] A few studies showed that fractional sodium (FENa) and potassium (FEK) excretions were higher in enuretic children.[5,6] These studies suggested a possible benign hereditary and/or postural disorder in renal tubular handling of Na^+ and K^+ in those children.[4-6]

There are various K^+ channels in the kidney. The voltage-gated K^+ channels are important in stabilization of cell membrane potential, and expressed in a variety of nephron segments. The Ca-activated maxi K^+ channel plays a role in flow-dependent K^+ secretion in the distal nephron,[7-9] while the other K^+ channel, the renal outer medullary K^+ channel (ROMK), is also found along the collecting duct in principal cells, where ROMK mediates K^+ secretion into urine to remove it from the body.[10]

Kir 4.1 and Kir 1.1, potassium transporter members, are expressed in distal tubules and they function as the key molecules for renal ion transport.[11] KCNJ10 channel protein is a member of Kir 4.1 family.[12] KCNJ10 gene (MIM602208) which is located on chromosome 1q22-23, consists of two exons, spans 33 kb, and several single nucleotide polymorphisms (SNPs) including exons, introns and promoter region.[13] Functional studies demonstrated that a few of these SNPs in KCNJ10 gene could affect the protein expression.[8]

As we mentioned above, urinary electrolyte levels, especially K^+, have been found different in enuretics,[4-6,14,15] whereas some other studies have found similar Na^+ and K^+ excretion compared to controls.[16,17] In the present study, we firstly investigated whether KCNJ10 gene polymorphisms are associated with MNE in Turkish children, and searched the relationship between studied polymorphisms, and Na^+ / K^+ excretion, FENa, FEK, frequency of bedwetting, duration of enuresis, the presence of wake-up problem (deep sleep), and family history.

Material and Methods

Forty-seven male and 50 female children with MNE (mean age 9.5 ± 2.7 years) referred to the pediatric outpatient clinic of Gaziantep University, Department of Pediatrics and Pediatric Nephrology were included in the study. Healthy controls comprised of 100 age- and sex-matched volunteers with no known disease affecting electrolyte concentrations, renal functions, and none of them were relatives of the enuretics. A patient is considered to have MNE if the involuntary voiding at least 2 nights per week occurs, beyond the age at which bladder control was normally achieved in the absence of congenital or acquired defects of the urinary tract.

The study was approved by the local ethical commitee (report# 02.07.2007/40), and informed consents were obtained from the patients and/or parents.

All patients and controls had detailed physical examination, tested negative for urinary protein, blood, and nitrate by dipstick and urine culture. None of the enuretics had ever been dry, and none had daytime incontinence or symptoms suggesting bladder-bowel dysfunction. Parents were asked about family history and the presence of wake-up problem (deep sleep). Although the subjects had similar eating habits, the diet was checked over 5 days before specimen collection to avoid excessive Na^+ and K^+ intake. None of the enuretics was taking any medication during the study period. All children had normal blood urea nitrogen (BUN) and creatinine levels, normal urinary ultrasonographic findings, and were normotensive at the time of study. The families not willing to give urine and blood samples were excluded (18 of 115 families). Blood and urine samples, *first urine in the morning*, were obtained to determine BUN, creatinine, phosphorus, Na^+, K^+, and urinary creatinine and electrolytes.

BUN, creatinine, electrolytes, as well as urinary creatinine, electrolytes, and urinary density

were determined by routine methods. FE_{Na}, and FE_K were calculated from below formulae:

FE_{Na} (%) = [(Urine Na^+/Plasma Na^+) / (Urine creatinine / Plasma creatinine] x100

FE_K (%) = [(Urine K^+/Plasma K^+) / (Urine creatinine / Plasma creatinine] x100

Genotyping

All children (97 MNE patients and 100 healthy controls) were analyzed for three SNPs in KCNJ10 gene. These SNPs were G to A transversion in intron 1 (SNP1) and G to A transversion in exon 2 (SNP2), and T to C transition in promoter (SNP3). Genomic DNA was extracted from peripheral blood samples.[18] All SNPs were genotyped by polymerase chain reaction- restriction fragment lenght polymorphism (PCR-RFLP) using the appropriate primers and restriction enzymes.[13] The digested products were resolved on 3% agarose gels and visualized under U.V.

Statistical Analysis

Results are given as mean ± SD, while allele frequencies and the distribution of genotype are given as %. Clinical and laboratory data of study groups compared by using Independent sample t and Mann- Whitney U tests. Genotype frequencies among patients and controls were calculated by using Graphpad Instat Version 3, and deviation from Hardy–Weinberg equilibrium (HWE) was examined by De-finetti program. We calculated odds ratios and 95% confidence intervals using binary logistic regression. The analyses of data were performed by use of a statistical software (SPSS for Windows, version 11.5). Max type-I error was accepted as 0.05.

Results

Since we found no statistically significant differences in serum and urinary electrolyte levels and the distribution of SNPs between boys and girls (p>0.05), we evaluated all children without sex differences.

BUN, creatinine, electrolytes, and tubular reabsorption of phosphorus were within normal limits in enuretics and controls. Urinary densities of enuretics and controls were 1019.7 ± 5.6 and 1021.1 ± 4.8, respectively (p>0.05). Serum K values were within normal limits in the patient and control groups (4.46 ± 0.35 mEq/L vs 4.50 ± 0.47 mEq/L, p > 0.05), while urinary K^+ excretion was higher in enuretics than controls

Table I. The demographic features of enuretic children and controls.

		Enuretics (n=97)	Controls (n=100)	p
Mean age (years)		9.5 ± 2.7	9.8 ± 2.5	>0.05
Gender	Male	47	45	>0.05
	Female	50	55	>0.05
Enuretic relatives	Present	77	17	<0.01
	Absent	20	83	<0.01
Frequency of bedwetting (night per week)		5.9 ± 1.7	-	-

Table II. Urinary electrolytes of enuretic children and controls.

Spot Urine (first urine in the morning)	Enuretics (n=97)	Controls (n=100)	p
Na^+ (mmol/ L)	130.82 ± 68.20	146.10 ± 71.36	>0.05
K^+ (mmol/ L)	55.72 ± 42.08	36.44 ± 22.30	<0.01
FE_{Na} (%)	0.67 ± 0.58	0.75 ± 0.83	>0.05
FE_K (%)	7.87 ± 7.70	5.55 ± 4.38	<0.01

FE_{Na}: fractional sodium excretion, FE_K: fractional potassium excretion.

(p <0.01). The demographic characteristics of children are shown in Table I and the urinary excretions of Na^+ and K^+ are shown in Table II.

KCNJ10 gene SNP1 in intron 1 and SNP2 in exon 2 were noninformative for Turkish children. SNP3 in promoter was informative and digestion of SNP3 PCR product by AciI enzyme generated two fragments for T allele (Fig. 1).

SNP3 in promoter showed a strong association in enuretic children for either distribution of genotype and allele frequency (Table III).

For SNP3, the distribution of TT, TC, and CC genotypes was 66%, 26.8% and 7.2% respectively in MNE compared with 38%, 59% and 3% respectively in the controls (p <0.0001), and TT genotype was higher in enuretics. The allele frequencies of T /C were 79.4% / 20.6% in MNE and 67.5%/ 32.5 % in the controls (p= 0.003). The observed genotype counts were not deviated from those expected according to the HWE in patient and control groups (p= 0.561, and p= 0.339, respectively).

Potassium excretion (mmol/L), and FE_K (%) in the first urine sample of enuretics with TT genotype were higher than controls (58.07 ± 42.87 vs 35.21 ± 22.79, *P* <0.01, and 7.65 ± 7.49 vs 5.53 ± 4.42, p= 0.034, respectively).

There was no correlation between the bedwetting episodes per week and duration of enuresis with FE_{Na}, FE_K, urinary Na^+ and K^+ excretion, and the distribution of genotypes, and allele frequencies (P>0.05). No statistically significant relationship was found between the gender and presence of deep sleep (p= 0.758). Because of the small number of enuretics with CC genotype, we compared the relationship between the presence of deep sleep and TT and 'TC +CC' genotypes. Enuretic children with TT

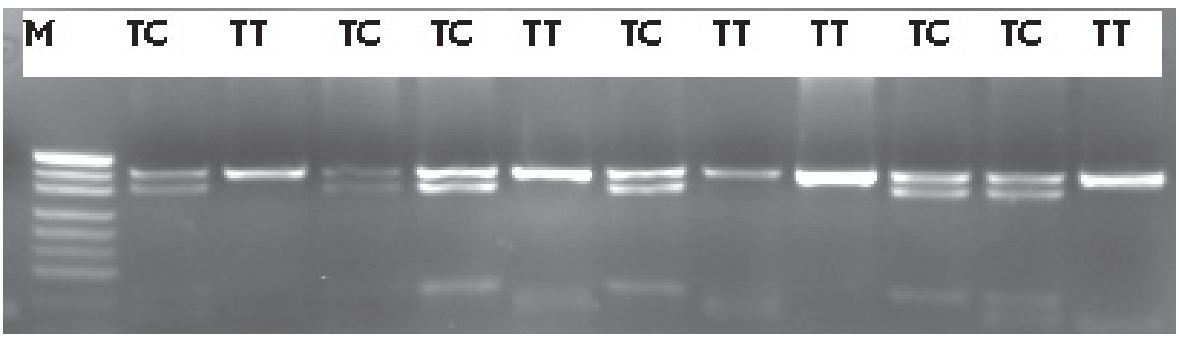


Fig. 1. KCNJ10 gene promoter polymorphism (M: pUC 19 MspI DNA leader).

Table III. The genotype distributions and allele frequencies of KCNJ10 gene promoter polymorphism in enuretic children and controls.

	Enuretics n (%)	Controls n (%)	Odds Ratio (95% CI)	*P* value
Genotype				
TT	64 (66)	38 (38)	3.16 (1.76-5.66)	<0.0001
TC	26 (26.8)	59 (59)	0.25 (0.13-0.46)	<0.0001
CC	7 (7.2)	3 (3)	2.51 (0.63-10.02)	0.08*
Total	97	100		
Allele				
T	154 (79.4)	135 (67.5)	1.85 (1.17-2.92)	0.003
C	40 (20.6)	65 (32.5)	0.53 (0.34-0.85)	0.003

*Fisher exact test

genotype had 4 times higher increased risk for the presence of deep sleep (odds=4,015 95%CI [1,562-10,320], while patients with T allele had 3.8 times higher risk compared to having C allele (odss=3,813, 95%CI [1,834-7,926].

Discussion

Current data suggest that underlying mechanism of enuresis is mostly physiological reasons, rather than psychiatric factors. Some studies stressed a possible renal tubular maturation defect,[4-6,14] and there are conflicting results in the literature on urinary electrolytes of enuretics. Some studies demonstrated higher FE_{Na} and FE_{K} excretions in enuretic children,[5] and a significant increase in FE_{Na} and FE_{K} during the day and at night,[6] while some others have found similar urinary Na^+ and K^+ values to controls.[16,17] In this study, we also found significantly increased K^+ excretion in enuretics, similarly to the previous studies.[5,6,14] Urinary K^+ predominantly derived from distal K^+secretion, since filtered K^+ is reabsorbed almost entirely in proximal segments of the nephron.[19] Therefore, increased K^+ excretion in those children suggests that there may be a problem in distal tubular regulation of K^+, like a failure of some K^+-regulating mechanisms. Excessive K^+ in the distal tubule together with the low ADH (which could not be shown in some studies) in enuretics may cause less tubular fluid reabsorption, and insufficiently reabsorbed K^+ remains in the distal tubule concomitantly with water. According to above hypothesis, all of these events may result in increased K^+ excretion (together with or without nocturnal polyuria) in those children,[14] indicating that there may be a problem in renal regulation of K^+ in MNE. We investigated the relationship between urinary electrolytes, especially K^+, and KCNJ10 gene promoter polymorphism which plays an important role in K^+ secretion in the distal nephron. This relationship has not been investigated previously.

Bockenhauer et al.[20] demonstrated that Kir 4.1 is expressed in the kidney with high specificity only in the distal convoluted tubule (DCT) on the basolateral membrane, and speculated that Kir 4.1 is critical for K^+ recycling across the basolateral membrane of DCT cells. Potassium is taken up by the basolateral Na^+, K^+-ATPase and must be recycled to maintain Na^+ / K^+ pump activity, and loss of Kir 4.1 function may reduce the function of Na^+, K^+-ATPase.[20,21] The exact role(s) of the inward-rectifying K channel Kir 4.1 in the distal nephron would be better understood by the continuing researches. Recently, an experimental study demonstrated that even modest reduction in Kir 4.1 expression results in impaired K conservation, which appears to be mediated by reduced expression of activated Na/Cl cotransporter expression.[22]

In the present study, SNP3 in promoter of KCNJ10 gene was strongly associated with either distribution of genotype and allele frequency in enuretics, and TT genotype was associated with higher urinary K^+ excretion. We suggest that TT genotype and T allele of KCNJ10 gene may have an important role on renal tubular handling of K^+ in nephron. It would be better to determine also the relationship between KCNJ10 gene polymorphism and polyuria in enuretic children. Unfortunately, this parameter could not be evaluated because the families mostly refused to weigh overnight diaper(s) + the amount of first voided urine. This is one of the limitations of our study.

Although there are only a few studies regarding the sleep patterns of enuretic children,[23,24] it is generally believed that they are deep-sleepers with impaired arousability.[25] We found that enuretic children with TT genotype had 4 times higher increased risk in terms of deep-sleep. KCNJ10 channel plays an important role in the central nervous system functioning.[26,27] It is a possible candidate gene for Autism Spectrum Disorders, and also linked to seizure susceptibility both in humans and in experimental studies.[28-32] However, there is no study evaluating the possible relationship between sleep disorders and KCNJ10 in literature. One of the limitation of this study is that the presence of deep sleep is based on only

anamnesis, and polysomnography could not be performed. This may be an interesting finding to be tested in the future.

The present study demonstrated that KCNJ10 gene promoter polymorphism may be associated with MNE in Turkish children, and enuretics with TT genotype are prone to high urinary K^+ excretion, and the presence of deep sleep. However, this does not mean that KCNJ10 gene polymorphism is the cause of MNE in children, but it may partially explain the different urinary K^+ excretions found in several studies. Future studies investigating the other SNPs, mutations or altered regulation of Kir4.1 in larger samples may help to learn the main role of KCNJ10 gene in enuresis.

Acknowledgements

The financial support of this study is the poster award of *another study, by Balat A et al., at the 4th International Congress of Uremic Researches and Toxicity, September 14-17, 2005, İzmir, Turkey.

[*Alaşehirli B, Balat A, Barlas O, Kont A, Şahinöz S. Neuronal nitric oxide gene polymorphism in children with minimal change nephrotic syndrome].

REFERENCES

1. Nevéus T, von Gontard A, Hoebeke P, et al. The standardization of terminology of lower urinary tract function in children and adolescents: report from the Standardization Committee of the International Children's Continence Society. J Urol 2006; 176: 314-324.
2. Mark SD, Fracs JD. Nocturnal enuresis. Br J Urol 1995; 75: 427-434.
3. Wille S, Anveden I. Social and behavioral perspectives in enuretics, former enuretics and non-enuretic controls. Acta Paediatr 1995; 84: 37-40.
4. Rittig S, Knudsen UB, Norgaard JP, Gregersen H, Pedersen EB, Djurhuus JC. Diurnal variation of plasma atrial natriuretic peptide in normals and patients with enuresis nocturna. Scand J Clin Lab Invest 1991; 51: 209-217.
5. Vurgun N, Gümüs BH, Ece A, Arı Z, Tarhan S, Yeter M. Renal functions of enuretic and nonenuretic children: hypernatriuria and kaliuresis as causes of nocturnal enuresis. Eur Urol 1997; 32: 85-90.
6. Vurgun N, Yiditodlu MR, Ozcan A, Arı Z, Tarhan S, Balkan C. Hypernatriuria and kaliuresis in enuretic children and the diurnal variation. J Urol 1998; 159: 1333-1337.
7. Wang W. Renal potassium channels: recent developments. Curr Opin Nephrol Hypertens 2004; 13: 549-555.
8. Shang L, Lucchese CJ, Haider S, Tucker SJ. Functional characterisation of missense variations in the Kir4.1 potassium channel (KCNJ10) associated with seizure susceptibility. Brain Res Mol Brain Res 2005; 139: 178-183.
9. Lu Z. Mechanism of rectification in inward-rectifier K+ channels. Annu Rev Physiol 2004; 66: 103-129.
10. Wang WH, Giebisch G. Regulation of potassium (K) handling in the renal collecting duct. Pflugers Arch 2009; 458: 157-168.
11. Isomoto S, Kondo C, Kurachi Y. Inwardly rectifying potassium channels: their molecular heterogeneity and function. Jpn J Physiol 1997; 47: 11-39.
12. Lehman-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. Physiol Rev 1999; 79: 1317-1372.
13. Farook VS, Hanson RL, Wolford JK, Bogardus C, Prochazka M. Molecular analysis of KCNJ10 on 1q as a Candidate gene for type 2 diabetes in pima Indians. Diabetes 2002; 51: 3342-3346.
14. Balat A, Cekmen M, Yürekli M, et al. Adrenomedullin and nitrite levels in children with primary nocturnal enuresis. Pediatr Nephrol 2002; 17: 620-624.
15. Natochin YV, Kuznetsova AA. Defect of osmoregulatory renal function in nocturnal enuresis. Scan J Urol Nephrol Suppl 1999; 202: 40-43.
16. Aceto G, Penza R, Delvecchio M, Chiozza ML, Cimador M, Caione P. Sodium fraction excretion rate in nocturnal enuresis correlates with nocturnal polyuria and osmolality. J Urol 2004; 171(6 Pt 2): 2567-2570.
17. Unüvar T, Sönmez F. The role of urine osmolality and ions in the pathogenesis of primary enuresis nocturna and in the prediction of responses to desmopressin and conditioning therapies. Int Urol Nephrol 2005; 37: 751-757.
18. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1998; 16: 1215.

19. Satlin LM, Holliday MA. Potassium. In: Holliday MA, Barratt TM, Avner ED (eds) Pediatric Nephrology (3rd edt). Baltimore: Williams and Wilkins, 1994: 215-221.

20. Bockenhauer D, Feather S, Stanescu HC, et al. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. N Engl J Med 2009; 360: 1960-1970.

21. Wagner CA. New roles for renal potassium channels. J Nephrol 2010; 23: 5-8.

22. Malik S, Lambert E, Zhang J, et al. Potassium conservation is impaired in mice with reduced renal expression of Kir4.1. Am J Physiol Renal Physiol 2018; 315: F1271-F1282.

23. Hunsballe JM. Increased delta component in computerized sleep electroencephalographic analysis suggests abnormally deep sleep in primary monosymptomatic nocturnal enuresis. Scand J Urol Nephrol 2000; 34: 294-302.

24. Bader G, Neveus T, Kruse S, Sillen U. Sleep of primary enuretic children and controls. Sleep 2002; 25: 573-577.

25. Wolfish NM, Pivik RT, Busby KA. Elevated sleep arousal thresholds in enuretic boys: clinical implications. Acta Paediatr 1997; 86: 381-384.

26. Neusch C, Rozengurt N, Jacobs RE, Lester HA, Kofuji P. Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. J Neurosci 2001; 21: 5429-5438.

27. Djukic B, Casper KB, Philpot BD, Chin LS, McCarthy KD. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci 2007; 27: 11354-11365.

28. Ferraro TN, Golden GT, Smith GG, et al. Fine mapping of a seizure susceptibility locus on mouse Chromosome 1: nomination of Kcnj10 as a causative gene. Mamm Genome 2004; 15: 239-251.

29. Buono RJ, Lohoff FW, Sander T, et al. Association between variation in the human KCNJ10 potassium ion channel gene and seizure susceptibility. Epilepsy Res 2004; 58: 175-183.

30. Sicca F, Imbrici P, D'Adamo MC, et al. Autism with seizures and intellectual disability: possible causative role of gain-of-function of the inwardly-rectifying K (+) channel Kir4.1. Neurobiol Dis 2011; 43: 239-247.

31. Phani NM, Acharya S, Xavy S, et al. Genetic association of KCNJ10 rs1130183 with seizure susceptibility and computational analysis of deleterious non-synonymous SNPs of KCNJ10 gene. Gene 2014; 536: 247-253.

32. Zhang SP, Zhang M, Tao H, et al. Dimethylation of histone 3 lysine 9 is sensitive to the epileptic activity, and affects the transcriptional regulation of the potassium channel Kcnj10 gene in epileptic rats. Mol Med Rep 2018; 1: 1368-1374.

The Turkish Journal of Pediatrics 2020; 62: 215-223
https://doi.org/10.24953/turkjped.2020.02.007

Original Article

Clinical manifestation and outcomes of children with hypertrophic cardiomyopathy in Kosovo

Ramush A. Bejiqi[1,2], Ragip Retkoceri[1], Naim Zeka[1], Armend Vuçiterna[1], Aferdita Mustafa[1], Arlinda Maloku[1], Rinor Bejiqi[2]

[1]Pediatric Clinic, University Clinical Center of Kosovo, Prishtina; [2]University of Gjakova "Fehmi Agani", Gjakova, Republic of Kosovo.

ABSTRACT

Background and objectives. Identification of the manifestations, assessment and follow up of children with hypertrophic cardiomyopathy (HCM) by transthoracic echocardiography may be important for clinical management and our understanding of pathogenesis.

Methods. We present a comprehensive analysis of 43 children seen in Kosovo, 23 were male, aged between 4 months and 9 years at first presentation (median of 2 years and 3 months).

Results. Cardiac failure, seen in almost half of them, was the most common presenting feature. At admission, the chest x-ray revealed an increased cardiothoracic ratio, to a mean of 72% in 6 infants and to 65% in 37 older children. Measured by transthoracic echocardiography, 28 children had asymmetric hypertrophy of left ventricle while 15 had concentric hypertrophy. Left ventricular ejection fraction was depressed in 21 children. Patients with cardiac failure received various combinations of diuretics, B-blockers, ACE inhibitors and anticoagulant therapy (aspirin). Death occurred in 8 children, in 4 of them shortly after admission, the other 4 left Kosovo and continued examination and treatment abroad Kosovo; their death has been confirmed by family members. The remaining 32 were followed- up for a mean 42 months, with a range from 5 to 115 months. Surgical intervention was not performed to any of them, despite the clinical and echocardiography indications due to a limitation of resources. Recovery was noted in 14 children but still requiring anti-heart failure medications. Slightly over two-fifths died. Of those with asymmetric form, 45% died, half of those presenting in infancy, and 89% of those who presented at admission with signs of cardiac failure.

Conclusion. The results of our study show that similar to many centers, the etiology of HCM is often uncertain. In the absence of etiology, treatment aimed at the cause is either impossible or, at best, empirical.

Key words: hypertrophic cardiomyopathy, left ventricular hypertrophy, heart failure, myectomy transthoracic echocardiography.

Hypertrophic cardiomyopathy (HCM) is defined as the presence of hypertrophied, non-dilated ventricle in the absence of a hemodynamic disturbance that is capable of producing the existent magnitude of wall thickening (e.g., hypertension, aortic valve stenosis, hyperthyroidism, catecholamine secreting tumors, etc.).[1] It is the most common inherited cardiovascular disease, with diverse etiology, affecting populations worldwide and the leading cause of sudden cardiac death in young people. While sarcomeric gene defect has been reported to be the primary cause of HCM in adults, in children the disease is seen in a wide variety of multisystem and cardio specific disorders. It is common to group these diseases as familial, syndromic, neuromuscular, and metabolic (storage disease and mitochondrial disorders).[1,2] HCM in childhood is a heterogeneous disease with variable progression. The disease has been reported from several centers and countries, and described for several groups. Incidentally,

✉ Arlinda Maloku
arlindamaloku@yahoo.com

Received 14th October 2018, revised 11th January 2019, accepted 14th February 2019.

Table I. Summary of some publications on childhood hypertrophic cardiomyopathy.

Leadauthor	Year	Country	Etiology	N° of patients
Heinrich K	1995	Germany, USA	mixed	600
CecchiFs	1995	Italy	mixed	202 (134 >older 15 years)
Alan W. N	1996	Australia	mixed	80
Sanae Mi	1998	Japan	mixed	309
Barry J. Maron	2000	USA, Italy	mixed	744
Steven D. Colan	2007	USA	mixed	855
Cristina Basso	2009	Italy, USA	mixed	115
JoseOliva-Sandoval	2010	Spain	mixed	152
Georgios K Efthim	2010	Greece	mixed	380

most of the reports have drawn attention to the generally severe course of the disease, especially to its unsatisfactory response to standard anti-heart failure therapy (Table I).[3] Reports have mostly come from tertiary centers, raising the possibility of a selection bias in favor of very sick children. Unfortunately, reports from Balkan countries on the disease are still scanty.

The aim of this article is to present a diagnostic approach, treatment and outcome of children with HCM in Kosovo, as a small country with limited technical and human resources, and to compare our findings with recent publications in this field. The objective of this study was to provide an account of children with HCM as seen in the country of Kosovo, diagnosed by echocardiography and analyzing the data of 43 patients, registered at the Unit of Cardiology, from January 2007 to December 2017, aged from 21 days (3 weeks) to 9 years.

Material and Methods

Pediatric Clinic in Prishtina, part of the University Clinical Center of Kosovo in Prishtina, Kosovo, provides pediatric cardiology services, of secondary and tertiary level. Practically, all children with known or suspected cardiac disease are referred to our institution from the regional hospitals for cardiac evaluation and care. This system has enabled us to build a database and provide services to virtually all patients with pediatric cardiac disease in Kosovo. This project is part of data relating to HCM that forms the material for this study, which aimed to provide an account of the disease as seen in Kosovo. The study protocol was approved by the Medical Ethics Committee of the University Clinical Center of Kosovo in Prishtina (1098/26.07.2018). Written informed consent was obtained from all patients.

The field of the research

We commenced our study in January 2007, where summaries of patients with cardiac disease were recorded in the database of the cardiology department. Two pediatric cardiologists in the Department of Cardiology evaluated cardiological examinations, where 43 children fulfilled the standard criteria for the diagnosis of HCM, and they present the subjects of this study. Prior to the examination, weight and stature were recorded and the body surface area was calculated by the Dubois and Dubois formula. None of the patients were receiving cardiovascular medication at the time of admission. The evaluation of each child comprised a short familiar and personal history, physical examination, chest radiography, electrocardiogram, and trans thoracic echocardiography. Determination of levels of the cardiac enzymes in serum was not considered a critical investigation. Echocardiography evaluation was performed in all patients, at presentation and during follow-up visits by the same two pediatric cardiologists. Follow-up investigations, comprising mostly of electrocardiograms and trans thoracic

echocardiographs, were performed as often as the clinical state warranted. The results obtained are shown in absolute and relative numbers.

Results

Both sexes were affected, with a non-significant predomination of males, there being 23 males (53%) and 20 females (47%). At initial presentation, all patients were aged between 3 weeks and 9 years, the median age being 13 months and mean 22.33 months. Of this number, 8 children(18%) were less than 12 months old, 23 children (53%) aged between one and 5 years, and 12 (28%) were more than 5 years old. To half of them, the reason for cardiac examination was a systolic heart murmur. At admission, the chest x-ray revealed an increased cardiothoracic ratio, to a mean of 72% in 6 infants and to 65% in 38 older children. All of the patients were Kosovar Albanians and citizens of Kosovo.

Twelve children in our study manifested echocardiography signs of LVOT, half of them with asymmetric HCM. Using continual and color Doppler imaging technique, in 11 children at the level of OTLV the pressure gradient (PG) was registered, measuring from 3.2m/s (PG = 41mmHg) to 5.3m/s (PG =112 mmHg).

Of our overall group, 4 children died shortly after admission, and 4 children died while being treated abroad, with signs of arrhythmia or sudden death, despite therapy coverage. Five of them were with positive family history of cardiomyopathy.

The children who died in Kosovo were 3 males and 1 female, the youngest child died at age 26 months, the other at age 32 months, 3yaers and 4 months and 6 years and 2 months (Table II) . Four 4 children died while being treated abroad, with signs of arrhythmia or sudden death, despite therapy coverage. Five of them were with positive family history of cardiomyopathy.

Etiology

In 18 children (41%), siblings had reportedly suffered from the same disease, and these patients were categorized as being familial. Four of them (three males and one female) were cousins, 16 members of this family were suffering from HCM. Eight of them died during this period (one child and six adults),

Table II. Age at diagnosis, standard and antiarrhythmic drugs and age at death of children with HCM at our study.

Initials of children	Age at diagnosis	Standard medical therapy	Antiarrhythmic therapy	Age at death
B.L. (male)	19 months	Kaptopril/Enalapirl Furosemide, Aspirin Spironolactone	Flecainide (start) Amiodarone (continue)	32 months
B. A (female)	11 months	Enalapirl, Furosemide, Aspirin Spironolactone	Sotalol (start) Felcainide (continue)	26 months
A.S (male)	8 months	Kaptopril Furosemide, Aspirin Spironolactone	Atenolol (start) + Amiodarone	3 years 4 months
S.D. (male)	33 months	Enalapirl, Furosemide, Aspirin Spironolactone	Sotalol (start) + Amiodarone	6 years 2 months
R.D. (male)	4 years 5 months	Enalapirl, Furosemide	Flecainide	Left Kosovo
I.R. (male)	6 years	Kaptopril Furosemide, Aspirin	Amiodarone (start) Propafenone (continue)	Left Kosovo
T. R. (female)	5 years 6 months	Kaptopril Furosemide	Sotalol	Left Kosovo
O. B. (female)	3 years 8 months	Enalapirl, Furosemide	Propafenone (start) Amiodarone (continue)	Left Kosovo

and all had sudden death. Four children (9%) had LEOPARD syndrome disease, but without pulmonary hypertension. In the remaining patients, etiology of HCM was unknown.

Clinical state at presentation

At presentation, we found evidence of congestive heart failure in 4 children, in 12 children an atypical heart murmur was noted during the routine examination, while, the remaining 27 were referred for cardiac examination following radiographic findings and disturbances in cardiothoracic ratio. The cardiothoracic ratio in this group of children ranged from 44 to 76% with a mean of 58%. In 26 other children, in whom the ratio could accurately be evaluated, it ranged from 59 to 77, with a mean of 65%. In Fig. 1. we presented the echocardiography of the patient with hypertrophic cardiomyopathy.

Medication

All 4 children who presented with cardiac failure received various combinations of standard anti-heart failure drugs. Later in the course of the disease, 3 children received infusions of Amiodarone when they developed ventricular rhythm disturbances and became critically ill. Furosemide and Spironolactone

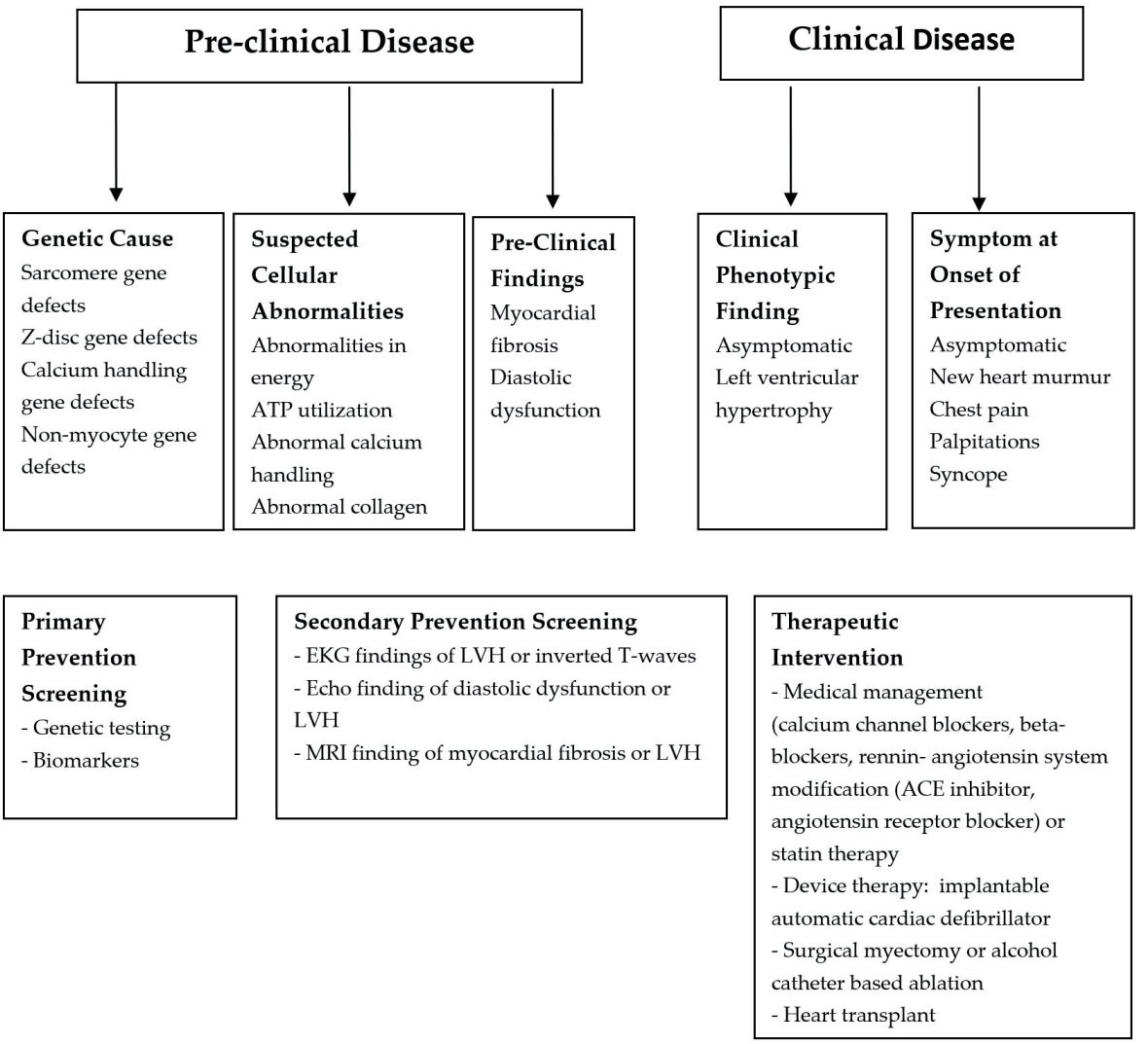


Fig. 1. Screening strategies for hypertrophic cardiomyopathy at each stage of disease.[10]

were standard part of the therapy during the whole time of hospitalization. Twenty-eight children, including 4 children with signs of cardiac failure, received Captopril and 8 of them developed a Captopril-induced intolerable cough, which necessitated the drug replacement by Enalapril. Initially, all patients were treated with a ß-blockers (Propranolol, Atenolol or Sotalol), while Carvedilol was additionally administrated in 4 children, because intolerable hypotension developed. Standard oral antiarrhythmic therapy was based on the using one of oral antiarrhythmic medication (Propafenone, Flecainide, Sotalol or Amiodarone). In 28 patients we instituted empirical treatment with Aspirin.

Discussion

Hypertrophic cardiomyopathy (HCM) is an important disease affecting populations worldwide. It is the most common inherited cardiovascular disorder and the leading cause of sudden death in young people. Several community-based epidemiologic studies have estimated the risk of sudden cardiac death in teenagers and young adults with HCM to be approximately 1% per year.[4] Associated risk factors for sudden cardiac death include: A family history of HCM related premature death, unexplained syncope, a hypotensive or attenuated blood pressure response to exercise, recurrent no sustained ventricular tachycardia and massive left ventricular hypertrophy (a wall thickness > 3 cm). The prevalence of HCM has been estimated to be at most 0.2% in the United States, affecting about 1 in every 500 adults. Based on the data of the high incidence of HCM and often cause of the sudden death some countries have applied screening for HCM as routine examination.[5]

The primary purpose of screening for HCM is to identify affected children before they experience sudden death. Early recognition of the disease, either in the pre-clinical stage (before left ventricular hypertrophy develops) or in the clinical stage (after left ventricular hypertrophy has developed) may allow for earlier treatment with the potential to alter disease progression. A secondary aim of screening would be to identify family members with either pre-clinical or clinical disease, thus offering them the same therapeutic benefits as offered to the index case. As a result of these phenotypic and age-related variations, any diagnostic or screening strategy for HCM must include a variety of components. These range from simple measures such as personal and family history, the physical examination, electrocardiography or trans thoracic echocardiography (Fig.2).[5,6] More

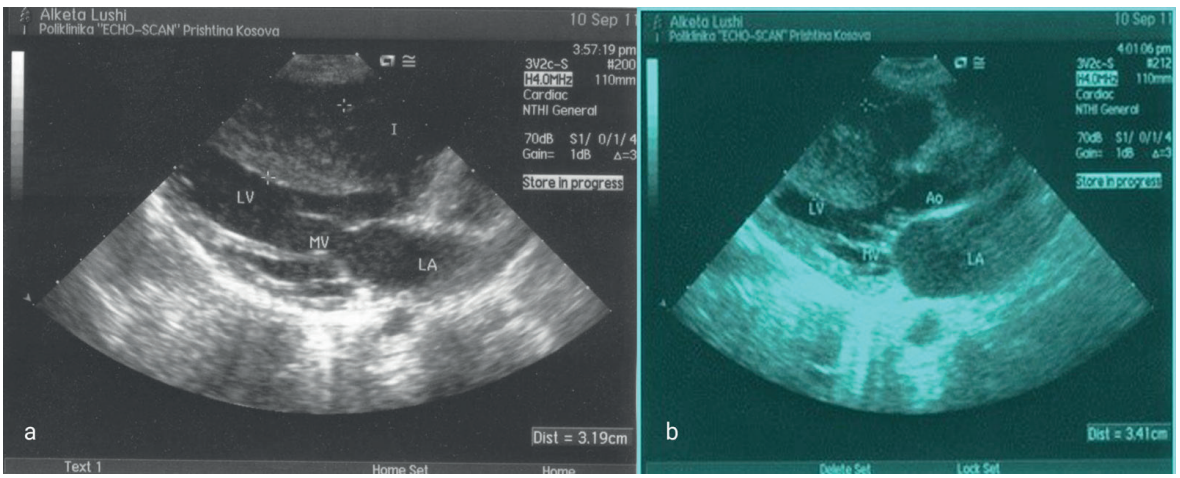


Fig. 2. Parasternal left ventricular long-axis echocardiografic section obtained a patient in diastole (a) and systole (b) with hypertrophic cardiomyopathy.

LV- left ventricle, LA- left atrium, Ao- Aorta, MV- mitral valve.

complex examinations such as cardiac magnetic resonance imaging, biomarkers, and genetic analyses may be appropriate for diagnosis and care in specific cases. The best application of these many modalities is yet to be determined, and will undoubtedly vary between locations, population and availability.[7] During a mean follow-up of 46 months, approximately one-fifth of the children died, others improved but continued to require anti-heart failure and anti-arrhythmic medications. Also, these figures raise the very important question on why the standard medical treatment of the disease is so frequently.

Management of symptoms

Hypertrophic cardiomyopathy is a complex disease with variation in presentation, symptoms, severity, and response to therapy. Medications are often prescribed to treat symptoms and prevent further complications. Medications such as beta-blockers and calcium channel blockers relax the heart muscle, allowing it to fill better and pump more effectively. Other medications may be prescribed as needed to control heart rate or decrease the occurrence of arrhythmias. The goals of the therapy in HCM are symptom control and prolongation of survival. Symptoms such as chest pain, dyspnea, and exercise intolerance can often be managed medically, and surgery has been successful in certain patient groups.[8] The clinical importance of outflow obstruction to the natural history of HCM and the associated symptoms has been highly controversial. The presence of outflow tract obstruction has not been found to be associated with an increased risk of sudden death; patients with outflow tract obstruction are at greater risk for symptoms and progression to death due to heart failure. Although the ability to define the etiology of HCM has improved over time, this goal still remains elusive.[9]

Despite the recent progress in treating children with HCM therapy based on the use of beta-blockers, diuretics, calcium channel blockers, antiarrhythmic drugs (Sotalol, Flecainide, Propafenone, Amiodarone), pacemaker therapy (asynchronous ventricular pacing), implantable cardioverter defibrillator (ICD), in extremely severe forms surgical myectomy and percutaneous radiofrequency septal reduction, survival rate is still low.[3] Our account of HCM, based on our experience at the National Referral Center in Prishtina corroborates the dismal accounts of the disease which have been published previously from other centers.

While many children with HCM are asymptomatic, some typical prognostic profiles are well recognized. One group of patients has symptoms of cardiac failure, including exertional dyspnea, orthopnea, chest pain, and general fatigue. This group of patients has normal or hyper contractile left ventricular function, with or without obstruction of the left ventricular outflow tract (LVOT). While significant obstruction typically causes symptoms, there are also asymptomatic patients who do not have obstructed outflow tract, and symptoms are due to factors such as diastolic dysfunction of the left ventricle, mitral regurgitation or micro vascular dysfunction.[10,11]

A second well recognized group is made up of the patients with atrial fibrillation and its complications, such as embolic stroke. The final group with a typical clinical profile is made up of those who are at risk of sudden cardiac death. Most children in our study with clinical manifestation of HCM belong to the first group (29 children), no child were registered in the second group, and 14 children were registered in the third group, all having family HCM.[12]

Most of the anatomical abnormalities in HCM can be assessed reliably by trans thoracic echocardiography. These include: Abnormal mitral valve motion, a reduction of the anteroposterior dimension of the left ventricular outflow tract and of the left and right ventricular cavities, increased thickness of the interventricular septum and the posterior left ventricular wall. Comparison of the hemodynamic and echocardiography data showed that some degree of abnormal

mitral valve motion during systole may occur in the absence of left ventricular outflow tract obstruction. Other, hitherto unrecognized, abnormalities in HCM detected by this technique are: Aortic valve regurgitation in 1/3 of children with evidence of left ventricular outflow tract obstruction at cardiac catheterization, left ventricular inflow tract obstruction at the mitral valve level associated with gross septal hypertrophy and abnormal forward displacement of the posterior mitral valve leaflet and of the chordae tendineae during systole.[5,6]

Hypertrophic obstructive cardiomyopathy is an uncommon cause of left ventricular outflow tract obstruction in children. In symptomatic patients, with a severe form of LVOT obstruction, open heart surgical myectomy has been the only therapeutic option. Recent data in treating patients with obstructive form of the HCM, using percutaneous radiofrequency septal reduction, as an alternative to surgical myectomy, from many centers showed enviable results, especially after having failed pharmacological therapy.[4,13] Transthoracic and transesophageal Doppler echocardiography is a gold standard to document the degree of myocardial septal hypertrophy and the gradient at rest across the left ventricular outflow tract. Twelve children from our study group developed severe form of obstructive cardiomyopathy and, due to technical limitation; none of the children were treated surgically or by using radiofrequency procedure.[14,15]

Cardiac magnetic resonance imaging (CMRI), is considered the gold standard for determining the physical properties of the left ventricular wall and can serve as an alternative screening tool when an echocardiogram provides inconclusive results, especially in the identification of segmental lateral ventricular hypertrophy where echocardiography cannot be accomplished alone.[16] A limitation of our study is the lack of CMRI application in examination and diagnosis of children with HCM as a result of the deficiency of MRI equipment at our institutional the time of study.

Currently, cardiac transplantation is the ultimate surgical resort for patients who do not respond to medical or surgical treatment. But the option is available only in relatively few centers, most of them in United States of America and in Europe. For the pediatric cardiologist who has no recourse to cardiac transplantation, caring for the child with HCM and treatment-resistant cardiac failure remains a very challenging assignment.[17] Quite often, the choice must be made between continuing treatment with barely effective conventional drugs, adding Carvedilol and Amiodarone, implantation of the implantable cardioverter defibrillator, despite their ill-defined pediatric dosing and lingering uncertainties about efficacy in children. In all probability, the choice will be influenced as much by the available resources as by the embraced philosophies of care.[17,18]

A limitation of our study relates to the diagnosis of pathohistological type of HCM which, for the technical reason, was completely based on the clinical and transthoracic thoracic echocardiography examination. Indeed, the recent statement of the American Heart Association on cardiomyopathies does not recommend endomyocardial biopsy as a test for the diagnosis of disease even though endomyocardial biopsy, evaluated using the Dallas criteria, remains the gold standard for diagnosis. Endomyocardial biopsy is not feasible in most centers that provide care for children with cardiac disease, including HCM.[19,20] The clinical implication of all these factors is that in many centers, including ours, the etiology of HCMis often uncertain. In the absence of etiology, treatment aimed at the cause is either impossible or, at best, empirical.

A limitation of our study with regard to the treatment was also the inability to apply pulse Holter monitoring for accurate diagnosis of the type of heart rhythm disturbances. Pathological findings with pulse Holter monitoring contribute to the prevention of sudden death or to determining the indication for implantation of the ICD. Despite the strong indication for this

treatment and sudden death of 7 members from the same family, none of the children from our study group was treated with the ICD.[21,22]

HCM is often severe in our patients. While the clinical diagnosis is usually easy, and the hemodynamic severity can be ascertained fairly accurately, the etiology is frequently uncertain. The response to standard anti-failure medical and surgical treatment is often unsatisfactory and cardiac transplantation is not feasible. For the time being, the hope for improved survival in our center, and similar centers, are hinged on on-going international efforts to manipulate multifactor mechanisms implicated in HCM.

REFERENCES

1. Lipshultz SE, Sleeper LA, Towbin JA, et al. The incidence of pediatric cardiomyopathy in two regions of the United States. N Engl J Med 2003; 348: 1647-1655.
2. Maron BJ. Hypertrophic cardiomyopathy in childhood. Pediatr Clin North Am 2004; 51: 1305-1346.
3. Colan SD, Lipshultz SE, Lowe AM, et al. Epidemiology and cause-specific outcome of hypertrophic cardiomyopathy in children: findings from the Pediatric Cardiomyopathy Registry. Circulation 2007; 115: 773-781.
4. Bos JM, Towbin JA, Ackerman MJ. Diagnostic, prognostic and therapeutic implications of genetic testing for hypertrophic cardiomyopathy. J Am Coll Cardiol 2009; 54: 201-211.
5. Maron MS, Olivotto I, Betocchi S, et al. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. N Engl J Med 2003; 348: 295-303.
6. Maron BJ. Echocardiographic assessment of left ventricular hypertrophy in patients with obstructive or nonobstructive hypertrophic cardiomyopathy. Eur Heart J 1983; 4(Suppl F) 73-91.
7. Dimitrow PP, Podolec P, Grodecki J, et al. Comparison of dual-chamber pacing with nonsurgical septal reduction effect in patients with hypertrophic obstructive cardiomyopathy. Int J Cardiol 2004; 94: 31-34.
8. Nishimura RA, Holmes DR Jr. Clinical practice. Hypertrophic obstructive cardiomyopathy. N Engl J Med 2004; 350: 1320-1327.
9. Colan SD. Treatment of hypertrophic cardiomyopathy in childhood. Progr Pediatr Cardiol 2011; 31: 13-19.
10. Dadlani GH, Harmon WG, Perez-Colon E, Sokoloski MC, Wilmot I, Lipshultz SE. Diagnosis and screening of hypertrophic cardiomyopathy in children. Progr Pediatr Cardiol 2011; 31: 21-27.
11. Maron BJ. Sudden death in hypertrophic cardiomyopathy. J Cardiovasc Trans Res 2009; 2: 368-380.
12. Hindieh W, Adler A, Weissler-Snir A, Fourey D, Harris S, Rakowski H. Exercise in patients with hypertrophic cardiomyopathy: a review of current evidence, national guideline recommendations and a proposal for a new direction to fitness. J Sci Med Sport 2017; 20: 333-338.
13. Minakata K, Dearani JA, Schaff HV, O'Leary PW, Ommen SR, Danielson GK. Mechanisms for recurrent left ventricular outflow tract obstruction after septal myectomy for obstructive hypertrophic cardiomyopathy. Ann Thorac Surg 2005; 80: 851-856.
14. Semsarian C, Richmond DR. Sudden cardiac death in familial hypertrophic cardiomyopathy: an Australian experience. Aust N Z J Med 1999; 29: 368-370.
15. Elliott P, Spirito P. Prevention of hypertrophic cardiomyopathy-related deaths: theory and practice. Heart 2008; 94: 1269-1275.
16. Spirito P, Autore C, Rapezzi C, et al. Syncope and risk of sudden death in hypertrophic cardiomyopathy. Circulation 2009; 119: 1703-1710.
17. Maron MS, Olivotto I, Zenovich AG, et al. Hypertrophic cardiomyopathy is predominantly a disease of left ventricular outflow tract obstruction. Circulation 2006; 114: 2232-2239.
18. Hess OM, Sigwart U. New treatment strategies for hypertrophic obstructive cardiomyopathy: alcohol ablation of the septum: the new gold standard? J Am Coll Cardiol 2004; 44: 2054-2055.
19. Maron BJ, Yacoub M, Dearani JA. Benefits of surgery in obstructive hypertrophic cardiomyopathy: bring septal myectomy back for European patients. Eur Heart J 2011; 32: 1055-1058.
20. Emmel M, Sreeram N, de Giovanni JV, Brockmeier K. Radiofrequency catheter septal ablation for hypertrophic obstructive cardiomyopathy in childhood. Z Kardiol 2005; 94: 699-703.

21. Elliot PM, Gimeno JR, Tome MT, et al. Left ventricular outflow tract obstruction and sudden death risk in patients with hypertrophic cardiomyopathy. Eur Heart J 2006; 27: 1933-1941.

22. Kofflard MJ, Ten Cate FJ, van der Lee C, van Domburg RT. Hypertrophic cardiomyopathy in a large community-based population: clinical outcome and identification of risk factors for sudden cardiac death and clinical deterioration. J Am Coll Cardiol 2003; 41: 987-993.

The Turkish Journal of Pediatrics 2020; 62: 224-232
https://doi.org/10.24953/turkjped.2020.02.008

Original Article

Does magnetic resonance imaging increase core body temperature in children? Results of the administration of propofol and ketofol: a randomized clinical study

Çiğdem Akyol Beyoğlu[1], Pınar Kendigelen[1], İlhan Beyoğlu[1], Fatiş Altındaş[1], Sebuh Kuruoğlu[2], Güner Kaya[1]

Departments of [1]Anesthesiology and Reanimation and [2]Radiodignostic and Interventional Radiology İstanbul University Cerrahpasa Faculty of Medicine, İstanbul, Turkey.

ABSTRACT

Background and objectives. Magnetic resonance imaging (MRI) may cause a temperature increase in the imaging area, while intravenous anesthetics may develop a tendency for hypothermia, especially in the pediatric population. The effect of different anesthetics on core body temperature in children during these procedures remains controversial. We examined the effect of propofol and ketofol on core body temperatures in a pediatric population during MRI. Our hypothesis was that the increase in body temperature will be more prominent in pediatric patients receiving ketofol than in those receiving propofol.

Methods. This was a randomized, prospective, double-blind study in pediatric patients aged 6 months to 10 years. The patients were American Society of Anesthesiologist (ASA) physical class I–II who had undergone MRI under anesthesia at the Cerrahpasa School of Medicine, MRI Area, between August 2014 and February 2016. Patients were assigned to one of two groups: Group I (propofol group) and Group II (ketofol group). MRIs were performed with a 1.5 Tesla (T) device. Bilateral tympanic membrane temperature measurements before and after the procedure were performed.

Results. Body temperature decreased in both groups after MRI. Clinically significant hypothermia or hyperthermia was not observed in any of the patients.

Conclusion. Temperature monitoring is not necessary for every patient being imaged. However, temperature changes should be closely monitored in high-risk patients.

Key words: magnetic resonance imaging, body temperature, ketofol, propofol, sedation.

Magnetic resonance imaging (MRI) is a commonly used imaging modality for diagnosis in pediatric patients. The imaging area must remain stationary to improve the image quality. Therefore, MRIs are frequently performed on children under general anesthesia or sedoanalgesia.[1] During imaging, the body absorbs radiofrequency (RF) waves emitted from the MRI device, resulting in a temperature increase in the imaging area. This increase can cause a significant rise in body temperature since the ratio of body surface area (BSA) to weight is high in children.[1] However, body temperature can also drop because the environmental temperature during the MRI should be low (20 °C) for the device to work effectively. Active heating devices are not used since they are incompatible with the MRI device, and intravenous (IV) anesthetics negatively affect thermoregulation.[1]

Sedation with IV anesthetics disrupts thermoregulation.[2,3] Most IV anesthetics cause peripheral vasodilatation, resulting in a redistribution of body temperature from central to peripheral body compartments, thereby

✉ Çiğdem Akyol Beyoğlu
akyolbeyoglu@gmail.com

Received 15th September 2018, revised 9th March 2019, 5th April 2019, 18th May 2019, accepted 20th May 2019.

leading to hypothermia.[4-6] Thermodysregulation is frequently observed after the induction of anesthesia with IV propofol, which has a prominent peripheral vasodilation effect.[7-10]

Ketamine is different from other IV anesthetics and does not impair thermoregulation because it stimulates the sympathetic nervous system and increases peripheral vascular resistance.[7,8] It has been shown that the use of ketamine in combination with propofol for maintenance of anesthesia inhibits the development of hypothermia.[7,11]

Our hypothesis was that the increase in body temperature that occurs during MRI will be more prominent in pediatric patients receiving ketofol than in those receiving propofol and, in turn, the risk of hypothermia will be lower in these patients. Our first aim was to evaluate the effects of a ketamine and propofol mixture (ketofol) on body temperature in pediatric patients undergoing imaging with a 1.5 Tesla (T) MRI device. Our second aim was to determine factors, such as age, gender, BSA, imaging area, and shot duration, that may affect body temperature.

Sedative drugs and general anesthetics applied to pediatric patients for immobilization and absorption of RF waves emitted from the MRI device have varying effects on body temperature. By comparing the effects of ketamine and propofol, we think our study can bring new insight to the current literature concerning the confusing effects general anesthetics have on body temperature during MRIs.

Ketamine is different from other general anesthetics as it does not affect body temperature. Unfortunately, to date, there is very little information in the literature concerning the effects of ketamine on body temperature during MRI.

Material and Methods

The study was approved by Cerrahpasa Medical Faculty Ethics Committee on 1 July 2014 (83045809/604.01/01/118099) with written informed consent from parents. The study was registered clinicaltrials.gov with registration number NCT02931786. Then, the study was conducted prospectively, randomly, double-blindly in pediatric patients aged 6 months to 10 years with ASA physical class I-II who underwent MRI under anesthesia. Allergy to IV anesthetics, severe cardiac or pulmonary disease, high intracranial pressure or epilepsy, initial body temperature of 37.5 °C and above were exclusion criteria.

Parents accompany children in the sedation area and anesthesia induction is started with inhalation anesthesia in the meantime parents move to the waiting hall. Sevoflurane inhalation anesthesia was performed after the patients were monitored in the sedation area (HR, SPO2, NIBP). Hemodynamic monitoring continued during MR imaging. All patients underwent iv cannulation together with sevoflurane inhalation induction before entering the MRI unit. 0.1 mg^{-kg} midazolam (Zolamid, Defarma, Turkey) and 0.01 mg^{-kg} atropine (Atropine Sulfate, Biofarma, Turkey) were administered intravenously. Patients were divided into two groups according to the sealed envelope method: Group I (propofol group) and Group II (ketofol group). Propofol 1 mg^{-kg} (Propofol 1%, Fresenius, Germany) was administered intravenously to Group I, and 0.1 ml^{-kg} of a mixture (ketofol) of propofol and ketamine (Ketalar 500 mg/10 ml, Pfizer, USA) was administered intravenously to Group II.

There is 5 mg propofol and 5 mg ketamine in 1 ml of ketofol solution we used in the study.

The sedation level of the patients was evaluated according to the Children's Hospital of Wisconsin Sedation Scale[12] (Table I), and the patients with a sedation score of 3 and below were taken to the MRI unit. Evaluation of sedation level and record of hemodynamic parameters were done by the same anesthesiologist blinded to the study groups. Magnetic resonance imaging was performed with the Siemens MAGNETOM Avanto 1.5T (Siemens Healthcare

Table I. Children's Hospital of Wisconsin Sedation Scale.

6 Spontaneous agitated, anxious, in pain without stimulus
5 Spontaneous awake and calm without stimulus
4 Drowsy with eyes open or closed, easily aroused with mild to moderate verbal stimulus
3 Drowsy, arousable with moderate tactile or loud verbal
2 Can be aroused to consciousness but slow with sustained painful stimulus
1 Can be aroused but not to consciousness with sustained painful stimulus
0 Unresponsive to painful stimuli

Sector, Henkestraße, Erlangen, Germany). For the patients with a sedation score of 4 and above, 0.5 mg^{-kg} propofol was administered intravenously to Group I, and 0.05 ml^{-kg} of a mixture (ketofol) of 5 mg/ml propofol and 5 mg/ml ketamine was administered intravenously to Group II. Bilateral tympanic membrane temperature measurements (Genius TM 2, Covidien MN, USA) before entering the MRI unit were performed and recorded by an anesthesiologist blinded to study groups. Patients were taken to the magnetic room with the same type of single-layer cotton clothes, and headphones were plugged to cover both ears. No patients were actively warmed, and all the patients were covered with a hospital blanket up to their shoulders. The temperature of the magnetic room was kept at 20°C, and the humidity level was <50%. 4 lt/min oxygen was given to the patients with the appropriate type of facemask. Heart rate and peripheral oxygen saturation (SpO2) were monitored during MRI. Noninvasive blood pressure values at the start and end of the procedure were recorded. Also, the additional warning was avoided during MRI.

After MRI was completed, the patients were removed from the magnetic room and bilateral tympanic membrane temperature measurements were repeated. During temperature measurement, the child's auricle was slightly pulled backward and upward by holding its superior part, and thermometer sensor was slightly pushed into the ear. A few seconds after pressing the button which initiates the measurement, it was removed from the ear and the measured value was recorded. Temperature measurements before and after imaging and patient follow-up were performed by the same anesthesiologist. When there was a difference of 1 °C between temperature measurements, the measurement was repeated and the two values were averaged. After the measurement was completed, the sedation score was re-evaluated and re-recorded.

Age, gender, body surface area, imaging area and shot duration of the patients were recorded.

Statistical Data Analysis

Data obtained in the study were analyzed using SPSS (Statistical Package for Social Sciences) for Windows 22.0 program. The number, percentage, mean, standard deviation was used as descriptive statistical methods in the evaluation of the data.

The t-test was used to compare continuous (or quantitative) data between two independent groups. The difference between repeated measures was analyzed by the paired t-test.

The obtained findings were evaluated within a 95% confidence interval and at a significance level of $p < 0.05$.

Sample size estimation:

On the basis of a prior study[1] we considered a difference of 0.5 °C between the two groups after general anesthesia in MRI unit. We decided to include at least 27 patients for each group in the study with a confidence interval of 95% identifying at a two-tailed alpha level of 0.05 (G* Power 3.1.9.4). Therefore, we planned to study a minimum of 60 patients predicting the loss of follow up.

Results

Our study was performed in 90 patients undergoing MRI in the MRI unit of our hospital for various reasons between June 2014 and February 2016. During this time, 5 children with intracranial mass, 6 children with epilepsy, and 4 children with a body temperature of 37.5°C before imaging were excluded from the study. Seventy-five children who fulfilled the inclusion criteria were evaluated in the final analysis (Fig. 1).

There were no significant differences between groups' demographic data in terms of gender distribution, age and body surface area (p= 0.01, p= 0.06, p= 0.06) (Table II).

There was no significant difference between groups in means of peripheral oxygen saturations and heart rates in any time intervals (p>0.5, p>0.5).

There was no significant difference in sedation scores before and after MRI according to groups (p= 0.12, p= 0.55).

While systolic blood pressure (SBP) before MRI was significantly higher in Group II than in Group I, there was no significant difference between the groups in terms of SBP after MRI (p= 0.001, p= 0.809).

While diastolic blood pressure (DBP) before MRI was significantly higher in Group II than

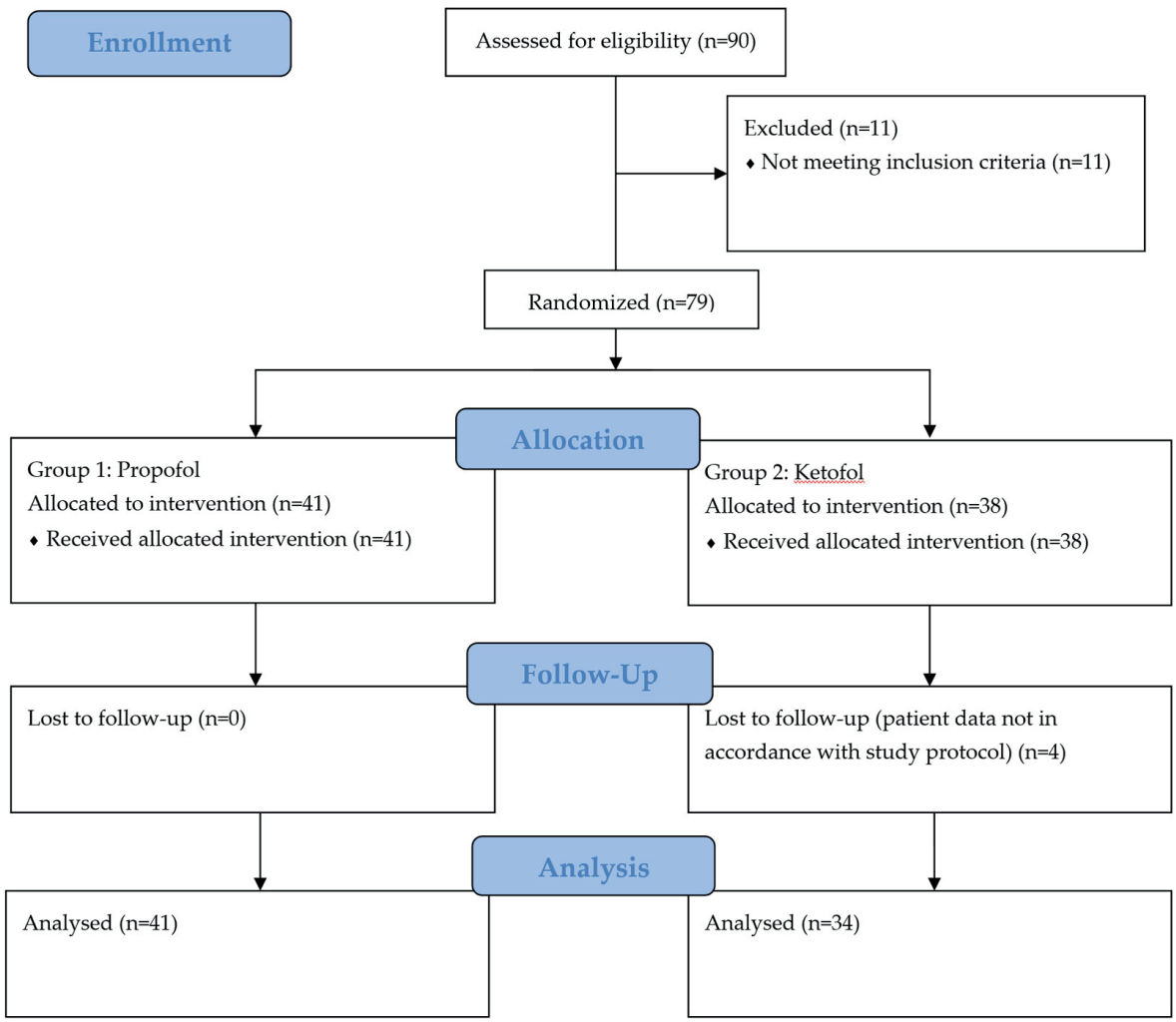


Fig. 1. Flow diagram of the study.

Table II. Demographic data.

	Group I (n: 41) (propofol)	Group II (n: 34) (ketofol)	p
Gender (M/F) *	19/22	21/13	0.01
Age (month)	38.76 ± 19.47	39.94 ± 21.24	0.06
Body Surface Area (kg/m^2)	0.57 ± 0.11	0.62 ± 0.12	0.06

*M: Male, F: Female 15

in Group I, there was no significant difference between the groups in terms of DBP after MRI (p= 0.001, p= 0.068).

While 30 patients underwent MR imaging of the brain and 11 patients underwent MR imaging of the other regions (spine, abdomen, pelvis) in Group I, 14 patients underwent MR imaging of the brain and 20 patients underwent MR imaging of the other regions (spine, abdomen, pelvis) in Group II. The mean shot duration was 21.15 ± 8.7 minutes in Group I and

20.24 ± 6.3 minutes in Group II, respectively. There was no significant difference between the groups in terms of mean shot duration (p= 0.7).

In Group I, the mean right tympanic membrane temperatures before MRI were significantly higher than the mean right tympanic membrane temperatures after MRI (p <0.0001). In Group II, the mean right tympanic membrane temperatures before MRI were significantly higher than the mean right tympanic temperatures after MRI (p <0.022). We found a statistically significant difference between the temperatures of two groups after MRI

(p= 0.001). The temperature decrease was more prominent in Group I compared to Group II (Table III).

In Group I; mean of SBP was 88.4 ± 10.2 before MRI and was 84.5 ± 9.9 after MRI. In Group II; mean of SBP was 96.4 ± 6.7 before MRI and was 85.2 ± 14.4 after MRI.

In Group I; mean of DBP was 49.8 ± 8.5 before MRI and was 48.8 ± 9.1 after MRI. In Group II; mean of DBP was 58.2 ± 13 before MRI and was 52.1 ± 6.9 after MRI.

Propofol or ketofol was additionally given to 3 patients with a sedation score of 4 and above in Group I and to 4 patients with a sedation score of 4 and above in Group II. All the patients were asked to remain completely inactive to obtain high-quality images. None of the patients developed any respiratory or cardiac complications.

Discussion

When 75 pediatric patients included in our study were examined, the tympanic membrane temperatures were significantly decreased after MRI in both groups. However, clinically significant hypothermia or hyperthermia was not observed in any of the patients.

The incidence of hypothermia in children undergoing MRI has not been clearly established to date and thus, remains a subject of debate. Studies on this subject have been mostly performed with oral or rectal sedatives. However, some studies have reported a decrease in body temperature of 21-52% in children under anesthesia during MRI.[9,13,14] Hypothermia should be avoided, especially in premature infants. Preterm infants are more sensitive to the negative effects of cold stress

Table III. The mean right tympanic membrane temperatures before and after MRI according to groups.

Right tympanic membrane temperature (°C)	Group I	Group II	p
Before MRI	36.08 ± 0.45	36.24 ± 0.36	0.09
After MRI	35.67 ± 0.46	36.11 ± 0.44	0.001
p	0.0001	0.022	

due to having thinner skin and limited fat stores.[15]

The results of our study are similar to a study done by Acar et al.[13]. In both studies, body temperatures decreased under MRI, but the decreases were not clinically important.

The results we obtained in our study are not compatible with some previous studies that investigated the effect of MRI on body temperature.[1,16-18] Machata et al.[1] evaluated children aged between 1 month and 6 years, and reported increases in core body temperature with MRI. The reason behind this increase may have been due to the younger age of the patients. Another case by Kussman et al.[18] examined a child scanned for 95 minutes for a cardiac MRI. In this case, it seems that the long scanning duration was responsible for hyperthermia in this patient. Another explanation for this difference may be due to the fact that the magnetic field strength of the MRI device used in some studies is 3 T.[1,9] The MRI device emits RF radiation (RFR) to the body region to be imaged in a strong magnetic field and works on the principle that the tissues absorb these RF waves and return the energy they receive. In a 3 T MRI device with a high magnetic field strength, the imaging area is more rapidly exposed to a stronger magnetic field and thus, the absorption of RF waves by the body and the increase in temperature are more intense in 3 T MRI devices than in 1.5 T MRI devices.[1] In our study, the tympanic membrane temperatures did not increase, possibly because the imaging was performed by an MRI device with a magnetic field strength of 1.5 T.

In a previous study investigating the effects of MRIs performed under anesthesia on body temperature in pediatric patients, there was a body temperature decrease after imaging.[14] Although this is consistent with the results of our study, 1.5 T and 3 T MRI devices were used together in the previous study, and their effects on body temperature were not compared between the 1.5 T and 3 T MRI devices. In our study we used a 1.5 T MRI device on all patients, a fact we believe is important in terms of standardization of the study.

To ensure complete inactivity in the MRI units, IV anesthetics, especially propofol, are frequently used.[19] In some studies where IV anesthetics were not used for sedation, it was reported that body temperatures increased during MRI.[17,18] The thermoregulatory center is depressed with the induction of anesthesia. Moreover, peripheral vasodilatation occurs with other IV anesthetics, except for ketamine, resulting in a redistribution of body temperature from central to peripheral body compartments.[7,8] We found a statistically significant difference between temperature changes of the two groups after MRI. This result suggests that propofol is more effective in producing a body temperature decrease during MRI than ketofol. This finding is in line with the previous literature.[21,22]

The expected effect after the introduction of anesthesia is a decrease in body temperature that is dependent on the drug used and its dose.[7,8] In the current study, we evaluated the level of anesthesia depth according to the Children's Hospital of Wisconsin Sedation Scale and sent only children under deep sedation to the imaging room. We think that since body temperatures decreased in most of the patients in our study, this decrease may be related to the sedation level. The level of anesthesia depth was not recorded in previous studies that showed increases in body temperature after MRIs.[15-17] Young children under deep sedation are more susceptible to hypothermia and thus, body temperature should be monitored in such patients.[22] There is no risk of hypothermia in children undergoing moderate sedation.[22]

The studies in which chloral hydrate and inhalation anesthetics were used for sedation under MRI have typically concluded that MRI increases core body temperature.[14,17] We feel this result, which is incompatible with our study, may be due to the use of chloral hydrate and inhalation anesthetics instead of IV anesthetics for sedation in the past two studies.[14,17] All general anesthetics impair autonomic thermoregulatory

control.[24] However, most studies investigating the effects of inhalation anesthetics on thermoregulation have been performed with halothane, desflurane, and isoflurane.[24-30] In addition, Ozaki et al.[31] showed that age had more of an effect on thermoregulation compared to sevorane/nitrous oxide anesthesia. It has also been reported that isoflurane is more likely to impair thermoregulation compared to sevoflurane.[32] Furthermore, volatile anesthetics do not affect the peripheral shunt flow, which plays a role in heat regulation.[26-33]

Lo et al.[14] did not report how long and under which inhalation anesthetic they used in their study, and they concluded that the decrease in body temperature was associated with a younger age and lower temperature before the MRI. The other patients in their study had an increase in body temperature.[14] Moreover, the mean age of patients was lower in both studies compared to our study.[14,17] The increase in BSA-to-weight ratio, which is inversely proportional to age, may increase the absorption of RF energy from the MRI device.[1,17,18] The IV anesthetics we used in our study may mask the temperature increase resulting from this energy absorption. However, there were only three infants in Group I and four in Group II in our study. Therefore, we think that the higher mean age of the children in our study may have caused a lower RF energy absorption compared to other studies.[14,17]

All IV anesthetics, except for ketamine, disrupt thermoregulation.[7,11] In some studies, the effects of ketamine and other IV anesthetics on the thermoregulatory center were found to be similar.[34,35] In our study, body temperature did not increase in the ketofol group, which may have been due to the low strength of the 1.5 T MRI device. However, another explanation may be that we used a single bolus dose of ketamine and not an infusion. Therefore, we were able to prevent children from having significant hyperthermia in both groups.

In this study, the average age of the children was 38 and 39 months in the ketofol and propofol groups, respectively. The older age may have caused a decreased absorption of RF energy compared to younger children due to the BSA-to-weight ratio, resulting in a more pronounced drop in body temperature under anesthesia.[1] The shot duration was short and similar in both groups. This may be the reason for the lack of profound decreases in temperature in both groups. Blankets were enough to keep body temperatures within safe limits in children undergoing MRI in our study.

One of the limitations of our study is that we did not measure the temperature in body regions other than the head and neck in cases where the cranial region was imaged. The necessity of using devices compatible with the MRI device in imaging units increases the cost and limits the use of certain devices, such as rectal thermometers, that measure temperatures from various body regions. Another limitation of our study is that the strength of the MRI device was 1.5 T. Previous studies on this subject have shown that a 3 T MRI device has a more pronounced effect on body temperature.[1,9,18]

In our study, the effects of propofol and ketofol on temperature changes during MRI were not clinically significant. Although factors such as the characteristics of the MRI device, the age of the patient, and the imaging area are more effective on temperature change, this issue needs to be further evaluated. Temperature monitoring may not be necessary for every patient being imaged; however, temperature changes should be closely monitored, especially in high risk patients. Cold stress causes an increase in oxygen consumption and metabolic acidosis. Therefore, hypothermia brings a potential risk for newborns and infants, especially if they are preterm, due to their thinner skin and limited fat stores. Hyperthermia is also deleterious for brain injury patients.[22] We suggest temperature monitoring of newborns, preterm infants, and brain injury patients during MRI.

REFERENCES

1. Machata AM, Willschke H, Kabon B, Prayer D, Marhofer P. Effect of brain magnetic resonance imaging on body core temperature in sedated infants and children. Br J Anaesth 2009; 102: 385-389.
2. Bissonnette B, Sessler DI. Thermoregulatory thresholds for vasoconstriction in pediatric patients anesthetized with halothane or halothane and caudal bupivacaine. Anesthesiology 1992; 76: 387-389.
3. Sessler DI. Perioperative heat balance. Anesthesiology 2000; 92: 578-596.
4. Matsukawa T, Sessler DI, Sessler AM, et al. Heat flow and distribution of during induction of general anesthesia. Anesthesiology 1995; 82: 662-673.
5. Vassilieff N, Rosencher N, Sessler DI, Conseiller C, Lienhart A. Nifedipine and intraoperative core body temperature in humans. Anesthesiology 1994; 80: 123-128.
6. Kurz A, Sessler DI, Annadata R, Dechert M, Christensen R, Bjorksten AR. Midazolam minimally impairs thermoregulatory control. Anesth Analg 1995; 81: 393-398.
7. Mufti TM. Changes in body temperature during anaesthesia. J Pak Med Assoc 2002; 52: 220-224.
8. Ikeda T, Kazama T, Sessler DI, et al. Induction of anesthesia with ketamine reduces the magnitude of redistribution hypothermia. Anesth Analg 2001; 93: 934-938.
9. Isaacson DL, Yanosky DJ, Jones RA, Dennehy N, Spandorfer P, Baxter AL. Effect of MRI strength and propofol sedation on pediatric core temperature change. J Magn Reson Imaging 2011; 33: 950-956.
10. Tsui BC, Wagner A, Usher AG, Cave DA, Tang C. Combined propofol and remifentanil intravenous anesthesia for pediatric patients undergoing magnetic resonance imaging. Paediatr Anaesth 2005; 15: 397-401.
11. Kinoshita T, Suzuki M, Shimada Y, Ogawa R. Effect of low-dose ketamine on redistribution hypothermia during spinal anesthesia sedated by propofol. J Nippon Med Sch 2004; 71: 9298.
12. Thevaraja AK, Batra YK Rakesh SV, et al. Comparison of low-dose ketamine to midazolam for sedation during pediatric urodynamıc study. Pediatr Anesth 2013; 23: 415-421.
13. Acar HV, Yarkan Uysal H, Gunal Eruyar S, Dikmen B. Body temperature decreases in pediatric patients undergoing magnetic resonance imaging under general anaesthesia. 10AP3-4. Eur J Anaesthesiol 2012: 29: 160.
14. Lo C, Ormond G, McDougall R, Sheppards SJ, Davidson AJ. Effect of magnetic resonance imaging on core body temperature in anaesthetised children. Anaesth Intensive Care 2014; 42: 333-339.
15. Miller RD. Miller's Anesthesia (8th ed). Philadelphia: Saunders, 2015: 2763.
16. Patel A, Davidson M, Aziz R, Taylor T. Pediatric Temperature Variation in the MRI Scanner Under General Anesthesia (Abstract) PA/AAP Pediatric Anesthesiology 2011- San Diego CA: Winter Meeting, 2011.
17. Bryan YF, Templeton TW, Nick TG, Szafran M, Tung A. Brain magnetic resonance imaging increases core body temperature in sedated children. Anesth Analg 2011; 102: 1674-1679.
18. Kussman BD, Mulkern RV, Holzman RS. Iatrogenic hyperthermia during cardiac magnetic resonance imaging. Anesth Analg 2004; 99: 1053-1055.
19. Jenkins E, Hebbar KB, Karaga KK, et al. Experience with the use of propofol for radiologic imaging in infants younger than 6 months of age. Pediatr Radiol 2017; 47: 974- 983.
20. Leslie K, Sessler DI, Bjorksten AR, et al. Propofol causes a dose-dependent decrease in the thermoregulatory threshold for vasoconstriction but has little effect on sweating. Anesthesiology 1994; 81: 353-360.
21. Matsukawa T, Kurz A, Sessler DI, Bjorksten AR, Merrifield B, Cheng C. Propofol linearly reduces the vasoconstriction and shivering thresholds. Anesthesiology 1995; 82: 1169-1180.
22. Diaz M, Becker DE. Thermoregulation: physiological and clinical considerations during sedation and general anesthesia. Anesth Prog 2010; 57: 25-33.
23. Miller RD. Miller's Anesthesia (8th ed). Philadelphia: Saunders, 2015: 1624.
24. Xiong J, Kurz A, Sessler DI, et al. Isoflurane produces marked and nonlinear decreases in the vasoconstriction and shivering thresholds. Anesthesiology 1996; 85: 240-245.
25. Sessler DI, McGuire J, Hynson J, Moayeri A, Heier T. Thermoregulatory vasoconstriction during isoflurane anesthesia minimally decreases cutaneous heat loss. Anesthesiology 1992; 76: 670-675.
26. Kurz A, Xiong J, Sessler DI, Dechert M, Noyes K, Belani K. Desflurane reduces the gain of thermoregulatory arteriovenous shunt vasoconstriction in humans. Anesthesiology 1995; 83: 1212-1219.

27. Kurz A, Plattner O, Sessler DI, Huemer G, Redl G, Lackner F. The threshold for thermoregulatory vasoconstriction during nitrous oxide/isoflurane anesthesia is lower in elderly than in young patients. Anesthesiology 1993; 79: 465-469.

28. Lenhardt R, Negishi C, Sessler DI, Ozaki M, Tayefeh F, Kurz A. Paralysis only slightly reduces the febrile response to interleukin-2 during isoflurane anesthesia. Anesthsiology 1998; 89: 648-656.

29. Lenhardt R, Negishi C, Sessler DI, et al. The effect of pyrogen administration on sweating and vasoconstriction thresholds during desflurane anesthesia. Anesthesiology 1999; 90: 1587-1595.

30. Negishi C, Lenhardt R, Sessler DI, et al. Desflurane reduces the febrile response to administration of interleukin-2. Anesthesiology 1998; 88: 1162-1169.

31. Ozaki M, Sessler DI, Suzuki H, Ozaki K, Atarashi K, Negishi C. The threshold for thermoregulatory vasoconstriction during nitrous oxide/sevoflurane anesthesia is lower in elderly than in young patients. Ann N Y Acad Sci 1997; 15: 789-791.

32. Saito T. A comparison of the body temperature during sevoflurane anesthesia and isoflurane anesthesia. Ann N Y Acad Sci 1997; 15: 786-788.

33. Sessler DI. Perioperative thermoregulation and heat balance. Lancet 2016; 387: 2655-2664.

34. Son SC, Yoon SH. Effects of propofol and ketamine on body temperature during induction of general anesthesia in children. A-618. Eur J Anaesthesiol 2004; 21: 151.

35. López KR, Gibbs PH, Reed DS. A comparison of body temperature changes due to the administration of ketamine-acepromazine and tiletamine-zolazepam anesthetics in cynomolgus macaques. Contemp Top Lab Anim Sci 2002; 41: 47-50.

The Turkish Journal of Pediatrics 2020; 62: 233-243
https://doi.org/10.24953/turkjped.2020.02.009

Original Article

Aortic balloon valvuloplasty and mid-term results in newborns: a single center experience

Birgül Varan[1], Kahraman Yakut[1], İlkay Erdoğan[1], Murat Özkan[2], Kürşat Tokel[1]

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

ABSTRACT

Background and objectives. Aortic balloon valvuloplasty (ABV) has become the first-line treatment for critical aortic valve stenosis in infants. We aimed to evaluate the short- and mid-term results of patients who underwent ABV during neonatal period, the factors affecting the success and complications of the procedure.

Methods. We retrospectively examined 65 patients who underwent ABV during the neonatal period between 1998 and 2017. All hospital records including cardiac catheterization reports, echocardiographic information, and angiographic views were reviewed.

Results. Forty five (69.2%) of the patients were male and mean follow-up was 6.2 ± 4.9 years (range: 6 months - 19 years). The mean age of the patients at the first ABV was 14.5 ± 10.6 days (range: 1-30 days) and body weight was 3.25 ± 0.6 kg (range: 1.5-4.8 kg). The peak systolic gradient measured during pre-valvuloplasty cardiac catheterization was 73.3 ± 22.7 mmHg (range: 30-142 mmHg), and it decreased to 29.2 ± 12.2 mmHg (range: 5-55 mm Hg) after the procedure. Valvuloplasty was successful in 59 (90.7%) patients. There was no more than mild aortic regurgitation in any patient before valvuloplasty. There was mild aortic regurgitation in 21 patients before the valvuloplasty. In the acute phase after valvuloplasty, 30 patients had mild, 15 had moderate and two had severe aortic regurgitation. There was a significant increase in the degree of aortic regurgitation related to valvuloplasty ($p < 0.05$). The most important complication of ABV was increased aortic regurgitation (26.2%). Another important complication was femoral artery occlusion; and was detected early after valvuloplasty (61.6%). There was no serious complication or death in the acute phase.

Conclusions. In newborns with valvular aortic stenosis, balloon valvuloplasty has become the first choice in many centers due to its high success rate, low mortality and morbidity, and increased clinical experience. Aortic regurgitation and femoral artery occlusion were the most important complications. Although reintervention for residual or recurrent aortic valve stenosis is common during the first year after valvuloplasty, these patients are able to reach advanced ages without the need for surgical intervention. Surgical valvotomy is a good alternative treatment for a small number of patients in whom valvuloplasty fails.

Key words: balloon valvuloplasty, aortic valve stenosis, complication.

Aortic balloon valvuloplasty (ABV) is the first-line treatment for neonatal critical or severe aortic stenosis in many centers. Many studies have shown that balloon valvuloplasty effectively reduces the valvular gradient in aortic valve stenosis and the rate of short-term restenosis is low.[1-3] Especially in critically ill newborns, it is a palliative approach that can be applied with lower morbidity and mortality compared with aortic valve surgery. However, it is not always possible to completely eliminate morbidity and mortality with interventional therapy. Aortic valve morphology, the severity of the stenosis, left ventricular structure and function and the degree of regurgitation after valvuloplasty are possible factors determining the outcome.[1,2] The most common complication of valvuloplasty is the increase in aortic regurgitation. Although development of mild to moderate aortic regurgitation after valvuloplasty is frequently reported, severe regurgitation is rare.[1,2]

✉ Birgül Varan
birgulv@hotmail.com

Received 20th February 2019, accepted 15th April 2019.

In this study, we evaluated the efficacy of valvuloplasty in newborns with aortic valve stenosis, complications, factors affecting the development of aortic regurgitation, and the necessity and outcome of repeat valvuloplasty or aortic valve replacement in follow-up. As our series cover babies smaller than or 30 days old it is different from most of the studies which also include babies more than one month old.

Material and Methods

We retrospectively reviewed the data of 65 newborns with a critical or severe aortic valve stenosis who underwent ABV during the first 30 days of life between 1998-2017. All hospital records of the patients, including physical examination findings, cardiac catheterization reports, echocardiography information, and angiographic views were reviewed. Patients who underwent first ABV after the first 30 days of life, patients with missing catheter measurements, or patients with congenital heart disease who were not eligible for biventricular repair were excluded from the study. Both angiographic and echocardiographic examinations were used to classify the aortic regurgitation associated with the procedure. Criteria defined by the American Echocardiography Society were used for echocardiographic classification; 1) absence of aortic regurgitation, 2) mild grade aortic regurgitation, 3) moderate grade aortic regurgitation, and 4) severe grade aortic regurgitation.[4] Grade of aortic regurgitation is assessed by aortic root angiography before and after valvuloplasty and was graded at the time of the procedure based on Seller's criteria.[5,6] Grade 1+ (mild): A small amount of contrast material enters the left ventricle in diastole; it is essentially cleared with each beat and never fills the entire ventricular chamber. Grade 2+ (moderate): contrast enters the left ventricle with each diastole resulting in faint opacification of the entire chamber. Grade 3+ (moderate to severe): The left ventricle is well opacified and equal in density with the ascending aorta. Grade 4+ (severe): Complete dense opacification of the left ventricle in one beat and appears more densely opacified than the ascending aorta.

Aortic valve morphology was classified by echocardiography as monocuspid, bicuspid (functional or anatomic bicuspid) and tricuspid. The presence of endocardial fibroelastosis was assessed by echocardiography. Left ventricular systolic functions were classified according to ejection fraction and shortening fraction data. If the ejection fraction is below 30%, left ventricular systolic function is considered to be severely impaired, between 31-57% moderately impaired, and above 58% normal left ventricular function. The diameter of the aortic annulus was measured by two-dimensional echocardiography and angiography in the left ventricular mid-systolic phase. Valvuloplasty procedure was started with balloons with balloon diameter/annulus diameter ratio of 0.75-0.9. The procedure was continued with the balloon diameter/annulus diameter ratio of 1-1.2 maximum, taking into account the residual transvalvular gradient and the grade of aortic regurgitation and, if necessary, increasing the balloon diameter by 1 mm. Valvular aortic gradient was determined by echocardiography by measuring peak gradient and mean gradient with continuous flow Doppler. In addition, systolic valvular gradient was measured during catheterization. Cardiac catheterization was performed when transthoracic echocardiography revealed a mean gradient of ≥50 mmHg in the aortic valve, ST-T wave change in patients with gradient <50 mm Hg, presence of left ventricular systolic dysfunction, and/or decreased antegrade flow in the aortic valve. The success criteria for aortic valvuloplasty was 30% reduction in systolic pressure gradient, gradient <50 mm Hg in patients with normal cardiac output, decreased left ventricle end diastolic pressure, increased forward flow from the valve, termination of prostaglandin E1 treatment and valvuloplasty-associated moderate or less aortic regurgitation. In all patients with sheath insertion in the femoral artery regardless of the presence of pulse after the procedure, heparin infusion was continued

at the dose of 20 units/kg/hour for at least six hours. Heparin was used as an anticoagulant in the treatment of patients with disturbed circulation of the extremity after venous access. Fibrinolytic therapy (streptokinase or tissue plasminogen activator) was started in patients without femoral pulse after 6 hours of heparin infusion. The loading dose of heparin was 50 units/kg (intravenous), and the maintenance dose was 20 units/kg/hour. For streptokinase, the intravenous loading dose was 3000 units/kg, and the maintenance dose was 1500-2000 units/kg/hour. Tissue plasminogen activator infusion was continued at the dose of 0.2 mg/kg/hour for five hours. During heparinization, activated partial thromboplastin time value was kept between 60-80 seconds. If the fibrinogen level dropped below 100 mg/dl after streptokinase or tissue plasminogen activator treatment, patients were administered fresh frozen plasma (10 ml/kg). Patients with absent pulse and/or disrupted blood flow in the extremities underwent Doppler ultrasonography.

This study was approved by the ethics committee of our university (KA18/338 – 06/11/2018).

Statistical analysis: Statistical analysis was performed using the PASW version 17.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed in mean ± standard deviation (SD), and frequency. A p value less than 0.05 was considered statistically significant.

Results

Diagnosis of aortic stenosis was based on auscultation of a cardiac murmur in 46 patients during a routine visit, 12 were diagnosed after cardiac examination due to respiratory distress and/or cyanosis, five were diagnosed during antenatal period and two were diagnosed after deterioration of the general condition and development of metabolic acidosis. Aortic valve morphology was monocuspid in four patients and bicuspid in 61. A total of 85 aortic balloon valvuloplasties were performed in 65 patients.

At the first valvuloplasty procedure, the mean age of the patients was 14.5 ± 10.6 days (1-30 days) and body weight was 3.25 ± 0.6 kg (1.5-4.8 kg). Before the valvuloplasty, the aortic valve annulus was 6.5 ± 0.9 mm (4-8.8 mm), z score was -0.53 ± 1.59 (-5.43 - 3.06), the mitral valve annulus was 11.1 ± 2 mm (6-15.7 mm), z score was -0.37 ± 1.18 (-4.39 - 2.05). The mean balloon diameter/annulus diameter ratio was 0.96 ± 0.1 (0.75-1.2) (Fig. 1) and the balloon diameter was 6.1 ± 0.6 mm (3.5-7 mm). The stenosis of the aortic valve was critical in 26 (40%) patients. Five patients underwent predilatation with 3.5-4 mm (2 cm length) coronary angioplasty balloons followed by dilatation with 5-6 mm (2 cm) valvuloplasty balloons. A patient with critical valvular aortic stenosis concomitant with aortic arch hypoplasia was dilated with a 3.5 mm (2 cm) coronary angioplasty balloon. In this patient, the procedure was not continued

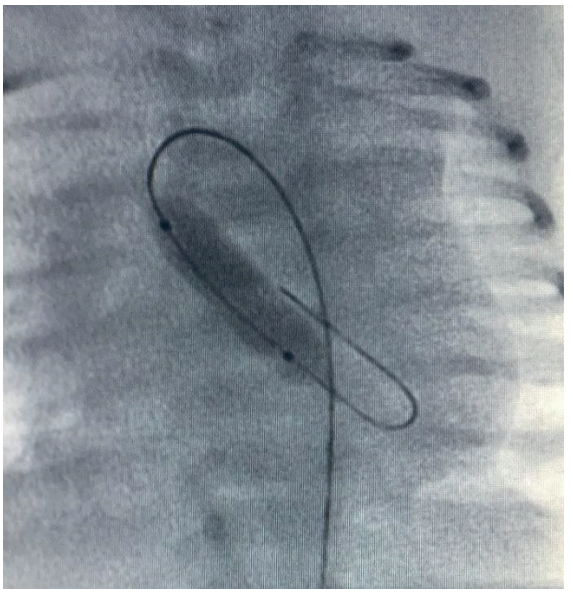


Fig. 1. The angiogram of a 6 day-old newborn with bicuspid aortic valve and severe valvular aortic stenosis. Aortic annulus was measured 6.5 mm. Balloon aortic valvuloplasty was performed by using 6 mm (2 cm) balloon (ballon diameter/annulus diameter: 0.92; Tyshak-II, NuMed Canada Inc., Cornwall, Canada). The peak systolic gradient in the aortic valve regressed from 98 mmHg to 32 mmHg; there was no aortic regurgitation in the aortogram performed after the procedure.

because the aortic annulus was 4 mm and hypoplasic aortic arch was present. Twenty (30.8%) of the patients also had stenosis in other left heart structures. The most common pathologies were aortic coarctation (21.5%), aortic arch hypoplasia (15.6%) and mitral valve stenosis (10.7%). Nine (13.8%) patients had multiple left-sided obstructive lesions in addition to aortic valve stenosis (Table I).

ABV was performed in all patients via femoral artery. In all patients with sheath insertion in the femoral artery regardless of pulse status after the procedure, heparin infusion was continued for at least six hours. Twenty four of 30 patients without palpable pulse received streptokinase, whereas six received TPA. No serious bleeding or circulatory disturbance in the extremities was observed during these treatments. Despite the anticoagulation treatment, there was no palpable femoral pulse in eight of these patients during discharge. There was no circulatory compromise in their extremities. Dysrhytmia developed in three patients during the procedure; supraventricular tachycardia which resolved with adenosine treatment occurred in one patient and spontaneously resolved nonsustained ventricular tachycardia occurred in two patients during the procedure.

The mean peak systolic gradient before and after the procedure, and the mean decrease in the gradient is shown in (Table II). Eight patients (12.3%) had significant heart failure symptoms before the intervention. Seventeen (26.2%) patients received inotropic drug treatment for 8.1 ± 5.8 days (range: 3-23 days) and eight (12.3%) patients received prostaglandin E1 treatment for 3.2 ± 2.1 days (range: 1-7 days). There was no significant association between the decrease in the gradient and the final balloon diameter/annulus diameter ratio ($p > 0.05$). Similarly, there was no significant association between the increase in aortic regurgitation degree and the final balloon diameter/annulus diameter ratio ($p > 0.05$).

There was no significant association between aortic valve morphology and gradient determined before valvuloplasty, decrease in postoperative gradient and increase in aortic regurgitation degree ($p > 0.05$). There was no significant association between aortic valve morphology and severity of aortic stenosis ($p > 0.05$) and with the need for repeat ABV ($p > 0.05$).

Thirty-eight (58.5%) patients had endocardial fibroelastosis. Endocardial fibroelastosis was present in 76.9% of patients with critical aortic stenosis and in 44.7% of patients with non-critical aortic stenosis. There was a significant association between critical aortic stenosis and endocardial fibroelastosis ($p < 0.05$). Patients

Table I. Demographic and clinical characteristics of the patients (N=65).

Characteristics	Results
Female/male, n (%)	20 (30.8) / 45 (69.2)
Age, days	14.5 ± 10.6 (range: 1-30)
Body weight, kg	3.25 ± 0.6 (range: 1.5-4.8)
Follow-up time, years	6.2 ± 4.9
Aortic valve morphology, n (%)	
Bicuspid	61 (93.8)
Monocuspid	4 (6.2)
Aortic arch hypoplasia, n (%)	10 (15.6)
Mitral valve stenosis, n (%)	7 (10.7)
Multiple left-sided obstructive lesions, n (%)	9 (13.8)
Endocardial fibroelastosis, n (%)	38 (58.5)
Moderate left ventricular systolic dysfunction, n (%)	16 (24.6)
Severe left ventricular systolic dysfunction, n (%)	22 (33.8)

Table II. Data and complications related to aortic balloon valvuloplasty procedure.

Features	Results
Pre-ABV catheter peak systolic gradient, mmHg	73.3 ± 22.7 (range: 30-142)
Post-ABV catheter peak systolic gradient, mmHg	29.2 ± 12.2 (range: 5-55)
Decrease in the gradient, mmHg	44.1 ± 20.8 (range: 10-127)
Pre-ABV aortic valve annulus, mm	6.5 ± 0.9 (range: 4-8.8)
Balloon diameter, mm	6.1 ± 0.6 (range: 3.5-7)
Balloon diameter / annulus diameter ratio	0.96 ± 0.1 (range: 0.75-1.2)
Pre-ABV aortic regurgitation moderate and severe	None
Post-ABV aortic regurgitation moderate and severe, n (%)	17 (26.2)
Repeat ABV, n (%)	17 (26.2)
Surgical valvotomy, n (%)	4 (6.2)
Aortic valve replacement, n (%)	7 (10.7)
Ross procedure, n (%)	1 (1.5)
ABV success rate, n (%)	59 (90.7)
Femoral artery occlusion, n (%)	30 (46.2)
Dysrhythmia, n (%)	3 (4.6)

ABV: aortic balloon valvuloplasty

with endocardial fibroelastosis had decreased ejection fraction [47.9 ± 18.8% (range: 18-76%) versus 68.8 ± 11.7% (range: 45-85%), $p < 0.05$)] and shortening fraction value [23.2 ± 11% (range: 8-42%) versus 36.9 ± 9.1% (range: 21-55%), ($p < 0.05$)].

There was no significant difference in the increase of aortic regurgitation degree related to valvuloplasty between the patients with critical aortic valve stenosis and the other patients ($p > 0.05$). Valvuloplasty was repeated at least once in 53.3% of patients with critical aortic valve stenosis and in 20.7% of other patients. The rate of repeat valvuloplasty in patients with critical aortic valve stenosis was significantly higher than the other patients ($p < 0.05$). Surgical intervention was performed in 15.4% of patients with critical aortic valve stenosis and in 15.4% of other patients. There was no significant difference between two groups for requirement of surgical intervention.

In 18.5% of the patients, surgical intervention was performed once to the aortic valve and in 6% of the patients a second operation was performed. In six patients, aortic valve replacement was performed due to severe aortic regurgitation. Vegetation resection and aortic valve replacement was made in one patient due to infective endocarditis. Four patients underwent surgical valvotomy due to unsuccessful valvuloplasty, two patients underwent repeat surgical valvotomy due to recurrence of aortic valve stenosis, two patients underwent reoperation for aortic valve replacement due to prosthetic valve dysfunction, and one patient underwent Ross procedure. In addition, three patients underwent coarctation repair and one patient underwent repair of ascending aorta aneurysm. The first surgery was performed at a mean age of 3.1 ± 3.8 years (range: 26 days-10 years) and the second at a mean age of 10.5 ± 2.5 years (range: 8-14 years).

In patients with critical aortic valve disease, the mean ejection fraction was 44.9 ± 20.6% (range: 18-77%) and the mean shortening fraction was 21.7 ± 11.9% (range: 8-42%). In other patients, the mean ejection fraction was 65.2 ± 13.5% (range: 30-85%) and the mean shortening fraction was 34.1 ± 9.7% (range: 13-54%). In patients with critical aortic valve stenosis, both ejection fraction and shortening fraction values were significantly lower than the other patients ($p < 0.05$).

There was no significant difference in the annulus diameter, valve morphology, balloon diameter/annulus diameter ratio, aortic gradient, reduction in gradient with valvuloplasty procedure, and aortic annulus Z-score between patients with mild to moderate aortic regurgitation related to valvuloplasty and patients with severe aortic regurgitation related to valvuloplasty ($p > 0.05$).

In 14 patients with severe left ventricular systolic dysfunction (ejection fraction ≤30% before valvuloplasty), complete recovery was achieved within the first six months after the procedure. Six patients died without improvement in the ejection fraction, no data were available for two patients. In 16 patients with moderate left ventricular systolic dysfunction (ejection fraction: 31-57% before valvuloplasty), complete recovery was achieved within the first six months after the procedure. Aortic regurgitation before valvuloplasty was mild in 32.8% of patients, and absent in 67.2%. Aortic regurgitation after valvuloplasty was mild in 46.9% of patients, moderate in 23.1% and severe in 3.1%. There was a significant increase in the degree of aortic regurgitation after valvuloplasty ($p < 0.05$). ABV was successful in 59 patients (90.7%). Surgical valvotomy was performed in two patients with critical aortic stenosis because the guidewire and catheter could not be advanced from the valve. Two patients underwent surgical valvotomy as there was no significant reduction in aortic gradient. The procedure was considered unsuccessful in two patients because severe aortic regurgitation developed early after the procedure. Femoral artery occlusion developed in 40 (61.5%) patients, increase in aortic regurgitation in 36 (55.3%), hematoma at the entrance site in one patient, respiratory depression requiring short duration respiratory support in one patient, and convulsion developed in one patient. In the late period, moderate dilatation of the ascending aorta developed in four patients and aneurysmatic dilatation requiring surgical repair was present in one patient. There was no serious complication or death associated with ABV procedure.

Seventeen patients developed re-stenosis during follow-up, second valvuloplasty was performed at a mean of 27.2 ± 45.8 months of age (range: 5 days - 13 years). In one patient, third valvuloplasty was performed at 16 months of age. Freedom from reintervention after valvuloplasty was 71.7% in the first year, 58.8% in the third year, 53.1% in the fifth year, and 26.9% in the tenth year of follow-up.

Six patients died in the first year. One died due to sepsis and multiorgan failure in the fifth day after valvuloplasty. One died due to low cardiac output and cardiac arrest in the early period after the Ross procedure and coarctation repair. The other four patients who died had Shone complex. None died during valvuloplasty. It was determined that all deceased patients had critical valvular aortic stenosis, severe left ventricular dysfunction and other stenotic lesions in left heart structures.

Discussion

Aortic balloon valvuloplasty in neonatal patients is safely performed as a first-line treatment in many centers around the world.[2,7-9] Until the first half of 1980s, the standard treatment approach was surgical valvotomy. Lababidi et al.[10] performed ABV for the first time in 1984. Subsequent studies have reported that ABV is an alternative and effective method to surgical valvotomy.[1,2,11-13] In accordance with the literature, we determined that most patients were diagnosed with cardiac examination on the basis of murmur during routine examination. We have shown that ABV is an effective intervention to reduce the aortic valve gradient in patients with congenital aortic valve stenosis. Many similar studies have reported an effective reduction of the gradient with valvuloplasty and a low rate of restenosis in the early period.[1-3,14,15] Sullivan et al.[2] reported that the most common lesion associated with aortic valve stenosis was aortic coarctation (21%) and mitral valve stenosis (7.8%). In this study, 5.8% of patients had multiple left-sided obstructive lesions in addition to aortic valve

stenosis. In accordance with the literature, the most common obstructive lesions associated with valvular aortic stenosis in our study were aortic coarctation, aortic arch hypoplasia and mitral valve stenosis.

Few studies have reported that ABV was performed with access through the femoral vein, umbilical artery or umbilical vein.[2,14,16] As in our study, valvuloplasty was performed via femoral artery in many studies.

Sullivan et al.[2] reported that mean peak systolic gradient was 61 ± 23 mmHg before, and 18 ± 9 mmHg after valvuloplasty. The acute decrease in the gradient was 43 ± 21 mmHg. We also found that the residual gradient was at acceptable level after valvuloplasty. In our patients, valvuloplasty was successful at a high rate (90.7%). In six patients the procedure was considered unsuccessful. There was no significant decrease in aortic gradient in two patients. We failed to advance the guidewire through the valve in two and they underwent surgery. Severe aortic regurgitation developed in two patients after the procedure. Surgical valvotomy was performed successfully in four patients. Surgical valvotomy is a good treatment option for thick, dysplastic valves through which a guidewire can not be advanced.

Several complications related to ABV such as aortic regurgitation, femoral artery thrombosis, left ventricular perforation, pericardial tamponade, aortic perforation, excessive blood loss, life-threatening arrhythmia and death have been reported.[1,2,11,17,18] Balloon valvuloplasty-related mortality is reported in critical aortic valve stenosis, severe left ventricular systolic dysfunction, or accompanying complex anomalies.[19,20] In studies comparing surgical valvotomy with percutaneous valvuloplasty, early mortality rate in the surgical group is reported as 10-28.5% whereas it was 0-16.6% in the valvuloplasty group, although the long-term results of both procedures are similar.[1,12,13] In recent years, early mortality related to surgical valvotomy in newborns has been reported at lower rates, but there are also reports giving rates as high as 20-30% and this is extremely high.[12,21] Torres et al.[1] reported no death because of valvuloplasty in their multicenter study. Sullivan et al.[2] also reported no death in 76 newborns. High mortality rates are reported in case of accompanying stenotic left heart pathologies.[22,23] The mortality rate was 9.3% in our study. All of the patients who died had critical aortic stenosis, severe left ventricular dysfunction or had stenotic left heart lesions. In our study, the most important complications of ABV was increased aortic regurgitation and femoral artery occlusion. There was no serious complication or death associated with ABV.

Vascular injury or obstruction are the most common problems in these patients. Vascular complications are significantly reduced with anticoagulant and/or fibrinolytic treatment. We observed that anticoagulant and/or fibrinolytic drugs can be safely used in newborns for the treatment of obstruction in the femoral artery during interventional procedures.

Severe dysrhythmia related to valvuloplasty may be seen in patients with impaired left ventricular systolic function. Ventricular tachycardia, ventricular fibrillation, supraventricular tachycardia, and atrioventricular block are frequently reported. The incidence of dysrhythmia has been reported as 1-13% in different series.[14,21] There was no serious dysrhythmia resulting in mortality or morbidity in our series.

The increase in valvuloplasty-associated aortic regurgitation was reported as 43% in the series of Labadibi, 7-59% in different studies.[1,2,10,11] The incidence of moderate and severe aortic regurgitation was 2.2-29%.[1-3,11,14] The development of aortic regurgitation after valvuloplasty in newborns is more frequent.[2,14,24] Fratz et al.[24] reported that the incidence of moderate and severe aortic regurgitation after valvuloplasty was 29% in newborns and 19% in babies older than one months of age. Sullivan et al.[2] also reported that the incidence of moderate and severe aortic regurgitation after valvuloplasty was 16% in newborns and

9% in others. While the effect of large diameter balloons on aortic regurgitation in neonates is not yet clear, balloons with a final balloon/annulus diameter ratio of 0.9-1 have been used in many studies.[2,8] Lewin et al.[26] reported that there was a significant correlation between frequency of moderate-severe aortic regurgitation and the ratio of balloon diameter/annulus diameter. However, Hamidi-Manesh et al.[25] showed that there was no significant correlation. Reich et al.[17] reported that a functional bicuspid aortic valve is an independent risk factor for the appearance of aortic regurgitation after valvuloplasty. In our study, the aortic regurgitation related to valvuloplasty was moderate in 23.1% and severe in 3.1% of infants. There was no significant association between the increase in aortic regurgitation and the balloon diameter/annulus diameter ratio. This may be so because we kept the balloon diameter as low as possible. To prevent moderate-to-severe aortic regurgitation it is important to perform a careful measurement of the annulus diameter by both echocardiography and angiography, to initiate valvuloplasty with a smaller diameter balloon, to check for pressure and aortic regurgitation after each procedure and, if necessary, to use a larger diameter balloon. In order to choose the balloon diameter we use both echocardiographic and angiographic measurements. Considering a smaller measurement may be useful in reducing aortic regurgitation.

Endocardial fibroelastosis causes considerable volume and size loss with diastolic dysfunction in the left ventricle.[3,27] Although biventricular repair can be performed in patients with borderline left ventricular structure[19], there are studies reporting that this is an important risk factor for mortality.[28,29] In our study, we determined left ventricular systolic dysfunction, borderline left ventricular structure and endocardial fibroelastosis as important risk factors for mortality.

Soulatges et al.[3] reported freedom from surgical intervention and transcatheter intervention as 72.9% and 54%, respectively in 37 newborns at a mean follow-up of 11 years. Rossi et al.[30] also reported freedom from surgical and transcatheter intervention as 47% and 59%, respectively at a mean follow-up of 10 years. In many studies, freedom from reintervention (surgery or transcatheter) after valvuloplasty was reported as 40-60%.[3,9,30-33] In our study, the results were similar to the literature. In accordance with previous studies, severe aortic regurgitation and recurrent aortic valve stenosis were the most important reasons for late surgical intervention in our series.[2,24,27]

It has been reported that the presence of endocardial fibroelastosis in patients with congenital aortic valve stenosis causes left ventricular systolic and diastolic dysfunction and increased mortality.[34-36] Same was true for our study. We determined that left ventricular systolic dysfunction was both more frequent and more severe in patients with critical aortic valve stenosis. The need for inotropic support and length of stay in hospital were significantly prolonged in patients with left ventricular systolic dysfunction after valvuloplasty.

The success of valvuloplasty was found to be associated with the diameter of the valve annulus.[37,38] In our study, we found no association with annulus diameter, but there was an association between the need for reintervention and the diameter of the annulus in the mid-term follow-up. Many studies have reported that the duration of hospital stay after valvuloplasty was shorter, morbidity and mortality were less, but the need for reintervention was higher.[19,21] In our series, the duration of hospital stay of patients undergoing valvuloplasty who had no additional cardiac pathology was very short.

Aortic valve replacement is required due to progressive severe aortic regurgitation during or after valvuloplasty.[2,24] The incidence of severe aortic regurgitation in the long-term has been reported as 21-38%.[2,39] Nine of our patients (13.8%) developed severe aortic regurgitation at follow-up. In six of them, aortic valve replacement was performed due to progressive increase in aortic regurgitation and left

ventricular dilatation. Three patients remained in follow-up. The data show that valvuloplasty has a low mortality risk in the mid- to long-term. However, it has significant risks such as valve dysfunction and aortic valve replacement in the long-term.[2,9] Sullivan et al.[2] reported that aortic valve replacement was not required in 45% of newborns at 15 years of follow-up after ABV. Maskatia et al.[9] also reported that aortic valve replacement was not required in 70% and 61% of patients at 10 and 15 years of follow-up after ABV, respectively. In our series, six patients (9.2%) underwent aortic valve replacement due to severe aortic regurgitation, one (1.5%) underwent Ross procedure and coarctation repair at a mean follow-up of 6.2 ± 4.9 years after valvuloplasty. In addition, five of these patients underwent Konno procedure and one patient underwent repair of ascending aorta aneurysm.

In many studies residual aortic stenosis and acute aortic regurgitation after valvuloplasty are found to be the most important risk factors for aortic valve replacement in the long-term.[2,9,31] Therefore, it is very important to identify correctable risk factors determining long-term valve functions. Risk factors associated with aortic valve replacement should be considered when deciding whether to continue procedure in the residual aortic stenosis. In our study, we determined that the most important risk factor associated with aortic valve replacement was moderate-severe aortic regurgitation, which developed after the procedure and continued during the follow-up. We prefer to terminate the procedure in the gray zone of 40-50 mmHg gradient for residual aortic stenosis in order to avoid increase in the aortic regurgitation.

One of the most important problems for the operator is the persistence of high residual AS after valvuloplasty in case of dysplastic and thick valves. Should the procedure continue with larger balloons, with the risk of aortic regurgitation or should the procedure be terminated? Sullivan et al.[2] found that patients with moderate or severe acute aortic regurgitation and a residual AS gradient <30 mmHg after valvuloplasty had an approximately three times greater risk of requiring AVR compared to those patients with a residual AS gradient ≥30 mmHg and mild or less aortic regurgitation. In addition, all patients with acute moderate or severe aortic regurgitation underwent aortic valve replacement at 15 years of follow-up while aortic valve replacement was not required in 52% of cases with high residual gradient and mild or less aortic regurgitation.[2] In our study, 45% of cases with residual gradient <40 mmHg in the acute period and moderate-severe aortic regurgitation underwent aortic valve replacement. On the other hand, aortic valve replacement was performed in 6.2% of cases with residual gradient ≥40 mmHg in the acute period and mild or no aortic regurgitation. However, we think that there is insufficient data to determine risk factors for aortic valve replacement and re-intervention types because the number of cases was low and the mean follow-up period was 6.2 ± 4.9 years. We believe that follow-up with tolerable residual aortic stenosis after valvuloplasty is better than severe acute post-valvuloplasty aortic regurgitation to avoid aortic valve replacement in the mid and long-term.

A retrospective study is a significant disadvantage in the collection and evaluation of data. The change of the pediatric cardiologist and the surgical team performing the interventional procedures during 18 years may have caused differences in patient selection and treatment strategy. At the same time, the progress in medical equipment technology during this period might have affected the results. The number of patients (65 newborns) was a significant advantage, but the number of patients undergoing surgical valvotomy was low and comparison of surgical valvatomy with balloon valvuplasty was not possible.

In conclusion, the choice of valvuloplasty or surgical intervention in valvular aortic stenosis depends on the experience of the center. Recently, the initial treatment of choice in newborns is ABV because of the lower mortality. The prognosis of the patient is closely related to the left ventricular structure and the

development of aortic regurgitation. Although valvuloplasty is a safe and effective procedure in the treatment of congenital aortic stenosis, it should be known that it involves significant risks such as regurgitation, requirement for reintervention, and aortic valve replacement.

REFERENCES

1. Torres A, Vincent JA, Everett A, et al. Balloon valvuloplasty for congenital aortic stenosis: multicenter safety and efficacy outcome assessment. Catheter Cardiovasc Interv 2015; 86: 808-820.
2. Sullivan PM, Rubio AE, Johnston TA, Jones TK. Long-term outcomes and re-interventions following balloon aortic valvuloplasty in pediatric patients with congenital aortic stenosis: a single-center study. Catheter Cardiovasc Interv 2017; 89: 288-296.
3. Soulatges C, Momeni M, Zarrouk N, et al. Long term results of balloon valvuloplasty as primary treatment for congenital aortic valve stenosis: a 20-year review. Pediatr Cardiol 2015; 36: 1145-1152.
4. Zoghbi WA, Enriquez-Sarano M, Foster E, et al; American Society of Echocardiography. Recommendations for evaluation of the severity of native valvular regurgitation with two dimensional and Doppler echocardiography. J Am Soc Echocardiogr 2003; 16: 777-802.
5. Michel PL, Vahanian A, Besnainou F, Acar J. Value of qualitative angiographic grading in aortic regurgitation. Eur Heart J 1987; 8: 11-14.
6. Sellers RD, Levy MJ, Amplatz K, Lillehei CW. Left retrograde cardioangiography in acquired cardiac disease: technique, indications and interpretations in 700 cases. Am J Cardiol 1964; 14: 437-447.
7. Stapleton GE. Transcatheter management of neonatal aortic stenosis. Cardiol Young 2014; 24: 1117-1120.
8. Ewert P, Bertram H, Breuer J, et al. Balloon valvuloplasty in the treatment of congenital aortic valve stenosis-a retrospective multicenter survey of more than 1000 patients. Int J Cardiol 2011; 149: 182-185.
9. Maskatia SA, Ing FF, Justino H, et al. Twenty-five year experience with balloon aortic valvuloplasty for congenital aortic stenosis. Am J Cardiol 2011; 108: 1024-1028.
10. Lababidi Z, Wu JR, Walls JT. Percutaneous balloon aortic valvuloplasty: results in 23 patients. Am J Cardiol 1984; 53: 194-197.
11. Maskatia SA, Justino H, Ing FF, Crystal MA, Mattamal RJ, Petit CJ. Aortic valve morphology is associated with outcomes following balloon valvuloplasty for congenital aortic stenosis. Catheter Cardiovasc Interv 2013; 81: 90-95.
12. McCrindle BW, Blackstone EH, Williams WG, et al; and the members of the Congenital Heart Surgeons Society. Are outcomes of surgical versus transcatheter balloon valvotomy equivalent in neonatal critical aortic stenosis? Circulation 2001; 104(12 Suppl 1): I152-I158.
13. Zain Z, Zadinello M, Menahem S, Brizard C. Neonatal isolated critical aortic valve stenosis: balloon valvuloplasty or surgical valvotomy. Heart Lung Circ 2006; 15: 18-23.
14. Akdeniz C, Erdem A, Demir F, et al. Our medium-term results with aortic balloon valvuloplasty and factors affecting development of aortic regurgitation. Anadolu Kardiyoloji Dergisi 2011; 11: 329-335.
15. Tokel NK, Ekici E, Kutsal A, İkizler C. Konjenital aort darlıklarında balon valvüloplasti: Erken ve orta dönemli sonuçlar. Türk Kardiyoloji Derneği Arşivi 1997; 25: 108-113.
16. Nişli K, Karaca S, Dindar A. Balloon valvuloplasty for aortic stenosis using umbilical vein access in a newborn: first experience in Turkey. Turk Kardiyol Dern Ars 2016; 44: 521-523.
17. Reich O, Tax P, Marek J, et al. Long term results of percutaneous balloon valvoplasty of congenital aortic stenosis: independent predictors of outcome. Heart 2004; 90: 70-76.
18. Balmer C, Beghetti M, Fasnacht M, Friedli B, Arbenz U. Balloon aortic valvoplasty in paediatric patients: progressive aortic regurgitation is common. Heart 2004; 90: 77-81.
19. McElhinney DB, Lock JE, Keane JF, Moran AM, Colan SD. Left heart growth, function, and reintervention after balloon aortic valvuloplasty for neonatal aortic stenosis. Circulation 2005; 111: 451-458.
20. Latiff HA, Sholler GF, Cooper S. Balloon dilatation of aortic stenosis in infants younger than 6 months of age: intermediate outcome. Pediatr Cardiol 2003; 24: 17-26.
21. Hill GD, Ginde S, Rios R, Frommelt PC, Hill KD. Surgical valvotomy versus balloon valvuloplasty for congenital aortic valve stenosis: a systematic review and meta-analysis. J Am Heart Assoc 2016; 5: e003931.
22. Crespo D, Miró J, Vobecky SJ, et al. Experience in a single centre with percutaneous aortic valvoplasty in children, including those with associated cardiovascular lesions. Cardiol Young 2009; 19: 372-382.

23. Brown DW, Dipilato AE, Chong EC, et al. Sudden unexpected death after balloon valvuloplasty for congenital aortic stenosis. J Am Coll Cardiol 2010; 56: 1939-1946.

24. Fratz S, Gildein HP, Balling G, et al. Aortic valvuloplasty in pediatric patients substantially postpones the need for aortic valve surgery: a single-center experience of 188 patients after up to 17.5 years of follow-up. Circulation 2008; 117: 1201-1206.

25. Hamidi-Manesh L, Tibby SM, Herman R, Rosenthal E, Qureshi SA, Krasemann T. Influence of balloon size on aortic regurgitation in neonates undergoing balloon aortic valvuloplasty-a retrospective study over an 11-year period. J Interv Cardiol 2013; 26: 200-207.

26. Lewin RF, Dorros G, King JF, Seifert PE, Schmahl TM, Auer JE. Aortic annular tear after valvuloplasty: the role of aortic annulus echocardiographic measurement. Cathet Cardiovasc Diagn 1989; 16: 123-129.

27. Tuo G, Khambadkone S, Tann O, et al. Obstructive left heart disease in neonates with a "borderline" left ventricle: diagnostic challenges to choosing the best outcome. Pediatr Cardiol 2013; 34: 1567-1576.

28. Lofland GK, McCrindle BW, Williams WG, et al. Critical aortic stenosis in the neonate: a multi-institutional study of management, outcomes, and risk factors. J Thorac Cardiovasc Surg 2001; 121: 10-27.

29. Han RK, Gurofsky RC, Lee KJ, et al. Outcome and growth potential of left heart structures after neonatal intervention for aortic valvestenosis. J Am Coll Cardiol 2007; 50: 2406-2414.

30. Rossi RI, Manica JL, Petraco R, Scott M, Piazza L, Machado PM. Balloon aortic valvuloplasty for congenital aortic stenosis using the femoral and the carotid artery approach: a 16-year experience from a single center. Catheter Cardiovasc Interv 2011; 78: 84-90.

31. Brown DW, Dipilato AE, Chong EC, Lock JE, McElhinney DB. Aortic valve reinterventions after balloon aortic valvuloplasty for congenital aortic stenosis intermediate and late follow-up. J Am Coll Cardiol 2010; 56: 1740-1749.

32. d'Udekem Y, Siddiqui J, Cameron CS, et al. Long-term results of a strategy of aortic valve repair in the pediatric population. J Thorac Cardiovasc Surg 2013; 145: 461-469.

33. Petit CJ, Maskatia SA, Justino H, Mattamal RJ, Crystal MA, Ing FF. Repeat balloon aortic valvuloplasty effectively delays surgical intervention in children with recurrent aortic stenosis. Catheter Cardiovasc Interv 2013; 82: 549-555.

34. Miyamoto T, Sinzobahamvya N, Wetter J, et al. Twenty years experience of surgical aortic valvotomy for critical aortic stenosis in early infancy. Eur J Cardiothorac Surg 2006; 30: 35-40.

35. Friedman KG, Schidlow D, Freud L, Escobar-Diaz M, Tworetzky W. Left ventricular diastolic function and characteristics in fetal aortic stenosis. Am J Cardiol 2014; 114: 122-127.

36. Han RK, Gurofsky RC, Lee KJ, et al. Outcome and growth potential of left heart structures after neonatal intervention for aortic valve stenosis. J Am Coll Cardiol 2007; 50: 2406-2414.

37. Podnar T, Berden P, Vesel S. Balloon dilation of neonatal critical aortic valvar stenosis via the umbilical artery. Cardiol Young 2009; 19: 278-281.

38. Kim DW, Raviele AA, Vincent RN. Use of a 3 French system for balloon aortic valvuloplasty in infants. Catheter Cardiovasc Interv 2005; 66: 254-257.

39. Moore P, Egito E, Mowrey H, Perry SB, Lock JE, Keane JF. Midterm results of balloon dilation of congenital aortic stenosis: predictors of success. J Am Coll Cardiol 1996; 27: 1257-1263.

The Turkish Journal of Pediatrics 2020; 62: 244-251
https://doi.org/10.24953/turkjped.2020.02.010

Original Article

Clinically isolated syndrome and multiple sclerosis in children: a single center study

Gonca Bektaş[1], Melis Ulak Özkan[2], Edibe Pembegül Yıldız[2], Tuğçe Aksu Uzunhan[2], Serra Sencer[3], Nur Aydınlı[2], Mine Çalışkan[2], Meral Özmen[2]

[1]Department of Pediatric Neurology, Bakırköy Dr. Sadi Konuk Research and Training Hospital, İstanbul; Departments of [2]Pediatric Neurology and [3]Neuroradiology, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey.

ABSTRACT

Background and objectives. This study was conducted to determine the differences in clinical and radiological features at the first demyelinating event in children with clinically isolated syndrome (CIS) and multiple sclerosis (MS).

Methods. This was a single center retrospective cohort study of the children with CIS followed-up at Istanbul University Faculty of Medicine, Department of Pediatric Neurology, between 2010 and 2018. Children with CIS who were assessed at 3, 6, 12 and 24 months following their first identified demyelinating event were included. Demographic data, mode of presentation and the presence of the oligoclonal band in the cerebrospinal fluid (CSF) were abstracted from the medical records. Magnetic resonance imaging of the brain and spinal cord was analyzed for the location, number, size and gadolinium enhancement of the lesions.

Results. A total of 51 patients' data was assessed, 38 patients at a mean age of 12.3 years were enrolled in the study. Twenty-seven children (71%) evolved into clinically definite MS after a mean follow-up of 11 months. Older age at first demyelinating event and the presence of the oligoclonal band in CSF were tended to be more common in patients with MS than patients with CIS ($p < 0.05$). The increased number of T2-hyperintense lesion and the presence of the lesion in periventricular, infratentorial and corpus callosum were associated with a tendency for development of MS ($p < 0.05$).

Conclusion. Older age at first demyelinating event, the presence of the oligoclonal band in CSF, the number and localization of T2-hyperintense lesion were associated with a tendency for development of MS.

Key words: child, clinically isolated syndrome, multiple sclerosis.

Multiple sclerosis (MS), an acquired inflammatory demyelinating disease of the central nervous system (CNS), has its onset during childhood in 3-5% of MS patients.[1] The treatment approach focuses on prevention of demyelinating event recurrence, which may lead to severe physical disability. The International Pediatric Multiple Sclerosis Study Group (IPMSSG) has suggested initiating immunomodulatory therapy promptly in children diagnosed with MS to prevent disease progression.[2]

Clinically isolated syndrome (CIS), an inflammatory demyelinating disease of the CNS, refers to the first clinical event of the CNS leading to symptoms and signs for at least 24 h.[3] The studies on adult patients with CIS stated the positive prognostic effect of early initiation of immunomodulatory therapy prior to MS diagnosis.[4,5] However, immunomodulatory treatment has not been suggested in children with CIS. Treatment approaches may improve by identifying children with a higher risk for developing MS.

✉ Gonca Bektaş
goncabektas@gmail.com

Received 28th February 2019, revised 16th May 2019, accepted 21st June 2019.

In this study, we aimed to determine the differences in clinical and radiological features at the first demyelinating event in children with CIS and MS and to identify children with CIS at higher risk of developing MS.

Material and Methods

This was a single center retrospective cohort study of the children with CIS followed-up at the Istanbul University Faculty of Medicine, Department of Pediatric Neurology, in Turkey between January 1, 2010, and January 31, 2018.

Participants

Children with CIS according to the revised International Pediatric Multiple Sclerosis Study Group criteria who had a clinical and radiological assessment at 3, 6, 12 and 24 months following their first identified demyelinating event were included.[6,7] CIS was diagnosed according to following criteria; (1) clinical CNS event with presumed inflammatory demyelinating cause which could be monofocal or polyfocal, (2) the first CNS demyelinating disease, (3) no encephalopathy, (4) baseline MRI which has not fulfilled the requirements to diagnose MS.[7] The exclusion criteria was the presence of clinical and radiological features resembling neuromyelitis optica disorder at the first demyelinating event (e. g. bilateral optic neuritis, longitudinally extensive transverse myelitis). Children were diagnosed with clinically definite MS in case of 2 or more nonencephalopathic clinical events with presumed inflammatory cause occurring in a 30 day interval in presence of MRI evidence of dissemination in space, or 1 nonencephalopathic clinical event with MRI evidence of dissemination in space and in which follow-up MRI emerges at least one new lesion.[6,7] None of the patients with CIS received immunomodulatory drugs prior to MS diagnosis.

The study was reviewed and approved by the Institutional Ethics Committee at the Istanbul University Faculty of Medicine (Date: 08.11.2017, No: 13). All participants and/or their legal representatives provided a written informed consent and assent.

Data collection

Demographic and clinical data including age, sex, mode of presentation, the season of the first event, and cerebrospinal fluid (CSF) oligoclonal band status were abstracted from the medical records. The season of the first event was classified as winter (Dec 1–Feb 28), spring (March 1–May 30), summer (June 1–Aug 30), or autumn (Sept 1– Nov 31). All CSF specimens were analyzed at Istanbul University Faculty of Medicine by isoelectric focusing.

The required magnetic resonance imaging (MRI) protocol was 1.5 Tesla brain MRI which consisted of T2-weighted/FLAIR, pre- and post- gadolinium T1-weighted sequences in the sagittal and axial planes. Spinal cord MRI scans were in the axial and sagittal planes on Tl-weighted and T2-weighted imaging. MRI of the brain and spinal cord was analyzed by an experienced neuroradiologist blinded to the clinical features for the location, number, size and gadolinium enhancement of the lesions.[6] White matter hyperintensities greater than 5 mm in diameter on T2-weighted or FLAIR MRI images were counted. Lesions greater than 2 cm in diameter were classified as giant lesions, and lesions involving more than two adjacent gyri were considered as confluent lesions.

Statistical analysis

Quantitative variables were described as mean and standard deviation. Frequencies and percentages were calculated. Qualitative clinical and MRI characteristics were compared using Fischer's exact test, and quantitative clinical and MRI characteristics were compared using the Student's t-test. $p < 0.05$ was accepted as statistically significant. All statistical analyses were performed using SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA).

Results

A total of 51 patients' data was assessed for eligibility, 38 patients at a mean age of 12.3 years (range 3 to 17, standard deviation [SD] 3.4) were enrolled in the study. Twenty-seven children (71%) evolved into clinically definite MS after a mean follow-up of 11 months (range 1 to 61 months, SD 13.3). The earliest MS development occurred in a boy aged 12-years-old who presented with diplopia and horizontal right conjugate gaze palsy. The baseline axial FLAIR MRI showed the hyperintense lesions in the periventricular white matter and brainstem and diffusion-weighted and apparent diffusion coefficient imaging showed high signal intensity resembling stroke in the brain stem. Therefore, we performed a follow-up MRI. He was diagnosed with MS by emerging new T2-hyperintense lesions on brain MRI 1 month after the first event, type II pattern was observed by isoelectric focusing of the CSF (Fig. 1).

The remaining 11 children (29%) showed disease progression neither clinically nor radiologically during a total of 7 years of follow-up (mean 4.9 years, SD 1.8). Demographic and clinical features of children with CIS and MS are presented in Table I.

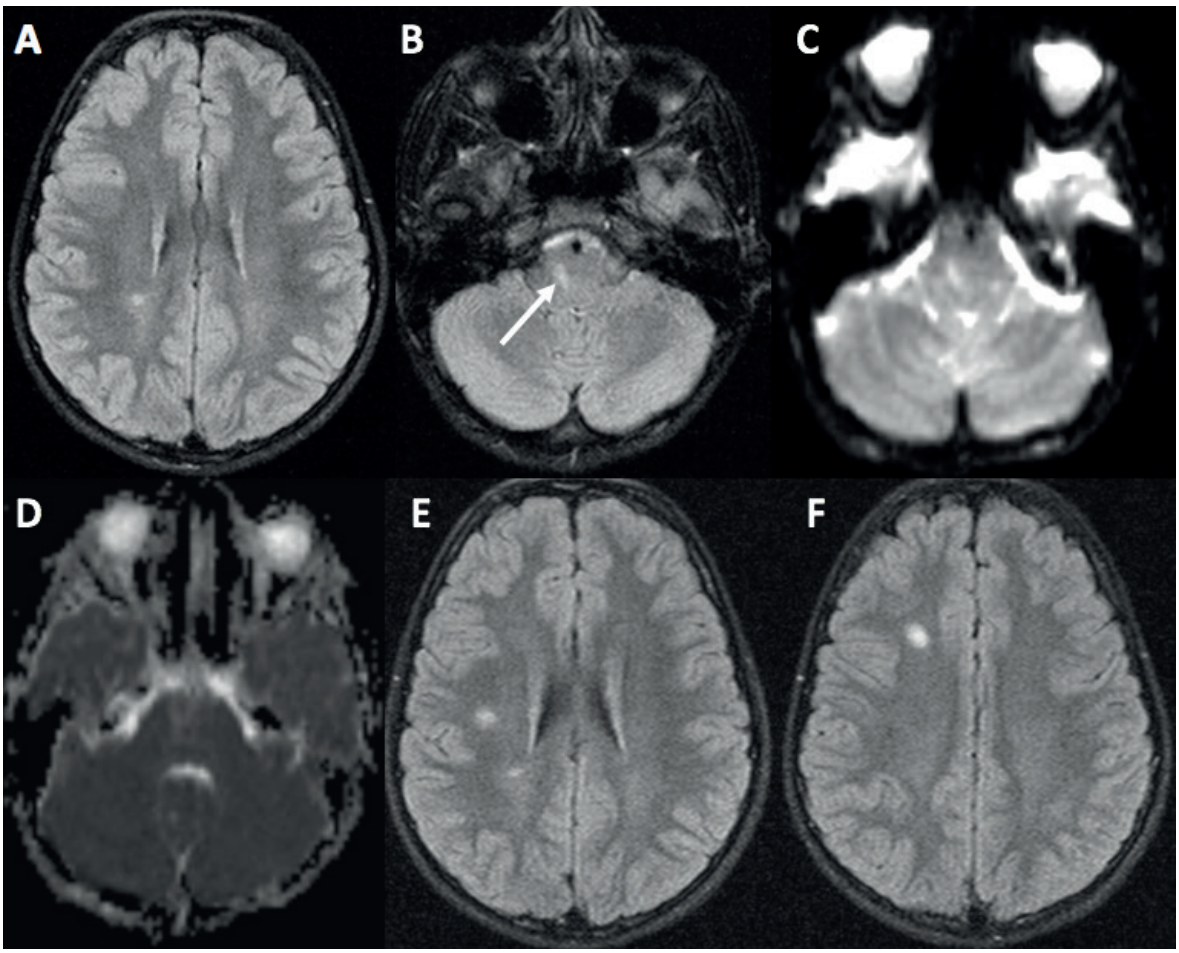


Fig. 1. The brain magnetic resonance imaging (MRI) of an 11-year-old boy presenting with diplopia who was diagnosed with MS 1 month after the first event. (**A, B**) Baseline axial FLAIR MRI of the brain revealing the hyperintense lesions in the periventricular white matter and brainstem (white arrow). (**C, D**) Diffusion-weighted imaging and ADC showing high signal intensity resembling stroke in the brain stem. (**E, F**) One month after the first event, FLAIR MRI showing new hyperintense lesions in the periventricular white matter.

Table I. Demographic and clinical characteristics of patients.

	MS, at first event	CIS	*p*
n	27	11	
Mean age at first event, years ± SD (range)	13.2 ± 2.6 (7.1-17)	10.1 ± 4.2 (3-16.9)	0.021
Female/male, *n* (%)	17/10 (63/37)	9/2 (82/18)	0.44
Presentation			0.06
Optic neuritis, *n* (%)	5 (45)	6 (55)	
Brain stem / cerebellar, *n* (%)	12 (86)	2 (14)	
Spinal cord, n (%)	1 (50)	1 (50)	
Cerebral hemispheres, *n* (%)	8 (89)	1 (11)	
Multifocal, *n* (%)	1 (50)	1 (50)	
CSF OCB positive, *n* (%)	23/24 (96)	5/9 (56)	0.013

CSF= cerebrospinal fluid, CIS=clinically isolated syndrome, MS=multiple sclerosis, OCB= oligoclonal band.

Clinical features

The age was younger in children with CIS than children with MS (p= 0.021). The age at first event was not statistically different between patients with different modes of presentation (p= 0.2). The mean age at first event was 11,3 ± 3 years (range 6.7 to 14.7 years) in patients with optic neuritis. Female preponderance was present in both children with CIS and MS. The first event presented mostly in spring (34%, n=13) and winter (29%, n=11) than in summer (18%, n=7) and autumn (18%, n=7). The season of the first event did not differ between patients with CIS and MS (p= 0.72).

The modes of presentation were monofocal including brainstem/cerebellar syndrome (n=14), optic neuritis (n=11), dysfunction of cerebellar hemispheres (n=9), spinal cord syndrome (n=2) and multifocal (n=2). The ratio for developing MS among patients with brainstem/cerebellar syndrome, optic neuritis, dysfunction of cerebellar hemispheres, spinal cord syndrome, and multifocal involvement were respectively 86%, 45%, 89%, 50%, and 50% during the follow-up period. Patients with dysfunction of cerebellar hemispheres and brainstem/cerebellar syndrome were more likely to develop clinically definite MS compared to patients with optic neuritis, spinal cord syndrome, and multifocal presentation. However, in terms of clinical presentation at onset, there was no statistically significant difference between patients with CIS and MS (p >0.05).

The oligoclonal band status was available for 33 patients. The CSF oligoclonal bands were positive in 23 children with MS (96%, n=25) and in 5 children with CIS (56%, n=8). The children with brain lesions in the first brain MRI (92%, n=24/26) were more likely to be positive for CSF oligoclonal bands than children without brain lesion (57%, n=4/7) (p= 0.021). Children having CSF oligoclonal bands were more likely to have MS than CIS (p= 0.013) (Table I). The isoelectric focusing of CSF showed pattern I in 4 children with CIS and one with MS; pattern II in 4 children with CIS and 18 children with MS; pattern III in one with CIS and 5 children with MS. Isoelectric focusing pattern was significantly different between children with CIS and MS (p= 0.026).

Neuroimaging features

Brain MRI of 38 patients and spinal cord MRI of 34 patients were available for assessment. Baseline MRI findings of the brain and spinal cord are shown in Table II. Significantly higher numbers of T2-hyperintense lesions were detected in patients with MS (mean 6.6 ± 5.9, range 0 to 26) compared to patients with CIS (mean 2.5 ± 2.9, range 0 to 8) (p= 0.015). The number of T1-hypointense lesions was not significantly different between children with MS (mean 1.3 ± 3, range 0 to 15) and children

Table II. Baseline magnetic resonance imaging findings in patients with clinically isolated syndrome and multiple sclerosis.

	MS, at first event	CIS	p
n	27	11	
Number of T2-hyperintense lesions, mean (range)	6.6 (0-26)	2.5 (0-8)	0.015
Presence of periventricular lesion, n (%)	23 (85)	4 (36)	0.005
Presence of juxtacortical lesion, n (%)	7 (26)	4 (36)	0.69
Presence of infratentorial lesion, n (%)	16 (60)	2 (18)	0.03
Presence of lesion of the corpus callosum, n (%)	12 (44)	0 (0)	0.008
Presence of T1-hypointense lesion, n (%)	12 (44)	2 (18)	0.16
Number of T1-hypointense lesions, mean (range)	1.3 (0-15)	0.4 (0-2)	0.15
Presence of gadolinium-enhancing lesion, n (%)	3 (11)	2 (18)	0.61
Number of gadolinium-enhancing lesions, mean (range)	0.3 (0-4)	0.5 (0-4)	0.54
Presence of confluent lesion, n (%)	5 (19)	0 (0)	0.29
Presence of giant lesion, n (%)	3 (11)	1 (9)	1.00
n	26	8	
Presence of spinal lesion, n (%)	7 (27)	2 (33)	1.00

with CIS (mean 0.4 ± 0.8, range 0 to 2) (p= 0.15). The number of gadolinium-enhancing lesions did not differ between patients with MS (mean 0.3 ± 0.9, range 0 to 4) and children with CIS (mean 0.5 ± 1.3, range 0 to 4) (p= 0.54).

In the baseline MRI, the ratio of the presence of periventricular, infratentorial, corpus callosum, juxtacortical, spinal, and T1-hypointense lesions were 85%, 60%, 44%, 26%, 27%, 44% in children with MS and 36%, 18%, 0%, 36%, 33%, 18% in children with CIS, respectively. The patients with periventricular, infratentorial, and corpus callosum lesions were more likely to have MS than CIS (p= 0.005, p= 0.03, p= 0.008, respectively). The presence of juxtacortical, spinal, T1-hypointense was not significantly different between patients with CIS and MS (p >0.05).

The presence of gadolinium-enhancing, confluent, and giant lesions were 11%, 19%, 11% in children with MS and 18%, 0%, 9% in children with CIS, respectively. The presence of gadolinium-enhancing, confluent and giant lesions did not differ between patients with CIS and MS (p >0.05).

Discussion

A total of 38 children with CIS aged 3-17 years were retrospectively evaluated. Age, sex, mode of clinical presentation, the status of the oligoclonal band in CSF, and baseline MRI findings of the brain and spinal cord were evaluated to determine their relevance to the development of MS.

Clinically definite MS developed in 71% of children after a mean follow-up of 11 months. The previous studies specified a risk from 15% to 62% for MS conversion during the follow-up duration of 2.2-7.6 years.[8-13] The exclusion of children with CIS who had clinical and radiological features resembling other demyelinating diseases rather than MS (e. g. bilateral optic neuritis, longitudinally extensive transverse myelitis) may be responsible for a higher conversion rate to MS in our cohort.

In agreement with other studies of children with CIS, the older age at first demyelinating event and the presence of the oligoclonal band in the CSF tended to be more common in patients with MS than patients with CIS.[14-17] It has been

suggested that the change in cerebrospinal fluid components and hormonal profile with age were associated with an increased risk of progression to MS in older children.[16,18] The CSF oligoclonal bands were more likely to be present in children with brain lesion in the first brain MRI than children without brain lesion (92% vs. 57%). The presence of oligoclonal bands in the CSF which was found to be associated with a higher lesion load in the brain MRI has been suggested as an earlier finding of MS progression.[19] Also, between children with CIS and MS, the isoelectric focusing pattern was different, which could be interpreted as either indicating different diseases or different stages of the same disease. Female sex was predominant in children with CIS and MS, in line with a previous study.[20]

The rate of conversion to MS differed according to the initial mode of clinical presentation. The brainstem/cerebellar or cerebral involvement was associated with the greatest risk of subsequent MS diagnosis. These clinical presentations were determined as the predictors of progression to MS in previous studies with long-term follow-up.[8,21] The preponderance of brainstem dysfunction as the initial symptom of MS was also emphasized in children.[21,22] The likelihood of MS was 45% in children presenting with optic neuritis, that is a greater ratio compared to previous studies (17-36%). The mean age of children with optic neuritis was similar in our study to in previous studies (11.3 years vs. 10.2 to 12.2 years). However, the age ranges were broader in these studies, ranging from 2 to 17 years, than in our study (6.7 to 14.7 years).[13,23-26] It was suggested that the risk of developing MS is correlated with age based on the differentiation of the immune system.[27] This could elucidate why the ratio for the likelihood of MS was higher in our study than previous studies.

In terms of initial MRI findings, the number and localization of T2-hyperintense lesion were related with the development of MS. The increased number of T2-hyperintense lesion and the presence of the lesion in periventricular, infratentorial and corpus callosum were associated with a tendency for the development of MS, which is consistent with previous findings.[14,16,20,28-30] The MRI lesion load, which has been suggested as an indicator of immune activation, were defined as a predictor for developing MS.[28] However, there was no difference observed between children with MS and CIS in the number of T1-hypointense lesions and gadolinium-enhancing lesions, and the presence of juxtacortical, spinal, T1-hypointense, gadolinium-enhancing, confluent, and giant lesions.

The limitations of our study are the small sample size, relatively short-term follow-up period, and the absence of data regarding serum 25-hydroxyvitamin D status, Epstein-Barr-virus infection, and genotype which were suggested risk factors of progression to MS.[20] The first event emerged mostly in spring and winter than in summer and autumn, that could be caused by the seasonal variation of the serum 25-hydroxyvitamin D status. However, we did not find a significant difference in the season of the first event between patients with CIS and MS. The data regarding antibodies against myelin oligodendrocyte glycoprotein was not available in our cohort. However, it has been suggested that these antibodies were absent in pediatric patients with relapsing-remitting MS.[31]

In conclusion, we identified that older age at first event, the presence of CSF oligoclonal bands, a higher number of T2 lesions and periventricular, infratentorial and corpus callosum lesions were associated with a tendency for development of MS. These clinical and radiological features may be suggestive of development of MS in children with CIS.

Acknowledgment

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Banwell B, Ghezzi A, Bar-Or A, Mikaeloff Y, Tardieu M. Multiple sclerosis in children: Clinical diagnosis, therapeutic strategies, and future directions. Lancet Neurol 2007; 6: 887-902.
2. Chitnis T, Tenembaum S, Banwell B, et al; International Pediatric Multiple Sclerosis Study Group Consensus statement: Evaluation of new and existing therapeutics for pediatric multiple sclerosis. Mult Scler J 2012; 18: 116-127.
3. Miller DH, Chard DT, Ciccarelli O. Clinically isolated syndromes. Lancet Neurol 2012; 11: 157-169.
4. Tintore M, Rovira A, Rio J, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. Brain 2015; 138(Pt 7): 1863-1874.
5. Kappos L, Freedman MS, Polman CH, et al; BENEFIT Study Group Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: A 3-year follow-up analysis of the BENEFIT study. Lancet 2007; 370: 389-397.
6. Sadaka Y, Verhey LH, Shroff MM, et al; Canadian Pediatric Demyelinating Disease Network 2010 McDonald criteria for diagnosing pediatric multiple sclerosis. Ann Neurol 2012; 72: 211-223.
7. Krupp LB, Tardieu M, Amato MP, et al; International Pediatric Multiple Sclerosis Study Group International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: Revisions to the 2007 definitions. Mult Scler J 2013; 19: 1261-1267.
8. Peche SS, Alshekhlee A, Kelly J, Lenox J, Mar S. A long-term follow-up study using IPMSSG criteria in children with CNS demyelination. Pediatr Neurol 2013; 49: 329-334.
9. Krupp LB, Banwell B, Tenembaum S; International Pediatric MS Study Group Consensus definitions proposed for pediatric multiple sclerosis and related disorders. Neurology 2007; 68(16 Suppl 2): S7-S12.
10. Dale RC, Pillai SC. Early relapse risk after a first CNS inflammatory demyelination episode: Examining International consensus definitions. Dev Med Child Neurol 2007; 49: 887-893.
11. Neuteboom RF, Boon M, Catsman Berrevoets CE, et al. Prognostic factors after a first attack of inflammatory CNS demyelination in children. Neurology 2008; 71: 967-973.
12. Alper G, Heyman R, Wang L. Multiple sclerosis and acute disseminated encephalomyelitis diagnosed in children after long-term follow-up: Comparison of presenting features. Dev Med Child Neurol 2009; 51: 480-486.
13. Tantsis EM, Prelog K, Brilot F, Dale RC. Risk of multiple sclerosis after a first demyelinating syndrome in an Australian Paediatric cohort: Clinical, radiological features and application of the McDonald 2010 MRI criteria. Mult Scler J 2013; 19: 1749-1759.
14. Van der Vuurst de Vries RM, van Pelt ED, Mescheriakova JY, et al. Disease course after clinically isolated syndrome in children versus adults: A prospective cohort study. Eur J Neurol 2017; 24: 315-321.
15. Dale RC, Brilot F, Banwell B. Pediatric central nervous system inflammatory demyelination: Acute disseminated encephalomyelitis, clinically isolated syndromes, neuromyelitis optica, and multiple sclerosis. Curr Opin Neurol 2009; 22: 233-240.
16. Waldman AT, Stull LB, Galetta SL, Balcer LJ, Liu GT. Pediatric optic neuritis and risk of multiple sclerosis: Meta-analysis of observational studies. J AAPOS 2011; 15: 441-446.
17. Mikaeloff Y, Suissa S, Vallee L, et al; KIDMUS Study Group. First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability. J Pediatr 2004; 144: 246-252.
18. Chabas D, Ness J, Belman A, et al; For the US Network of Pediatric MS Centers of Excellence Younger children with MS have a distinct CSF inflammatory profile at disease onset. Neurology 2010; 74: 399-405.
19. Tur C, Montalban X. CSF oligoclonal bands are important in the diagnosis of multiple sclerosis, unreasonably downplayed by the McDonald criteria 2010: No. Mult Scler J 2013; 19: 717-718.
20. Banwell B, Bar-Or A, Arnold DL, et al. Clinical, environmental, and genetic determinants of multiple sclerosis in children with acute demyelination: A Prospective National Cohort Study. Lancet Neurol 2011; 10: 436-445.
21. Ghezzi A, Pozzilli C, Liguori M, et al. Prospective study of multiple sclerosis with early onset. Mult Scler J 2002; 8: 115-118.
22. Ghezzi A, Deplano V, Faroni J, et al. Multiple sclerosis in childhood: Clinical features of 149 cases. Mult Scler J 1997; 3: 43-46.

23. Wilejto M, Shroff M, Buncic JR, Kennedy J, Goia C, Banwell B. The clinical features, MRI findings, and outcome of optic neuritis in children. Neurology 2006; 67: 258-262.

24. Alper G, Wang L. Demyelinating optic neuritis in children. J Child Neurol 2009; 24: 45-48.

25. Heussinger N, Kontopantelis E, Rompel O, Paulides M, Trollmann R. Predicting multiple sclerosis following isolated optic neuritis in children. Eur J Neurol 2013; 20: 1292-1296.

26. Lucchinetti CF, Kiers L, O'Duffy A, et al. Risk factors for developing multiple sclerosis after childhood optic neuritis. Neurology 1997; 49: 1413-1418.

27. Chabas D, Ness J, Belman A; US Network of Pediatric MS Centers of Excellence Younger children with MS have a distinct CSF inflammatory profile at disease onset. Neurology 2010; 74: 399-405.

28. Kuhle J, Disanto G, Dobson R, et al. Conversion from clinically isolated syndrome to multiple sclerosis: A large multicenter study. Mult Scler J 2015; 21: 1013-1024.

29. Fisniku LK, Brex PA, Altmann DR, et al. Disability and T2 MRI lesions: A 20-year follow-up of patients with relapse onset of multiple sclerosis. Brain 2008; 131(Pt 3): 808-817.

30. Brex PA, Ciccarelli O, O'Riordan JI, Sailer M, Thompson AJ, Miller DH. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. N Engl J Med 2002; 346: 158-164.

31. Pröbstel AK, Rudolf G, Dornmair K, et al. Anti-MOG antibodies are present in a subgroup of patients with a neuromyelitis optica phenotype. J Neuroinflammation 2015; 12: 46.

The Turkish Journal of Pediatrics 2020; 62: 252-258
https://doi.org/10.24953/turkjped.2020.02.011

Original Article

Outcomes and prognostic factors for pediatric cancer patients admitted to an intensive care unit in a university hospital

Adem Dursun, Serkan Özsoylu, Başak Nur Akyıldız

Division of Pediatric Intensive Care Unit, Department of Pediatrics, Erciyes University Faculty of Medicine, Kayseri, Turkey.

ABSTRACT

Background and objectives. The aim of this study was to investigate the factors predicting Pediatric Intensive Care Unit (PICU) mortality and the outcomes in cancer patients admitted to PICU.

Methods. We conducted a retrospective study in 48 consecutive cancer patients admitted to the PICU between January 1, 2015 and January 1, 2018. A total of 48 patients (21 males and 27 females) were enrolled in this study.

Results. The median age was 77 (33,5-149) months. The median duration of PICU stay was 5 (2-9) days. Patients were classified according to their stage of disease. Ten (20.8%) patients were in the remission group, 9 (18.8%) patients were in the induction period and 29 (60.5%) patients were in the progressive diseasegruops. Thirty-nine patients (81.2%) had hematological malignancies, 6 (12.5%) had extracranial solid tumors and 3 (6.3%) had intracranial solid tumors. Thirty-seven patients died and the mortality rate was found to be 77.1%. mortality rates were 11%, 88% and 93% for patients in remission,during induction period and in the progressive disease group, respectively ($p < 0.01$).The most frequent reasons of PICU admission were respiratory failure in 29 (60.4%), sepsis in 12 (25%), circulatory collaps in 2 (4.2%), and other reasons in 5 patients (10.4%). The median PRISM III among survivors was significantly lower than non-survivors (13.1 ± 6.4; vs. 20.7 ± 5.2; $p < 0.001$). At a cut-off value of 13, the sensitivity of the PRISM III was 94.4% and the specificity was 58.3% (AUC: 0.821). OSDwas present in 41 (85%) patients, 82% of them died (34/41). The presence of MOF, the use of mechanical ventilation and inotrop support were significantly related with mortality. Univariate logistic regression analysis showed that male gender [odds ratio (OR)=5.588, P= 0.041, 95% confidence interval (95%CI) 1.070-29.191], presence of organ system dysfunction[OR=12.143, P= 0.008, 95%CI 1.947- 75.736], need for mechanical ventilation[OR=34.000, P= 0.001, 95%CI 5.272-219.262], IS [OR=8.5, P= 0.001, 95%CI 1.318-54.817]were the predictors ofhigh mortality in pediatric cancer patients. PRISM III score ≥ 13 was a predictive criteria of PICU mortality.

Conclusion. We conclude that the key to improving survival rates is to pick up on this group of patients as soon as possible.We, believe that cancer patients could be saved by earlier evaluation and intervention by the PICU team when they have a less severe disease.

Key words: cancer, pediatric, PICU.

Over the past few decades, development in current treatment protocols for cancer patients have resulted in a significant increase in survival rates. Thereby, an increased number of patients with cancer are requiring admission to pediatric intensive care units (PICU). Disease-related complications or treatment-associated side effects may lead to severe and life threatening complications such as tumor lysis syndrome, sepsis, and respiratory and cardiovascular insufficiency. These complications may require prompt initiation of intensive care treatment. Therefore, identification of children whose admission to PICU will improve their survival areso very crucial.

Although there have been improvements in supportive care, previous published studies

✉ Adem Dursun
dr.ademdursun@gmail.com

Received 22nd February 2018, revised 20th February 2019, 12th March 2019, accepted 13th March 2019.

reported poor outcomes of children with cancer who required PICU admission specifically when invasive ventilation, inotropic support and continuous renal replacement therapy are needed.[1] Outcomes and risk factors associated with mortality in PICU are needed to establish the optimal clinical management of cancer patients.

The aim of our study was to investigate incidence, causes, outcomes and prognostic factors associated with mortality in cancer patients transferred to PICU.

Material and Methods

This retrospective, observational study was carried out in the 12- bed medical PICU of the Erciyes University Child Hospital in Kayseri, Turkey. We reviewed the clinical records of all cancer patients (<18 years old) who required PICU admission between January 1, 2015 and January 1, 2018. Only the first admission was recorded in patients with multiple PICU admissions. Patients who stayed in the PICU for shorter than 24 hours were also excluded.

The following information was abstracted from the medical charts of the patients: sex and age, underlying primary disease, reason for admission, thrombocytopenia, neutropenia, therapeutic interventions (positive inotropic support, mechanical ventilation, and dialysis), PRISM III score, length of PICU stay, number of organ failures, and outcome (survivors vs. nonsurvivors at the time of leaving the PICU). For organ system dysfunctions (OSD) and sepsis, International Pediatric Sepsis Consensus Conference Report of ''Definitions for sepsis and organ dysfunction in pediatrics'' on January 2005 was used in this study.[2]

Patients admitted to PICU were evaluated from the medical charts of the patients with a pediatric hematology-oncology fellow and they were classified according to stage of disease in 3 treatment groups as remission, induction period and progressive disease groups.

Infection was defined as a suspected or proven infection. Pathogenic organisms were proved by positive culture or polymerase chain reaction test. The definition also included clinical syndromes associated with a high probability of infection, such as petechiae and purpura in a child with hemodynamic instability, or fever, cough, and hypoxemia in a patient with leukocytosis and pulmonary infiltrates on chest radiograph. Additionally, an elevation of C-reactive protein and procalcitonin were also presumed to be an infection. Invasive fungal infections were defined according to "Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group". Thrombocytopenia was defined as a platelet count below the lower limit of normal(<150,000/microL) and neutropenia was defined as absolute neutrophil count less than 1500/mL. The patients who had arterial blood pressure less than 2 standard deviations of normal value for age and whom received any vasopressor or inotropic drug within 24 hours of admission were defined as positive inotropic support. The patients who were unable to maintain adequate oxygenation or ventilation (PaO2<70 mm Hg PaCO2>65 mm Hg when FiO2>0.60), received mechanical ventilation.

Patients were discharged from the PICU after documented hemodynamic/respiratory and neurological stability lasting >48 and 24 hours, respectively. Hemodynamic stability was defined as no need for inotropic drugs for continuous volume expansion; diuresis >1 mL/kg/h, and no need for renal replacement therapy. Respiratory stability was defined as off MV for >48 hours, no need for noninvasive ventilatory support, and oxygen saturation (SpO2) >95% with FiO2.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 (IBM, Armonk, NY). The normality

of parametric data was analyzed by the Shapiro Wilk test. Numerical variables were expressed as mean ± SD or median (25-75p). Comparisons between groups for data with a normal distribution were performed using Student's t-test, and the comparisons between groups for data that did not show a normal distribution were performed using the Mann- Whitney U test. Categorical variables were compared using the X^2 test. The bivariate correlation tests were used to analyze the correlations. Whether PRISM III value was a significant marker that differentiated survivors from non-survivors was explored using 95% confidence intervals and the area under ROC curve. When a significant area under the curvewas obtained, the maximum possible sum of the sensitivity and specificity levels was considered the best cut-off point. The statistically significant risk factors were analyzed by univariate logistic regression analysis. A p value less than 0.05 was considered statistically significant

Results

During the study period,a total of 48 patients (21 males and 27 females) were enrolled in this study. Forty-three patients were transferred from the Haematology Department and 5 patients were admitted from the emergency department.The median age at the time of admission was 77 (33,5-149) months. The median duration of PICU stay was 5 (2-9) days. Table I shows the clinical characteristics of all patients included in the study. Thirty-nine patients (81.2%) had hematological malignancies, 6 (12.5%) had extracranial solid tumors and 3 (6.3%) had intracranial solid tumors.

Thirty-seven patients died and the mortality rate was found to be 77.1%, higher than the yearly overall PICU mortality rate (16%) (p <0.001). In estimation of overall PICU mortality rate, patients were excluded who died of cancer. Fourteen of the study cohort were followed because of hematopoietic stem cell transplantation. Among these patients, 6 received allogeneic, 5 haploidentical and 3 autologous and 11 of them (78%) died.

The most frequent reasons of PICU admission were respiratory failure in 29 (60.4%), sepsis in 12 (25%), circulatory collaps in 2 (4.2%), and other in 5 patients (10.4%). For the 2 patients who were admitted to PICU due to circulatory collapse, mortality rate was found to be 100%, and it was 96.6% for respiratory failure, 58.3% for sepsis and 40% for other diagnoses.

On admission, the mean PRISM III score was 18.8 (± 6.4). The mean PRISM III among survivors was significantly lower than among non-survivors (13.1 ± 6.4; vs. 20.7 ± 5.2; p <0.001). At a cut-off value of 13, the sensitivity of PRISM III was 94.4% and the specificity was 58.3% (AUC: 0.821). ROC curve of the PRISM III differentiating survivors from non- survivors is presented in Figure 1.

According to their stage of disease we classified the patients into 3 treatment groups: remission (n=10, 20.8%), induction period (n=9, 18.8%) and progressive disease (n=29, 60.4%). Patients admitted to the PICU in the remission

Table I. Characteristics of pediatric cancer patients admitted to the Pediatric Intensive Care Unit.

Variables	N (%)
Sex	
Male	21 (43.8)
Female	27 (56.2)
Diagnosis of Patients	
Hematological malignancy	39 (81.3)
Intracranial solid tumors	3 (6.3)
Extracranial solid tumors	6 (12.5)
Reason for admission	
Sepsis	12 (25)
Respiratory failure	29 (60.4)
Circulatory Collaps	2 (4.2)
Other	5 (10.4)
Stage of Disease	
Remission	10 (20.8)
Progressive disease	29 (60.5)
Induction period	9 (18.8)
Outcome	
Survival	11 (22.9)
Non-Survival	37 (77.1)

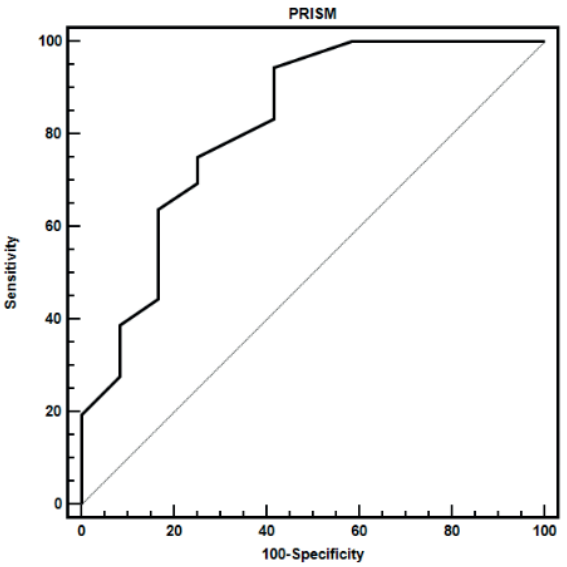


Fig. 1. ROC curve of the PRISM III differentiating survivors from non- survivors.

group had the highest survival rate (89%) compared topatients in the induction period and progressive disease groups (12% and 7%, respectively; p <0.01). In the remission group the mean PRISM III score was 11.4 (±6.5), s 20.8 (±5.3) in the progressive disease group and 19.4 (±5.2) in the induction period group (p <0.01).

Three therapeutic modalities used in the ICU were mechanical ventilation, inotropic support and renal replacement therapy (RRT). Mechanical ventilation (invasive or noninvasive) was applied to 39 patients (3 patients in the remission group, 27 patients in the progressive disease group and 8 patients in the induction period group) and the median day of mechanical ventilation was 3 days (2-6). Thirty-four of these were both mechanically ventilated and received positive inotropic support. RRT was used in 5 patients (3 patients inthe induction period group and 2 patients in the progressive disease group). Among these, 4 needed inotropic support (IS) and mechanical ventilation (MV). Five patients were applied only IS and 3 patients were performed neither MV, IS, nor RRT. Among mortality rates in therapeutic interventions, it was highest in the patients where all the 3 interventions were performed together (100%), followed by patients who required both mechanical ventilation and positive inotropic support (Table II).

OSD was present in 41 (85%) of patients, 82% of them died (34/41). Mortality was significantly correlated to the number of organ failure (p-value <0.001). The presence of OSD was detected in 4 (40%)patients inremission group, 8 (88%) patients in progressive disease group and 29 (100%) patientsininduction period. Inthe remission group the presence of OSD rate was significantly lower when compared with patients in the induction period and progressive disease group (p <0.01)

When survivors are compared with nonsurvivors, no significant differences were found in primary underlying disease, presence of neutropenia, presence of thrombocytopenia, presence of fungal infection and RRT.Mortality rate was significantly related to gender, presence of OSD, MV and IS. (Table III. Univariate logistic regression analysis showed that it was higher in males [OR=5.588, p= 0.041, 95%CI 1.070-29.191], with a presence of OSD [OR=12.143, P= 0.008, 95%CI 1.947-75.736], mechanical ventilation [OR=34.000, P= 0.001, 95%CI) 5.272-219.262], and IS [OR=8.5, P= 0.001, 95%CI 1.318-54.817].

Table II. Mortality rates according to therapeutic interventions.

Therapeutic interventions	N(%)	Mortality	Overall Mortality
		N (%)	(%)
MV	1 (2)	0 (0)	0
MV+IS	34 (70)	2 (94)	86
MV+IS+RRT	4 (8)	4 (100)	11

Five patients were applied IS and 1 patient was applied RRT. Three patients were performed neither MV, IS, nor RRT
IS: indicates inotropic support, MV: mechanical ventilation, RRT: renal replacement therapy.

Table III. Risk factors related to survival for pediatric hematology/oncology patients admitted to the Pediatric Intensive Care Unit.

Risk Factors	No. Patients (%)	No. Mortality (%)	p
Gender			0.03
Male	21 (43.8)	19 (90)	
Female	27 (56.3)	17 (62)	
Primary diagnosis			ns
Hematologic malignancy	39 (81)	29 (74)	
Solid tumors	9 (19)	7 (78)	
Presence of neutropenia			ns
Yes	37 (77)	28 (75)	
No	11 (23)	8 (72)	
Presence of Thrombocytopenia			ns
Yes	42 (88)	33 (79)	
No	6 (12)	3 (50)	
Presence of OSD			0.007
Yes	41 (85)	34 (83)	
No	7 (15)	2 (29)	
Presence of fungal infection			ns
Yes	17 (36)	14 (82)	
No	31 (64)	22 (64)	
Mechanical ventilation			0.001
Yes	38 (80)	34 (89)	
No	10 (20)	2 (20)	
Positive inotropic support			0.028
Yes	42 (88)	34 (81)	
No	6 (12)	2 (33)	
Renal replacement therapy			ns
Yes	5 (10)	4 (80)	
No	43 (90)	32 (74)	
PRISM score			0.03
<13	4 (8)	0	
>13	44 (92)	36 (81)	

No. Patients: Number of patients, No. Mortality: Number of mortality, ns: non spesifik, OSD : organ system dysfunction, PRISM: Pediatric Risk of Mortality

Discussion

The present study is one of the small number of retrospective studies on pediatric cancer patient populations who need PICU admission. In this study, we evaluated the data of 48 cancer patients to identify the prognostic factors that affect the outcome of PICU admission. Despite improvement of intensive care support, our results indicated that the mortality rate of advanced stage cancer patients is still high. In our study cohort, while the overall PICU mortality rate in pediatric cancer patients was found to be much higher than non cancer patients, it was similar to overall PICU mortality rates in the remission group. The observed mortality rate is relatively high compared with the rates reported in recently published studies.[3,4] However, it is important to emphasize a fundamental difference between patient groups. Compared to the population of patients in the study of Akhtar et al.[4] most of our patients presented with advanced disease (Higher PRISM III score; 18.8 versus 7). Additionally children admitted

postoperatively are far less immunosuppressed compared with those with hematological malignancies. Our cohort did not include the patients who needed PICU admission for routine postsurgical management and majority of the patiens were in theprogressive disease group in which survival is expected to be low. These factors may explain this discrepancy.

In our study, hematological malignancies were the most prominent diagnosis among patients admitted to our PICU with solid tumors accounting for only 19% of the cases. Hematologic malignancy was not found as a risk factor in survival and this was consistent with previous studies.[5]

Mortality is influenced by the reason for admission. Respiratory failure is a major cause of PICU admission and patients admitted because of respiratory failure and circulatory collapsehad the worst outcomes (96.5%, 100%, respectively). Our findings concur with the findings of Dursun et al.[3] who reported higher mortality rate in patients with circulatory collapse and respiratory failure. Additionally, the present study investigated that the mortality was significantly influenced by the patients' stage of disease. Children in remission group had a lower mortality rate when compared with those in induction period and progressive disease groups.High Mortality rate is expected in progressive disease groups.However, in our study, mortality rate was found to be high (88%) also in the induction group.The low survival rates in the induction group can be explained by the severity of patients on admission (the mean PRISM III scoreon admission was 19.4 (±5.2)). This indicates adelayed presentation to the PICU in the induction period. Due to sepsis, respiratory failure and need forIS, these patients may requre intensive care. Therefore patients should be consulted with PICU immediately.

A variety of prognostic factors has been described in patients requiring PICU. In our study we found a high incidence of OSD (85%) and the presence of OSD was found to be a risk for mortality, with a mortality rate of 83% in patients with two or more organ failures against a 29% mortality rate , similar to the findings of others.[5] Furthermore,in univariate analysis, our results showed that the presence of OSD increases the mortality with an odds ratio of 12.143. Similar to the present results, previous studies have also demonstrated a significant correlation between the number of organ failure and mortality in pediatric cancer patients admitted to the PICU.[1,6,7]

The PRISM III score evaluates the mortality risk based on data collected during the first 24 hours in the PICU.[8] Thus creating the Oncological-PRISM score, some authors have proposed to add important prognostic factors for children posthematopoietic stem cell transplantationand a score> 10 pointsis accepted as high. However, it has not yet been validated.[9] Akhtar et al.[4] demonsrated the mortality rates 51.6% and 18.6% in patients with high (>10 points) and low (<10 points) PRISM III, respectively. This concept is supported by the finding of Dursun et al.[3] who reported the sensitivity and specificity of estimatingoutcome using PRISM III score (cut-off value for poor survival >10 points) were 90% and 50%, respectively. Our analysis confirmed the reported relationship between survival and PRISM III scores in cancer patients. However, the present study found that patients with PRISM III score ≥13 had very poor outcome and it was a good indicator for death in PICUwith a sensitivity of 94.4% and specificity of 58.3%. Our results could be used to better analyze cohorts of cancer patients admitted to PICU and in the evaluation of new treatment strategies.

For a better understanding of which cancer patients were at higher risk for mortality, we described three therapeutic modalities and analyzed the risk factors separately. The use of mechanical ventilation, inotropic support and renal replacement therapy were found to be associated with poor prognosis.Their combination was associated with a worse prognosis with the mortality rate reaching from 94% to 100%. In univariate analysis, the use of mechanical ventilation showed the strongest association with unfavorable

prognosis after PICU admission, with an almost 34-fold increase in mortality risk and we also found thatinotropic support increased 8.5 folds.These data are supported by previous findings.[10,11] We also reported that no significant differences were seen in presence of leukopenia, thrombocytopenia, fungal infection and RRTwhen survivors were compared with nonsurvivors.

The limitations of our study are its retrospective nature and its single-center design. Furthermore, the relatively small sample size leads us to refrain from drawing solid conclusions. Nevertheless, our study investigated the significant conclusions about risk factors. Because of variations in the underlying disease composition, ICU admission, and discharge criteria it is rather difficult to compare the mortality in different studies.

In conclusion, the mortality rate of cancer patients in the induction period and progressive disease groups was high. However, it was similar to non cancer patients' mortality rate in the remission group. Factors associated with mortality after PICU admission may prove particularly useful for clinicians to inform patients and families. PRISM III score ≥13 was predictive criteria of PICU mortality. As we mentioned before, most of our patients were referred late to PICU. From these data, we conclude that the key to improve survival rates is to pick upon this group of patients as soon as possible. We believe that cancer patients could be saved by earlier evaluation and intervention by the PICU team when they have a less severe disease.

The study was approved by the "Medical Research Local Ethics Committee" of the Erciyes University with a number of 2018/51. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. We obtained informed consent from the parents.

REFERENCES

1. Heying R, Schneider DT, Korholz D, Stannigel H, Lemburg P, Göbel U. Efficacy and outcome of intensive care in pediatric oncologic patients. Crit Care Med 2001; 29: 2276-2280.
2. Goldstein B, Giroir B. Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005; 6: 2-8.
3. Dursun O, Hazar V, Karasu GT, Uygun V, Tosun O, Yesilipek A. Prognostic factors in pediatric cancer patients admitted to the pediatric intensive care unit. J PediatrHematol Oncol 2009; 31: 481-484.
4. Akhtar N, Fadoo Z, Panju S, Haque A. Outcome and prognostic factors seen in pediatric oncology patients admitted in PICU of a developing country. Indian J Pediatr 2011; 78: 969-972.
5. Ali AM, Sayed HA, Mohammed MM. The outcome of critically Ill pediatric cancer patients admitted to the Pediatric Intensive Care Unit in a Tertiary University Oncology center in a developing country: A 5-year experience. J Pediatr Hematol Oncol 2016; 38: 355-359.
6. Haase R, Lieser U, Kramm C, et al. Management of oncology patients admitted to the paediatricintensive care unit of a general children's hospital - a single center analysis. Klin Padiatr 2011; 223: 142-146.
7. van Gestel JP, Bollen CW, Bierings MB, Boelens JJ, Wulffraat NM, van Vught AJ. Survival in a recent cohort of mechanically ventilated pediatric allogeneic hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant 2008; 14: 1385-1393.
8. Pollack MM, Ruttimann UE, Getson PR. Pediatric risk of mortality (PRISM) score. Crit Care Med 1988; 16: 1110-1116.
9. Gonzalez-Vicent M, Marin C, Madero L, Sevilla J, Diaz MA. Risk score for pediatric intensive care unit admission in children undergoing hematopoietic stem cell transplantationand analysis of predictive factors for survival. J Pediatr Hematol Oncol 2005; 27: 526-531.
10. Haase R, Mathony U, Lieser U, Nagel F, Sitka U, Burdach S. Oncology patients in a pediatric intensive care unit–a 7-year experience. Klin Padiatr 2003; 215: 234-240.
11. Hallahan AR, Shaw PJ, Rowell G, O'Connell A, Schell D, Gillis J. Improved outcomes of children with malignancy admitted to a pediatric intensive care unit. Crit Care Med 2000; 28: 3718-3721.

The Turkish Journal of Pediatrics 2020; 62: 259-266
https://doi.org/10.24953/turkjped.2020.02.012

Original Article

Ambulatory arterial stiffness index is increased in obese children

Mehmet Taşdemir[1], Ergin Erginöz[2], Özlem Bostan Gayret[3], Ilmay Bilge[1]

[1]Division of Pediatric Nephrology, [2]Department of Pediatrics, Koç University School of Medicine, İstanbul; [3]Department of Pediatrics, Health Sciences University, Bağcılar Training and Research Hospital, İstanbul, Turkey.

ABSTRACT

Background and objectives. One way to measure arterial stiffness is the ambulatory arterial stiffness index (AASI), which is the relationship between diastolic and systolic ambulatory blood pressure (BP) over 24-hours.

Methods. We studied the difference in AASI between obese and lean children. AASI was calculated from 24-hour ambulatory blood pressure monitoring in 53 obese children (33 girls) and compared with age-matched 42 healthy subjects (20 girls). Hypertension was defined according to the criteria of the American Heart Association. To evaluate inflammation, the blood level of high-sensitive C-reactive protein was measured.

Results. The mean age was 10.6 ± 2.83 years in obese children and 11.3 ± 3.17 years in healthy subjects. Hypertension was determined in three (5.6%) obese children. The median heart rate-SDS, pulse pressure and blood pressure values did not differ between the two groups. The mean AASI was significantly higher in obese children compared to healthy subjects (0.42 ± 0.15 vs. 0.29 ± 0.18, p <0.001). AASI significantly correlated with nighttime SBP-SDS, nighttime SBP-load, systolic and diastolic nocturnal dipping, with no independent predictor.

Conclusion. This study confirms that AASI is increased in obese children. AASI calculation is a useful, cost-effective, and an easy method to evaluate arterial stiffness. Early detection of increased arterial stiffness can help clinicians come up with preventive measures in the management of patients.

Key words: arterial stiffness, blood pressure measurement, cardiovascular risk, children, obesity.

In recent years, childhood obesity has become an epidemic health problem. The prevalence of obesity is more common in developed countries (16-30%) compared to underdeveloped countries (6-13%).[1] The increase in prevalence of obesity has led to the emergence of comorbidities associated with it. Hypertension is one of the most important comorbidities that is associated with increased body mass index (BMI) in children and its presence in adulthood is an important risk factor for early cardiovascular disease (CVD).[2-4] Cardiovascular (CV) risk factors including hypertension, dyslipidemia, metabolic syndrome, and obesity itself have effects on vascular function and structure such as endothelial dysfunction, increased carotid artery intima media thickness (IMT), and increased arterial stiffness.[5,6]

To evaluate vascular function and structure, different techniques have been used in the past. These include measurements of carotid artery IMT, pulse wave velocity (PWV), and flow mediated dilatation. Arterial stiffness is a measure of vascular elastic behavior which can be altered by different etiologies such as atherosclerosis, hypertension, dyslipidemia and obesity. Decreases in elastic mechanical features of arteries lead to increased arterial stiffness in obese children.[7] Since measurements of vascular

✉ Mehmet Taşdemir
mtasdemir@kuh.ku.edu.tr

Received 19th March 2018, revised 3rd June 2018, 20th August 2018, 10th September 2018, 27th November 2018, accepted 4th March 2019.

This study has been presented as a poster at the 50th Anniversary Meeting of the European Society for Pediatric Nephrology (ESPN), 6-9 September 2017, Glasgow, Scotland.

structure and function by IMT, PWV, or other ways are required by experienced persons and expensive tools, ambulatory arterial stiffness index (AASI) as a less complex method was developed in 2006.

AASI is the relationship between diastolic and systolic ambulatory blood pressure (BP) over 24-hours, which can be obtained by performing a linear regression analysis of diastolic BP over systolic BP and subtracting the slope from 1.[8] Cardiovascular risk, in particular stroke, was predicted by AASI in different adult populations.[9,10] In childhood, increased AASI has been stated in children with hypertension and diabetes mellitus.[11] Besides AASI, ambulatory blood pressure monitoring can be used to calculate pulse pressure and these two parameters have been suggested as markers of arterial stiffness and predictors of cardiovascular disease.[11] To assess arterial stiffness, few studies have investigated AASI in obese children.[12]

In this prospective trial, we have studied the difference of AASI between obese and lean children.

Material and Methods

Study population

In this prospective cohort 53 obese children (33 girls) aged between 4-16 years were investigated at pediatric outpatient clinic, Ministry of Health Bağcılar Training and Research Hospital, Istanbul, Turkey. The control group consisted of age-matched 42 healthy subjects (20 girls) aged between 5-18 years. Obesity was defined as body mass ındex (BMI) ≥95th percentile according to height- and sex-specific BMI charts by Centers for Disease Control and Prevention (CDC).[13] The control group included lean children with a normal BMI percentile (<85th percentile). Weight and height were measured and recorded in all subjects.

Informed consent was obtained from all individual participants (and/or their parents or legal guardians) included in the study.

Laboratory evaluation

In the obese group blood samples were collected in the morning after an overnight fast for the assessment of metabolic parameters which included fasting glucose level, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, insulin, 25-hydroxyvitamin D [25(OH)D], and high sensitive C-reactive protein (hsCRP). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the equation HOMA-IR = [Fasting plasma insulin (miU/mL) × Fasting plasma glucose (mg/dl)] / 405. HOMA-IR cut-off values for insulin resistance in the prepubertal period were accepted to be 2.67 in boys and 2.22 in girls, and in the pubertal period, 5.22 in boys and 3.82 in girls.[14] Dyslipidemia was defined as serum levels of total cholesterol ≥200 mg/dl and/or LDL ≥130 mg/dl and/or triglycerides ≥100 mg/dl in the 6-9 years of age group and ≥130 mg/dl in the 10-18 years of age group according to the National Heart, Lung, and Blood Institute (NHLBI) criteria.[15] Vitamin D deficiency was defined as <30 nmol/L.[16] The normal limits of hsCRP were accepted as 0-5 mg/dl.

Blood pressure assessments

Casual blood pressure (BP) measurement was performed three times with 10 min intervals by an oscillometric device (Nihon Kohden, Vismo, Germany) and was averaged; indexed systolic (SBP) and diastolic (DBP) BP were calculated by dividing 95th percentile according to height and gender.[17] 24-hour ambulatory blood pressure monitoring (ABPM) was performed by using a portable device (SpaceLabs 90217A-1 oscillometric device, Spacelabs Healthcare, UK). We used different cuff sizes according to the patient's arm circumference. Width of the inflatable bladder of the cuff was chosen as 40% of upper arm circumference, and length of the inflatable bladder was decided as 80% of upper arm circumference (almost long enough to encircle the arm). The non-dominant arm served for cuff placing. Blood pressure recordings were obtained with 20-min intervals in the wake

period and 30-min intervals in the sleep period. Keeping a diary (particularly sleep disturbances) was asked to participants for evaluating daytime and nighttime BP measurements. ABPM recordings were considered sufficient if it contained ≥75% successful measurements. Patients with insufficient (less than 75%) blood pressure measurements (i.e., due to the manual application of the device or a software problem) were excluded from the study. Hypertension was defined according to the criteria of American Heart Association (AHA).[18]

Ambulatory arterial stiffness index

Twenty-four-hour blood pressure measurements were entered into the Statistical Package for the Social Sciences (SPSS) software program (IBM SPSS Statistics, Armonk, NY) for each patient. Linear regression was performed on the 24-hour BP recordings, with diastolic BP as the dependent variable and the systolic BP as the independent variable. The unstandardized regression slope (B) was considered in this study since the 24-hour BP recordings were unchanged. AASI was calculated by subtracting the regression slope from 1.

Other parameters that were recorded from the BP device included the 24-hour, day and night heart rates, systolic and diastolic BP values and loads, and pulse pressures (PP). Standard deviation score (SDS) was calculated for the mean 24-hour, day and night heart rate, systolic and diastolic BP values by the LMS method using height-specific normative values [degree of skewness (L), median (M) and coefficient of variation (S)].[19]

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at which the studies were conducted (Ministry of Health Bağcılar Training and Research Hospital, approval number 2015/366) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statistical analysis

Data analysis was carried out using SPSS software version 21 (IBM SPSS Statistics, Armonk, NY). All parameters were examined in terms of distribution of parameters by the adjusted Fisher-Pearson standardized moment coefficient. Continuous data were expressed as the mean ± SD if the distribution was normal and/or median (IQR) otherwise. Student-t test was used for the comparison of normally distributed variables, and Mann–Whitney U test was used if data was not normally distributed. Categorical variables were presented as a number and compared using the Chi-squared test and the Fisher's exact test. Associations among AASI, PP, and other parameters were assessed by Pearson correlation analysis if data was normally distributed and by Spearman correlation analysis if data was not normally distributed. The variables that showed a p value of <0.20 in the univariate analysis and parameters which were reported to be associated in the previous studies were tested in a multivariate linear regression analysis to identify the important independent factors influencing AASI values. The abnormally distributed parameters were transformed into a normal distribution by logarithmic transformation. Significance was allowed at p <0.05.

Results

Demographic features of obese and healthy groups are summarized in Table I. Weight and BMI-SDS of the obese group were significantly higher than the healthy group (p <0.001). Metabolic features of obese participants are presented in Table II. Dyslipidemia was determined in 21 obese children (39.6%) [including increased total cholesterol (n: 1), increased LDL-cholesterol (n: 5), increased triglycerides levels (n: 11), at least two abnormal lipid values (n: 2), and at least three abnormal lipid levels (n: 2)]. Increased HOMA-IR rate was calculated in 30 obese children (56.6%). A total of 47 obese children (88.7%) were described

Table I. Characteristics of healthy and obese participants.

Characteristics	Healthy participants (n: 42)	Obese participants (n: 53)	*p* value
Age, years	11.3 ± 3.17	10.6 ± 2.83	0.48
Male/Female, n (%)	22 (52) / 20 (48)	20 (37) /33 (63)	0.15
Height, m	1.50 ± 0.17	1.46 ± 0.16	0.36
Height-SDS	0.51 ± 1.41	0.63 ± 1.04	0.57
Weight, kg	45.5 ± 15.1	65.0 ± 21.8	<0.001
Weight-SDS	0.42 ± 1.17	2.86 ± 0.83	<0.001
BMI, kg/m^2	19.6 ± 3.49	29.1 ± 4.42	<0.001
BMI-SDS	0.23 ± 1.03	2.71 ± 0.57	<0.001

BMI: body mass index, SDS: standard deviation score.

Table II. Metabolic features of obese participants.

Features	Obese participants (n: 53)
eGFR, ml/min/1.73m^2*	120 (21)
Total cholesterol, mg/dl	162.1 ± 26.9
LDL-C, mg/dl	90.1 ± 26.1
HDL-C, mg/dl	53.2 ± 17.1
Triglycerides, mg/dl*	101 (80.5)
Fasting glucose, mg/dl	90.2 ± 9.9
Insulin, miU/ml*	17.5 (15)
HOMA-IR*	3.69 (3.87)
Vitamin D, ng/ml*	17.8 (12.6)
hsCRP, mg/L*	1.05 (1.17)

eGFR: estimated glomerular filtration rate, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, hsCRP: high sensitive C reactive protein, HOMA-IR: homeostasis model assessment of insulin resistance.
* Non-parametric values are presented as median (IQR).

as vitamin D deficient. Increased hsCRP level (>5mg/dl) was found in five obese children (9.4%).

Hypertension was found in three obese participants (5.6%) including masked HT (n: 2) and ambulatory HT (n: 1). Five obese children had prehypertension and 17 had white coat hypertension. All subjects were normotensive in the healthy group. Indexed casual SBP and DBP, 24-hour, daytime and nighttime SBP-SDS, DBP-SDS, and MAP-SDS did not significantly differ between the obese and healthy groups. Although a statistically significant difference was found in the mean value of heart rate between the groups, there was no significant between-group difference in the median values of 24-h, day- and nighttime heart rate-SDS (Table III).

While the mean pulse pressure values did not significantly differ between the groups, the mean AASI was significantly higher in obese children compared to healthy subjects (0.42 ± 0.15 versus 0.29 ± 0.18, p <0.001; Table III). The clinical parameters (age, gender, height-SDS and BMI-SDS) blood pressure parameters (logarithmic-indexed-casual SBP and DBP, 24-h, MAP-SDS, nighttime SBP-SDS and load, systolic and diastolic nocturnal dipping) and laboratory findings (vitamin D and LDL-,cholesterol, levels) were separately analyzed in the obese and healthy subjects by the univariate analysis to determine the factors influencing AASI. All variables with a p value of <0.20 and parameters which were reported to be associated in previous studies (age, gender, height-SDS, BMI-SDS, log-indexed-casual SBP, nighttime SBP-SDS, nighttime SBP-Load, systolic and diastolic nocturnal dipping) were included in the enter model of the multivariate linear regression analyses. As shown in Table IV, AASI significantly correlated with indexed-casual SBP, nighttime SBP-SDS and nighttime SBP load, systolic and diastolic nocturnal dipping, with no independent predictor. Furthermore, AASI was evaluated with the presence of dyslipidemia, increased HOMA-IR and metabolic abnormality, no significant difference was noted.

Table III. Results of arterial stiffness indexes, casual and 24-h ambulatory blood pressure measurements of participants.

Measurements	Healthy participants n=42	Obese participants n=53	*p* value
Indexes of arterial stiffness			
AASI	0.29 ± 0.18	0.42 ± 0.15	<0.001
Pulse pressure, mmHg*	44.5 (9)	44 (10)	0.23
24-h HR, bpm	83.2 ± 8.4	88.2 ± 7.5	0.002
24-h HR-SDS *	0.10 (0.01)	0.10 (0.00)	0.48
Daytime HR-SDS *	-0.50 (1.00)	-0.10 (1.06)	0.058
Nighttime HR-SDS *	0.21 (1.55)	0.60 (1.15)	0.46
Casual SBP, indexed	0.91 ± 0.08	0.95 ± 0.10	0.73
Casual DBP, indexed	0.81 ± 0.08	0.84 ± 0.12	0.27
24-h SBP-SDS	-0.15 ± 0.92	-0.24 ± 1.15	0.54
24-h SBP-load, % *	5.5 (12.0)	7.0 (18.0)	0.80
24-h DBP-SDS	-0.28 ± 0.83	-0.63 ± 0.99	0.73
24-h DBP-load, % *	8.0 (10.0)	9.0 (16.0)	0.39
24-h MAP-SDS	0.06 ± 0.77	0.02 ± 0.90	0.72
Daytime SBP-SDS	-0.45 ± 0.93	-0.49 ± 1.06	0.27
Daytime SBP-load, % *	3.5 (9.9)	6.9 (13.7)	0.59
Daytime DBP-SDS*	-0.86 (1.00)	-0.60 (1.23)	0.85
Daytime DBP-load, % *	5.2 (9.1)	7.1 (12.1)	0.08
Nighttime SBP-SDS	0.27 ± 0.79	0.32 ± 1.12	0.12
Nighttime SBP- load, % *	10.2 (29.6)	10.0 (31.6)	0.53
Nighttime DBP-SDS*	0.61 (0.98)	0.21 (1.44)	0.051
Nighttime DBP- load, % *	13.8 (27.6)	12.5 (31.6)	0.83
Non-dippers for SBP (n, %)	33 (78.6)	34 (64.2)	0.12
Non-dippers for DBP, n (%)	15 (35.7)	12 (22.6)	0.13

AASI: ambulatory arterial stiffness index, DBP: diastolic blood pressure, HR: heart rate, MAP: mean arterial pressure, SBP: systolic blood pressure, SDS: standard deviation score, NS: not significant.
* Non-parametric values are presented as median (IQR).

Discussion

The important finding of this study was that the AASI was significantly increased in obese children, with no significant difference in the pulse pressure.

Arterial stiffness has been noted as one of the most important hemodynamic factors contributing to the development of cardiovascular complications.[20] Increased arterial stiffness can predict the risk for cardiovascular death and development of cardiovascular disease at an early stage before vascular lesions induce symptoms.[21] The last decade has observed increased interest in the methods of measuring arterial elasticity, either directly or by the way of surrogate measures. The important question that needs to be answered is which methods are the best options for assessing arterial stiffness. The answer should include cost-effective, accessible, and easy methods.

The most common available techniques for evaluating arterial stiffness are those measuring carotid artery IMT (with M-mode measurements), flow-mediated dilatation, PWV and augmentation index. These methods have been shown to predict cardiovascular events in patients with hypertension, kidney failure,

Table IV. Univariate and multivariate analysis of AASI, clinical and laboratory parameters of obese participants.

Parameters	Univariate analysis		Multivariate analysis		
	Correlation *r*	*p* value	95% CI	Beta value	*p* value
Age	0.018	0.86	-0.016 to 0.012	-0.034	0.80
Gender	-0.196	0.80	-0.151 to 0.023	-0.205	0.14
Height-SDS	-0.088	0.40	-0.021 to 0.055	0.115	0.37
BMI-SDS	-0.122	0.39	-0.093 to 0.048	-0.084	0.52
Log-indexed-casual SBP	0.251	0.07	-0.049 to 1.561	0.247	0.065
Log-indexed-casual DBP	-0.012	0.93			
24-h MAP-SDS	0.023	0.82			
Nighttime SBP-SDS	0.220	0.032	-0.066 to 0.057	-0.033	0.88
Nighttime SBP-load	0.199	0.068	-0.001 to 0.004	0.215	0.29
Systolic nocturnal dipping	-0.335	0.001	-0.019 to -0.001	-0.356	0.074
Diastolic nocturnal dipping	-0.271	0.008	-0.007 to 0.004	-0.133	0.41
LDL-C	0.031	0.82			
Vitamin-D	0.022	0.87			

CI: confidence interval, DBP: diastolic blood pressure, LDL-C: low-density lipoprotein cholesterol; Log: logarithm; MAP: mean arterial pressure, SBP: systolic blood pressure, SDS: standard deviation score.

and diabetes mellitus.[22,23] However, all these techniques require experienced persons and expensive tools. AASI, which is an accessible, cost-effective, and easy method derived from ABPM, was introduced as an index that predicts cardiovascular risk and particularly stroke in different populations.[8,9] In some studies, AASI has been found to be correlated with PWV and carotid-IMT.[24] However, some researchers have found only a weak correlation between AASI and PWV and have suggested AASI only as a surrogate measure of arterial stiffness.[25] Until now there has been no study to validate AASI and other measurement techniques of arterial stiffness. Increased AASI has been previously shown in patients with hypertension, diabetes mellitus, and end-stage kidney disease (ESKD).[11,26,27] In the obese population, AASI has been investigated in very few studies and was found to be significantly higher.[12,28] Similarly, the important finding in our study was that AASI increased significantly in obese children compared to matched lean children.

Over the last decade, researchers have focused on measures of subclinical atherosclerosis in childhood obesity to predict cardiovascular risk in adulthood. In previous studies, it has been shown that adolescents whose LDL-cholesterol levels were elevated, showed increased carotid artery IMT and endothelial dysfunction. Obesity itself, along with insulin resistance, dyslipidemia, and hypertension, may contribute to increased cardiovascular morbidity and mortality.[13] In our study dyslipidemia was noted in 39.6% of the obese children, with no significant correlation between AASI and LDL-cholesterol. A greater number of patients are needed to assess this relationship.

Pulse pressure is another marker to evaluate arterial stiffness. In previous studies, pulse pressure was assessed together with AASI and found to be significantly increased in hypertensive obese population.[12,29] Also, increased pulse pressure has been suggested to be related with aging and decreased vascular compliance.[30] On the contrary, there was no significant difference in pulse pressure between obese and lean children in our study. It may be associated with the younger age and lower rate of hypertension in our participants.

Previously, AASI was studied in a large group of hypertensive adults and was determined to be significantly increased.[8,9] The data on AASI in children with hypertension are insufficient.

Some researchers reported that hypertension was the most important and independent factor affecting AASI.[12] Simonetti et al.[26] showed that hypertensive children had an increased AASI regardless of their BMI. On the contrary, Stergiou et al.[29] stated a significant relationship between AASI and BMI with no correlation to hypertension. Our study which included obese children who were nearly all normotensive showed no significant correlation between AASI and BMI-SDS. On the other hand, AASI significantly correlated with indexed-casual SBP, nighttime SBP-SDS and -load, systolic and diastolic nocturnal dipping, with no independent predictor. The cause of increased AASI in normotensive obese individuals needs to be clarified. We do not have an accurate explanation concerning increased arterial stiffness in normotensive obese population. Some theories have been asserted to explain the causes of increased AASI including inflammation, oxidative stress, and activation of the sympathetic nervous system. In our study, 94% of the participants were normotensive, and 9.4% had elevated hsCRP levels which were measured as an inflammatory marker. In univariate correlation analysis, AASI was not associated with hsCRP.

The strength of this study comes from the evaluation of mostly normotensive obese children and the comparison to a matched control group of lean, healthy children. Furthermore, it was essential to show the significant correlation between AASI and indexed-casual SBP, nighttime SBP-SDS and load, systolic and diastolic nocturnal dipping.

The limitations of this study includes a low sample size, lack of waist circumference, lack of correlation with PWV and carotid distensibility, and lack of other inflammation markers.

In conclusion, this study confirms that AASI increased significantly even in normotensive obese children and that AASI is mainly influenced by systolic BP. This method is a cheaper, easier, and a more accessible method than others to assess arterial stiffness. Further prospective studies are needed to explain the causes of increased AASI in normotensive populations, to investigate the role of inflammation, oxidative stress, and activation of the sympathetic nervous system, and to evaluate the association between AASI and cardiovascular disease. Early detection of increased arterial stiffness can help clinicians come up with preventive measures in the management of their patients.

Acknowledgments

We thank the patients and their families taking part in this study.

REFERENCES

1. World Obesity Federation, World Map of Obesity. Available at: http://www.worldobesity.org/aboutobesity/world-map-obesity/?map=children. (Accessed on April 2, 2017)
2. Rosner B, Cook NR, Daniels S, Falkner B. Childhood blood pressure trends and risk factors for high blood pressure: The NHANES experience 1988-2008. Hypertension 2013; 62: 247-254.
3. Theodore RF, Broadbent J, Nagin D, et al. Childhood to early-midlife systolic blood pressure trajectories: Early-life predictors, effect modifiers, and adult cardiovascular outcomes. Hypertension 2015; 66: 1108-1115.
4. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998; 338: 1650-1656.
5. Beauloye V, Zech F, Tran HT, Clapuyt P, Maes M, Brichard SM. Determinants of early atherosclerosis in obese children and adolescents. J Clin Endocrinol Metab 2007; 92: 3025-3032.
6. Skilton MR, Celermajer DS. Endothelial dysfunction and arterial abnormalities in childhood obesity. Int J Obes (Lond) 2006; 30: 1041-1049.
7. Aggoun Y, Farpour-Lambert NJ, Marchand LM, Golay E, Maggio AB, Beghetti M. Impaired endothelial and smooth muscle functions and arterial stiffness appear before puberty in obese children and are associated with elevated ambulatory blood pressure. Eur Heart J 2008; 29: 792-799.

8. Dolan E, Li Y, Thijs L, et al. Ambulatory arterial stiffness index: rationale and methodology. Blood Press Monit 2006; 11: 103-105.

9. Dolan E, Thijs L, Li Y, et al. Ambulatory arterial stiffness index as a predictor of cardiovascular mortality in the Dublin Outcome Study. Hypertension 2006; 47: 365-370.

10. Kikuya M, Staessen JA, Ohkubo T, et al. Ambulatory arterial stiffness index and 24-hour ambulatory pulse pressure as predictors of mortality in Ohasama, Japan. Stroke 2007; 38: 1161-1166.

11. Sulakova T, Janda J, Cerna J, Janstova V, Feber J. Assessment of arterial stiffness from ambulatory blood pressure monitoring in children with diabetes mellitus type-1 (DMT1). J Hum Hypertens 2012; 26: 357-364.

12. Saner C, Simonetti GD, Wuhl E, Mullis PE, Janner M. Increased ambulatory arterial stiffness index in obese children. Atherosclerosis 2015; 238: 185-189.

13. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC growth charts for the United States: methods and development. National Center for Health Statistics. Vital Health Stat 11 2002; 246: 1-190.

14. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu M, Kendirici M, Keskin M, Kondolot M. Insulin resistance in obese children and adolescents: HOMA–IR cut–off levels in the prepubertal and pubertal periods. J Clin Res Pediatr Endocrinol 2010; 2: 100-106.

15. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung and Blood, Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 2011; 128(Suppl 5): S213-S256.

16. Munns CF, Shaw N, Kiely M, et al. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. J Clin Endocrinol Metab 2016; 101: 394-415.

17. Neuhauser HK, Thamm M, Ellert U, Hense HW, Rosario AS. Blood pressure percentiles by age and height from nonoverweight children and adolescents in Germany. Pediatrics 2011; 127: e978-e988.

18. Flynn JT, Daniels SR, Hayman LL, et al. Update: ambulatory blood pressure monitoring in children and adolescents: a scientific statement from the American Heart Association. Hypertension 2014; 63: 1116-1135.

19. Wuhl E, Witte K, Soergel M, Mehls O, Schaefer F; German Working Group on Pediatric Hypertension. Distribution of 24-h ambulatory blood pressure in children: normalized reference values and role of body dimensions. J Hypertens 2002; 20: 1995-2007.

20. Laurent S, Cockcroft J, Van Bortel L, et al; European Network for Non-invasive Investigation of Large Arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006; 27: 2588-2605.

21. Domanski M, Norman J, Wolz M, Mitchell G, Pfeffer M. Cardiovascular risk assessment using pulse pressure in the first national health and nutrition examination survey (NHANES I). Hypertension 2001; 38: 793-797.

22. Mancia G, De Backer G, Dominiczak A, et al; Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2007; 25: 1105-1187.

23. Wang KL, Cheng HM, Sung SH, et al. Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study. Hypertension 2010; 55: 799-805.

24. Li Y, Wang JG, Dolan E, et al. Ambulatory arterial stiffness index derived from 24-hour ambulatory blood pressure monitoring. Hypertension 2006; 47: 359-364.

25. Leoncini G, Ratto E, Viazzi F, et al. Increased ambulatory arterial stiffness index is associated with target organ damage in primary hypertension. Hypertension 2006; 48: 397-403.

26. Simonetti GD, Von Vigier RO, Wuhl E, Mohaupt MG. Ambulatory arterial stiffness index is increased in hypertensive childhood disease. Pediatr Res 2008; 64: 303-307.

27. Degi A, Kerti A, Cseprekal O, et al. Ambulatory arterial stiffness index in children after kidney transplantation. Pediatr Transplant 2013; 17: 598-604.

28. Prochotska K, Kovacs L, Vitariusova E, Feber J. Is arterial stiffness predicted by continuous metabolic syndrome score in obese children? J Am Soc Hypertens 2016; 10: 47-54.

29. Stergiou GS, Kollias A, Giovas PP, Papagiannis J, Roussias LG. Ambulatory arterial stiffness index, pulse pressure and pulse wave velocity in children and adolescents. Hypertens Res 2010; 33: 1272-1277.

The Turkish Journal of Pediatrics 2020; 62: 267-273
https://doi.org/10.24953/turkjped.2020.02.013

Original Article

The corrected QT interval prolongation in adolescents with cardiac iron overload β-thalassemia major

Najib Advani, Ahmad Kautsar, Murti Andriastuti

Department of Child Health, Faculty of Medicine Universitas Indonesia-Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

ABSTRACT

Background and objectives. Iron-induced cardiomyopathy remains the leading cause of mortality in β-thalassemia major patients. The T2* magnetic resonance imaging (MRI) technique is the gold standard for iron load detection, yet it is expensive and not widely available especially in the developing countries. Some previous studies showed that QTc interval could be used as an early detection of cardiac iron overload. This study aimed to evaluate the diagnostic value of QTc interval as a marker of early detection of cardiac iron overload in adolescent beta thalassemia major patients.

Methods. We prospectively evaluated electrocardiography (ECG) parameter of QTc interval in 50 β-thalassemia major patients aged 10-18 years. All participants had a 12-lead ECG evaluation, echocardiogram and cardiac MRI T2* examination within three months (average 15 days). They were categorized as cardiac iron overload (MRI T2* <20 millisecond) and non-cardiac iron overload (MRI T2* >20 millisecond).

Results. Of the 50 patients, the male to female ratio was 1.08:1 and the mean age was 13.7 ± 2.43 years. All participants showed normal systolic and diastolic function using conventional echocardiography. The mean QTc interval was significantly different between cardiac iron overload group (464.44 ± 20.35 ms) and non-cardiac iron overload group (431.09 ± 32.29) (p= 0.001). Diagnostic study of QTc interval resulted in AUC 0.8 (p= 0.002). Calculated sensitivity and specificity of QTc interval were 0.88 and 0.73 respectively, with cut-off point of 449 ms.

Conclusion. Cardiac iron overload is associated with QTc prolongation in adolescents. QTc interval of 449 ms could be considered as a cut-off point of cardiac iron overload.

Key words: adolescent, β- thalassemia major, cardiac MRI T2*, QTc interval.

Thalassemia is the most prevalent single gene-disorder which has 4.5% prevalence of carrier gene and 300.000-500.000 homozygotes born each year.[1] Regular blood transfusion is inevitable in beta thalassemia major due to its nature of chronic hemolysis and impose the risk of having iron overload.[2] Routine blood transfusion in addition to increase of intestinal iron absorption will lead to iron loading in many organs.[2,3] Iron overload or in combination with immunogenetic factors will lead to the development of cardiomyopathy in beta-thalassemia major.[4-6] Despite the advances in iron chelating agent, iron overload cardiomyopathy remains the most important cause of mortality and morbidity.[7-9] Hence, it is of utmost important to detect iron overload early as adequate agent could reverse the disease process.[10-12]

Echocardiography study is the standard monitoring of cardiac function in thalassemia patients yet systolic and diastolic dysfunction are late signs.[13,14] Rhythm monitoring using 12-lead electrocardiography (ECG) was previously considered nonspecific although recent meta-analysis of case control studies has proven otherwise.[15-17] Cardiac magnetic resonance imaging T2-star (T2*) is currently becoming the gold standard of cardiac iron overload

✉ Najib Advani
najib.advani@gmail.com

Received 20th December 2018, revised 17th March 2019, 10th June 2019, accepted 20th September 2019.

evaluation and has a good correlation with cardiac iron concentration.[18-20] However, it is quite expensive and not widely available in the developing countries.

The incidence of QT prolongation and sudden cardiac death are increased among thalassemia major patients.[21-30] A previous study revealed that increased iron stores are associated with QT prolongation independent of hemochromatosis genotype and inflammation.[17] Therefore, we evaluated the current literature to evaluate the association of QTc interval prolongation and cardiac iron loading. However, to the best of our knowledge there is no such study done exclusively in the adolescent group. The aim of this study was to determine the use of QTc interval prolongation in adolescent beta-thalassemia major to detect cardiac hemosiderosis assessed by MRI T2*.

Material and Methods

Study population

Fifty adolescent patients, aged 10-18 years (27 males, 23 females) with β-thalassemia major were enrolled in this study during June until November 2017. Inclusion criteria were asymptomatic β-thalassemia major patients with pre-transfusion hemoglobin level above 7 g/dl. Patients with clinical sign and symptoms of heart failure, impaired renal and liver function were excluded. All subjects were receiving regular blood transfusions every 2-3 weeks and chelation therapy, which was started before the age of 5 years. At the time of MRI examination, 32 patients were receiving deferiprone (DFP) chelation therapy, 8 deferasirox (DFX), 4 combination therapy of DFP and DFX, 3 combination therapy of DFP and deferoxamine (DFO), and 3 combination therapy of DFP and DFX.

All subjects were evaluated for 12-lead electrocardiography and echocardiography within 3 months after cardiac MRI T2* examination. The local ethics committee approved the study protocol (decision number 424, dated may 8th, 2017) and written informed consent was obtained from all patients and/or parents.

Ferritin measurement

Ferritin levels were determined by an electrochemiluminescence technique using the Roche e 411 Cobas immunoassay analyzer (Roche Diagnostics). The mean serum ferritin value was derived from the mean obtained at 3-months interval over the previous year.

Cardiac iron concentration

T2* MRI examination was performed by 1.5 Tesla MRI scanner (Siemens Avanto Germany). Myocardial T2* was analyzed using dedicated software (Thalassemia-Tools; Cardiovascular Imaging Solutions, London, United Kingdom) with regions of interest in ventricular septum. Each image was acquired during 11-13 s breath-hold, using a gradient echocardiogram sequence. The repetition time was 200 millisecond, the flip angle used was 20^0, echo times was 1.3-23 ms, the base resolution matrix was 128 pixels, the field of view was 39.7 cm and 19.7 cm, and the sampling bandwidth was 125 kHz. Results of cardiac T2* were categorized as severe (T2* < 10 ms), mild to moderate (10 ms <T2*< 20 ms), and acceptable (T2* >20 ms). All investigators involved in the study were blind of any information regarding patient's medical records.

Echocardiography

Systolic function (fractional shortening and ejection fraction) and diastolic function (ratio of the Early (E) to late (A) ventricular filling velocity) were recorded. Normal left systolic function was defined as ejection fraction between 56%-78% and fraction shortening between 28%-44%; while normal diastolic function was defined as E/A ratio 1.9 ± 0.5.[31] Normal right ventricular systolic function was defined as tricuspid annular plane systolic excursion (TAPSE) > 16 mm.[32]

ECG Measurement

We performed 12-lead ECG examinations on all subjects with speed set at 25 mm/second and gain at 10 mm/mV. The ECG was scanned and interpreted manually blinded to the MRI results. Conduction parameters, including PR, QRS, and QT intervals were measured from the average of three consecutive beats mostly in lead II. The QT interval were measured from the start of QRS complex until the end of T wave. The end of QT interval was selected using tangential method.[26] Correction of QT interval for heart rate was calculated using Bazett's formula (QTc = QT/√RR). All interval measurements were presented in milliseconds. ECG was measured prior to cardiac MRI examination.

Statistical analysis

All tests were carried out using SPSS (Statistical Package for Social Sciences) version 23 software (IBM Corp., NY, USA). Sample size was calculated using formula for comparing two independet means. Data are expressed as mean ± standard deviation as indicated. Saphiro-Wilk test was performed for testing normality. Statistically significant differences between two groups of continous variables were determined by using independent t-test or Mann-Whitney test as appropriate. Diagnostic study and cut-off were set using receiver operating curve analysis. A P-value < 0.05 was considered statistically significant.

Results

Subjects comprised of 50 patients (mean age 13.7 ± 2.43 years, 54% [n=27] male, 46% [n=23] female) with transfusion dependent β-thalassemia major. Patient's demographic and baseline characteristics are presented in Table I. There were 41 (82%) patients without evidence of cardiac hemosiderosis (T2* >20 ms), while 9 (18%) had cardiac hemosiderosis, (T2* < 20 ms), of whom 2 had severe cardiac hemosiderosis (T2*<10 ms). Mean ferritin serum levels were increased in patients with cardiac hemosiderosis (5185 ± 2247 ng/ml) compared to non-siderosis (4339 ± 2011 ng/ml) but it was statistically not significant (p= 0.32).

Table I. Baseline characteristics of patients.

Characteristics	n=50
Gender n (%)	
Male	27 (54)
Female	23 (46)
Age, mean ± SD, years	13.71 ± 2.43
Age at diagnosis, mean ± SD, months	8.19 ± 8.24
Body weight, mean ± SD, kg	33.07 ± 10.05
Frequency of transfusion/year, mean ± SD, times/years	16 ± 3.7
Pre-transfusion Hb, mean ± SD, g/dl	9 ± 1.32
Chelation	
Monotherapy	
DFP	32 (64%)
DFX	8 (16%)
Combination	
DFP+DFX	4 (8%)
DFP+DFO	3 (6%)
DFO+DFX	3 (6%)

SD: Standard deviation, DFP: deferipron, DFX: desferoxamine, DFO: desferral.

Echocardiography examination of all patients revealed no abnormalities either in systolic or diastolic parameters as shown in Table II. Of all assessed ECG parameters, only mean QTc interval showed significant difference between cardiac iron overload (CIO) and no-CIO groups (p= 0.001) (Table III). We could not find other causes of long QTc in subjects with prolonged QTc. Further ROC analysis of QTc interval demonstrated an area under curve (AUC) value of 0.835 (p= 0.02 CI 95% 0.705-0.965) for the presence of cardiac iron (Fig. 1). The optimal cut-off point of diagnosis CIO was selected at 449 ms and yielded a sensitivity of 88.9% and a specificity of 73.1%. Negative predictive value (NPV) and positive predictive value (PPV) for this value were 42.1% and 96.7%, respectively.

Discussion

To our knowledge, this is the first study assessing ECG parameters exclusively done in adolescent

Table II. Echocardiography results.

Echo Results	T2* < 20 (n=9)	T2* > 20 (n=41)	p
Left Ventricle Systolic Function			
LVEF, mean ± SD, %	67.2 ± 6.5	66.03 ± 6.4	0.53[a]
FS, mean ± SD, %	35.7 ± 6.7	35.98 ± 4.99	0.92[a]
Right Ventricle Systolic Function			
TAPSE, mean ± SD, mm	22.64 ± 2.91	23.54 ± 4.05	0.55[b]
Diastolic Function			
E/A, mean ± SD	1.78 ± 0.3	1.7 ± 0.26	0.48[a]

[a] Mann-whitney test [b] independent t-test
LVEF: Left ventricular ejection fraction, SD: Standard deviation
FS: Fractional shortening, TAPSE: Tricuspid annular plane systolic excursion
E/A: Ratio of the early to late ventricular filling velocity, mm: millimeter.

Table III. Difference of ECG parameters between CIO and no-CIO patients.

ECG Parameters (mean ± SD)	T2*<20 (n=9)	T2*>20 (n=41)	p
Heart Rate, (BPM)	95 ± 13	86 ± 18	0.09[a]
PR Interval (ms)	134.25 ± 32.77	147.61 ± 21.02	0.13[b]
QRS duration (ms)	77.75 ± 10.21	78.82 ± 13.32	0.921[b]
QT Interval (ms)	417.8 ± 57	387 ± 38.4	0.052a
QTc Interval (ms)	464.44 ± 20.35	431.09 ± 32.29	0.001[a]

[a] T-test [b] Mann-Whitney Test
BPM: beat per minute, CIO: Cardiac Iron Overload, ms: millisecond.

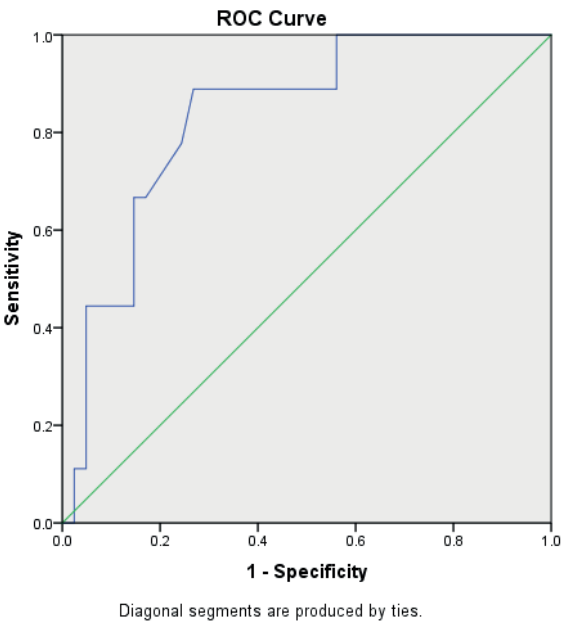


Fig. 1. ROC curve of QTc interval.

β-thalassemia patients. The most important finding of the present study is the difference of QTc interval in CIO and no-CIO adolescent β-thalassemia patients. Furthermore, we demonstrated that QTc interval had relatively good diagnostic value to diagnose CIO.

Patients with iron overload cardiomyopathies experience heart failure with systolic and diastolic dysfunction often in combination with electrical rhythm disturbances, including slowed electrical conduction, heart block, and the increase likelihood of having atrial fibrillation.[14,27] Iron overload cardiomyopathies are marked by early diastolic dysfunction which precedes systolic dysfunction.[14,27] Conventional echocardiography examination in this study was all normal indicating that all patients were at the early stage of the disease.

Electrical rhythm disturbances recorded in CIO in previous studies are mostly non-specific, typically associated with repolarization abnormalities and relative bradycardia.[25] QT interval as a mean of identifying depolarization and repolarization abnormalities are highlighted in our study. Recent meta-analysis showed

that thalassemia patients have prolonged QT interval compared to healthy controls.[17] The relationship between QT parameters and iron overload measured by serum ferritin had been studied previously with conflicting results.[24,28] Ulger et al.[28] found no correlation of QT parameters (QT interval, QTc interval, and QT dispersions) with serum ferritin levels while Faruqi et al.[24] showed QT interval, QTc interval, and QT dispersion are prolonged in patients with high serum ferritin level (>2500 ng/ml). Although serum ferritin was traditionally used as a marker for iron overload, it does not necessarily represent tissue's iron content since its level is influenced by many factors including infections. Furthermore, ferritin level is measured from the blood and not from any organ such as the heart, therefore it may not reveal its real level in the heart. In this study we measured directly focused to the heart by ECG and cardiac MRI, which shows obvious correlation between MRI and ECG results.

Measuring cardiac iron loading has become possible using MRI T2*, and well corellated with cardiac iron loading in vivo.[19,20] The relationship between electrocardiographic recording and cardiac MRI T2* has been prevously studied.[25] Detterich et al.[25] found that repolarization indices were the most sensitive discriminators and QT interval was greater in patients with CIO.[25] In our study, mean QT interval was not statistically different but QTc interval was significantly increased in CIO patients. Although Detterich et al.[25] had also found a significantly longer QTc in patients with detectable cardiac iron they did not exclusively include adolescents. Our study specified in adolescent patients which may or may not be valid for other age groups. Besides QT parameters, other repolarization abnormalities such as ST and T wave abnormalities may be found in CIO patients which was not focused in our study. QT prolongation in thalassemia patients may be secondary to compensatory ventricular dilation and increased circulatory oxidative species caused by labile plasma iron.[29,30] In an animal model, CIO was characterized by a decrease of the overshoot and duration of action potential and impairment of delayed rectifier potassium channel.[33] CIO is also associated with bradycardia which may arise from altered intrinsic sinoatrial node function.[34] The number of patients with bradycardia was not different between two groups in our study which may be due to small sample size or relatively early stage of the disease.

The relatively good diagnostic value of QTc interval with high sensitivity might lead to the use of QTc interval as a screenig tool. The QTc interval prolongation perhaps could be regarded as a surrogate marker of MRI T2* to detect cardiac iron overload especially in the adolescent group. It is indeed much cheaper, widely available and could be done by the majority of health personnel. It is needed in areas where thalassemia is of high prevalence with limited resources. However, larger sample size and multi center study may be needed to support the results

Cardiac iron overload is associated with QTc prolongation in the adolescent. The QTc interval value of 449 ms might be used as a cut off point of cardiac iron overload.

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008; 86: 480-487.
2. Hershko C. Iron loading and its clinical implication. Am J Hematol 2007; 82(Suppl 12): 1147-1148.
3. Martin A, Thompson AA. Thalassemias. Pediatr Clin N Am 2013; 60: 1383-1391.
4. Kremastinos DT, Tiniakos G, Theodorakis GN, Katritsis DG, Toutouzas PK. Myocarditis in beta-thalassemia major. A cause of heart failure. Circulation 1995; 91: 66-71.
5. Kremastinos DT, Flevari P, Spyropoulou M, Vrettou H, Tsiapras D, Stavropoulos-Giokas CG. Association of heart failure in homozygous beta-thalassemia with the major histocompatibility complex. Circulation 1999; 100: 2074-2078.

6. Economou-Petersen E, Aessopos A, Kladi A, et al. Apolipoprotein E epsilon-4 alelle as a genetic risk factor for left ventricular failure in homozygous thalassemia. Blood 1998; 92: 3455-3459.

7. Modell B, Khan M, Darlison M. Survival in beta thalassemia major in the UK: data from the UK Thalassemia register. Lancet 2000; 355: 2051-2052.

8. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Hematologica 2004; 89: 1187-1193.

9. Felker GM, Thompson RE, Hare JM, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med 2000; 342: 1077-1084.

10. Tanner MA, Galanello R, Dessi C, et al. A randomized, placebo-cotrolled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. Circulation 2007; 115: 1876-1884.

11. Farmaki K, Tzoumari I, Pappa C, Chouliaras G, Berdoukas V. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complication of thalassemia major. Br J Haematol 2010; 148: 466-475.

12. Porter JB. Optimizing iron chelation strategies in beta-thalassemia major. Blood Rev 2009; 23(Suppl 1): S3-S7.

13. Davis BA, O'Sullivan C, Jarrit PH, Porter JB. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. Blood 2004; 104: 263-269.

14. Wood JC, Enriquez C, Ghugre N, et al. Physiology and pathophysiology of iron cardiomyopathy in thalassemia. Ann N Y Acad Sci 2005; 1054: 386-395.

15. De Chiara B, Crivellaro W, Sara R, et al. Early detection of cardiac dysfunction in thalassemic patients by radionuclide angiography and heart rate variability analysis. Eur J Haematol 2005; 74: 517-522.

16. Qureshi N, Avasarala K, Foote D, Vichinsky EP. Utility of holter echocardiogram in iron-overloaded hemoglobinopathies. Ann N Y Acad Sci 2005; 1054: 476-480.

17. Henriksen LF, Petri AS, Hasselbach HC, Kanters JK, Ellervik C. Increased iron stores prolong the QT interval-a general population study including 20 261 individuals and meta-analysis of thalassemia major. Br J Haematol 2016; 174: 776-785.

18. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001; 22: 2171-2179.

19. Kirk P, He T, Anderson LJ, et al. International reproducibility of single breathhold T2* MR for cardiac and liver iron assessment among five thalassemia centers. J Magn Reson Imaging 2010; 32: 315-319.

20. Carpenter JP, He T, Kirk P, et al. On T2* magnetic resonance and cardiac iron. Circulation 2011; 123: 1519-1528.

21. Garadah TS, Kassab S, Mahdi N, Abu-Taleb A, Jamsheer A. QTc interval and QT dispersion in patients with thalassemia major: electrocardiographic (EKG) and echocardiographic evaluation. Clin Med Insights Cardiol 2010; 4: 31-37.

22. Oztarhan K, Delibas Y, Salcioglu Z, et al. Assessment of cardiac parameters in evaluation of cardiac functions in patients with thalassemia major. Pediatr Hematol Oncol 2012; 29: 220-234.

23. Farahani B, Abbasi MA, Khaheshi I, Paydary K. Evaluation of QT interval in β thalassemia major patients in comparison with control group. Heart Views 2012; 13: 42-45.

24. Faruqi A, Ahmad SI, Ahmed ST. Evaluation of QT parameters in patients of thalassemia majors with iron overload. J Pak Med Assoc 2016; 66: 799-802.

25. Detterich J, Noetzli L, Dorey F, et al. Electrocardiographic consequences of cardiac iron overload in thalassemia major. Am J Hematol 2012; 87: 139-144.

26. Postema PG, De Jonas SSG, Van der Bilt IAC, Wilde AAM. Accurate electrocardiographic assessment of the QT interval: teach the tangent. Heart Rhythm 2008; 5: 1015-1018.

27. Pennell DJ, Udelson JE, Arai AE, et al; American Heart Association Committee on Heart Failure and Transplantation of the Council on Clinical Cardiology and Council on Cardiovascular Radiology and Imaging. Cardiovascular function and treatment in β-thalassemia major a consensus statement from the American heart association. Circulation 2013; 128: 281-308.

28. Ulger Z, Aydinok Y, Levent E, Gurses D, Ozyurek AR. Evaluation of QT dispersion in β thalassaemia major patients. Am J Hematol 2006; 81: 901-906.

29. Claster S, Wood JC, Noetzli L, et al. Nutritional deficiencies in iron overloaded patients with hemoglobinopathies. Am J Hematol 2009; 84: 344-348.

30. Westwood MA, Anderson LJ, Maceira AM, et al. Normalized left ventricular volumes and function in thalassemia major patients with normal myocardial iron. J Magn Reson Imaging 2007; 25: 1147-1151.

31. Haas NA, Kleideiter U. Pediatric Cardiology. Stuutgart: Thieme, 2015: 31.

32. Koestenberger M, Ravekes E, Everett AD, et al. Right ventricular function in infants, children and adolescents: reference values of the tricuspid annular plane systolic excursion (TAPSE) in 640 healthy patients and calculation of z score values. J Am Soc Echocardiogr 2009; 22: 715-719.

33. Kuryshev YA, Brittenham GM, Fujioka H, et al. Decreased sodium and increased transient outward potassium currents in iron-loaded cardiac myocytes. Implications for the arrhythmogenesis of human siderotic heart disease. Circulation 1999; 100: 675-683.

34. Rose RA, Sellan M, Simpson JA, et al. Iron overload decreases CaV1.3-dependent L-type Ca2+ currents leading to bradycardia, altered electrical conduction, and atrial fibrillation. Circ Arrhythm Electrophysiol 2011; 4: 733-742.

The Turkish Journal of Pediatrics 2020; 62: 274-279
https://doi.org/10.24953/turkjped.2020.02.014

Case Report

Tetanus; a forgotten infection disease: a report of two cases

Ülkem Koçoğlu Barlas[1], Hasan Serdar Kıhtır[1], Osman Yeşilbaş[1], Mey Talip Petmezci[1], Nihal Akçay[1], Ercüment Petmezci[1], Nevin Hatipoğlu[2], Esra Şevketoğlu[1]

[1]Division of Pediatric Intensive Care Unit, [2]Department of Pediatrics, University of Health Science, Bakırköy Dr. Sadi Konuk Health Training and Research Center, İstanbul, Turkey.

ABSTRACT

Background. Tetanus is an infectious disease that can be seen in all age groups in underdeveloped and developing countries, where vaccination programs are inadequate. In developed countries, it is reported more frequently in the adult age group, where the protection of vaccination is diminished and the doses are delayed.

Case. In this report, we present generalized tetanus, which was observed in two male patients aged 12 and 6 years, admitted at different times, together with clinical course and treatment approaches. Both patients belong to different nationalities, who immigrated a couple of months before their application to our hospital. They applied with similar histories and complaints and were not vaccinated during infancy.

Conclusion. With the development of vaccination programs, this disease with high morbidity and mortality can be prevented.

Key words: clostridium tetani, tetanus, trismus.

Tetanus is an acute, often fatal disease that is caused by an exotoxin (tetanospasmin) produced by the anaerobic, gram-positive spore-forming bacterium Clostridium tetani.[1] It can enter the body through any type of wound, such as a scratch or deep cut.[2] Tetanospasmin is attached to the neuromuscular junction and migrates to motor neurons in the central nervous system (CNS) by retrograde axonal transport and disrupts the coordination of muscle movements by inhibiting the release of neurotransmitters.[2] The microorganism is difficult to isolate and does not cause any obvious laboratory abnormality. Diagnosis is usually based on the trauma and tetanus immunization history, typical clinical findings and the exclusion of diseases that cause similar conditions.[3] As a result of successful vaccination programs, the incidence of tetanus has been reduced to 0.02 per 100,000 population in Turkey.[3] We present these two cases (three years apart) to draw attention to this disease, since it's diagnosis and treatment approaches are not well known by a new generation of physicians and it is more likely to be seen in our country with the increase in the immigrant population.

✉ Ülkem Koçoğlu Barlas
ulkemkocoglu@yahoo.com

Received 20th July 2017, revised 19th August 2017, 19th September 2017, 19th November 2017, 29th December 2018, accepted 6th April 2019.

Case 1

A 12-year-old refugee male patient, who had a history of a punture on his right foot base after stepping on a nail ten days prior to admission, was admitted to the pediatric emergency department with complaints of difficulty in opening the jaw and back pain. On physical examination, in addition to lock-jaw, he had facial and abdominal muscle rigidity. He was hospitalized in the Pediatric Intensive Care Unit (PICU) with a preliminary diagnosis of tetanus. On admission to the intensive care unit, he was conscious, aware of his surroundings and agitated due to pain; he also had difficulty in

speaking. The Glasgow Coma Scale (GCS) score was 15/15. The respiratory and cardiovascular system examinations were normal. The patient's laboratory tests were normal. His medical history revealed that he had not been vaccinated against tetanus in childhood. 3000 International Units (IU) of tetanus immunoglobulin (TIG) was administered intramuscularly to the patient, who underwent tetanus vaccination in the emergency department, so that a half dose was applied to the area around the wound. His wound was superficial and clean, therefore no surgical wound debridement was required. Osteomiyelitis was also not considered because of normal imaging and negative infection markers as a result of orthopedics opinion. Diazepam was administered in combination with dexmedetomidine and fentanyl infusions for sedation and analgesia. Metronidazole, vancomycin and ceftazidime were started together because the bone and soft tissue infections could not be distinguished in the injury area. Metronidazole and Ceftazidime were used for 10 days, Vancomycin was used for 14 days. On the second day of hospitalization, 3000 IU of TIG was readministered intramuscularly to the patient because diazepam did not reduce contractions. Then, intravenous magnesium sulphate and midazolam infusions were started. He was monitored in a silent and dark room. On the fifth day of hospitalization, he had difficulty in breathing because his body was stiff and therefore a total atelectasis developed in the left lung, which led him to be intubated. During his follow up treatment with deep sedation and analgesia it was decided to add muscle relaxant to his therapy. After intubation, analgesia was provided with fentanyl infusion whereas sedation was continued with midazolam and sodium thiopental infusions. Rocuronium infusion was started as a muscle relaxant. Baclofen was used enterally via the optimal dose-escalation strategy. On the tenth day of hospitalization, a sudden cardiac arrest developed in the patient while on mechanical ventilation and spontaneous circulation was achieved through approximately 10 minutes of cardiopulmonary resuscitation. After rocuronium and sedative agents were discontinued weekly, he was awakened. However, treatment was maintained because severe contractions were observed again. At the end of the first month, the patient who still required mechanical ventilation underwent tracheostomy due to prolonged intubation. In subsequent follow-ups, the contractions did not decrease, and he was not conscious at the time when awakened. Common ischemic areas were seen on brain magnetic resonance imaging (MRI). The current status of the patient was considered as spastic tetraplegia. He was followed by tracheostomy during the next two months. On the 110th day of hospitalization, he died due to multiple organ failure secondary to septic shock. Trismus is shown in Figure 1, contraction of the patient is shown in Figure 2.

Case 2

A 6-year-old refugee male patient was admitted to the pediatric emergency department of another hospital with a history of a punture on his left foot base after stepping on a nail ten days prior to admission. Tetanus vaccine plus 500 IU of TIG were given and then he was included into a vaccination schedule. His wound was superficial and clean, so no antibiotic treatment and surgical wound debridement was required. He was readmitted to the pediatric emergency department with complaints of backpain and difficulty in opening the mouth and swallowing

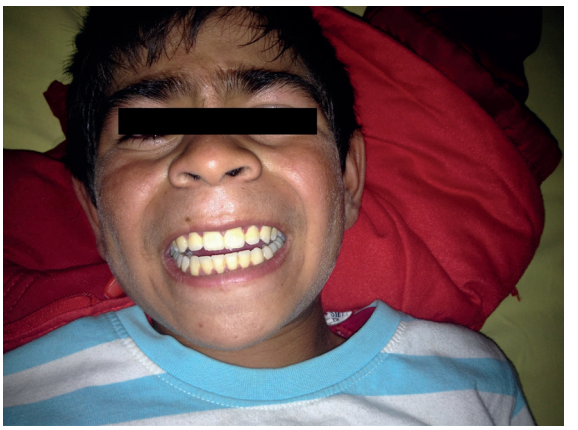


Fig. 1. Trismus of the patient.

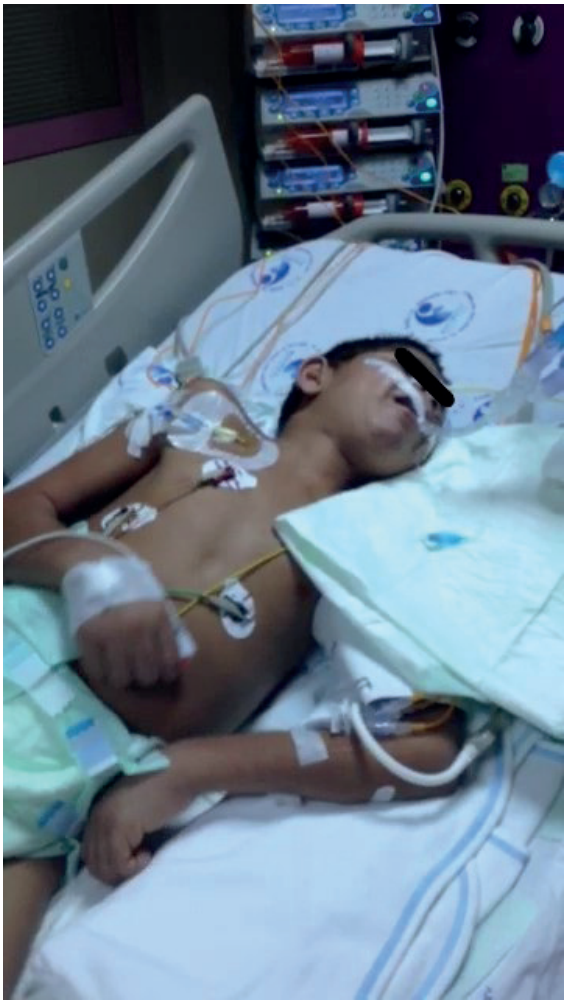


Fig. 2. Contraction of the patient.

for the last few days. At presentation, he was conscious and agitated. His physical examination revealed muscle contractions within all body and lock-jaw. The Glasgow Coma Scale (GCS) score was 13/15. Because of the contraction of the neck and back muscles, he had opisthotonus and respiratory difficulty. Other system examinations and laboratory tests were normal. His medical history indicated that he had not been vaccinated against tetanus. He was hospitalized in the PICU with a preliminary diagnosis of tetanus. Intravenous ampicillin-sulbactam and metronidazole were started and 2500 IU of TIG was administered intramuscularly to the patient that a half dose was injected around the wound areas. The patient was intubated and mechanical ventilation was started due to difficulty of breathing. After intubation, midazolam was started as a sedative, fentanyl was started as an analgesic agent, and rocuronium and magnesium sulphate were started as a muscle relaxant. Baclofen was used enterally via the optimal dose-escalation strategy. The sedation level was monitored by *the bispectral index* (BIS). Midazolam and thiopental infusions were dynamically regulated so that BSI value remained between 40 and 60. Brain regional oxygenation was monitored by *near-infrared regional spectroscopy* (NIRS) receivers attached to the right and left forehead regions. Respiration and circulatory support were provided, so that NIRS value remained over 60%. Brain MRI of the patient was assessed as normal. Despite deep sedation, rocuronium, magnesium sulphate and baclofen treatments, 500 IU of TIG was administered intrathecally to the patient due to resistant contractions observed during daily sedation vacation. Periodic hypertension and autonomic dysfunction were observed. The patient's blood pressure was controlled using amlodipine with esmolol and dexmedetomidine infusions. Sedative and muscle relaxant drugs were discontinued weekly. During the third week of hospitalization, the contractions of the patient were considerably reduced. Therefore, while rocuronium was stopped, sedative and analgesic agents were reduced. On the 26th day of hospitalization, the contractions were rather diminished and the muscle strength was sufficient for spontaneous breathing, so the patient was extubated. Non-invasive mechanical ventilation was primarily used and then oxygen was administered by the mask. On the 32nd day of hospitalization, he was conscious and aware of his surroundings and spontaneously breathed at room air and was well-fed orally. The patient was discharged when the contractions had completely disappeared.

Demographics and treatment approaches used in our patients are shown in detail in Table I.

Written consent was obtained from the parents of the patients participating in this study.

Table I. Demographics and treatment approaches used both patients.

	Case 1	Case 2
Demographics		
Age (years)	12	6
Sex (M/F)	M	M
Sedatives		
Dexmedetomidine	BI+AI	BI+AI
Diazepam	BI	-
Sodium thiopental	AI	AI
Midazolam	AI	AI
Analgesic Treatment		
Fentanyl	BI+AI	AI
Muscle Relaxants		
Rocuronium	AI	AI
Magnesium sulphate	Bolus	Infusion
Other Treatments		
Baclofen(enteral)	+	+
Antibiotics	Metronidasole, Vancomycin, Ceftazidime	Ampisilin-Sulbactam, Metronidasole
TIG(IU)	6000	3500
Mechanical Ventilation(days)	105	26

AI: after intubation, BI: before intubation, TIG: tetanos immunglobulin.

Discussion

Tetanus is one of the oldest known human diseases. Although there is a great accumulation of information about such an important disease, high mortality rates are not surprising even with the best treatment approaches. Preventative medical approaches for tetanus are far more important than therapeutic approaches.[4] The best example for this situation could be that both cases were not vaccinated in childhood.

Tetanus is always diagnosed with the history and accompanying clinical findings.[3] Both cases had a history of "puncture to the foot base by a dirty nail". This condition is usually associated with tetanus and requires urgent hospitalization. However, in our first case, there was no history of hospital admission and tetanus vaccination following the event. Our second case was vaccinated after the event, but the disease still developed. This suggests that childhood vaccinations are more important than post-event vaccinations and TIG application in suspected contaminated injuries, especially in unvaccinated cases, must be made at an early stage.

Although tetanus is an infectious disease, antibiotic treatment takes part at the end in the guidelines and recommended first-line antibiotic is metronidazole.[5] TIG is one of the most important drugs in the treatment of tetanus, and the recommended dose is 3000–6000 IU given intramuscularly.[6] In the first case, a total of 6000 IU of TIG was given with two different application methods. Although a total of 3000 IU of TIG was administered in the second case, and 500 IU of the total amount intrathecally, there was no significant difference in clinical course in terms of tetanus. Intrathecal administration of TIG has been a well-known method for many years, but it is still controversial and has not been definitely accepted.[7] Considering that the second case underwent intrathecal TIG, developed subsequent clinical findings and had an expected recovery time of 4-6 weeks, it is difficult to argue that intrathecal administration

of TIG led to a significant improvement in clinical course and the recovery period.

Sedative and muscle relaxant drugs are the essential parts of tetanus treatment. Diazepam is frequently used in adults, but its' use in children is limited because it cannot be given by continuous infusion and preservatives have possible toxic effects.[8] The first case was attempted to be followed with intensive sedation treatment prior to intubation. However, the need for intubation within days had led to the decision of earlier intubation in the management of the second case. Intubation of the patients allows using muscle relaxants and the formation of deeper sedation which leads to the comfort and protection of the patient from damage caused by contractions. Despite the positive clinical benefits, mechanical ventilation can contribute to death and permanent damage due to its' possible complications. In the first case, the development of cardiac arrest may be due to the involvement of the autonomic nervous system in tetanus[2] and may also be considered as a complication of mechanical ventilation. This can be regarded as the most important "event" for the negative outcome of the case. The involvement of the autonomic nervous system is condition reported in tetanus cases[2], and hypertension and tachycardia were seen in both cases. Dexmedetomidine is a drug which has sedative, analgesic and sympatholytic effects since it is a centrally acting alpha-2 agonist.[9] In the second case, we observed esmolol infusion showed positive effects on the attacks of hypertension and tachycardia.

Magnesium used to prevent contraction and stiffness in the treatment of tetanus prevents the effect of calcium at the neuromuscular junction. There are publications in the literature showing that magnesium is used in tetanus contractions, which do not respond to anticonvulsant and muscle relaxant drugs and as a result contractions were significantly suppressed.[10,11] We used magnesium sulphate to control refractory contractions in both cases.

The development of monitoring techniques in intensive care units seems to increase the chances of successful patient management.[12] We think that the BIS and NIRS monitors, which created differences in the management of these two cases are three years apart, may have had a critical effect on the positive outcome of the second case. The BIS monitoring, increases the patient's comfort, but also prevents the administration of excessive sedation. Similarly, the NIRS monitor gives information on cerebral blood flow and oxygen delivery and thus enables appropriate hemodynamic and ventilatory support and makes it possible to follow their results.[12] For these reasons, we believe that advanced monitoring techniques in tetanus cases may improve treatment success compared with standard approaches

Consequently, it should be kept in mind that tetanus with high mortality and morbidity rates can be seen in countries where immigration takes place. Although it has been presented as a forgotten disease in the title, it should be known that the disease will be seen more frequently if children are not vaccinated. Also injury cases should be carefully questioned in terms of the need for tetanus vaccination and TIG, and they should be implemented without delay. Identification of unvaccinated people and administration of vaccination programs could reduce the possibility of seeing a new case. We think that the use of early intubation and advanced monitoring techniques can provide positive contributions to reducing death and illness in addition to vaccination, TIG, sedation and muscle relaxant treatments.

REFERENCES

1. Stock I. Tetanus and clostridium tetani-a brief review. Med Monatsschr Pharm 2015; 38: 57-60.
2. Yalcın I, Salman N, Somer A. Bakteri enfeksiyonları. İçinde: Neyzi O, Ertuğrul T (eds). Pediatri Cilt 1-2 (4. Baskı), Ankara: Nobel Tıp Kitabevi, 2010: 613-616.
3. Aydın TT, Bayhan GI, Afsarlar CE, Oz FN, Akansel AR, Tanir G. A report of two pediatric tetanus cases. Turk J Pediatr 2011; 53: 437-440.

4. Tetanus vaccines: WHO position paper-February 2017. Wkly Epidemiol Rec 2017; 92: 53-76.

5. Rodrigo C, Fernando D, Rajapakse S. Pharmacological management of tetanus: an evidence based review. Crit Care 2014; 18: 217.

6. American Academy of Pediatrics. Tetanus (Lockjaw). In: Pickering LK, Baker CJ, Kimberlin DW, Long SS (eds). Red Book: 2012 Report of the Committee on Infectious Diseases (29th ed). Elk GroveVillage, IL: American Academy of Pediatrics, 2012: 707-712.

7. Kabura L, Ilibagiza D, Menten J, Van den Ende J. Intrathecal vs. intramuscular administration of human antitetanus immunoglobulin or equine tetanus antitoxin in the treatment of tetanus: a meta-analysis. Trop Med Int Health 2006; 11: 1075-1081.

8. McTague A, Martland T, Appleton R. Drug management for acute tonic-clonic convulsions including convulsive status epilepticus in children. Cochrane Database Syst Rew 2008; CD001905.

9. Burns J, Jackson K, Sheehy KA, Finkel JC, Quezado ZM. The use of dexmedetomidine in pediatric palliative care: a preliminary study. J Palliat Med 2017; 20: 779-783.

10. Katı I, Goktas U, Cagan E, Guzel A, Bartın S. The effect of magnesium sulfate on uncontrollable contractions in a tetanus case. Pediat Emerg Care 2012; 28: 366-367.

11. Attygalle D, Rodrigo N. Magnesium as first line therapy in the management of tetanus: a prospective study of 40 patients. Anaesthesia 2002; 57: 811-817.

12. Hayashida M, Kin N, Tomioka T, et al. Cerebral ischaemia during cardiac surgery in children detected by combined monitoring of BIS and near-infrared spectroscopy. Br J Anaesth 2004; 92: 662-669.

The Turkish Journal of Pediatrics 2020; 62: 280-283
https://doi.org/10.24953/turkjped.2020.02.015

Case Report

Clinical and laboratory awareness for an under recognized pathogen in newborn meningitis: *Mycoplasma Hominis*: a case report

Sinem Gülcan Kersin[1], Turgut Bozan[2], Hülya Özdemir[1], Hülya Selva Bilgen[1], Güner Söyletir[2], Aslı Memişoğlu[1], Eren Özek[1]

[1]Division of Neonatology, Departments of Pediatrics and [2]Department of Medical Microbiology, Marmara University Faculty of Medicine, İstanbul, Turkey.

ABSTRACT

Background. *Mycoplasma hominis* is a well-known bacterium colonizing the genito-urinary tract. It may cause pneumonia, bacteremia, abscesses, chronic lung disease, and rarely meningitis during the newborn period.

Case. A preterm infant with a birth weight of 885 grams was born at 27 weeks of gestation and had respiratory distress syndrome needing mechanical ventilation. Spontaneous intestinal perforation and grade four intraventricular hemorrhage was diagnosed on day three.

Conclusion. *M. hominis* was accepted as the causative agent of meningitis in this case report.

Key words: *Mycoplasma hominis*, newborn, meningitis, neonate, preterm.

Mycoplasma hominis is a well-known bacterium colonizing the genito-urinary tract. *M. hominis* has been associated with a variety of conditions that may affect the pregnancy period, the developing fetus and the newborn. This organism may contribute to chorioamnionitis, growth restriction, spontaneous abortion, stillbirth, premature labor and postpartum endometritis. In addition, an exposed neonate may develop pneumonia, bacteremia, meningitis, abscesses and chronic lung disease.[1] *Mycoplasma hominis* meningitis is a rare but life-threatening infection in both full term and preterm infants that might result in neurological deficits.[2,3] Survivors frequently have long-term morbidity including severe neurodevelopmental impairment. In this case report, *M. hominis* meningitis in a preterm infant is discussed.

✉ Sinem Gülcan Kersin
sinemgulcan@hotmail.com

Received 14th April 2019, revised 16th May 2019, accepted 27th May 2019.

Case Report

A 27-year-old G2P1 female at 27 weeks of gestation was admitted to the hospital with fever and coughing. On admission she developed acute respiratory distress and was transferred to the intensive care unit with the preliminary diagnosis of acute respiratory failure and sepsis. The patient was placed on mechanic ventilation, cardiac inotropic agents, large spectrum antibiotics and steroids were administered. No evidence of infection in blood and endotracheal aspirate (ETA) cultures was obtained except Candida albicans in the urine culture. As the patient clinically deteriorated despite the supportive measures and she had premature rupture of membranes, the infant was delivered with induction of labor in the intensive care unit. A female infant with a birth weight of 885 grams was born at 27 weeks of gestation with Apgar scores of 2, 3 and 5 at 1, 5 and 10 minutes respectively. Surfactant was administered and the infant was admitted to the neonatal intensive care unit (NICU). Blood and ETA cultures were obtained and vancomycin and meropenem

therapy were started due to the high-risk birth history. She was on fluconazole prophylaxis, caffeine and total parenteral nutrition and because of hypercapnia and low blood partial oxygen saturation; respiratory support was changed to high frequency oscillatory ventilation. On day two of life, liposomal amphotericin B was added to the treatment due to progressively increasing acute phase reactants, newly developed thrombocytopenia and inadequate clinical improvement under current therapy. The infant subsequently developed sinus tachycardia and morphine infusion was initiated for pain management. However, the infant's tachycardia persisted. On postnatal day three, greenish discoloration of the abdomen and general pallor evolved within minutes and spontaneous intestinal perforation was observed on abdominal X-Ray. The infant became hypotensive, with prolonged capillary filling time and bolus saline infusions were administered. Hemoglobin value was found to be 6.9 g/dl and and erythrocyte transfusion was given. Grade IV intraventricular hemorrhage was detected on transfontanel ultrasonography. The infant developed seizures on the third day and phenobarbital was administered, and levetiracetam was added to the therapy for persistent seizures. Bilateral extraventricular drains were placed by pediatric neurosurgery on day seven of life. On day 10 of triple antimicrobial therapy, there was still significant increase in acute phase reactants. Blood, ETA, cerebrospinal fluid (CSF) and urine cultures were obtained and colistin was added to therapy. No growth was detected in the blood, ETA, urine and CSF cultures. Colistin and liposomal amphotericin B were discontinued. However; acute phase reactants including CRP levels and leucocyte counts, continued to increase from 8 mg/L to 113 mg/L and 7600/μL to 33900/μL, respectively. On the 17th of life, second CSF culture was submitted to the microbiology laboratory. Leucocytes and erythrocytes with no microorganism were seen on Gram stained preparation of the sample. CSF was also inoculated onto blood agar, chocolate agar and thioglycollate broth and inoculated media were followed up daily for five days. Translucent and pinpoint colonies weakly grew on chocolate agar and blood agar on fifth day of incubation. No organism was observed on the Gram stained slide prepared from those colonies. Upon this, subculture from thioglycollate broth was performed. Two days later, translucent colonies were seen again on chocolate agar and this microorganism was identified as *Mycoplasma hominis* with MALDI-TOF MS. Since *M. hominis* is known generally as a cell wall deficient bacterium and require special medium to grow, to confirm our result, 16S rRNA gene sequencing was also performed directly from CSF sample of the patient. Genomic DNA was extracted from CSF by using DNeasy Blood and Tissue kit (Qiagen). 16S rRNA gene was amplified by PCR using universal primers 8UA and 907B and subsequently sequenced with an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Inc.). The BLAST software was used to search for DNA nucleotide sequences against similar nucleotide sequences in the database.[4] The 16S rRNA gene sequence showed 99% nucleotide identity to that of the strain *M. hominis* (GenBank accession no: CP033021.1). *M. hominis* was accepted as the causative agent of meningitis and antibiotic therapy was changed to ciprofloxacin monotherapy. On the second day of ciprofloxacin treatment, acute phase reactants decreased and therapy was continued for 21 days. On day 50 of life, the MRI showed multicyctic porencephalic hydrocephaly. The Auditory Brainstem Response (ABR) test was within normal limits. Informed consent was received from the family for writing this case. The patient was discharged at the end of the third month with a transpyloric feeding catheter and ventriculoperitoenal (VP) shunt. He had moderate neurological impairment including headlag, truncal hypotonia, unresponsiveness to sounds and there was also social developmental delay according to the Denver II test.

Discussion

Mycoplasma hominis is a rare but life-threatening infection in both full term and preterm

infants. *M. hominis* may be the causative agent of bacteremia, pneumonia, meningitis in newborns, but its significance as a pathogen causing neonatal meningitis is still unclear. *M. hominis* can also be isolated in CSF from infants without signs of meningitis.[5] Therefore, the clinical findings in newborns with *M. hominis* isolated from the CSF are variable. It may produce abnormal CSF findings such as pleocytosis or sometimes there is no inflamatory reaction in CSF.

Mycoplasma species are small-sized microorganisms. There are more than 100 species of Mycoplasma and at least 13 Mycoplasma species have been known to infect humans. *Mycoplasma pneumoniae*, *M. hominis* and *Ureaplasma urealyticum* are the most well-known species.[6] The lack of bacterial cell wall components makes *M. hominis* undetectable by Gram staining. Growth on bacterial cultures is very slow, requires specific media and occasionally can be found as pinpoint colonies on conventional media.[7] In our case; cerebrospinal fluid culture obtained on postnatal day 17 revealed pinpoint, translucent colonies on fifth day of incubation on all inoculated media and subcultures and *Mycoplasma hominis* was identified with MALDI-TOF MS. Furthermore, 16S rRNA gene sequencing of the sample also revealed presence of *M. hominis*.

M. hominis is intrinsically resistant to many antibiotics such as B-lactams, glycopeptides, sulfonamides and macrolides. The major antibiotics active against *M. hominis* are the tetracyclines, lincosamides, chloramphenicol and fluoroquinolones.[8] Our case did not respond to the broad spectrum antimicrobials but responded to ciprofloxacin within two days with a significant decline in CRP levels and total leucocyte count.

M. hominis is found in the urogenital tract with a prevalence between 15-17.7% and is mainly involved in urogenital infections and neonatal infections.[9,10] In our case the mother did not have a good follow-up during pregnancy and neither vaginal cultures nor screening for GBS was performed. Approximately half of the women with this infection may be asymptomatic or may experience only mild symptoms. In our case, the mother developed acute respiratory failure and sepsis at the 27th week of gestation. All her cultures including blood, urine and ETA cultures during her ICU stay, remained sterile. Placental pathology revealed chorioamnionitis but the cultures remained sterile as she was already receiving broad spectrum antibiotics. The mechanism by which bacterial vaginosis may lead to preterm birth is unknown.[11]

Simhan et al.[12] explains that ascending intrauterine infection occurs because of alterations in host-defense mechanisms and microbial factors. Since individuals differ in their innate ability to create an inflammatory response to bacterial products, it has been suggested that women who are immunologically hyporesponsive may not be able to control a large bacterial burden and are prone to ascending infection with variable consequences. The history of the mother revealed that she had isolated watery vaginal discharge with fishy odor about one month prior to presentation which might be the reason for her preterm birth.

In the literature extragenital infections such as joint, bone and CNS infections by *M. hominis* have been described, especially in newborn infants and immunocompromised patients. However, there are few reported cases of *M. hominis* meningitis in either premature or full term infants.[13] Until now this is also our first case diagnosed with *M. hominis* meningitis in our NICU. Hata et al.[2] reported that about one third of their adult patients with *M. hominis* meningitis had CNS complications and 28% of them had permanent neurological sequelae and 28% of them died. In this case, our patient underwent VP-shunt operation at 90th day of life after being diagnosed with hydrocephalus due to intraventricular bleeding on the postnatal day 10. During this period, ventricular taps were performed twice a week to decrease intracranial pressure.

Vertical transmission to both premature and full term infants may occur during labor. In a study by Chua et al.[10,11] the transmission rate to infants was 30% for *M. hominis*. As the mother did not have any vaginal cultures or screening before birth and soon afterwards, we were not able to prove that it was transmitted from the mother.

In conclusion, as shown in our case report, in clinical practice, when neonates have clinical and laboratory signs of sepsis/meningitis and deteriorate despite broad spectrum antimicrobials, microbiological evaluation is of prime importance. Incubation period of culture media must be at least five days for CSF sample and colonies must be evaluated carefully for microorganisms which are difficult to grow in routine culture media.

REFERENCES

1. Waites KB, Schelonka RL, Xiao L, Grigsby PL, Novy MJ. Congenital and opportunistic infections: ureaplasma species and Mycoplasma hominis. Semin Fetal Neonatal Med 2009; 14: 190-199.
2. Hata A, Honda Y, Asada K, Sasaki Y, Kenri T, Hata D. Mycoplasma hominis meningitis in a neonate: case report and review. J Infect 2008; 57: 338-343.
3. Watt KM, Massaro MM, Smith B, Cohen-Wolkowiez M, Benjamin Jr DK, Laughon MM. Pharmacokinetics of moxifloxacin in an infant with Mycoplasma hominis meningitis. Pediatr Infect Dis J 2012; 31: 197-199.
4. Benson DA, Boguski MS, Lipman DJ, Ostell J. GenBank. Nucleic Acids Res 1997; 25: 1-6.
5. Wolthers KC, Kornelisse RF, Platenkamp GJJM, et al. A case of Mycoplasma hominis meningo-encephalitis in a full-term infant: rapid recovery after start of treatment with ciprofloxacin. Eur J Pediatr 2003; 162: 514-516.
6. Sethi S, Singh G, Samanta P, Sharma M. Mycoplasma genitalium: an emerging sexually transmited pathogen. Indian J Med Res 2012; 136: 942-955.
7. Siber GR, Alpert S, Smith AL, Lin JSL, McCormack WM. Neonatal central nervous system infection due to Mycoplasma hominis. J Pediatr 1977; 90: 625-627.
8. Reissier S, Masson R, Guérin F, et al. Fatal nosocomial meningitis caused by Mycoplasma hominis in an adult patient: case report and review of the literature. Int J Infect Dis 2016; 48: 81-83.
9. Sobouti B, Fallah S, Mobayen M, Noorbakhsh S, Ghavami Y. Colonization of Mycoplasma Hominis and Ureaplasma urealyticum in pregnant women and their transmission to offspring. Iran J Microbiol 2014; 6: 219-224.
10. Chua KB, Ngeow YF, Lim CT, Ng KB, Chye JK. Colonization and transmission of Ureaplasma urealyticum and mycoplasma hominis from mothers to full and preterm babies by normal vaginal delivery. Med J Malaysia 1999; 54: 242-246.
11. Chua KB, Ngeow YF, Ng KB, Chye JK, Lim CT. Ureaplasma urealyticum and Mycoplasma hominis isolation from cervical secretions of pregnant women and nasopharyngeal secretions of their babies at delivery. Singapore Med J 1998; 39: 300-302.
12. Simhan HN, Caritis SN, Krohn MA, de Tejada BM, Landers DV, Hillier SL. Decreased cervical proinflammatory cytokines permit subsequent upper genital tract infection during pregnancy. Am J Obstet Gynecol 2003; 189: 560-567.
13. Watson L, Min Pang Y, Mitchell S, Dodgson A. Mycoplasma hominis meningitis in a 24 week premature neonate: case report and short literature review. J Pediatr Pharmacol Ther 2008; 13: 251-254.

The Turkish Journal of Pediatrics 2020; 62: 284-288
https://doi.org/10.24953/turkjped.2020.02.016

Case Report

Children with lymphoma presenting with hemophagocytic lymphohistiocytosis

Tekin Aksu[1], Turan Bayhan[2], Belgin Gülhan[3], Selma Çakmakcı[2], Ayşe Selcen Oğuz[4], Neşe Yaralı[1], Namık Yaşar Özbek[1], İnci İlhan[2]

[1]Department of Pediatric Hematology and Oncology, University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital; [2]Department of Pediatric Hematology and Oncology, University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital; [3]Department of Pediatric Infectious Diseases, University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital; [4]Department of Pathology, University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital, Ankara, Turkey.

ABSTRACT

Background. Hemophagocytic lymphohistiocytosis (HLH) may precede malignancy, in particular lymphomas and leukemias. However, the causative factors, appropriate treatment and the prognosis of this association is not established.

Case. Herein, we present two patients, one with nodular sclerosing Hodgkin lymphoma (HL) and concomitant Epstein-Barr virus (EBV) infection, and the other with anaplastic large cell lymphoma (ALCL), presented as malignancy associated HLH.

Conclusion. In our patients, malignancy directed therapy was sufficient to treat HLH symptoms both at presentation and at recurrence in the second patient.

Key words: Hodgkin lymphoma, anaplastic large cell lymphoma, hemophagocytic lymphohistiocytosis.

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening, hyperinflammatory disorder demonstrated by activation of macrophages, cytotoxic T and natural killer (NK) cells. This uncontrolled immune response leading to macrophage activation and enhanced cytokinemia can be called a cytokine storm.[1] In HLH diagnosis is based on refractory fever, hepatosplenomegaly, cytopenias, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis, low/absent NK-cell-activity, hyperferritinemia, and high-soluble interleukin-2-receptor levels.[2] It is classified into two subgroups; genetic (familial) and acquired (secondary). The familial HLH is characterized by a primary defect in cytotoxic lymphocyte function (e.g., disrupted release of cytolytic granules) and autosomal recessive mode of inheritance.[3]

The diagnosis of HLH and especially primary or secondary HLH distinction may be challenging at the presentation of the patients. A search for underlying diseases should be performed for all patients, and initial treatment should not be delayed. Flow cytometric screening tests or molecular studies to detect the underlying genetic defects are available, but might be inconclusive due to insensitivity or unknown genetic defects. Familial HLH usually presents in infants or younger children where the trigger is often not apparent.[3] Recurrent HLH and family history suggest primary HLH. Secondary HLH may develop due to several disorders, such as infections, rheumatologic diseases, and malign disorders. Additionally, malignancy-associated HLH (M-HLH) can be divided into two forms, where the malignancy

✉ Tekin Aksu
tekinaksu@gmail.com

Received 18th October 2018, revised 3rd December 2018, 18th February 2019, accepted 28th May 2019.

triggers HLH via hyperinflammation and, persistent antigen stimulation by malignant cells or chemotherapy-associated HLH, where the infections or dysregulated immune system due to antineoplastic treatment provokes HLH.[4,5] Also, hypomorphic mutations in familial HLH causing genes carried by adult patients are associated with late-onset HLH in the presence of viral infection or environmental stresses.[6] Furthermore, a recent study suggested that monoallelic perforin gene (*PRF1*) mutations involved in lymphocyte survival and functional activity, may play a role in the development of lymphoid tumors.[7] Although causative factors, appropriate treatment and the prognosis of this association is not established, the symptoms related to HLH often improve with treatment of the malignancy or HLH-directed therapy. Herein, we present two patients, one with Hodgkin lymphoma (HL), and the other with anaplastic large cell lymphoma (ALCL), presented with M-HLH.

Case 1

A previously healthy 12-year-old boy was referred to hospital with fever, abdominal mass and splenomegaly. His recent history revealed fever, fatigue and night sweats for the last month, and abdominal distention appeared one week before admission. In a local hospital, his hemoglobin (Hb) level was found to be 7.1 g/dL, and he was transfused with erythrocyte suspension. Physical examination disclosed an abdominal mass (10x10 cm) palpable on the umbilical area and his spleen was also palpable 10 cm below the left costal margin. There was no consanguinity between parents. Complete blood count (CBC) revealed Hb 9 g/dl, white blood cell (WBC) count 6.6x10^9/L, and platelet (Plt) count 199x10^9/L. Peripheral smear, and bone marrow (BM) aspiration smear and biopsy were all normal. Abdominal and chest tomography disclosed mediastinal, and abdominal multiple conglomerated lymphadenopathies. Biopsy from abdominal lymphadenopathy was compatible with nodular sclerosing HL with Epstein-Barr virus (EBV) latent membrane protein (LMP) positivity. Before the initiation of chemotherapy, his fever continued, and petechia and ecchymosis were noticed on his trunk. At the time, a CBC showed Hb 7.7 g/dl, WBC count 3.4 x 10^9/L, Plt count 21 x 10^9/L, and a BM aspiration smear revealed hemophagocytic histiocytes (Fig. 1). His ferritin level was 1424 ng/ml, triglyceride (TG) level 181 mg/dl, fibrinogen level 415 mg/dl and EBV polymerase chain reaction (EBV PCR) was 46.023 copies/ml. He had fever, splenomegaly, pancytopenia, hyperferritinemia, and hemophagocytosis which was compatible with HLH criteria reported by Henter et al.[2] Finally, he was diagnosed with HLH secondary to HL and concomitant EBV infection. After one cycle of chemotherapy (ABVD; 25 mg/m^2 doxorubicin, 9 mg/m^2 bleomycin, 6 mg/m^2 vinblastine, and 375 mg/m^2 dacarbazine), the abdominal mass shrunk to about half of its size. His fever resolved and cytopenia improved, however histiocytes with hemophagocytosis persisted on a repeat BM examination. After the second course of chemotherapy, his mass could no longer be palpable on physical examination. BM aspiration revealed no hemophagocytosis and ferritin level was 252 ng/ml, TG level 219 mg/dl, fibrinogen level 252 mg/dl and EBV PCR were negative 45 days after the first examination. He is still in remission after 16 months. Informed

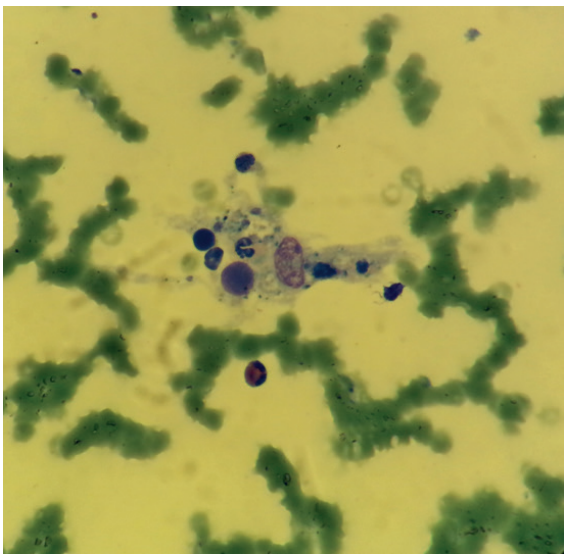


Fig. 1. Hemophagocytosis in bone marrow aspiration smear (magnification x100).

consent was received from the patient and the family.

Case 2

A previously healthy 15-year-old boy presented with persistent fever (>40 C°), fatigue, weight loss, skin rash and, hepatosplenomegaly. At admission, his CBC revealed Hb 7.9 g/dl, WBC count $0.3x10^9$/L, Plt count 52 x 10^9/L. A peripheral blood smear showed leukopenia and thrombocytopenia without blasts. Alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transferase levels were 163, 431, and 404 U/L, respectively. The direct bilirubin level was 2.71 mg/dl. Serum ferritin was 3087 ng/ml, TG level was 372 mg/dl, and fibrinogen was low (86 mg/dl). BM aspiration smear showed marked hemophagocytosis, without blast, parasites or lipid-laden macrophages. EBV and cytomegalovirus (CMV) infections were excluded by PCR analysis. He had fever, splenomegaly, pancytopenia, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, and hemophagocytosis which was compatible with HLH criteria reported by Henter et al.[2] His chest X-ray disclosed pneumonitis without a clear etiology. Ultrasonography revealed multiple abdominal lymphadenomegaly and splenic infarct which was confirmed with computed tomography without portal or splenic venous thrombosis. Simultaneously, his blood cultures showed *Candida albicans* sensitive to amphotericin B. Considering his age; he was assumed as secondary HLH. High dose intravenous immunoglobulin (IVIG) (2g/kg) therapy was implemented without success. Since he had a refractory fever (>40 C°), and septicemia, abdominal lymph node biopsy could not be performed. Due to our concern that steroids might impede the diagnosis of cancer, he received plasmapheresis for HLH for three consecutive days without clinical response. After completion of plasmapheresis, a nondiagnostic abdominal lymph node biopsy was attempted. Subsequently, high dose methylprednisolone (30 mg/kg, maximum dose 1g/day) was initiated together with cyclosporine A (5mg/kg/day). This HLH-directed therapy attained clinical response at once, but it was not long-lasting. Fever, cytopenia, coagulopathy, and elevation of ferritin levels recurred five days after the initiation of steroid treatment. We studied NK cell-mediated cytotoxicity, and NK and T cell degranulation did not suggest a primary defect in cytotoxic lymphocyte function. Three weeks after admission and ten days under the steroid treatment, he developed a maculopapular rash on his trunk. A skin biopsy showed anaplastic large cell kinase positive- ALCL. Therefore, he was diagnosed with HLH due to stage III ALCL four weeks after admission to our hospital. He received one course of dexamethasone (10 mg/m^2/day, five days), methotrexate (3 gr/m^2), ifosfamide (800 mg/m^2/day, five days), cytarabine (150 mg/m^2/dose, four doses), and etoposide (100 mg/m^2/day, two days). After the treatment, his fever resolved, and cutaneous lesions disappeared. He took this chemotherapy course with alternating dexamethasone (10 mg/m^2/day, five days), methotrexate (3 gr/m^2), cyclophosphamide (200 mg/m^2/day, five days), doxorubicin (25 mg/m^2/day, two days) for a total of six cycles. At the end of chemotherapy, he was in remission, however, two months after the cessation of therapy cutaneous lesions reappeared. Pathological examination of cutaneous lesions revealed ALCL. Interestingly, at the time of relapse, he had fever, cytopenia, hyperferritinemia, lymphadenopathies and splenomegaly, and hemophagocytosis in BM that we assumed recurrence of M-HLH. He is now in remission after three courses of chemotherapy and waiting for hematopoietic stem cell transplantation. Informed consent was received from the patient and the family.

Discussion

Existing evidence suggests that secondary HLH in patients who have cancer is multifactorial. The infections, severe inflammation triggered by malignancy, and loss of immune homeostasis due to anti-neoplastic treatment may lead to secondary HLH. Additionally hypomorphic

HLH causing gene mutations may act as a primary cofactor of Malignancy-associated HLH (M-HLH) in these patients.[5] Malignancy induced HLH is reported to have an incidence of 1.2%, and in particular, associated with lymphomas and leukemias.[4,8] Notably T cell and NK cell lymphomas cause HLH due to strong relation of these tumors with EBV infection.[9] A recent manuscript concerning pediatric and adolescent patients reported that malignancy was suspected in 8.4% of patients with HLH. Also, they found that most of the HLH presented before at the onset or of certain malignancies, mainly ALCL and HL as in our patients. Also, they found the median age was 12 years for malignancy-triggered HLH, and 5.5 years for chemotherapy-related HLH.[4] In our report, both of the patients were adolescents and HLH presented at the onset of HL in one of them, and before ALCL in the other. On the other hand, Strenger et al.[8] described 22 patients with M-HLH in which most of the patients developed HLH during hemato-/oncologic treatment.

Hodgkin lymphoma associated with HLH has been reported in a few case reports and case series.[9-18] Menard et al.[14] revealed EBV positivity in tumor cells via EBER and/or LMP-1 in 32 of the 34 adult patients with HLH associated with HL. They claimed that high expression of EBV LMP-1 in tumor cells might induce Th1 cells to produce large amounts of cytokines and initiate HLH process.[14] In our first patient, it seems EBV was a co-trigger contributing to cytokinemia and the development of M-HLH. HLH has been reported as an initial presentation of HL and associated with BM involvement possibly by inducing a cytokine storm.[9-15,17] However, our patient developed HLH after the initial presentation of HL, but before chemotherapy and without BM involvement.

Anaplastic large cell lymphoma, one of the most common pediatric large-cell lymphomas included in the mature T-cell lymphoma group usually admits with extranodal involvement, skin, and systemic symptoms.[19] It may also present with HLH.[20] Some clinical findings of ALCL are similar to HLH symptoms including fever, lymphadenopathy, skin rash, and hemophagocytosis. Studies have shown that proinflammatory cytokines which were elevated and possibly produced by malignant cells, may play a role in the clinical picture.[1,21] Additionally, Ciambotti et al.[7] suggest that mutations of *PRF1* in ALCL patients were missense mutations that impaired perforin function weakly, which were not enough to cause an HLH attack. Since our patient's T- and NK-cell functions were normal, we did not analyze *PRF1* mutations in our patients. However, at the time of relapse HLH also recurred that may occur due to the possible *PRF1* mutation. Also, *Candida* infection could be a co-trigger or co-infection for M-HLH in the patient. In our patients, treatment of malignancy and infection was sufficient to treat HLH symptoms both at presentation and at recurrence in the second patient.

In this report both of the patients met the HLH criteria reported by Henter et al.[2] However, the widely used HLH-2004 criteria may not be sufficient to diagnose HLH in a patient who also has active malignancy, as some features may be related to the malignancy itself. Daver et al.[5] proposed a schema containing 18 variables for adult M-HLH that incorporates a more accessible physical examination and laboratory variables. They claimed that patients who have any 5 of these 18 variables could be M-HLH. Despite the limitations, particularly for M-HLH, the HLH-2004 criteria are still the widely accepted definition.

In conclusion, we suggested secondary HLH for patients due to the late-onset presentation, normal cytotoxic lymphocyte function, and no recurrent disease or family history. HLH is an infrequent complication of HL and ALCL in children and may mask the primary tumor. Infections may be co-triggers for M-HLH, and should be treated expeditiously. Treatment should be directed to the malignant condition instead of HLH.

REFERENCES

1. Shimada A, Kato M, Tamura K, et al. Hemophagocytic lymphohistiocytosis associated with uncontrolled inflammatory cytokinemia and chemokinemia was caused by systemic anaplastic large cell lymphoma: a case report and review of the literature. J Pediatr Hematol Oncol 2008; 30: 785-787.
2. Henter JI, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48: 124-131.
3. Jordan MB, Allen CE, Weitzman S, Filipovich AH, Mcclain LK . How I treat hemophagocytic lymphohistiocytosis. Blood 2011; 118: 4041-4052.
4. Lehmberg K, Sprekels B, Nichols KE, et al. Malignancy-associated haemophagocytic lymphohistiocytosis in children and adolescents. Br J Haematol 2015; 170: 539-549.
5. Daver N, McClain K, Allen CE, et al. A consensus review on malignancy-associated hemophagocytic lymphohistiocytosis in adults. Cancer 2017; 123: 3229-3240.
6. Zhang K, Jordan MB, Marsh RA, et al. Hypomorphic mutations in PRF_1, $MUNC_{13-4}$, and $STXBP_2$ are associated with adult-onset familial HLH. Blood 2011; 118: 5794-5798.
7. Ciambotti B, Mussolin L, d'Amore ESG, et al. Monoallelic mutations of the perforin gene may represent a predisposing factor to childhood anaplastic large cell lymphoma. J Pediatr Hematol Oncol 2014; 36: e359-e365.
8. Strenger V, Merth G, Lackner H, et al. Malignancy and chemotherapy induced haemophagocytic lymphohistiocytosis in children and adolescents-a single centre experience of 20 years. Ann Hematol 2018; 97: 989-998.
9. Hagihara M, Inoue M, Hua J, Iwaki Y. Lymphocyte-depleted Hodgkin lymphoma complicating hemophagocytic lymphohistiocytosis as an initial manifestation: a case report and review of the literature. Intern Med 2012; 51: 3067-3072.
10. Abaza H, Ben Chaabene A, Sallami S, Boussen H, Rahal K, Guemira F. Hemophagocytic syndrome associated to a Hodgkin lymphoma. Tunis Med 2015; 93: 399-400.
11. Chan K, Behling E, Strayer DS, Kocher WS, Dessain SK. Prolonged hemophagocytic lymphohistiocytosis syndrome as an initial presentation of Hodgkin lymphoma: a case report. J Med Case Rep 2008; 2: 367.
12. Dominguez-Munoz MA, Morales-Camacho RM, Prats-Martin C, et al. Unusual co-occurrence of Hodgkin lymphoma and hemophagocytic lymphohistiocytosis in a bone marrow aspirate. Ann Hematol 2016; 95: 1019-1021.
13. Ichikawa S, Takahashi T, Katsushima H, Fukuhara N, Ichinohasama R, Harigae H. Advanced lymphocyte-rich classical Hodgkin lymphoma complicated with fatal hemophagocytic syndrome. Intern Med 2016; 55: 191-196.
14. Menard F, Besson C, Rince P, et al. Hodgkin lymphoma-associated hemophagocytic syndrome: a disorder strongly correlated with Epstein-Barr virus. Clin Infect Dis 2008; 47: 531-534.
15. Morita Y, Kenzaka T, Yoshimoto H, Ohno N. Hodgkin's lymphoma preceded by haemophagocytic lymphohistiocytosis. BMJ Case Rep 2013; 2013.
16. Roe C, Bennett J, Zhang L, et al. Hemophagocytic lymphohistiocytosis in malignant hematology: uncommon but should not be forgotten? Clin Lymphoma Myeloma Leuk 2015; 15: S147-S150.
17. Tatara R, Sato M, Fujiwara SI, et al. Hemoperfusion for Hodgkin lymphoma-associated hemophagocytic lymphohistiocytosis. Intern Med 2014; 53: 2365-2368.
18. Celkan T, Berrak S, Kazanci E, et al. Malignancy-associated hemophagocytic lymphohistiocytosis in pediatric cases: a multicenter study from Turkey. Turk J Pediatr 2009; 51: 207-213.
19. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016; 127: 2375-2390.
20. Li W, Repnikova E. ALK-positive anaplastic large-cell lymphoma with marked leukemoid reaction and hemophagocytic lymphohistiocytosis. Blood 2016; 127: 2041.
21. Al-Hashmi I, Decoteau J, Gruss HJ, et al. Establishment of a cytokine-producing anaplastic large-cell lymphoma cell line containing the t(2;5) translocation: potential role of cytokines in clinical manifestations. Leuk Lymphoma 2001; 40: 599-611.

The Turkish Journal of Pediatrics 2020; 62: 289-292
https://doi.org/10.24953/turkjped.2020.02.017

Case Report

Congenital afibrinogenemia in a 4-year-old girl complicated with acute lymphoblastic leukemia

Alper Özcan, Bahadır Samur, Şefika Akyol, Necdet Arda Erdoğmuş, Türkan Patıroğlu, Musa Karakükçü, Ekrem Ünal

Division of Pediatric Hematology Oncology, Department of Pediatrics, Erciyes University Faculty of Medicine, Kayseri, Turkey.

ABSTRACT

Background. Congenital fibrinogen deficiency is one of the rare inherited coagulation disorders. Congenital fibrinogen deficiency complicated with a hematological malignancy can be life threatening.

Case. We present a four-year-old girl with congenital fibrinogen deficiency complicated with acute lymphoblastic leukemia.

Conclusion. This case aims to highlight therapeutic approaches for the management of afibrinogenemia patients with acute leukemia.

Key words: acute lymphoblastic leukemia, child, congenital afibrinogenemia.

Congenital afibrinogenemia (CA) is a rare autosomal recessive bleeding disorder that is characterized by the undetectable low level of fibrinogen.[1] CA has an estimated prevalence of one for 1,000,000. The most common clinical symptoms are mucocutaneous, soft-tissue, joint and genito-urinary spontaneous bleeding, traumatic, or surgical bleeding.[2,3] Hemorrhagic diathesis and arterial and venous thromboembolic complications can develop in a patient with CA.[4] CA complicated with a hematological malignancy can be life threatening. Acute lymphoblastic leukemia (ALL) is the most common cancer in children and represents the leading cause of cancer-related mortality in children. Survival rate of ALL is approximately 85%.[5] Review of the literature has shown that the occurrence of hematological malignancies in patients with coagulation disorders are rare, with only some reported cases of leukemia in patients with haemophilia.[6] However, afibrinogenemia with ALL has never been reported. The coincidence of these two diseases together is important which has led to challenges in developing treatment strategies. We describe a four-year-old girl with CA complicated with ALL.

Case Report

A 4-year-old girl with a history of CA admitted to the emergency department with a complaint of bleeding in her mouth. From her medical history it was learned that the patient was diagnosed with CA because of intracranial hemorrhage at the age of 1 day. Her 22-year-old brother was also diagnosed with CA.

Her physical examination at the time of admission disclosed petechial rash and ecchymoses, pallor, and hepatosplenomegaly. Vital signs were normal. The white blood cell count of the patient was 94,530/mm^3 with 90% blasts, the measured hemoglobin level was 6,9 g/dl, and platelet count was 137,000/mm^3. Her

✉ Ekrem Ünal
drekremunal @yahoo.com.tr

Received 24th May 2018, revised 11th July 2019, accepted 27th August 2019.

This paper was presented in 11th Annual Congress of the European Association for Haemophilia and Allied Disorders (EAHAD) 2018 in Madrid, on February 7-9, 2018 (Poster presentation).

complete metabolic panel was normal except for an LDH of 440 u/L (135-225 u/L). Peripheral smear examination showed presence of 90% blasts which were bigger than lymphocytes with high nuclear-cytoplasmic ratio, moderate amount of cytoplasm, round to oval nucleus, fine chromatin and 0-1 nucleoli. Red blood cells were predominantly normocytic normochromic. Platelets were reduced. Bone marrow examination showed 85% L_1-type blasts which were smaller and twice the diameter of a red cell, high nuclear cytoplasmic ratio, regular shape of nucleus and some small nucleoli.

Immunophenotypical analyses showed that blasts were positive for CD19, CD10, CD38, CD58, CD79a, HLA-DR but were negative for myeloid markers. Flow cytometry findings were compatible with ALL. In addition, karyotypical analyses of peripheral blood, and bone marrow aspiration revealed no abnormalities. Cytogenetic analysis was normal. The cerebrospinal fluid examination showed no blasts. She received intensive chemotherapy for intermediate risk group ALL according to the Berlin-Frankfurt-Munich ALLIC 2009 protocol.

The Prothrombin time (PT)>100 (10-14 second), activated partial thromboplastin time (aPTT)>120 (25-36 second), International Normalized Ratio (INR)>10 were prolonged; fibrinogen level was not detectable. D dimer 190 (0-550 μg/L), antithrombin III 99.7% (83-118%) levels were in normal ratio. Levelsof fibrinogen and platelets count were influenced by her malignancy and chemotherapeutic treatment. Fibrinogen replacement therapy was given before the invasive procedures such as bone marrow aspiration, lumbar puncture, catheter insertion and removal. The target level of fibrinogen was 100 mg/dL. Thrombocyte suspension was given to the patient during active bleeding and before invasive procedures (platelet count <50,000/μL).

The bone marrow evaluations of 15^{th}-33^{rd} day were found to be in remission. As paradox arterial and venous thromboembolic complications can develop in patients with CA, low-molecular-weight heparin (enoxaparin sodium) injection, 1 mg/day, was started for the prophylaxis of thromboembolic complications of CA during the chemotherapy regimen. During the treatment protocol the patient's fibrinogen levels, hemogram, PT, aPTT, antifactorXa tests were evaluated not just during symptomatic times of bleeding and before invasive proceduresandthey were also checked periodically 2 times each week.

Finally, the patient finished the induction, consolidation, re-induction, and the continuation parts of the ALLIC 2009 protocol without any complications related to CA. During follow-up laboratory results revealed thePT>100 (10-14 second), PTT >150 (25-36 second), INR>5 were prolonged; D dimer 300 (0-550 μg/L), antithrombin III 90% (83-118%) levels were in normal ratio. Fibrinogen level was 99 (180-350 mg/dl). Fibrinogen level waselevated as the patient receivedfibrinojen concentrate before the catheter removal during the continuation therapy. Peripheral blood counts returned to normal she remained in clinical and laboratory remission without any bleeding diathesis symptoms or thrombosis. Written consent was obtained from the family for the publication of this case report.

Discussion

CA is a rare autosomal recessive disorder. Intracranial hemorrhage is a common site of bleeding in children with CA. CA may manifest as a neonatal intracranial hemorrhage due to traumatic delivery.[1] There are some challenges in management of the patients with CA resulting from both bleeding and thromboembolic complications.[2] This two-faced problem may be harder in patients with hematological malignancies. In the English and Turkish medical literature cases of acute childhood leukemia in a patient with hemophilia, von Willebrand syndrome were reported. To the best of our knowledge, this is the first reported case of ALL in patient with CA. It is important to show how to manage the

issues with the bleeding complications related to CA and acute leukemia.[3-6] In addition, the platelet numbers, and functions and factor levels that may also be affected by the ALL treatment. Thrombocytopenia can exacerbate the bleeding complications of CA; so when platelet transfusion is required the platelet count should be kept relatively higher than leukemic children without CA. Leukemic children with CA may require frequent platelet transfusions, and plasma derived fibrinogen concentrate. Children with CA complicated with acute leukemia may have long-term abnormalities such as inhibitor development from higher factor concentrate usage like hemophiliac patients. In addition the increased frequent of usage of blood products may result in increased adverse reactions including febrile reactions, bacterial & viral contamination, transfusion related acute lung injury, etc.[7-9] Thus, managing of bleeding disorders like afibrinogenemia/disfibrinogemia with ALL is extremely sophisticated.

The presented patient was regularly monitored with a detailed blood investigation before invasive procedures or in symptomatic bleeding times. As the half-life of fibrinogen is 2-4 days, the patient was evaluatedtwice a week using PT, aPTT, antifactorXa and fibrinogen levels tests. As described by Peyvandi et al.[10] fibrinogen replacement therapy was given before the invasive procedures such as bone marrow aspiration, lumbar puncture, catheter insertion and removal. The target level of fibrinogen was 100mg/dL. The critical level of transfusion for thrombocytopenia was detected according to Estcourt et al.[11] Thrombocyte suspension was transfused during active bleeding and before invasive procedures (platelet count <50,000/μL). With this methodology, the clinical phenotype of the presented child was moderate, and spontaneous bleeding was very rarely observed.

Furthermore, children with CA (in addition to these classic clinical features of bleeding episodes) may present with increased risk of thrombosis which often necessitating the concurrent use of anticoagulants and fibrinogen.[12] Because of our previous reported experience low-molecular-weight heparin, enoxaparin was started to balance the two sharp faces of the blade. It was speculated that, enoxaparin prophylaxis may be an effective way to control thrombosis which may result from CA or increased risk of hematologic malignancy such as catheter insertion, steroid and L-Asparaginase usage.[13,14]

Arterial and venous thromboembolic events have rarely been reported in patients with CA. But thrombosis is difficult to manage because of the bleeding tendency of the patients with CA.[12] Amri et al.[15] recommended the treatment of low-molecular weight heparin in patients with CA. In the experience of the presented case, we speculate that close monitoring, the usage of enoxaparin 1 mg/kg/day with fibrinogen replacement without any anti-agregant treatment seemed to be effective.

In conclusion, CA is known as a rare disease with different underlying disorders. To the best of our knowledge this is the first report of the co-occurrence of leukemia and CA in a pediatric patient. Thus, this strange togetherness led to a challenge in developing a treatment strategy and which may have resulted in bleeding complication. The togetherness of leukemia and bleeding disorders may require much more attention and the development of novel treatment strategies.

REFERENCES

1. Patiroglu T, Ozdemir MA, Unal E, et al. Intracranial hemorrhage in children with congenital factor deficiencies. Childs Nerv Syst 2011; 27: 1963-1966.
2. Ruiz-Sa´ez A. Thrombosis in rare bleeding disorders. Hematology 2012; 17(Supp 1): S156-S158.
3. Turner ML, Watson HG, Russell L, Langlands K, Ludlam CA, Parker AC. An HIV positive haemophiliac with acute lymphoblastic leukaemia successfully treated with intensive chemotherapy and syngeneic bone marrow transplantation. Bone Marrow Transplant 1992; 9: 387-389.
4. Kawakami K, Takezaki T, Nakazono S, et al. Acute childhood leukaemia in a patient with haemophilia: first report in Japan. Acta Paediatr Jpn 1994; 36: 91-94.

5. Kızmazoğlu D, Sarı S, EvimSezgin M, et al. Assessment of health-related quality of life in pediatric acute lymphoblastic leukemia survivors: perceptions of children, siblings, and parents. Turk J Haematol 2019; 36:112-116.

6. Dunn AL. Malignancy in patients with haemophilia: a review of the literature. Haemophilia 2010; 16: 427-436.

7. Patiroglu T, Murataldı S, Koklu E, Karakukcu M. Pleural empyema caused by Salmonella typhimurium in a patient with acute lymphoblastic leukemia. Erciyes Med J 2003; 25: 204-207.

8. Sinha R, Sodhi K, John B, Singh D. A rare case of acute lymphoblastic leukaemia with hemophilia A. Ital J Pediatr 2009; 35:40.

9. Zhang L, Li H, Zhao H, Zhang X, Ji L, Yang R. Retrospective analysis of 1312 patients with haemophilia and related disorders in a single Chinese institute. Haemophilia 2003; 9: 696-702.

10. Peyvandi F, Haertel S, Knaub S, Mannucci PM. Incidence of bleeding symptoms in 100 patients with inherited afibrinogenemia or hypofibrinogenemia. J Thromb Haemost 2006; 4: 1634-1637.

11. Estcourt LJ, Malouf R, Doree C, Trivella M, Hopewell S, Birchall J. Prophylactic platelet transfusions prior to surgery for people with a low platelet count. Cochrane Database Syst Rev 2018; 9: CD012779.

12. Ozdemir MA, Işik B, Patiroglu T, et al. A case of congenital afibrinogenemia complicated with thromboembolic events that required repeated amputations. Blood Coagul Fibrinolysis 2015; 26: 354-356.

13. Kose D, Paksoy Y, Koksal Y, Unal E. Neurological complication of non Hodgkin lymphoma in childhood: experience from a single center in Turkey. Childs Nerv Syst 2014; 30: 639-645.

14. Unal E, Yazar A, Koksal Y, Caliskan U, Paksoy Y, Kalkan E. Cerebral venous sinus thrombosis in an adolescent with Ewing sarcoma. Childs Nerv Syst 2008; 24: 983-986.

15. Amri Y, Kallel C, Becheur M, et al. Hypodysfibrinogenemia: a novel abnormal fibrinogen associated with bleeding and thrombotic complications. Clin Chim Acta 2016; 460: 55-62.

The Turkish Journal of Pediatrics 2020; 62: 293-309
https://doi.org/10.24953/turkjped.2020.02.018

Case Report

Mild encephalopathy with reversible splenial lesion associated with echovirus 6 infection: a case report and review of the literature

Enrico Masiello[1], Antonio Gatto[1], Ilaria Lazzareschi[1,2], Donato Rigante[1,2], Paolo Mariotti[3], Piero Valentini[1,2]

[1]Institute of Pediatrics, Fondazione Policlinico Universitario A. Gemelli IRCCS; [2]Università Cattolica Sacro Cuore; [3]Department of Pediatric Neurology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy.

ABSTRACT

Background. Mild encephalopathy with reversible splenial lesion (MERS), a clinic-radiological syndrome distinguished by reversible encephalopathy onset, has been increasingly recognized in Caucasian children.

Case. We describe a MERS case in a previously healthy 4-year-old girl admitted to the hospital with abnormal consciousness level, muscle weakness, dysphagia and dysarthria after a 4-day history of diarrhea and fever. Magnetic resonance imaging (MRI) of the brain showed hyperintensity in the corpus callosum splenium. Electroencephalogram was normal and cerebrospinal fluid (CSF) culture negative. The stool sample was positive for Echovirus 6 and serology test confirmed the infection. The child's condition gradually improved and the MRI, after 15 days, depicted a normal brain. Only a mild speech impairment was persistent at discharge, which disappeared one month later. We performed a literature review about pediatric MERS cases demonstrating that infectious agents have been rarely isolated in CSF.

Conclusion. MERS has an overall good prognosis independently from the treatment approach; this is confirmed by our case - one of the first reported with an Echovirus 6-related encephalopathy.

Key words: mild encephalopathy with reversible splenial lesion (MERS), echovirus, encephalopathy, child.

Mild encephalopathy with reversible splenial lesion (MERS) is a disorder characterized by prodromal symptoms such as fever, cough, vomiting or diarrhea, followed by mild encephalopathy 1-7 days later with a documented reversible splenial lesion. Lesions are typically hyperintense in T2-weighted brain magnetic resonance imaging (MRI) and demonstrate a transiently homogeneous low apparent diffusion coefficient (ADC). Takanashi et al.[1] proposed to classify this encephalopathy in MERS type I, describing patients with an isolated splenium corpus callosum (SCC) lesion and in MERS type II for patients with extensive white matter and/or lesions involving the entire corpus callosum (CC). The most relevant and common neurological MERS symptoms, which tend to completely recover within 1 month, are behavioral changes, consciousness disturbance and seizures.[2-5]

Numerous infectious diseases are associated with MERS in children: rotavirus (RV),[6-14] cytomegalovirus (CMV),[15-17] influenza A and B,[5,16-28] parainfluenza,[29] mumps[2-30,31] as well as other viral agents such as adenovirus,[16] human herpesvirus-6 (HHV-6),[32] human herpesvirus-1,[17] parvovirus B19,[33] enterovirus (EV)[17] and Epstein-Barr virus (EBV).[34] Also bacterial infections have been described related to MERS cases, namely *Escherichia coli*,[28] *Enterococcus faecalis*,[35] *Salmonella*,[16] *Campylobacter jejuni*[28] and *Mycoplasma pneumoniae*.[17,28,36-44]

✉ Antonio Gatto
antonio.gatto@policlinicogemelli.it

Received 9th April 2019, revised 15th June 2019, accepted 3rd July 2019.

Herein we describe a pediatric patient with Echovirus 6 infection and transient isolated SCC lesion on the brain MRI followed by extensive medical literature review about MERS in children.

Case Report

A previously healthy 4-year-old Caucasian girl with a 4-day-history of fever and diarrhea and mildl dehydration, was referred to our hospital in summer 2018. Patient's and family medical history was unremarkable. Physical examination on admission showed a body temperature of 38°C with a heart rate of 130 beats per minute, respiratory rate of 36 breaths per minute, oxygen saturation of 98% breathing room air and blood pressure of 103/64 mmHg. Chest and abdominal examination was normal. Neurological examination revealed an abnormal consciousness level, muscle weakness, dysphagia and dysarthria. The patient was responsive to painful stimuli with Glasgow Coma Scale score of 12 (eyes 3, verbal 4, motor 5), showed normal reflexes in the extremities; Kernig's and Babinski's signs were absent. Peripheral blood analysis showed a white blood cell count of 6970/μL, hemoglobin 10.6 g/dl, platelet count 325.000/μL and C-reactive protein 130 mg/L (normal value: <5). Biochemical parameters and blood gas analysis were normal. Lumbar puncture was performed, and cerebrospinal fluid (CSF) analysis revealed 125 white cell/ml, increased protein 50 mg/dl and normal glucose 52 mg/dl. Isotonic fluids and empirical intravenous antibiotic treatment with ceftriaxone and intravenous acyclovir were started. Real Time-Polymerase Chain Reaction (RT-PCR) to detect CMV, EBV, EV, influenza viruses and herpes viruses in CSF were negative. Bacterial CSF cultures were also negative. Specific serum IgM antibodies (ELISA) against EBV, influenza virus, parainfluenza viruses, parvovirus B19, herpes viruses and CMV were negative. Conversely, serological tests for influenza A, adenovirus, HHV-6 and varicella demonstrated previous infections. RV detection test was negative. RT-PCR was also negative for influenza A and B in the pharyngeal swab. Urine analysis was normal. Electroencephalogram (EEG) revealed high-voltage slow waves on both temporal regions, with no paroxysmal discharge activity. Brain MRI, on day 2 of hospitalization, showed a focal high-signal lesion of SCC on T2-weighted, fluid-attenuated inversion recovery (FLAIR) and diffusion-weighted images (Fig. 1). Specific serum IgM and IgA antibodies (ELISA) against Echovirus were positive and the stool culture confirmed an acute infection with Echovirus 6. We interrupted ceftriaxone and acyclovir therapy on day 5. In consideration of persistent neurological symptoms such as muscle weakness, dysphagia and dysarthria combined with drowsiness and ataxia, brain MRI results, we initiated intravenous methylprednisolone treatment (2 mg/kg/day) for 5 days and continued the treatment with prednisone per os (2 mg/kg/day) for 2 weeks and subsequent decalage. A progressive clinical improvement was already noted on day 7 of hospitalization and continued until neurological symptoms disappeared, when the girl turned to a normal consciousness level and restarted drinking fluids. Fifteen days after admission, follow-up brain MRI and EEG were normal. The patient was discharged without neurological sequelae, except for a residual speech impairment, which had disappeared at her one-month follow-up visit. Informed consent was obtained by the parents for all procedures and for the publication of this case.

Discussion

EV epidemic outbreaks occur in summer or early fall in temperate regions. Echovirus 6 is one of the most frequently detected EV worldwide.[45] EVs are classified in 4 species: (a) human enterovirus A (coxsackie virus A2-A8, A10, A12, A14, and A16; EV71, 76, 89, 90, and 91), (b) human enterovirus B (coxsackie virus A9 and CVB1-CVB6; echovirus 1-7, 9, 11-27, and 29-33; EV69, 73-75, 77-88, 97, 100, and 101), (c) human enterovirus C (coxsackie virus A1, A11, A13, A17, A19-A22, and A24; polioviruses 1-3;

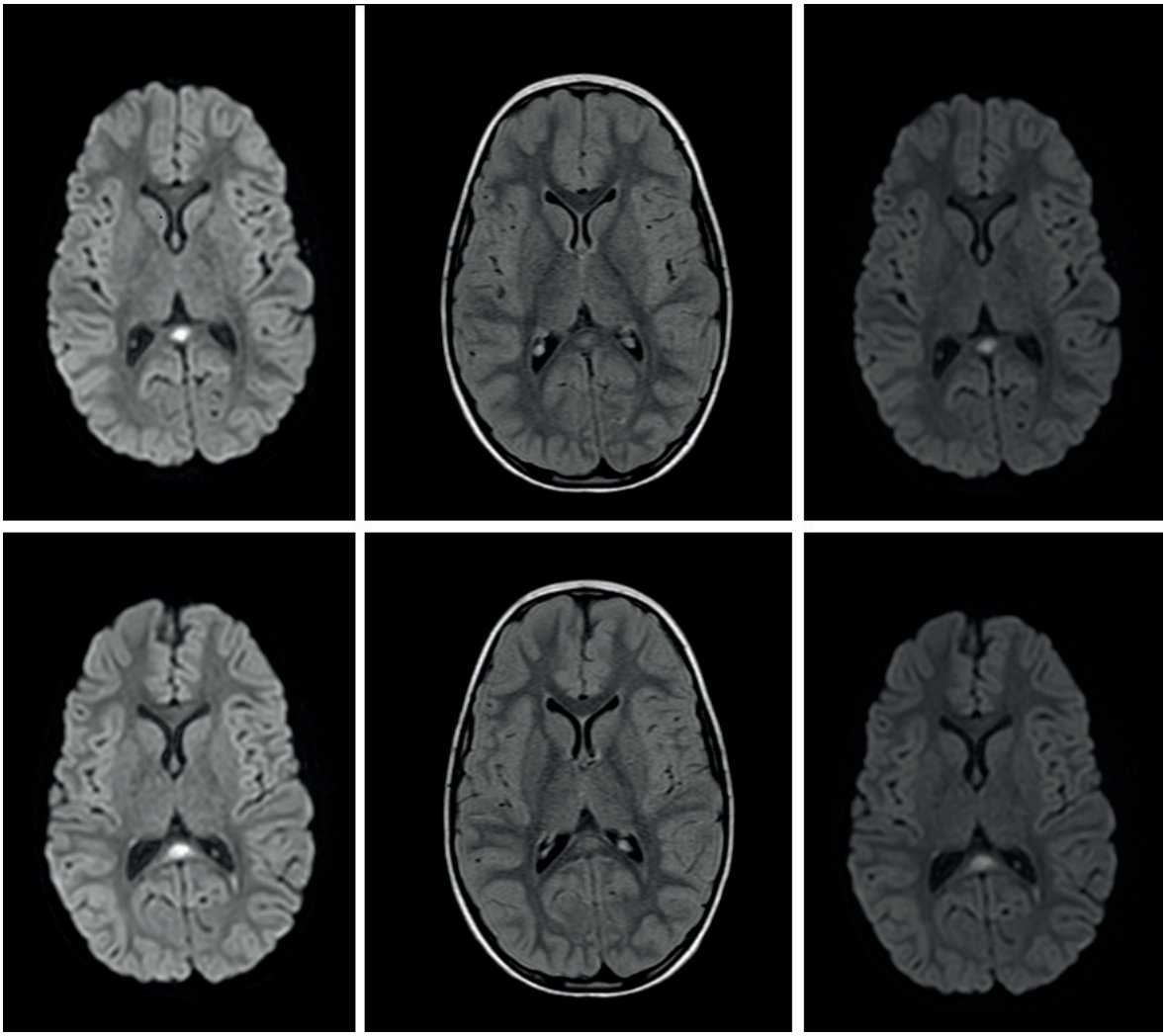


Fig. 1. MRI imagines on day 2 with hyperintensity in the SCC.

EV96), and (d) human enterovirus D (EV68 and EV70).[46] Echovirus (E) is the major causative agent of aseptic meningitis, and E6, E9, E11, E13, E19, E30 are the most common EV types detected in patients with aseptic meningitis. Consciousness disturbance, ataxia, acute muscle weakness, dysarthria and dysphagia, as reported in our patient, are typical neurological symptoms of EV-related encephalitis. Skin rashes and diarrhea are common non-neurological symptoms in EV infections.[45]

Brain MRI, in the presented case, revealed a transient splenial lesion, based on which we diagnosed MERS, a benign disorder characterized by homogenously reduced diffusion lesions (type I MERS), occasionally associated with extensive white matter and/or entire callosal lesions (type II MERS) on brain MRI.[1,2,47] Kobata et al.[6] eported for the first time a RV-related MERS case. In 2004, Tada et al.[2] described MERS as a new encephalopathy with a mild clinical course and good outcome.

We performed a literature review on pediatric MERS cases until July 2018 using Pubmed and Google database for English, Italian and Japanese language publications to clarify clinical features, etiology, neuroimaging and prognosis of this condition. The search was performed using the following keywords: ”enterovirus”, “echovirus”, “encephalitis”, ”encephalopathy”,

"mild encephalitis/encephalopathy with a reversible splenial lesion", "MERS" and "pediatric MERS". We identified 165 cases, including 52 English-language full reports (145 cases), 1 Japanese-language full report (1 case)[48] and 9 English-language abstracts (11 cases). Inclusion criteria were age less than 18 years and encephalopathy by means of brain MRI showing reversible hyperintensity of the splenium of the corpus callosum.

In the 165 pediatric MERS cases, mean age at time of diagnosis was 5.04 years (range 1 day-18 years) and male/female ratio (88/77) was 1.14. Clinical characteristics, laboratory data, neuroimaging results, and patient outcomes are summarized in Table I.

Considering the demographic distribution of patients, MERS occurs mostly in South East Asia, with 84 Japanese cases, 53 Chinese, 9 Australian and 9 Turkish cases, 3 Belgium cases, 1 case from Switzerland, United Kingdom, Poland, Malaysia, Korea, USA and Italy, respectively. Some Australian reports documented the syndrome predominantly in the Caucasian population, indicating an epidemiologic correlation to local virus circulation instead of genetic association.[16,19] Clinical surveillance in Europe demonstrated variable non-polio infection distribution across the years. An increased occurrence rate of 60% related to Echovirus 6 infection with neurological symptoms in patients younger than 7 years was documented in Netherlands from January until August 2016.[49]

Consciousness disturbance (CD; GCS<13) was the most common (93/165, 56.3%) neurological symptom in MERS patients, followed by seizures (77/165, 46.6%) and delirious behavior (DB) (55/165, 33.3%). As described by Kashiwagi et al.[5], DB symptoms are divided as follows: visual hallucination, nonvisual sensory misperceptions, emotional changes (such as laughter and fear), incoherent speech, purposeless movements, and impulsive behavior. Among the 55 cases with DB, clear delirium occurred in 13/55 (23.6%), irritability in 13/55 (23.6%), hallucinations in 5/55 (9.1%), purposeless movements in 4/55 (7.3%) and abnormal behavior in 3/55 (5.4%). Movement disorders were described in 16/165 patients (9.7%), mainly ataxia in 12/16 (75.0%), tremor in 3/16 (18.7%) and gait disturbance in 1/16 (6.2%). Speech impairment was reported in 16/165 patients (9.7%), dysarthria in 5/16 (31.2%), slurred speech in 5/16 (31.2%), abnormal speech in 5/16 (31.2%), and mutism in 2/16 (12.5%). Motor deficits were described in 11/165 (6.6%) patients, 5/11 (45.4%) showed signs of motor deterioration, 2/11 (18.2%) muscle weakness, 1/11 (9.1%) upper arm paresis, 1/11 (9.1%) lower arm sensorimotor polyneuropathy, 1/11 (9.1%) dominant hemiplegia and 1/11 (9.1%) akinetic mutism. Cranial nerve deficits such as eye movement disorders, ophthalmoplegia and strabismus were reported in 4/165 patients (2.4%), blindness in 3/165 (1.8%), dizziness in 2/165 (1.2%), facial palsy in 1/165 (0.6%) and pseudobulbar palsy in 1/165 (0.6%).

Fever (117/165, 70.9%) was the most common non-neurological prodromal symptom, followed by gastrointestinal symptoms such as abdominal pain, vomiting and diarrhea (77/165, 46.6%), cough (29/165, 17.6%) and headache (21/165, 12.7%). Only one patient had clinical signs of sepsis.[50]

MERS-associated infectious agents were identified in 106/165 patients (64.2%): RV in 26/165 (15.7%), *Mycoplasma pneumoniae* in 19/165 (11.5%), influenza A in 14/165 (8.5%), mumps virus in 8/165 (4.8%), adenovirus in 6/165 (3.6%), influenza B in 6/165 (3.6%), CMV in 3/165 (1.8%), *Enterococcus faecalis* in 3/165 (1.8%), EBV in 3/165 (1.8%) - one had also an EBV-related hemophagocytic lymphohistiocytosis - herpesvirus in 2/165 (1.2%), coxsackie virus in 2/165 (1.2%), Echovirus in 2/165 (1.2%), *Escherichia coli* in 2/165 (1.2%), parainfluenza virus, *Salmonella* gastroenteritis, HHV-6 infection, *Campylobacter jejuni*, *Klebsiella pneumoniae*, parvovirus B19, respiratory syncytial virus, dengue virus, *Streptococcus pneumoniae* and group B *Streptococcus* in only 1 patient (0.6%), respectively. MERS was

Table I. Pediatric Cases of Mild encephalopathy with reversible splenial lesion.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
Kobata R. et al. 2002	1	2	F	Consciousness disturbance	Fever, diarrhoea, vomiting	Rotavirus	NE	NE	Type I	DZP	CR
Takanashi J. 2004	2	7	F	Drowsiness, hallucination	Fever, cough, rinorrhea	Influenza A (H3)	N	N	Type I	Acetaminophen	CR
	3	11	M	Right sided dominant paralysis	Fever, cough, rhinorrhea	Influenza B	N	N	Type II	Acetaminophen, Amanthadin	CR
	4	3	F	Seizure, motor deterioration	Diarrhoea, vomiting	UK	Pleocytosis	SBA	Type I	Dex, PB	CR
	5	2	M	Seizure, drowsiness	Fever	UK	N	SBA	Type I	Diazepam	CR
	6	4	F	Seizure, blindness	Fever, diarrhoea	UK	NE	SBA	Type I	Diazepam	CR
	7	5	M	Seizure, delirium	Fever	Influenza A	N	SBA	Type I	-	CR
	8	5	M	Seizure, delirium	Fever	Adenovirus	N	SBA	Type I	IVIG	CR
Tada H. et al. 2004	9	7	M	Delirium	Fever, parotitis	Mumps	Pleocytosis	SBA	Type I	IVIG	CR
	10	8	M	Delirium, seizure	Headache, fever, vomiting	Mumps	Pleocytosis	N	Type I	-	CR
	11	4	F	Seizure, delirium	Fever	UK	N	NE	Type I	Antibiotic, Diazepam	CR
	12	9	F	Neck stiffness, rigor, tremor	Fever	UK	Pleocytosis	SBA	Type I	Acyclovir, Antibiotic, Dex	CR
	13	10	M	Drowsiness	Fever	UK	N	SBA	Type I	Antibiotic, IVIG	CR
Natsume et al. 2006	14	2	F	Seizure	Diarrhoea, vomiting	Rotavirus	ND	N	Type I	DZP,PB	CR
	15	6	M	Headache, delirious behavior	Fever, vomiting	UK	N	N	Type II	-	CR
	16	8	M	Drowsiness	Fever	UK	Pleocytosis	RD	Type II	Acyclovir	CR
Takanashi J. et al. 2006	17	4	F	Drowsiness, seizure	Fever	EBV	N	DSW	Type II	Acyclovir	CR
	18	5	F	Drowsiness, seizure	Fever	UK	N	N	Type II	PSL	Mental delay (Frontal Atrophy)
Matsubara K. et al. 2007	19	12	F	Headache, consciousness disturbance, muscle weakness	Fever	Influenza B	N	FSW	Type I	PSL	CR
Ganapath-y S. et al. 2008	20	12	M	Drowsiness, delirious behavior	Fever, headache	Influenza B	N	SBA	Type I	-	CR
Tokunaga Y. et al. 2008	21	8	F	Ataxia, muscle weakness, tremor	Fever, cough	M. Pneumoniae	NE	NA	Type I	-	CR
Hashimoto Y. et al. 2009	22	3	M	Drowsiness, up-deviation eight, hypotonia	Fever, vomiting, diarrhoea	UK	Pleocytosis, ▲P	DSW	Type I	Acyclovir	CR (SCC lesion until 154 d-Gliosis)
Ohgoshi Y. et al. 2009	23	10	F	Drowsiness	Cough	M. Pneumoniae	NE	NA	Type I	NA	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
Fukuda et al. 2009	24	2	M	Consciousness disturbance	Fever, vomiting, diarrhoea	Rotavirus	NA	DSW	Type I	PSL	CR
Kato et al. 2009	25	1	F	Seizure	Fever, vomiting, diarrhoea	Rotavirus	NA	IS	Type I	DZP	CR
Fluss J. 2009	26	2,5	F	Hipotonia, refusal of walking, irritability, dysartria consciousness disturbance, mutism, ataxia.	Fever, cough	Influenza A	Pleocytosis	SBA	Type I	Acyclovir	CR
Imamura T. et al. 2010	27	6	F	Seizure, consciousness disturbance	Fever, sore throat	Adenovirus	N	N	Type II	MDZ, PSL, Antibiotic,	CR
	28	2	M	Seizure, consciousness disturbance	Fever	UK	N	NA	Type II	Antibiotic, Dex	CR
Jang Y.Y. et al. 2010	29	2,5	F	Seizure	Vomiting, diarrhoea	Rotavirus	NE	N	Type I	Acyclovir	CR
Iwata A. et al. 2010	30	14	M	Dysarthria, dysphagia, mild ptosis (Pseudobulbar Palsy)	Fever, cough, headache, fatique	Influenza A (H1N1)	N	NA	Type II	Zanamivir	CR
Kubo K. et al. 2010	31	12	F	Abnormal behavior, drowsiness, seizure	Fever	M. Pneumoniae	NA	NA	Type I	Steroid	CR
Takanashi J. et al. 2010	32	10	M	Seizure, drowsiness	Fever	UK	N	FSW	Type II	Steroid, Acyclovir	CR
	33	6	M	Delirious behavior, drowsiness, consciousness disturbance	Fever, cough, rhinorrhea	UK	N	FSW	Type II	Zanamivir	CR
Arawaka et al. 2010	34	4	F	Consciousness disturbance	Diarrhoea, vomiting	Rotavirus	NA	NE	Type I	Supportive	CR
	35	3	M	Consciousness disturbance	Fever, vomiting	Rotavirus	NA	NE	Type I	Supportive	CR
Osuka S. et al. 2010	36	3	F	Drowsiness, seizure	Fever, vomiting, diarrhoea	M. Pneumoniae	N	NA	Type I	Antibiotic	CR
	37	8	M	Ataxia, mental confusion, drowsiness, lethargy	Fever	M. Pneumoniae	N	NA	Type I	Antibiotic, Steroid	CR
Hara M. et al. 2011	38	8	M	Delirious behavior	Fever, vomiting	Mumps vaccination	Pleocytosis	NA	Type II	mPSL	CR
Itamura S. et al. 2011	39	14	F	Delirious behavior	Fever, headache, chest pain	Kawasaki D.	N	N	Type I	IVIG	CR
Nakamoto T. et al. 2012	40	6	F	Delirium	Cough, fever	M. Pneumoniae	NE	NA	Type I	Antibiotic	CR
Morichi S. et al. 2012	41	9	M	Seizure, delirium	Fever, coughing, nasal discharge	Influenza A	N	DSW	Type II	Oseltamivir. mPSL	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
	42	8	M	Delirious behavior, drowsiness	Fever	Kawasaki D.	N	FSW	Type I	IVIG, CY, IFX	CR
	43	7	F	Delirious behavior, drowsiness	Fever	Kawasaki D.	N	N	Type I	IVIG	CR
Takanashi J. et al. 2012	44	10	F	Delirious behavior, drowsiness	Fever	Kawasaki D.	N	N	Type II	IVIG	CR
	45	2	F	Delirious behavior, drowsiness	Fever	Kawasaki D.	N	DSW	Type I	IVIG, mPSL	CR
	46	14	F	Delirious behavior, drowsiness	Fever	Kawasaki D.	Pleocytosis	DSW	Type I	IVIG	CR
	47	7	F	Delirious behavior, drowsiness	Fever	Kawasaki D.	N	DSW	Type I	IVIG	CR
Sato T. et al. 2012	48	7	F	Consciousness disturbance, delirious behavior	Right neck pain, fever, vomiting, diarrhoea	Kawasaki D.	N	DSW	Type I	Antibiotic, IVIG	CR
	49	1	F	Seizure	NA	UK	N	NA	Type I	Antiepilept	NA
	50	2	F	Seizure	NA	UK	N	NA	Type II	Antiepilept	NA
Miyata R.	51	2	M	Seizure	NA	Influenza A	N	NA	Type I	Antiepilept	NA
et al. 2012	52	3	F	Seizure	NA	UK	N	NA	Type II	Antiepilept	NA
	53	6	M	Seizure, delirious behavior	NA	RS Virus	N	NA	Type I	Antiepilept	NA
	54	13	F	Seizure	NA	Influenza A	N	NA	Type I	Antiepilept	NA
Uchida Y. et al. 2013	55	7	M	Abnormal speech, drowsiness	Cough, fever	M. Pneumoniae	Pleocytosis	N	Type II	Antibiot, Steroid	CR
Kometani H. et al. 2013	56	6	M	Consciousness disturbance, delirious behavior	Fever, headache	E. Faecalis Pyelonephritis	N	DSW	Type II	Antibiotic	CR
	57	9	M	Seizure, Consciousness disturbance, Delirious behavior	Fever, vomiting	E. Faecalis Pyelonephritis	Pleocytosis	NA	Type I	Antibiotic	CR
Matsuoka T. et al. 2013	58	4	M	Seizure, delirious behavior	Vomiting, diarrhoea,	Rotavirus	N	N	Type I	Antiepilept	CR
Okamoto T. et al. 2014	59	16	F	Delirium, consciousness disturbance	Fever, headache, back pain	K. Pneumoniae Pyelonephritis	N	NE	Type I	Antibiotic	CR
Haplern L.A. et al. 2013	60	5	M	Consciousness disturbance, seizure	Fever, cough	Parainfluenzae virus	Pleocytosis	SBA	Type II	Antibiotic	CR
Fuchigami T.et al. 2013	61	4	F	Consciousness disturbance, seizure	Vomiting, diarrhoea	Rotavirus	N	DSW	Type I	mPSL Diuretic	CR
Yokoyama et al. 2013	62	2	M	Seizure	Fever, vomiting, diarrhoea	Rotavirus	NA	N	Type I	PB	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
Notebaert A.et al. 2013	63	13	M	Abnormal speech, ataxia, confusion, drowsiness, lethargy	Abdominal pain, fever	M. Pneumoniae	Pleocytosis	DSW	Type II	NA	CR
Kashiwagi M. et al. 2014	64	2,75	M	Seizure, dysarthria, delirious behavior, consciousness disturbance	Fever	Influenza A	N	DSW	Type II	mPSL, MDZ, Mannitol, DZP, Oseltamivir	CR
	65	3,25	F	Seizure, dysarthria, delirious behavior, consciousness disturbance	UK	Influenza A	N	DSW	Type I	mPSL, Mannitol	CR
	66	7,75	F	Consciousness disturbance	UK	Influenza A	ND	DSW	Type I	mPSL, Mannitol	CR
	67	10,7	F	Delirious behavior, consciousness disturbance	UK	Kawasaki D.	N	DSW	Type II	PB, mPSL	CR
	68	6,25	M	Delirious behavior, consciousness disturbance	UK	Kawasaki D.	ND	N	Type I	mPSL	CR
	69	5	M	Delirious behavior, seizure, Consciousness disturbance	Enterocolitis, diarrhoea	Rotavirus	N	DSW	Type II	mPSL	CR
Suzuki H. et al. 2014	70	11	M	Abnormal behavior	Fever, (HLH)	Parvovirus B19	N	DSW	Type I	IVIG	CR
Hatanaka M.et al. 2014	71	17m	F	Seizure, consciousness disturbance	Fever, AESD	HHV-6	N	N	Type I	mPSL,MDZ, DZP,IVIG	CR
Shah S. et al. 2014	72	2	M	Seizure	Fever, cough	Influenza A	N	N	Type I	Oseltamivir. Clobazam	CR
Watanabe T.et al 2014	73	6m	M	Consciousness disturbance	Fever, renal dysfunct. (Fanconi s.)	UK	N	N	Type I	Supportive	CR
Mazur-Meleska K. et al. 2015	74	6	F	Seizure	Fever, diarrhoea	Rotavirus	ND	FSW	Type I	Dex	CR
Karampatsas K..et al. 2015	75	4	M	Consciousness disturbance	Vomiting, diarrhoea, fever	Rotavirus	N	DSW	Type I	Antibiotic	CR
Pan J.J. et al. 2015	76	18	F	Headache	Fever	UK	Pleocytosis	NA	Type I	mPSL+Dex, IVIG	CR
	77	2	F	Seizure	Fever	Rotavirus	NA	NA	Type I	mPSL+Dex, IVIG	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
	78	3	M	Irritability, lethargy, ataxia	Fever, cough	UK	NA	NA	Type II	Antibiotic, Acyclovir	CR
	79	6	F	Irritability, lethargy	Fever, coryza, headache, vomiting, abdominal pain	Kawasaki D.	NA	NA	Type I	IVIG, Aspirin, Antibiotic	CR
	80	5	F	Drowsiness, confusion, slurred speech, seizure, ataxia	Fever,vomiting	UK	NA	NA	Type I	mPSL, MDZ	CR
Ka A. et al. 2015	81	7	F	Drowsiness, confusion, visual hallucinations	Fever, headache, abdominal pain, vomiting, bloody diarrhoea	Salmonella	NA	NA	Type I	Antibiotic, Acyclovir	CR
	82	9	M	Irritability, slurred speech, confusion, ataxia	Fever, vomiting	CMV	NA	NA	Type II	mPSL, Antibiotic	CR
	83	5	F	Lethargy, ataxia	Fever, vomiting, cough	Influenza B	NA	NA	Type II	None	CR
	84	4	M	Confusion, slurred speech, hallucinations, ataxia	Fever, abdominal pain,cough, coryza, conjunctiv.	Adenovirus	NA	NA	Type I	Antibiotic, Acyclovir	CR
	85	9	M	Consciousness disturbance	Fever, vomiting	Mumps vaccination	Pleocytosis	FSW	Type II	NA	CR
	86	5	M	Delirium, seizure	Fever, vomiting, headache	Mumps vaccination	Pleocytosis	FSW	Type II	NA	CR
Takanashi J. et al.2015	87	2	M	Consciousness disturbance, dysarthria	Fever	Mumps vaccination	Pleocytosis	N	Type I	NA	CR
	88	8	M	Delirium	Fever, vomiting, headache	Mumps vaccination	Pleocytosis	N	Type II	NA	CR
	89	1	M	Consciousness disturbance, seizure	Fever, vomiting	Mumps vaccination	Pleocytosis	DSW	Type I	NA	CR
Kawagoshi R. et al. 2015	90	6	M	Drowsiness, irritability	Fever, cough, headache	M. Pneumoniae	Pleocytosis	DSW	Type I	Antibiotic	CR
	91	8	M	Drowsiness, consciousness disturbance	Fever	Influenza A	NA	DSW	Type I	IVIG, mPSL	CR
Azuma J. et al. 2016	92	3	F	Drowsiness, consciousness disturbance, seizure	Fever	E. faecalis	NA	DSW	Type I	Antibiotic	CR
	93	2	F	Consciousness disturbance, seizure	Fever, vomiting	Rotavirus	NA	FSW	Type I	mPSL	CR
Fong C.Y.et al. 2016	94	14	F	Delirium, ophtalmoplegia, consciousness disturbance	Fever	Dengue v. type II	UK	NA	Type I	Supportive	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
Fang Q.et al. 2016	95/123	4±3,6	M/F 17/12	Consciousness disturbance (18 pts), Seizure (18 pts)	Fever	Rotavirus 5 pts, M. Pneumoniae 4 pts, HSV 2 pts, Coxsackie 2 pts, Adenovirus 2 pts, Echovirus 2 pts, Influenza A 1 pt, CMV 1 pt, EBV 1 pt, UK 9 pts	Pleocytosis and ▲P in 3 pts	NA	Type I 24 pts; Type II 5 pts	Antiviral 3 pts, Steroid 7 pts, IVIG 2 pts, Antiepileptic 1 pt	CR
Hirayama Y.et al. 2016	124/126	4,2	M/F 2/1	NA	NA	Influenza 1 pt, UK 2 pts	NA	NA	NA	NA	CR
Hosoda A. et al.2016	127	3	F	Seizure	Clinical signs of sepsis	GBS	N	DSW	Type I	Antibiotic, Diazepam	Brain Atrophy, Temporal regression of motor function
Ikeno M. et al. 2016	128	14	M	Consciousness disturbance, CIP	Headache, gastric perforation,	UK	Pleocytosis, ▲P	DSW	Type I	mPSL	CR
Ueda N. et al. 2016	129	14	M	Abnormal speech, hallucinations, delirious behavior, drowsiness	Fever, cough	M. Pneumoniae	ND	NE	Type I	mPSL, Acyclovir	CR
	130	8	F	Drowsiness, lethargy, ataxia, tremors	Cough, headache, vomiting, diarrhoea	M. Pneumoniae	ND	N	Type II	Antibiotic	CR
Avcu G. et al. 2016	131	10	M	Consciousness disturbance, motor automatism	Fever, vomiting, lethargia	S. Pneumoniae	Pleocytosis, ▲P	N	Type I	Antibiotic, Acyclovir	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
	132	2,9	M	Seizure, irritability, motor deterioration, slurred speech	Fever, vomiting, abdominal pain	M. Pneumoniae	N	N	Type I	None	CR
	133	6,6	M	Headache, delirium, stupor, slurred speech, neck stiffness, drowsiness	Fever, vomiting, abdominal pain	M. Pneumoniae	N	SBA	Type I	mPSL, IVIG	CR
	134	3	F	Seizure, irritability, motor deterioration	Fever, vomiting, diarrhoea, cough	Rotavirus	N	SBA	Type I	Dex,PB, IVIG	CR
	135	2,3	F	Ataxia	Fever, vomiting, MLNE, diarrhoea, lip and knee-joints effusion	Rotavirus	N	N	Type I	Dex, IVIG	CR
	136	3,7	F	Seizure, hallucination	Cough, diarrhoea, vomiting, abdominal pain, MLNE	Rotavirus	N	SBA	Type I	-	CR
Chen W.X. et al. 2016	137	7	M	Drowsiness	Fever, vomiting, headache	Adenovirus	N	N	Type I	-	CR
	138	2,4	F	Seizure	Vomiting, diarrhoea, cough	BJ Campylobacter	N	N	Type I	DZP, PB	CR
	139a	10m	M	Seizure, irritability	Fever, cough, vomiting	UK	N	N	Type I	Dex, IVIG	CR
	139b	1	M	Seizure, irritability, stupor, drowsiness, motor deterioration	Diarrhea, fever, vomiting	UK	N	IS	Type II	LEV, PB	ID
	140	2,4	F	Seizure, irritability	Diarrhoea, vomiting	Rotavirus	N	SBA	Type I	mPSL	CR
	141	1,4	F	Seizure, irritability	Diarrhoea, cough, vomiting	Rotavirus	N	N	Type I	DZP	CR
	142	2	M	Seizure, irritability, motor deterioration	Fever, vomiting	UK	N	SBA	Type I	MDZ	CR
	143	10,5	M	Dizziness	Cough, fever	Influenza A	ND	NE	Type I	None	CR
	144	5	F	Seizure, abnormal behavior, consciousness disturbance	Fever, vomiting, abdominal pain, afbn	E. Coli	N	DSW	Type I	Antibiotic,Dex,	CR
Dong K. et al. 2016	145	14	F	Signs suggestive of transient ischemic attack (tia)	Flu-like symptoms	UK	N	DSW	Type II	-	CR
Yuan ZF et al. 2016	146	9	M	Drowsiness, left peripheral facial nerve paralysis, lethargy	Fever, headache, vomiting, macupapular rash	M. Pneumoniae	N	DSW	Type I	Dex,Antibiotic, Mannitol	CR
	147	12	M	Drowsiness, lethargy, dizziness	Cough, headache, vomiting	M. Pneumoniae	N	DSW	Type I	Dex, Antibiotic, Mannitol	CR

Table I. Continue.

Authors and year	n	Age (yrs)	Sex	Neurological symptoms	Prodromal symptoms	Etiology	CSF	EEG	MRI	Treatment	Prognosis
Kurokawa Y. et al. 2017	148	2	F	Seizure, consciousness disturbance	Fever	Kawasaki D.	NA	DSW	Type I	IVIG, mPSL, IFX	CR
Britton P. N. et al. 2017	149	5	F	Lethargy, unsteady gait	Fever, vomiting	Influenza B	ND	NE	Type I	-	CR
Sun D. et al. 2017	150	2d	M	Poor reactivity, tic of limbs	Decreased reactivity	UK	N	N	Type II	Antibiotic	CR
	151	3d	M	Gaze palsy and shaking of limbs	Decreased reactivity	UK	N	NA	Type II	Antibiotic,PB	CR
	152	12d	M	Limb hyperspasmia	-	UK	N	IS	Type II	Antibiotic, PB	CR
	153	3d	M	Seizure	-	UK	N	N	Type I	Antibiotic	CR
	154	1d	M	Groaning and irritability	-	UK	N	N	Type II	Antibiotic	CR
Yagamuci H. et al.2017	155	3	F	Gait disturbance, delirium, consciousness disturbance, irritability	Fever, abdominal pain	EBV-HLH	N	NA	Type II	HLH -2004 Regimen	CR
Feraco P.et al.2017	156	22m	M	Febril seizures, afebril seizures, drowsiness	Fever	CMV	N	DSW	Type I	Levetiracetam, Ganciclovir	CR
Vanderscheren G.et al 2018	157	16	M	Lethargy, akinetic mutism	Fever, cough	Influenza B	N	SBA	Type II	-	CR
Yildiz A.E.et al 2018	158/165	5,9	M/F 5/3	Seizure(4), Delirious Behavior (1), Drowsiness(1), Ataxia(1),Transient blindness(2),Abnormal speech(2), Headache(1)	Nausea and vomiting (6), Diarrhea(6), Fever(3)	Rotavirus 1 pt, E.Coli 1pt	N 6pts; NA in 2 pts	DSW in 1 pt	Type I 7 pts; Type II 1 pt	Ceftriaxone, Acyclovir 2 pt (for 2 days)	CR
Our case		4,9	F	Gait disturbance, lethargy, dysarthria, consciousness disturbance, strabismus, gaze paresis, muscle weakness	Fever, diarrhoea	ECHO-Virus 6	Pleocytosis, ▲P	SW	Type I	Antibiotic, Acyclovir Methilpred, Prednisolon	CR

A: abnormal, AESD: mild form of acute encephalopathy with biphasic seizures and late reduced diffusion, AFBN: acute focal bacterial nephritis, Antiepileptic: antiepileptic drugs, BA: background activity, CIP: critical illness polyneuropathy, CR: clinical recovery, CY: cyclosporine, d: days, Dex: Dexamethasone, DSW: diffuse slow wave, DZP: diazepam, FSW: focal slow wave, HLH: Hemophagocytic lymphohistiocytosis, ID: intellectual disability, IFX: infliximab, IVIG: intravenous immunoglobulin, IS: intermittent spikes, m: months, MDZ: midazolam, MNLE: mesenteric linphonode enlargement, mPSL: methylprednisolone, NA: not available, NE: not examined, N: normal, ND: not done, ▲P: high CSF proteins, PB: phenobarbital, PSL: prednisolone, pts: patients, RD: rolandic discharge, RS virus: Respiratory Syncytial virus, SBA: slow background activity, SCC: Splenium of the corpus callosum, UK: unknown.

associated with Kawasaki disease in 12/165 (7.3%) patients. Microbiologic and serologic examinations were negative in 59/165 (35.7%) patients, and no infectious pathogens were identified in these cases.

We analyzed the association between etiological agents and symptoms. CD symptoms 93/165 (56.4%) were most frequently related to *Mycoplasma pneumoniae* in 12/93 (12.9%) patients, RV in 8/93 (8.6%) and influenza virus in 6/93 (6.4%). About patients developing seizures (77/165, 46.7%), RV infection was identified in 14/77 (18.2%) and influenza in 6/77 (7.8%). Considering cases with DB symptoms (55/165, 33.3%) the most frequent etiological agents were influenza A virus in 7/55 patients (12.7%), RV in 6/55 (10.9%) *Mycoplasma pneumoniae* in 6/55 (10.9%) and mumps virus in 5/55 (9.0%). In 10/55 of patients with DB (18.2%) Kawasaki disease was confirmed. Movement disorders described in 16/165 cases (9.7%) occurred most frequently in patients with *Mycoplasma pneumoniae* infections (4/16, 25.0%), and all these cases had ataxia. Out of 16/165 cases characterized by speech impairment (9.7%), the most frequent pathogens identified were *Mycoplasma pneumoniae* in 5/16 (31.2%), influenza A virus in 4/16 (25.0%), influenza B in 1/16 (6.2%), CMV in 1/16 (6.2%), adenovirus in 1/16 (6.2%), and mumps virus in 1/16 (6.2%). In 4/5 cases (80.0%) with dysarthria an association with influenza A virus was demonstrated. Mutism was reported in 2 patients with influenza virus (A in 1 case and B in the other one).

Lumbar puncture was performed in 135/165 (81.8%) patients, and CSF pleocytosis (>10 WBCs/μl) was diagnosed in 24/165 (14.5%) patients, 7/24 (29.2%) showed increased protein levels. 17/24 (70.8%) CSF pleocytosis patients were described as MERS type I and 7/24 (29.2%) patients as type II. No correlation between pleocytosis and worse outcome could be observed. CSF pleocytosis patietns had the following infections: mumps virus in 8/24 (33.3%), *Mycoplasma pneumoniae* in 3/24 (12.5%), *Enterococcus faecalis* in 1/24 (4.2%), *Streptococcus pneumoniae* in 1/24 (4.2%) and human parainfluenza virus type 3 in 1/24 (4.2%). In 2 patients, persistent pleocytosis were described for a period of more than 5 months.[51-52] Identification of the infectious agent by RT-PCR on CSF specimens was performed in only 6 patients with mumps-related MERS,[31-32] while in other patients diagnosis was achieved with a blood serological test or RT-PCR with throat swab. CSF glucose was normal in all cases.

Considering EEG, in 60/165 patients (36.4%) EEG findings were abnormal: focal slow waves were described in 8/60 (13.3%) (occipital waves in 5, parieto-occipital, temporo-occipital and frontal waves in 1, respectively); EEG revealed diffuse slow waves in 31/60 (51.7%) (authors described high voltage slow wave in 7 cases), slow background activity in 17/60 (28.3%), intermittent spikes in 3/60 (5.0%), and rolandic discharge in 1/60 (1.7%). Analyzing the association between EEG results and etiological agents, influenza virus (both A and B) was observed in 8/60 (13.3%), RV in 7/60 (11.7%), mumps virus in 4/60 (6.7%), adenovirus, EBV, CMV, *Escherichia coli* and *Enterococcus faecalis* in 1/60 (1.7%), respectively. Analyzing the association between EEG results and clinical syndrome diagnosis of Kawasaki disease was found in 6/60 patients (10.0%) with EEG abnormalities. Between patients with abnormal EEG findings, 38/60 (63.3%) had CD symptoms, 26/60 (43.3%) seizures and 19/60 (31.7%) DB. In all patients, follow-up EEG was normal, as also in our case. Clinical follow-up in these 60 patients revealed a good prognosis, except for one MERS type II case reported by Chen et al.[28], who developed intellectual disability.

Considering neuroimaging, 121/165 (73.3%) were diagnosed as MERS type I. Diagnosis of MERS type II was reported in 44/165 (26.7%) with hyperintensity lesions of CC in association with hyperintensity in the semioval center and parietal white matter bilaterally, or associated with diffuse hyperintensity of white matter (6 patients), or with hyperintensity in the center semiovale and periventricular symmetric hyperintense lesions (8 cases). In 3 (2.0%) cases, authors did not specify the extension

of hyperintensity lesions in the CC. Fluss et al.[21] described a MERS type II case with an additional restricted diffusion area of the right dentate nucleus suffering clinical mutism. In 2006, Takanashi et al.[3] reported a MERS type II with hyperintense asymmetric lesion in the gray matter of the prefrontal cortex with subsequent frontal atrophy and intellectual disability. Hyperintensity lesions in the SCC and in other brain areas on neuroimaging disappeared within 30 days in all cases, except in 1 patient with MERS type I, in whom the lesion could be still detected on brain MRI follow-up in T2-weighted imagines after 134 day. CSF analysis showed pleocytosis and increased proteins.[51]

Data about treatments revealed that 35/165 patients (21.2%) received antibiotic therapy after MERS onset, and 21/165 (12.7%) antiviral treatment. Intravenous immunoglobulin was administered to 23/165 (13.9%) patients, in 9 cases in association with corticosteroids. Eleven patients received, as steroid therapy, only Dexamethazone; 23 patients, methil-prednisolone (authors in 21 cases had specified a pulsed-dosed therapy; in 2 cases was prescribed also Dexamethazone) and 2 patients prednisolone. For 11 cases the corticosteroids treatment has not been specified. In 2 patients, infliximab was also administered (Table I).

The median recovery time was 13.3 days for MERS type I (though data about recovery time was only for 103/121 (85.1%) patients available) and 12.6 days for MERS type II (data about recovery time was for 34/44 (77.3%) patients available).

Regarding prognosis, all patients completely recovered within 30 days, except for 4 patients. A long- term follow-up showed intellectual disability in 2 MERS type II cases.[3,28] Notably, one of these patients had recurrent MERS episodes; the other patient showed brain lesions in regions usually not involved in MERS, as the prefrontal cortex and white matter. Another patient with MERS type I associated with recurrent *Streptococcus* group B sepsis had mild brain atrophy and motor function regression, which improved gradually in the long-term.[50] Notebaert et al.[44] described one *Mycoplasma pneumoniae*-related MERS type II case with residual ataxia and speech impairment, who fully recovered 4 months later. Fluss et al.[21] described one case of influenza A-related MERS with a cerebellar lesion (in the right dentate nucleus) on MRI, presenting with dysarthria and ataxia; in this patient clinical recovery was obtained 1 month later.

Our literature review revealed two cases in which MERS and other encephalopathies overlapped. One case described, additional to MERS, febrile infection-related epilepsy syndrome (FIRES), characterized by intractable seizures and the other case acute encephalopathy with biphasic seizures and reduced diffusion (AESD), characterized by recurrent complex partial seizures.[30,53]

The exact relationship between radiological evidence and clinical condition of MERS patients is still unclear. In a retrospective study, Tada et al.[2] excluded any correlation between neuro-radiological features of brain lesions and neurological symptoms. Ueda et al.[36] reported a longer clinical recovery time in MERS type II related to *Mycoplasma pneumoniae* infection than in type I, suggesting also a less benign course.

MERS pathogenesis is still unclear. Researchers hypnotized intramyelinic edema as a possible cause, which however cannot explain neonatal MERS occurrence considering the incomplete myelination in newborns.[1,22,29,54,55] Kawasaki disease, an acute febrile systemic vasculitis, has been described in association with MERS, demonstrating a possible correlation to immune system abnormalities triggering the pathogenesis.[53,56,57] Kometani et al.[58] reported a IL-6 elevation in the CSF of MERS patients associated with focal bacterial nephritis caused by *Enterococcus faecalis*. RT-PCR rarely detected infections in the CSF, suggesting an immune-mediated mechanism in MERS pathogenesis. In our review, direct causative agents in CSF were identified only in 6 patients.[31,32] Moreover, unknown genetic factors could

be likely involved, and indeed recurrent and familial cases of MERS are described in medical literature. Neuroradiological features in familiar forms are also characterized by a more extensive brain involvement then in sporadic MERS cases.[59,60]

Recently, MERS has been increasingly recognized in Caucasian children, although none of cases had a recognized Echovirus 6 infection, as our patient. Infections (viral in 58% of cases), but also systemic inflammatory diseases (as Kawasaki disease) can be associated with MERS. Neurological manifestations occur shortly after prodromal symptoms and neuroimaging consents to substantiate MERS diagnosis. A short follow-up is necessary to confirm the transient nature of splenial lesions. The therapeutic approach in MERS may vary on a case-by-case basis; it remains unclear if a specific therapy might change the clinical history of the disease. Data of our review demonstrate that MERS is a disease with an overall good prognosis, as almost all patients reported a complete recovery of neurological symptoms within 30 days irrespective of treatment. Considering the low number of MERS cases and different severity degrees, multicentre studies are needed to clearly elucidate its pathogenesis and define treatment guidelines.

REFERENCES

1. Takanashi JI, Imamura A, Hayakawa F, Terada H. Differences in the time course of splenial and white matter lesions in clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS). J Neurol Sci 2010; 292: 24-27.
2. Tada H, Takanashi J, Barkovich AJ, et al. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion. Neurology 2004; 63: 1854-1858.
3. Takanashi J, Barkovich AJ, Shiihara T, et al. Widening spectrum of a reversible splenial lesion with transiently reduced diffusion. AJNR Am J Neuroradiol 2006; 27: 836-838.
4. Takanashi JI. Two newly proposed encephalitis/encephalopathy syndromes. Brain Dev 2009; 31: 521-528.
5. Kashiwagi M, Tanabe T, Shimakawa S, et al. Clinico-radiological spectrum of reversible splenial lesions in children. Brain Dev 2014; 36: 330-336.
6. Kobata R, Tsukahara H, Nakai A, et al. Transient MR signal changes in the splenium of the corpus callosum in rotavirus encephalopathy: value of diffusion-weighted imaging. J Comput Assist Tomogr 2002; 26: 825-828.
7. Karampatsas K, Spyridou C, Morrison IR, Tong CYW, Prendergast AJ. Rotavirus associated mild encephalopathy with a reversible splenial lesion (MERS)- case report and review of the literature. BMC Infect Dis 2015; 15: 446.
8. Yokoyama T, Yamada S, Doichi N, Kato E. Rotavirus-infected children with clinically mild encephalopathy with a reversible splenial lesion (MERS). BMJ Case Rep 2013: doi:10.1136/bcr-2013-008644.
9. Fuchigami T, Goto K, Hasegawa M, et al. A 4-year-old girl with clinically mild encephalopathy with a reversible splenial lesion associated with rotavirus infection. J Infect Chemother 2013; 19: 149-153.
10. Mazur-Melewska K, Jonczyk-Potoczna, Szpura K, et al. Transient lesion in the splenium of the corpus callosum due to rotavirus infection. Childs Nerv Syst 2015; 31: 997-1000.
11. Fukuda S, Kishi K, Yasuda K, Sejima H, Yamaguchi S. Rotavirus-associated encephalopathy with a reversible splenial lesion. Pediatr Neurol 2009; 40: 131-133.
12. Kato Z, Orii KE, Morimoto M, et al. A transient lesion in the corpus callosum during rotavirus infection. Pediatr Neurol 2009; 41: 467-469.
13. Arawaka C, Fujita Y, Imai Y, et al. Detection of group A rotavirus RNA and antigens in serum and cerebrospinal fluid from two children with clinically mild encephalopathy with a reversible splenial lesion. Jpn J Infect Dis 2011; 64: 204-207.
14. Matsuoka T, Yodoshi T, Sugai M, et al. A case of mild encephalopathy with a reversible splenial lesion associated with G5P[6]rotavirus infection. Case Rep Pediatr 2013: doi: 10.1155/2013/197163.
15. Feraco P, Porretti G, Marchiò G, Bellizzi M, Recla M. Mild encephalitis/encephalopathy with reversible splenial lesion (MERS) due to cytomegalovirus: case report and review of the literature. Neuropediatrics 2018; 49: 68-71.
16. Ka A, Britton P, Troedson C, et al. Mild encephalopathy with reversible splenial lesion: an important differential of encephalitis. Eur J Paediatr Neurol 2015; 19: 377-382.

17. Fang Q, Chen L, Chen Q, Lin Z, Yang F. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion of corpus callosum in Chinese children. Brain Dev 2017; 39: 321-326.

18. Takanashi JI, Barkovich AJ, Yamaguchi KI, Kohno Y. Influenza-associated encephalitis/encephalopathy with a reversible lesion in the splenium of the corpus callosum: a case report and literature. AJNR Am J Neuroradiol 2004; 25: 798-802.

19. Britton PN, Dale RC, Blyth CC, et al; ACE study investigators and PAEDS network Influenza-associated encephalitis/encephalopathy identified by the Australian childhood encephalitis study 2013-2015. Pediatr Infect Dis J 2017; 36: 1021-1026.

20. Vanderschueren G, Schotsmans K, Maréchal E, Crols R. Mild encephalitis with reversible splenial (MERS) lesion syndrome due to Influenza B virus. Pract Neurol 2018; 18: 391-392.

21. Fluss J, Ferey S, Menache-Starobinski C, Delavelle J, Van Bogaert P, Vargas MI. Mild influenza-associated encephalopathy/encephalitis with a reversible splenial lesion in a Caucasian child with additional cerebellar features. Eur J Paediatr Neurol 2010; 14: 97-100.

22. Ganapathy S, Ey EH, Wolfson BJ, Khan N. Transient isolated lesion of the splenium associated with clinically mild influenza encephalitis. Pediatr Radiol 2008; 38: 1243-1245.

23. Matsubara K, Kodera M, Nigami H, Yura K, Fukaya T. Reversible splenial lesion in influenza virus encephalopathy Pediatr Neurol 2007; 37: 431-434.

24. Morichi S, Kawashima H, Ioi H, Yamanaka G, Kashiwagi Y, Hoshika A. High production of interleukin-10 and interferon-γ in influenza-associated MERS in the early phase. Pediatr Int 2012; 54: 536-562.

25. Shah S, Keil A, Gara K, Gara K, Nagarajan L. Neurologic complication of influenza. J Child Neurol 2014; 29: NP49-NP53.

26. Iwata A, Matsubara K, Nigami H, Kamimura K, Fukaya T. Reversible splenial lesion associated with Novel Influenza A (H1N1) viral infection. Pediatr Neurol 2010; 42: 447-450.

27. Miyata R, Tanuma N, Hayashi M, et al. Oxidative stress in patients with clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS). Brain Dev 2012; 34: 124-127.

28. Chen WX, Liu HS, Yang SD, et al. Reversible splenial lesion syndrome in children: retrospective study and summary of case series. Brain Dev 2016; 38: 915-927.

29. Halpern LA, Agyeman P, Steinlin M, El-Koussy M, Grunt S. Mild encephalopathy with splenial lesion and parainfluenza virus infection. Pediatr Neurol 2013; 48: 252-254.

30. Hatanaka M, Kashiwagi M, Tanabe T, Nakahara H, Ohta K, Tamai H. Overlapping MERS and mild AESD caused by HHV-6 infection. Brain Dev 2015; 37: 334-338.

31. Hara M, Mizuochi T, Kawano G, et al. A case of clinically mild encephalitis with a reversible splenial lesion (MERS) after mumps vaccination. Brain Dev 2011; 33: 842-844.

32. Takanashi JI, Shiihara T, Hasegawa T, et al. Clinically mild encephalitis with a reversible splenial lesion (MERS) after mumps vaccination. J Neurol Sci 2015; 349: 226-228.

33. Suzuki H, Kusaka T, Okada H. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion caused by human parvovirus B19 infection: a case of two brothers with hereditary spherocytosis. Pediatr Neurol 2014; 51: 470-472.

34. Yagamguchi H, Ishida T, Yokoi T, et al. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion accompanied by Epstein-Barr virus hemophagocytic lymphohistiocytosis: a case report and review of the literature. J Pediatr Hematol Oncol 2017; 39: e92-e96.

35. Azuma J, Nabatame S, Katsura T, et al. Marked elevation of urinary β2-microglobulin in patients with reversible splenial lesions: a small case series. J Neurol Sci 2016; 368: 109-112.

36. Ueda N, Minami S, Akimoto M. Mycoplasma pneumoniae-associated mild encephalitis/encephalopathy with a reversible splenial lesion: report of two pediatric cases and a comprehensive literature review. BMC Infect Dis 2016; 16: 671.

37. Yuan ZF, Shen J, Mao SS, et al. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion associated with Mycoplasma pneumoniae infection. BMC Infect Dis 2016; 16: 230.

38. Kawagoshi R, Ono J. A case of Mycoplasma-associated encephalitis/encephalopathy with a reversible splenial lesion. Meiwa Igaku Zasshi 2015; 2: 35-40. [in Japanese].

39. Tokunaga Y. 1 girl case who showed transient of corpus callosum lesion with P-248 mycoplasmal diseases. No to Hattatsu 2008; 40(Suppl): 396. [in Japanese].

40. Ohgoshi Y, Sakai T, Nonaka S, Nakamura Y, Hosaki A, Bessho F. A case of mild encephalitis/encephalopathy with a reversible splenial lesion associated with Mycoplasma pneumonia. Nihon Shonika Gakkai Zasshi 2009; 113: 350. [in Japanese].

41. Nakamoto T, Tanaka K, Koga H, Kan N, Takahashi S. A case of mild encephalitis/encephalopathy with a reversible splenial lesion during Mycoplasma pneumoniae infection. Nihon Shonika Gakkasi Zasshi 2012; 116: 1255. [in Japanese].

42. Uchida Y, Morita H, Miyazaki K, Adachi S, Tatebayashi K, Kaneko H. A case of menimgoencephalitis with a reversible splenial lesion due to Mycoplasma pneumoniae infection. Shoni Kansen Menneki 2013; 25: 201-202. [in Japanese].

43. Osuka S, Imai H, Ishikawa E, et al. Mild encephalitis/encephalopathy with a reversible splenial lesion: evaluation by diffusion tensor imaging. Two case reports. Neorol Med Chir (Tokyo) 2010; 50: 1118-1122.

44. Notebaert A, Willems J, Coucke L, Van Coster R, Verhelst H. Expanding the spectrum of MERS type 2 lesions, a particular form of encephalitis. Pediatr Neurol 2013; 48: 135-138.

45. Jain S, Patel B, Bhatt GC. Enteroviral encephalitis in children: clinical features, pathophysiology and treatment advances. Pathog Glob Health 2014; 108: 216-222.

46. Pallansch MA, Roos R. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. . In: Knipe DM, Howley PM (eds). Fields Virology (5th edt).Philadelphia: Lippincott Williams & Wilkins, 2007: 839-894.

47. Hoshino A, Saitoh M, Oka A, et al. Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. Brain Dev 2012; 34:337-343.

48. Kurokawa Y, Masuda H, Kobayashi H, et al. Effective therapy with infliximab for clinically mild encephalitis/encephalopathy with a reversible splenial lesion in an infant with Kawasaki disease. Nihon Rinsho Meneki Gakkai Kaishi 2017; 40: 190-195.

49. Benschop KS, Geeraedts F, Beuvink B, et al; VIRO-TypeNed Increase in ECHOvirus 6 infections associated with neurological symptoms in the Netherlands, June to August 2016. Euro Surveil 2016; 21: 30351.

50. Hosoda A, Gatayama R, Moriyama S, et al. The first case of recurrent ultra late onset group B streptococcal sepsis in a 3-year-old child. IDCases 2017; 7: 16-18.

51. Hashimoto Y, Takanashi JI, Kaiho K, et al. A splenial lesion with transiently reduced diffusion in clinically mild encephalitis is not always reversible. A case report. Brain Dev 2009; 31 :710-712.

52. Ikeno M, Abe S, Kurahashi H, Takasu M, Shimizu T, Okumura A. Gastric perforation and critical illness polyneuropathy after steroid treatment in a patient with encephalitis/encephalopathy with transient splenial lesion. Brain Dev 2016; 39: 356-360.

53. Nozaki F, Kumada T, Miyajima T, et al. Reversible splenic lesion in a patient with febrile infection related epilepsy syndrome. Neuropediatrics 2013; 44: 291-294.

54. Pan JJ, Zhao YY, Lu C, Hu YH, Yang Y. Mild encephalitis/encephalopathy with a reversible splenial lesion: five cases and a literature review. Neurol Sci 2015; 36: 2043-2051.

55. Sun D, Chen WH, Baralc S, et al. Mild encephalopathy/encephalitis with a reversible splenial lesion (MERS): a report of five neonatal cases. J Huazhong Univ Sci Technol [Med Sci] 2017; 37: 433-438.

56. Itamura S, Kamada M, Nakagawa N. Kawasaki disease complicated with reversible splenial lesion and acute myocarditis. Pediatr Cardiol 2011; 32: 696-699.

57. Takanashi JI, Shirai K, Sugawara Y, Okamoto Y, Obonai T, Terada H. Kawasaki disease complicated by mild encephalopathy with a reversible splenial lesion (MERS). J Neurol Sci 2012; 315: 167-169.

58. Kometani H, Kawatani M, Ohta G, et al. Marked elevation of interleukin-6 in mild encephalopathy with a reversible splenial lesion (MERS) associated with acute focal bacterial nephritis caused by Enterococcus faecalis. Brain Dev 2013; 36: 551-553.

59. Imamura T, Takanashi JI, Yasugi J, Terada H, Nishimura A. Sisters with clinically mild encephalopathy with a reversible splenial lesion (MERS)-like features; familial MERS? J Neurol Sci 2010; 290: 153-156.

60. Yıldız AE, Maraş Genç H, Gürkaş E, Akmaz Ünlü H, Öncel İH, Güven A. Mild encephalitis/encephalopathy with a reversible splenial lesion in children. Diagn Interv Radiol 2018; 24: 108-112.

The Turkish Journal of Pediatrics 2020; 62: 310-314
https://doi.org/10.24953/turkjped.2020.02.019

Case Report

Wernicke's encephalopathy manifesting with diplopia after ileojejunostomy: report of a pediatric case with Hirschsprung disease

Beril Dilber[1], Tülay Kamaşak[1], İlker Eyüboğlu[2], Mehmet Kola[3], Ahmet Taner Uysal[3], Haluk Saruhan[4], Sonay Yalçın[4], Elif Sağ[5], Ali Cansu[1]

Departments of [1]Pediatric Neurology, [2]Radiology, [3]Ophthalmology, [4]Pediatric Surgery and [5]Pediatric Gastroenterology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey.

ABSTRACT

Background. Wernicke's encephalopathy (WE) is a coenzyme-induced disease with acute neuropsychiatric symptoms leading to high mortality and morbidity due to thiamine deficiency. WE is mostly caused by alcoholism in adult populations; however, it is often associated with gastrointestinal surgical procedures, recurrent vomiting, chronic diarrhea, cancer and chemotherapy treatment, systemic diseases, drugs, magnesium deficiency, and malnutrition in children. Although these predisposing factors are considered to be uncommon in children, they are actually highly frequent and can be fatal if not treated promptly.

Case. In this report, we present a patient who developed diplopia during total parenteral nutrition following surgical resection and was diagnosed with WE. The findings of the patient's cranial magnetic resonance imaging (MRI) findings were consistent with those of WE and the ocular findings of the patient resolved completely after thiamine treatment.

Conclusion. Although WE is rare in children it can be prevented by early diagnosis and treatment and oculomotor findings such as diplopia can be a warning sign.

Key words: Wernicke's encephalopathy, thiamine deficiency, diplopia, ileojejunostomy.

Wernicke's encephalopathy (WE) is an acute neurological condition first described by Carl Wernicke, characterized by mental state changes, ocular abnormalities, and cerebellar dysfunction.[1] Although WE is mostly caused by alcoholism in adult populations, it is often associated with gastrointestinal surgical procedures, recurrent vomiting, chronic diarrhea, cancer and chemotherapy treatment, systemic diseases, drugs, magnesium deficiency, and malnutrition in children.[2-5] Cranial imaging of WE often indicates symmetrical involvement of the periaqueductal region, the fourth ventricular base, and mammillary body in medial thalamus.[6-7] Mortality associated with WE is 17% and the symptoms are completely reversible with early diagnosis and treatment.[8] In this report, we present a patient who developed diplopia during total parenteral nutrition following surgical resection and was diagnosed with WE who then recovered rapidly and completely after thiamine support. By presenting this case, we aimed to emphasize that fatal situations such as WE, which is a rare condition in children, can be prevented by early diagnosis and treatment and that the oculomotor findings of WE can vary considerably, among which diplopia can be a warning sign.

✉ Beril Dilber
beriltem@gmail.com

Received 8th October 2019, revised 4th December 2019, 9th December 2019, accepted 15th December 2019.

Case Report

A 12-year-old girl who was being followed for Hirschsprung disease presented to the

emergency department with a five-day history of frequent vomiting. The patient was diagnosed with paralytic ileus and a jejunoileal resection and bridectomy was performed. A 55-cm section of the small intestine was removed. Total parenteral nutrition was provided to the patient for 2 weeks. On the 23rd day of hospitalization, the patient developed diplopia. On neurological examination, the patient was sleepy and was agitated in the presence of a stimulus, her speech was slow, and she had poor finger-tracking ability, diplopia, and restricted gaze. Visual acuity was 20/60 in the left eye and 20/70 in the right eye. Fundoscopy showed bilateral blurred optic discs and disc hemorrhage (Fig. 1). Deep tendon reflexes were reduced. There was no abnormality on cranial computed tomography (CT) and in blood parameters. On cranial magnetic resonance imaging (MRI), however, T2 and FLAIR sections showed symmetrical hyperintensity in medial thalamus, in the posterior part of the putamen, in the mesencephalon (tectum), in the periaqueductal region, at the level of facial colliculus, and in the mammillary body (Fig. 2-4). Based on these findings, a diagnosis of WE was made and the patient was given two doses of 200 mg/day thiamine support for five days. After the treatment, the visual complaints of the patient resolved completely. Serum thiamine (B1) level was measured by liquid chromatography/mass spectrometry and the level measured was found to be lower than the reference levels (9.2 [range, 25-75] nmol/L). A written consent was obtained from the parents.

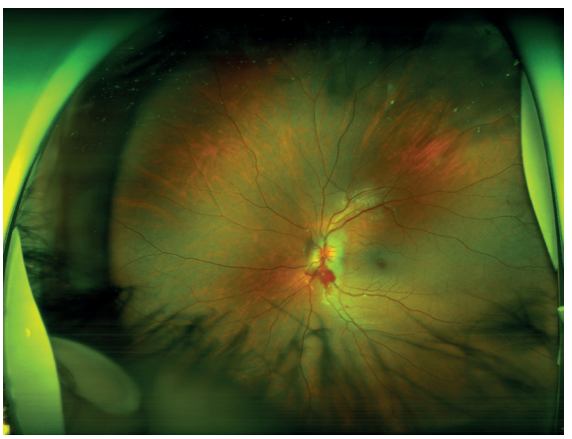


Fig. 1. Fundoscopy showed bilateral blurred optic discs and disc hemorrhage.

Discussion

Wernicke's encephalopathy is a disease characterized by acute neuropsychiatric symptoms as well as high mortality and morbidity associated with thiamine deficiency.[9] Thiamine is a vitamin essentially required as a coenzyme in the pathways in the brain and is absorbed from the duodenum through active carrier proteins and passes through the blood- brain barrier via active and passive mechanisms, thereby transforming to its active form in glial cells, thiamine pyrophosphate, and participating in the amino acid metabolism of carbohydrates, lipids, and glucose-dependent neurotransmitters.[9,10,13] The volume loss and cytotoxic edema in astrocytes resulting from thiamine deficiency appears within the first 4 days and leads to endothelial dysfunction and impaired blood-brain barrier function by days 7-10, ultimately resulting in neuronal necrosis and irreversible brain damage by day 14.[11-13] While the incidence of WE varies across communities, it has been reported to be 0.04-0.013% in clinical trials and to be higher in autopsy studies (0.8-2.8%), with similar rates in children.[6,10] Typical findings of WE such as mental status change, ophthalmoplegia, gait disturbances, and ataxia are seen in only 16% of patients. However, WE often manifests with headache and mild drowsiness and progresses gradually.[8,11] In our patient, diplopia developed in the fourth week after the onset of complaints.

Wernicke's encephalopathy is symmetrical in approximately 50% of the patients with the involvement of the periductal gray matter, mammillary body, and medial thalamus. Moreover, histopathological changes occur in bilateral dorsal thalamic nuclei in 100% of the patients. In our patient's MRI, symmetric hyperintensity was detected in medial thalamus, in the posterior part of the putamen, in the mesencephalon (tectum), in the periaqueductal

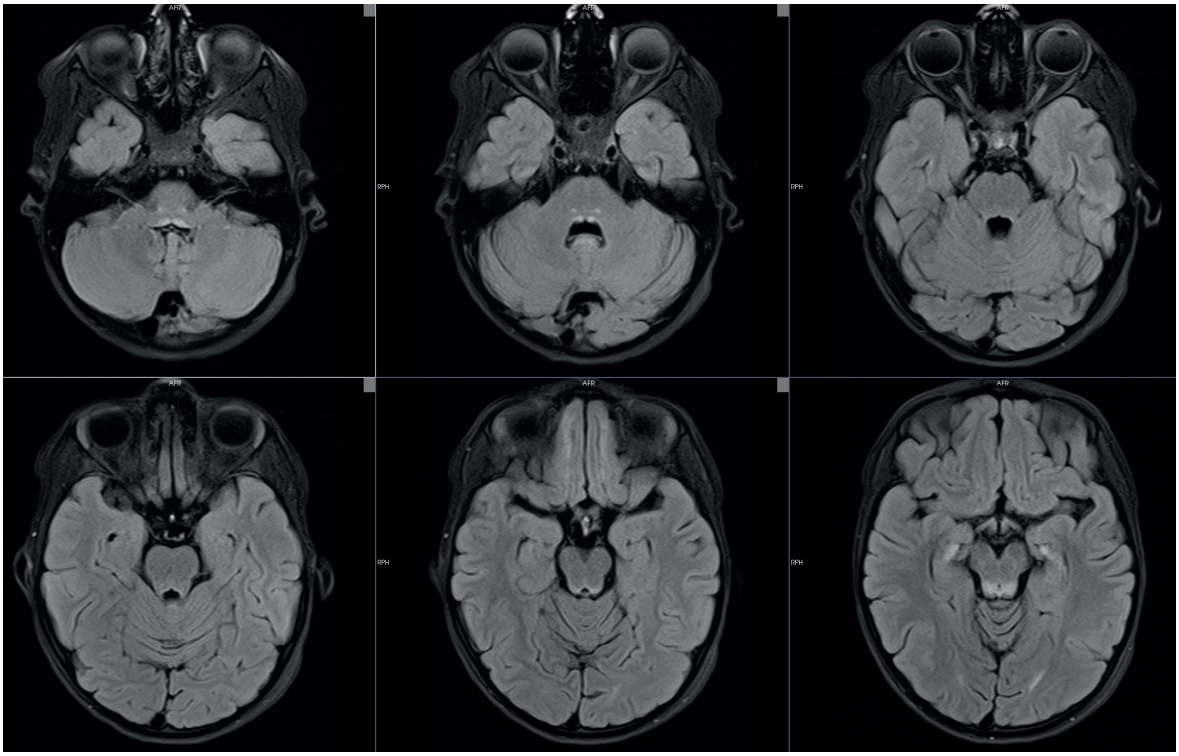


Fig. 2. ADC sections showed symmetrical diffusion restriction in medial thalamus, in the posterior part of the putamen, in the mesencephalon.

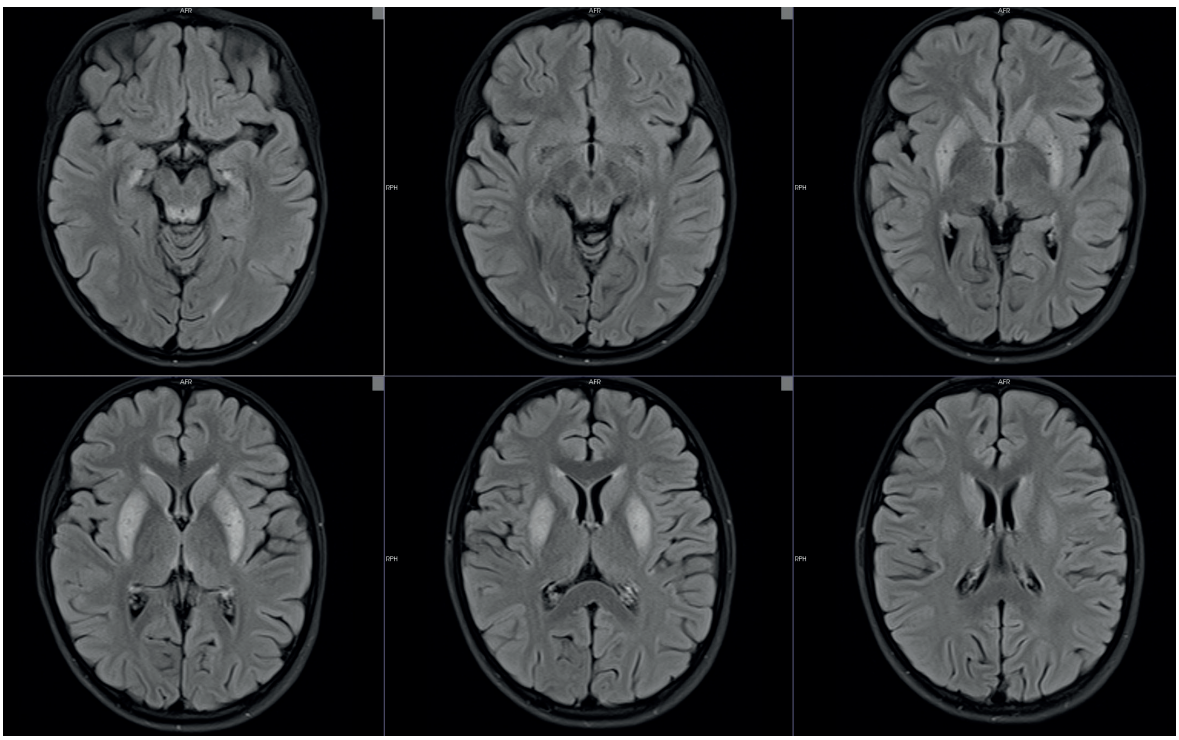


Fig. 3. DWI sections showed symmetrical hyperintensity in medial thalamus, in the posterior part of the putamen, in the mesencephalon (tectum), in the periductal region, at the level of facial colliculus, and in the mammillary body.

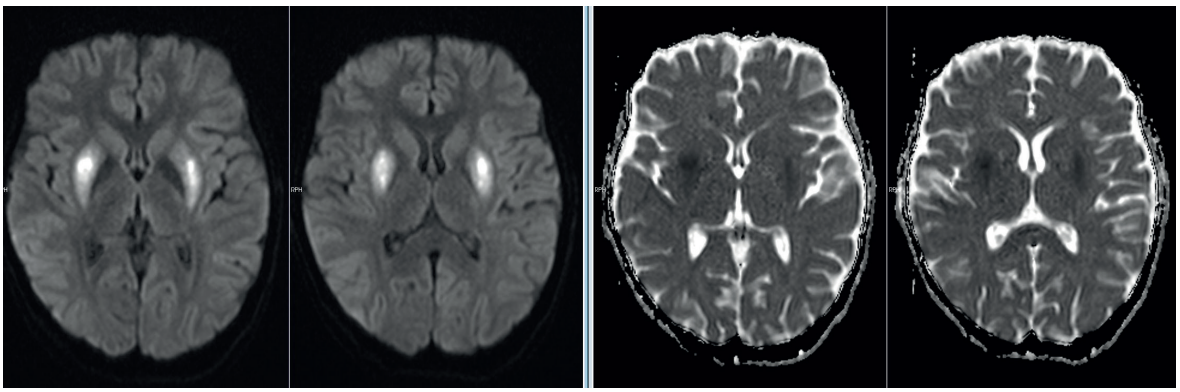


Fig. 4. Flair sections showed symmetrical hyperintensity in medial thalamus, in the posterior part of the putamen, in the mesencephalon (tectum), in the periductal region, at the level of facial colliculus, and in the mammillary body.

region, at the level of facial colliculus, and in the mammillary body. The ocular findings of WE such as disc edema and retinal bleeding, which are often unclear, can present as optic neuritis which is mostly seen in comatose and delayed patients.[15] However, in our patient, optic disc edema and retinal hemorrhage occurred in a period when the early changes in consciousness were not apparent. The initiation of empirical therapy in WE undergoing surgical intervention can be life-saving. The recommended thiamine dose in children is 100-200 mg/day twice daily or 1.8 mg per kilocalories and 100 mg/day, which is the optimal dosage needed for passing through the blood-brain barrier.[16-18] Thiamine was given in two doses of 200 mg/day intravenously since the patient was nonalcoholic, and in our patient, diplopia resolved on day 2 and all of the symptoms resolved by day 5 after the initiation of the thiamine therapy.

In conclusion, WE is a clinical condition that should be kept in mind by clinicians when vitamin absorption is prevented in children, particularly in those with predisposing intestinal system diseases such as Hirschsprung disease and those requiring long-term total parenteral nutrition following intestinal surgery. Early diagnosis of WE is highly important as it is a cause of treatable and preventable mortality. In this report, we presented a patient who was receiving postoperative treatment for Hirschsprung disease and was admitted with the complaint of diplopia. The patient received a prompt diagnosis of WE via MRI findings, and was treated completely after thiamine therapy.

REFERENCES

1. Wernicke C. Die akute haemorrhagische polioencephalitis superior. In: Lehrbuch der Gehirnkrankheiten fur Aerzte und Studirende, Bd II, Kassel: Fisher Verlag, 1881: 229-242.
2. Sechi G, Serra A. Wernicke's encephalopathy: new clinical settings and recent advances in diagnosis and management. Lancet Neurol 2007; 6: 442-455.
3. Kamaşak T, Kul S, Tuşat M, Ozgun N, Cansu A. A case of wernicke encephalopathy developing after ileal bypass surgery. Pediatr Emerg Care 2018; 34: e223-e225.
4. Acar Arslan E, Ekinci S, Zengin Akkuş P, Göçmen R, Haliloğlu G. Wernicke encephalopathy due to thiamine deficiency after surgery on a child with duodenal stenosis. Pediatr Neurol 2014; 51: 840-842.
5. Park SW, Yi YY, Han JW, Kim HD, Lee JS, Kang HC. Wernicke's encephalopathy in a child with high dose thiamine therapy. Korean J Pediatr 2014; 57: 496-499.
6. Cerase A, Rubenni E, Rufa A, et al. CT and MRI of Wernicke's encephalopathy. Radiol Med 2011; 116: 319-333.
7. Manzo G, Gennaro AD, Cozzolino A, Serino A, Fenza G, Manto A. MR imaging findings in alcoholic and nonalcoholic acute wernicke's encephalopathy: a review. Biomed Res Int 2014; 2014: 503596
8. Victor M, Adams RD, Collins GH. The Wernicke-Korsakoff syndrome: a clinical and pathological study of 245 patients, 82 with post-mortem examinations. Contemp Neurol Ser 1971; 7: 201-206.

9. Manzo L, Locatelli C, Candura SM, Costa LG. Nutrition and alcohol neurotoxicity. Neurotoxicology 1994; 15: 555-565.

10. Harper CG, Giles M, Finlay-Jones R. Clinical signs in the Wernicke-Korsakoff complex: a retrospective analysis of 131 cases diagnosed at necropsy. J Neurol Neurosurg Psychiatry 1986; 49: 341-345.

11. Vasconcelos MM, Silva KP, Vidal G, Silva AF, Domingues RC, Berditchevsky CR. Early diagnosis of pediatric Wernicke's encephalopathy. Pediatr Neurol 1999; 20: 289-294.

12. Lockman PR, McAfee JH, Geldenhuys WJ, Allen DD. Cation transport specificity at the blood-brain barrier. Neurochem Res 2004; 29: 2245-2250.

13. Chan F, Butterworth RF, Hazell AS. Primary cultures of rat astrocytes respond to thiamine deficiency-induced swelling by downregulating aquaporin-4 levels. Neurosci Lett 2004; 366: 231-234.

14. Desjardins P, Butterworth RF. Role of mitochondrial dysfunction and oxidative stress in the pathogenesis of selective neuronal loss in Wernicke's encephalopathy. Mol Neurobiol 2005; 31: 17-25.

15. Mumford CJ. Papilloedema delaying diagnosis of Wernicke's encephalopathy in a comatose patient. Postgrad Med J 1989; 65: 371-373.

16. Manzanares W, Hardy G. Thiamine supplementation in the critically ill. Curr Opin Clin Nutr Metab Care 2011; 14: 610-617.

17. Zuccoli G, Pipitone N. MR Imaging: an increasingly important tool in the early diagnosis of Wernicke encephalopathy. AJNR Am J Neuroradiol 2012; 33: E92.

18. Galvin R, Brathen G, Ivashynka A, Hillbom M, Tanasescu R, Leone MA. EFNS guidelines for diagnosis, therapy and prevention of Wernicke encephalopathy. Eur J Neurol 2010; 17: 1408-1418.

The Turkish Journal of Pediatrics 2020; 62: 315-319
https://doi.org/10.24953/turkjped.2020.02.020

Case Report

Kawasaki disease presented with status epilepticus and diffusion MRI abnormalities in the subcortical white matter

Dai Keino, Yuki Koto, Chisato Inuo

Department of Pediatrics, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.

ABSTRACT

Background. Kawasaki disease (KD) is an acute, self-limited vasculitis of unknown etiology that occurs predominantly in infants and young children. Encephalitis/encephalopathy is an extremely rare complication of KD.

Case. A previously healthy 8-month-old Japanese boy had a prolonged seizure after febrile illness for one day. On the fourth day, he had bilateral nonexudative conjunctivitis, changes in the extremities, rash and induration at the Bacillus Calmette-Guerin inoculation site. He was diagnosed with incomplete KD and treated with immunoglobulin. On the fifth day, he had cluster seizures. Brain magnetic resonance imaging (MRI) showed restricted diffusion in the left subcortical white matter, which was consistent with acute encephalopathy with biphasic seizures and late reduced diffusion (AESD). He was treated with controlled normothermia, pulsed-dose methylprednisolone, continuous infusion of midazolam, and edaravone. On the tenth day, he had a recurrent fever and was treated with a second course of immunoglobulin. Subsequently, he had defervescence, and the abnormal signal detected in the MRI disappeared. At the age of 11 months, he had normal growth and development for his age by the Denver Developmental Screening Test.

Conclusion. It is necessary to consider AESD as the differential diagnosis of prolonged seizure in infants with KD. Brain MRI led to early diagnosis and intervention in our patient. The neurological prognosis of our patient was relatively good, but the prognosis of KD with AESD is unknown. To clarify this, further case accumulation is warranted.

Key words: incomplete, Kawasaki disease, encephalopathy, acute encephalopathy with biphasic seizures and late reduced diffusion.

Kawasaki disease (KD) is an acute, self-limiting febrile illness of unknown etiology that predominantly affects children younger than five years of age. KD is classically diagnosed on the basis of the presence of fever for more than five days and five principal clinical features (bilateral nonexudative conjunctivitis, erythema of the lips and oral mucosa, changes in the extremities, rash, and cervical lymphadenopathy). Patients who do not fulfill these criteria are diagnosed with incomplete KD.[1]

✉ Dai Keino
d2keino@marianna-u.ac.jp

Received 17th July 2019, revised 6th September 2019, accepted 14th September 2019.

The most important complication of KD is coronary arterial aneurysm, which may cause ischemic heart disease and sudden death.[1] Complications such as febrile seizures and acute encephalopathy are extremely rare.[2,3] Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) occurs in children with infection. It is characterized by prolonged febrile seizures in the first phase and a cluster of seizures, deterioration of consciousness, and white matter lesions with reduced diffusion in the second phase.[4] Here, we report the clinical course of a pediatric case with incomplete KD, who presented with status epilepticus and characteristic brain MRI abnormalities. We explained the purpose of the report to the parents and obtained their informed consent.

Case Report

A previously healthy 8-month-old Japanese boy was admitted to our hospital, having experienced a prolonged generalized seizure on the first day of febrile illness. He was treated with three doses of midazolam (0.2 mg/kg), and the seizure lasted for approximately 50 minutes.

The blood count revealed no abnormalities. Hepatic enzyme, C-reactive protein, and procalcitonin levels were significantly elevated (Table I). The cerebrospinal fluid (CSF) examination revealed no abnormalities. The polymerase chain reactions for serum and CSF samples were negative for the influenza virus and the human herpes viruses (HHV) 6 and 7. Brain magnetic resonance imaging (MRI) revealed no abnormalities (Fig. 1, left column).

Thereafter, he was conscious with continued, mild lethargy. The laterality of deep tendon reflex response, muscle tonus, and motor function were normal. Paralysis was not observed. On the third day, he developed anterior uveitis and an induration at the Bacillus Calmette-Guerin inoculation site. By the fourth day, he developed bilateral nonexudative conjunctivitis, swelling in the extremities, and trunk rash. Blood biochemical findings revealed a progression of hypoalbuminemia and hyponatremia. Abdominal ultrasonography revealed gallbladder wall thickening. Therefore, he was diagnosed with incomplete KD and treated with immunoglobulin (2 g/kg) and aspirin (30 mg/kg).

On the fifth day, he had cluster seizures without laterality, and was somnolent during the intermittent phases. A brain MRI indicated hemicerebral lesions in the left subcortical white matter, which were most conspicuous in diffusion-weighted imaging (DWI; Fig. 1, middle column). An electroencephalography

Table I. Laboratory finding at admission.

Peripheral Blood								
WBC	7100	/μL	TP	5.3	g/dl	glucose	133	mg/dl
RBC	363	$\times 10^4$/μL	Alb	3.3	g/dl	NH3	82	μg/dl
Hb	10.0	g/dl	T-Bil	1.7	g/dl			
Hct	31.2	%	AST	420	IU/L	pH	7.22	
Plt	27.5	$\times 10^4$/μL	ALT	299	IU/L	pCO2	42.2	mmHg
			LDH	444	IU/L	HCO3	16.5	mmol/L
PT	42	%	γ-GTP	132	IU/L	BE	-10	mmol/L
PT-INR	1.60		CPK	57	IU/L			
APTT	56.6	sec	Cr	0.25	mg/dl	Cerebrospinal Fluid		
D-dimer	1.8	μg/ml	BUN	10.2	mg/dl	cell count	1	/μL
			UA	4.2	mg/dl	polynuclear	0	%
			Na	134	mEq/L	mononuclear	100	%
			K	3.4	mEq/L	protein	23.8	mg/dl
			Cl	107	mEq/L	glucose	83	mg/dl
			Ca	7.9	mg/dl			
			P	4.8	mg/dl			
			CRP	1.58	mg/dl			
			PCT	10.2	ng/dl			

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Hct: hematocrit, Plt: platelet, PT: prothrombin time, APTT: activated partial thromboplastin time, TP: total protein, Alb: albmin, T-Bil: total billirubins, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, γ-GTP: gamma-glutamyl transpeptidase, CPK: creatine phosphokinase, Cr: creatinine, BUN: blood urea nitrogen, UA: uric acid, CRP: C-reactive protein, PCT: procalcitonin, BE base excess.

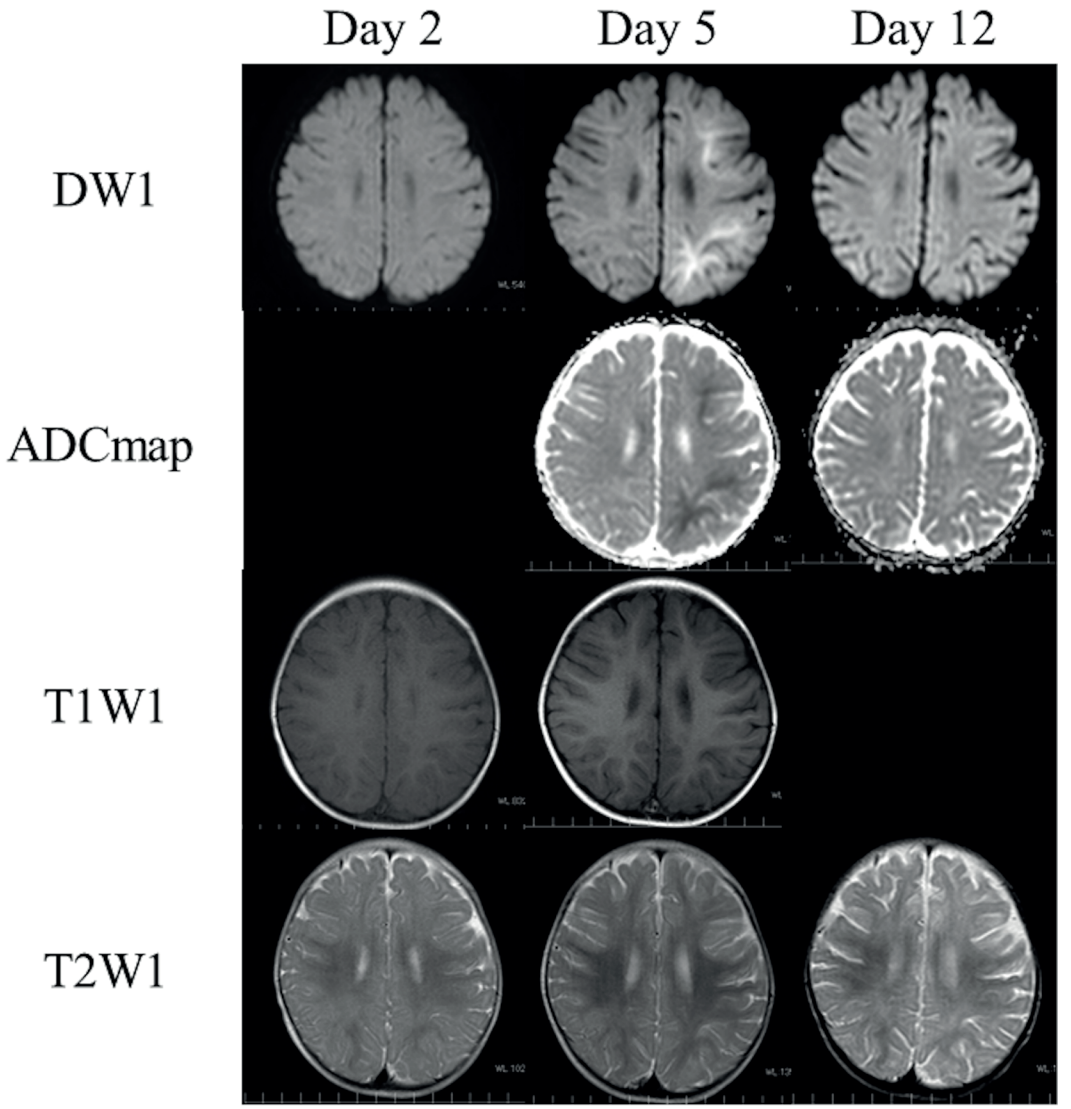


Fig. 1. Magnetic resonance images of the brain.

Left column: On the first day of febrile illness. Results of brain MRI were normal.

Middle column: On the fifth day. Brain MRI indicated left subcortical white matter of hemicerebrum lesions, which were most conspicuous with diffusion-weighted imaging (DWI).

Right column: On the twelfth day, the abnormal signal detected using the examination of brain MRI disappeared, and the MRI revealed mild left cerebral atrophy.

(EEG) performed during sleep demonstrated a left-right difference in the spindle waves and reduced activity in the left hemisphere (Fig. 2). He was diagnosed with AESD and treated with controlled normothermia for four days, high-dose methylprednisolone pulse therapy (30 mg/kg/day for three consecutive days), continuous midazolam infusion (0.5 mg/kg/h) alongside a 24-hour EEG for five days, and edaravone (1.0 mg/kg/dose twice for four days). On the tenth day, he had recurrent fever and was treated with a second course of immunoglobulin (2 g/kg). Subsequently, he had defervescence and periungual desquamation, and we confirmed the recovery of his consciousness. On the twelfth day, the abnormal signal on DWI had disappeared, and an MRI revealed mild left cerebral atrophy (Fig. 1. right column) and right

paresis. The EEG abnormality had improved. Echocardiography revealed a fusiform dilation (measuring 3.0 mm in diameter) of the right coronary artery. Thus, aspirin (3 mg/kg) was administered as an antiplatelet treatment for three months. At the third month, the fusiform dilation and right paresis improved. At the age of 11 months, although the mild left cerebral atrophy persisted, he could sit up on his own, pull himself up, and take his first steps, all of which are normal for his age according to the Denver Developmental Screening Test.

Discussion

Neurological involvements in KD have been infrequently reported. Transient unilateral facial nerve palsy, irritability, and lethargy are sometimes observed; however, encephalitis/encephalopathy is an extremely rare complication of KD.[5] Although clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) has been reported as a neurological complication,[6] there has been only one report of KD-related AESD.[7] Our patient had a typical clinical course, and MRI confirmed AESD, for which KD was the only likely etiology.

There have been several reports on the diffusion MRI abnormalities following status epilepticus.[4,7] Among them, there is an infantile subgroup that is characterized by a stereotypical clinical course and spatial distribution of the MRI lesions, designated as AESD. AESD is usually associated with infection, most often with that of the influenza virus, HHV 6, or HHV 7, and its incidence is high during infancy.[8,9] Rarely, AESD may occur even in cases of non-infectious illnesses, such as in traumatic brain injury.[10] The neurological outcomes of AESD vary from normal to mild or severe sequelae, including mental retardation, paralysis, and epilepsy.[4,8] Previously reported KD-related AESD involved severe neurological sequelae.[7]

The poor prognosis associated with the neurological outcomes of AESD is largely unknown, and an effective treatment for AESD has not been established. The effects of controlled normothermia, high-dose methylprednisolone pulse therapy, high-dose immunoglobulin therapy, continuous midazolam infusion alongside a 24-hour EEG, and edaravone administration were unclear in our patient. High-dose methylprednisolone pulse therapy is provided in acute encephalitis/

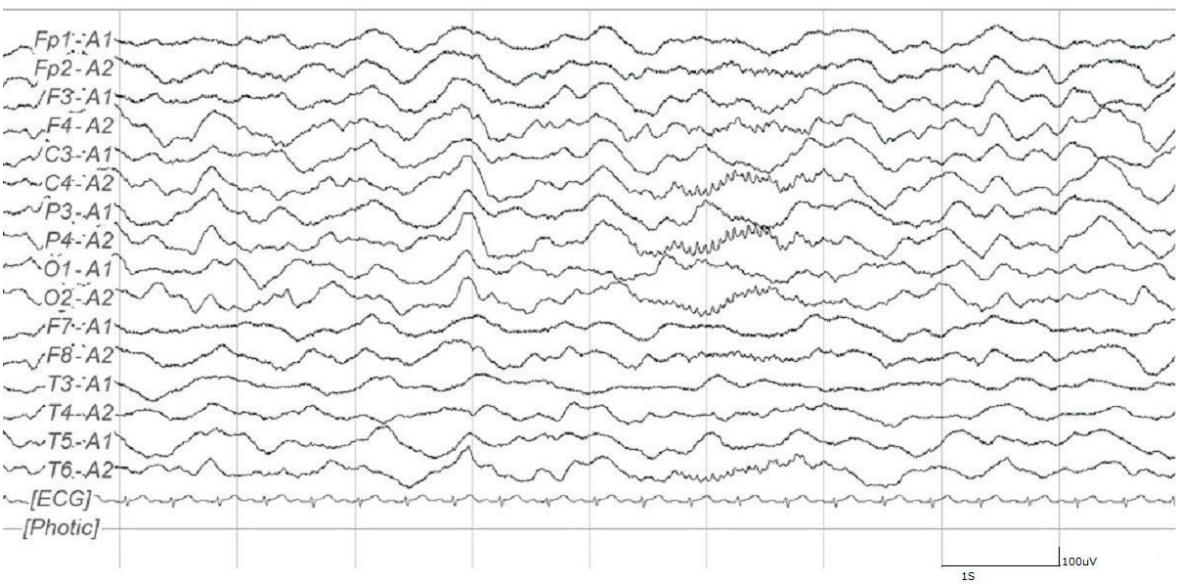


Fig. 2. Electroencephalography finding.
EEG during sleep demonstrates a left-right difference in the spindle waves and lazy activity in the left hemisphere.

encephalopathy (associated with influenza virus infection) and as the second line of treatment for KD. Controlled normothermia, continuous midazolam infusion alongside a 24-hour EEG, and edaravone administration are provided in acute encephalitis/encephalopathy (such as that associated with the influenza virus infection).[11] Edaravone is a free radical scavenger that interacts biochemically with a wide range of free radicals.[12] CSF-8-OHdG levels decreased after edaravone treatment, and this treatment is expected to be partially effective for AESD associated with HHV-6.[13] Since an established treatment for AESD is unavailable, we speculated that the above mentioned treatments may be effective for our patient.

The good neurological prognosis of our patient, as compared to that previously reported, was thought to be due to the unilateral nature of the lesion in the brain MRI. We must document more cases of KD-related AESD. We hope to develop a strategy for treatment and the analysis of neurological prognosis in such cases.

In conclusion, it is important that pediatricians acknowledge that encephalitis/encephalopathy, such as AESD and MERS, can occur in patients with KD, an acute febrile systemic vasculitis not directly associated with a pathogen. Further clinical, radiological, and immunological studies are necessary to clarify the frequency, mechanism, and prognosis of AESD-complicated KD.

REFERENCES

1. McCrindle BW, Rowley AH, Newburger JW, et al; American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Cardiovascular Surgery and Anesthesia; and Council on Epidemiology and Prevention. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American heart association. Circulation 2017; 135: e927-e999.
2. Yoshikawa H, Abe T. Febrile convulsion during the acute phase of Kawasaki disease. Pediatr Int 2004; 46: 31-32.
3. Tabarki B, Mahdhaoui A, Selmi H, Yacoub M, Essoussi AS. Kawasaki disease with predominant central nervous system involvement. Pediatr Neurol 2001; 25: 239-241.
4. Takanashi J, Oba H, Barkovich AJ, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006; 66: 1304-1309.
5. Terasawa K, Ichinose E, Matsuishi T, Kato H. Neurological complications in Kawasaki disease. Brain Dev 1983; 5: 371-374.
6. Takanashi JI, Shirai K, Sugawara Y, Okamoto Y, Obonai T, Terada H. Kawasaki disease complicated by mild encephalopathy with a reversible splenial lesion (MERS). J Neurol Sci 2012; 315: 167-169.
7. Shiba T, Hamahata K, Yoshida A. Acute encephalopathy with biphasic seizures and late reduced diffusion in Kawasaki disease. Pediatr Int 2017; 59: 1276-1278.
8. Takanashi J. Two newly proposed infectious encephalitis/encephalopathy syndromes. Brain Dev 2009; 31: 521-528.
9. Hoshino A, Saitoh M, Oka A, et al. Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. Brain Dev 2012; 34: 337-343.
10. Takase N, Igarashi N, Taneichi H, et al. Infantile traumatic brain injury with a biphasic clinical course and late reduced diffusion. J Neurol Sci 2018; 390: 63-66.
11. Morishima T, Okabe N, Namakura Y, et al. Guideline for influenza encephalopathy: revised edition. Jpn J Pediatr 2009; 62: 2483-2528 (in Japanese).
12. Itoh Satou T Nishida S, et al. Edaravone protects against apoptotic neuronal cell death and improves cerebral function after traumatic brain injury in rats. Neurochem Res 2010; 35: 348-355.
13. Tanuma N, Miyata R, Nakajima K, et al. Changes in cerebrospinal fluid biomarkers in human herpesvirus-6-associated acute encephalopathy/febrile seizures. Mediators Inflamm 2014; 1-8.

The Turkish Journal of Pediatrics 2020; 62: 320-325
https://doi.org/10.24953/turkjped.2020.02.021

Case Report

Acute necrotizing encephalopathy with organic psychosis: a pediatric case report

Leman Tekin Orgun[1], Ebru Arhan[1], Kürşad Aydın[1], Yasemin Taş Torun[2], Esra Güney[2], Ayşe Serdaroğlu[1]

Departments of [1]Pediatric Neurology and [2]Child and Adolescent Psychiatry, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Enteroviruses-associated acute necrotizing encephalopathy (ANE) of childhood is rarely reported in the literature. Clinico-radiological features of ANE are well-recognized and bilateral thalamic nuclei are frequently affected by ANE. Neuropsychiatric symptoms are rarely seen. Thalamic damage can cause psychosis.

Case. Herein, we present a pediatric case of enterovirus-associated ANE presenting with psychosis related to thalamus damage in whom a favorable response to treatment was achieved.

Conclusion. Thalamic damage occurs during the Enteroviruses-associated acute necrotizing encephalopathy and it can be related psychiatric sympthoms. Clinicians should be aware of uncommon presentations of ANE, and patients with thalamic damage should be followed for neuropsychiatric manifestations. Early recognition and appropriate treatment of ANE is important to obtain favorable outcomes.

Key words: childhood, encephalopathy, enterovirus, psychosis, thalamus damage.

Acute necrotizing encephalopathy (ANE) of childhood is a discrete form of acute encephalopathy induced by acute febrile diseases with a high morbidity and mortality rate, and predominantly affects infants and young children.[1] The main presenting symptoms of ANE include fever, vomiting, seizures, acute encephalopathy, and rapid alteration of consciousness after a non-specific viral illness.[2] Although the etiology and pathogenesis of ANE have not been explained clearly yet, it usually develops secondary to viral infections.[3] The influenza virus, parainfluenza virus, and human herpesvirus-6 (HHV-6) infections are the most common etiological agents, whereas enteroviruses are rarely reported to be the cause of ANE.[1]

✉ Leman Tekin Orgun
lemantekin15@yahoo.com

Received 18th February 2019, revised 6th April 2019, 14th May 2019, accepted 7th July 2019.

This case report was presented as a poster presentation at the 18th Annual Congres on Pediatric Neurology, 20-24 April 2016, Antalya, Turkey.

To date ANE has been recognized primarily as a clinic-radiological disorder the lesions involve multiple brain areas, including the brainstem, periventricular white matter, internal capsule, putamen, cerebellum and bilateral thalamic nuclei, which are known to be the most affected areas in ANE.[2-4] Thalamic damage may occur during various viral infections, vascular pathologies or tumoral infiltration and can cause perception of pain, movement disorders, insomnia and other sleep disorders, and psychosis.[5] However, neuropsychiatric symptoms during ANE are reported very rarely.[6]

In this report, we described a pediatric case of possible enterovirus-associated ANE presented with psychosis in whom a favorable response to immunotherapy was achieved.

Case Report

A 17-year-old, previously healthy male patient presented to our emergency department with a sudden-onset weakness of the left hand and

speech impairment. He was on paracetamol treatment for two days due to fever, rhinorrhea, and headache. His medical history was negative for recent travels and exposure to other drugs, and he was fully immunized. His family history revealed no neurological disorders. Physical examination findings were normal, except for pharyngeal hyperemia and fever. Neurological examination showed dysarthria, decreased muscle strength (4/5) on the left upper limb, and a Glasgow Coma Scale score of 13/15. He was hospitalized with the suspicion of encephalitis. He had a rapid deterioration of the muscle strength which decreased to 2/5 on the left upper limb and 4/5 on the right upper limb with a decreased level of consciousness and encephalopathy which developed within a few hours. He was taken to the intensive care unit, and treatment with oseltamivir, acyclovir, and intravenous immunoglobulin (IVIG) was initiated. Laboratory analysis showed normal blood counts, blood chemistry, electrolytes, thyroid hormone, thyroid antibodies, and vitamin B_{12} levels. Metabolic studies including blood pH, bicarbonate, lactate, ammonia, and tandem metabolic screening were normal. C-reactive protein level (CRP), erythrocyte sedimentation rate (ESR), and immunoglobulin profile, C3 and C4 levels, antinuclear antibody, and anti-DNA antibodies were also normal. Cerebrospinal fluid (CSF) examination showed normal glucose and protein levels without pleocytosis (protein: 30 mg/dL [reference:15-40 mg/dL], glucose: 60 mg/dL [reference: 40-70 mg/dL], and blood glucose level of 82 mg/dL). The CSF Gram staining and culture results were negative and the polymerase chain reaction (PCR) test of the CSF for herpes simplex virus-1 and -2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV), cytomegalovirus (CMV), non-polio enteroviruses and mycoplasma was negative. Electroencephalography showed diffuse slowing of 5-6 Hz theta wave without epileptiform activity. On brain magnetic resonance imaging (MRI), T2-weighted imaging (T2WI) revealed symmetric, heterogeneous hyperintense signal changes on the thalamic nuclei, and decreased signal intensities in the center which are surrounded by increased signal intensities on the anterior thalamus (Fig. 1). The susceptibility-weighted imaging (SWI) showed multiple petechial hemorrhagic areas in thalamus. However, MRI angiography and spinal cord MRI were normal. The diagnosis of ANE was established by clinical and radiological features. He was treated by immunotherapy (total 2 g/kg IVIG within five days and 1,000 mg/day intravenous [IV] pulse methylprednisolone). He showed a rapid recovery on the fourth day of treatment. His muscle strength became normal in all extremities, except for fine motor skills on his left hand. Throat swap PCR which was obtained on the first day of hospitalization was positive for enterovirus. PCR for influenza virus, parainfluenza virus, and HHV-6 were negative. On the fifth day of hospitalization, the patient developed nervousness, auditory and visual hallucinations, behavioral changes, intensive anxiety and treatment incompatibility. He was, then, evaluated by a child and adolescent psychiatrist and was diagnosed with psychosis (Brief Psychiatric Rating Scale [BPRS] score: 66) due to his overall condition, treatment with haloperidol 2 mg/day was initiated and the dose was titrated, if necessary.

On Day 17, he was discharged without any motor deficit and with mild psychiatric complaints (BPRS score: 12), with haloperidol 2 mg/day treatment. After five days of discharge, he was admitted to the emergency department due to nonsense speech, amnesia, severe agitation, and self-mutilation behaviors. Psychiatric consultation showed increased psychotic symptoms (BPRS score: 68), and dose of haloperidol was increased to 5 mg/day. On Day 22, follow-up MRI showed moderate regression in both thalamic lesions. In addition, psychiatric symptoms improved and dose of haloperidol treatment was reduced to 3 mg/day. On Day 8 of follow-up, he was discharged with significant clinical and psychiatric improvements (BPRS score: 8) and haloperidol 2 mg/day was prescribed. Haloperidol treatment was discontinued one month later. At

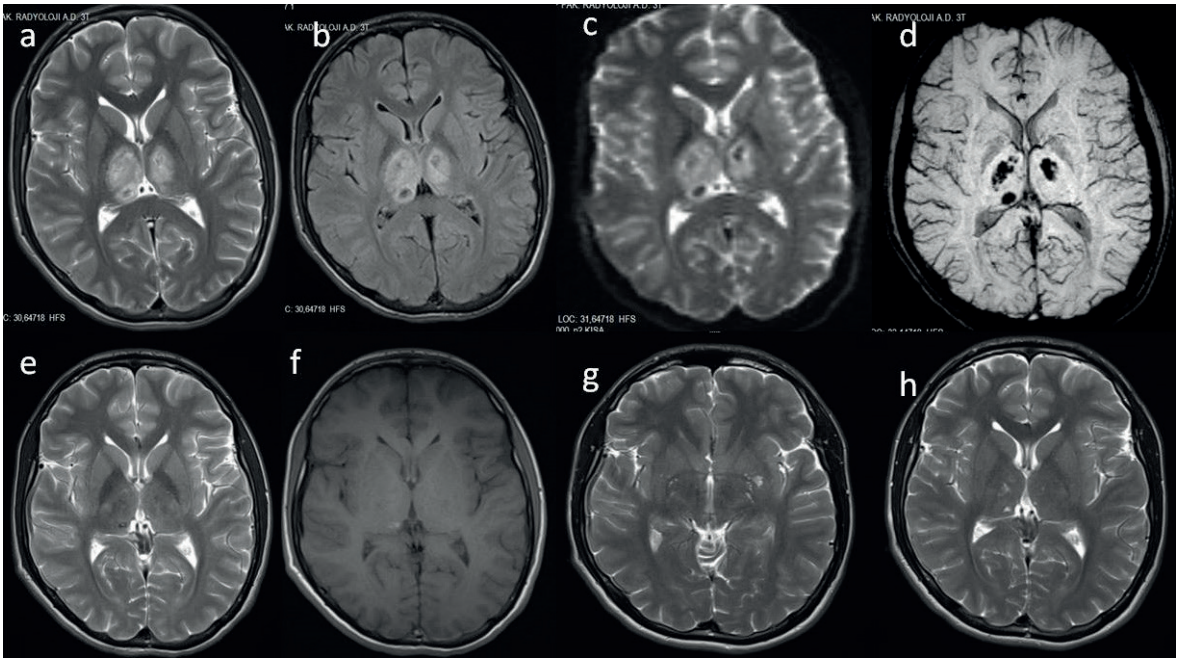


Fig. 1. Dynamic changes of magnetic resonance imaging (MRI) of the case . (a) and, (b) and (c) were, respectively, the T2-weighted image(T2WI), fluid attenuated inversion recovery(FLAIR), and diffusion-weighted imaging(DWI) at onset which revealed symmetric, heterogeneous hyperintense signal changes on the thalamic nuclei, and decreased signal intensities in the center which are surrounded by increased signal intensities on the anterior thalamus, and (d)susceptibility-weighted imaging(SWI) at onset which showed multiple petechial hemorrhagic areas in thalamus. (e), and (f) were, respectively, the T2WI, and T1WI imaging at 22th day, which revealed gradually regression of edema and mass effect in both thalamic lesions. (g) and (h) were T2WI imaging of fallow up at 18 months which showed mild sequelae and necrotic changes on bilateral thalamic nuclei.

two months, repeated MRI showed significant regression in both thalamic lesions with normal neurological and psychiatric examination findings. At 18 months, repeated MRI showed mild sequelae and necrotic changes on bilateral thalamic nuclei. At the final visit, his neurological and psychiatric examination findings were all normal. Informed consent was taken from patient and patient's parents.

Discussion

In this report, we described a pediatric case of possible enterovirus-associated ANE presenting with psychosis with a favorite response to immunotherapy. ANE frequently develops secondary to viral infections; influenza viruses, parainfluenza viruses, and HHV-6 infections are the most common etiological agents of ANE in children.[3] However, there are only few patients with ANE associated with enterovirus infection.[1]

Enterovirus is a well-known neurotropic virus which causes aseptic meningitis, encephalitis, brainstem encephalitis, and acute flaccid paralysis.[1,7] Although enterovirus-associated ANE may present with a wide range of clinical features, psychiatric symptoms have been rarely reported.[7] The clinical manifestations of encephalitis due to viral etiology are extremely variable and reflect the specific area and degree of brain involvement and inherent pathogenicity of the offending agent.[8] Enterovirus encephalitis (EVE) predominantly manifests as a generalized syndrome, with nonspecific alterations in consciousness ranging from lethargy and mild disorientation to coma. Clinical features of EVE are more commonly related to brainstem, frontal lobe, temporal lobe, and thalamus manifestations.[8] Other

viral agents involving thalamus are EBV, and influenza virus.[8] Among viral etiologic agents other than enterovirus, herpes simplex virus encephalitis (HSVE) presents itself with atypical manifestations in children. Besides, encephalitis associated with primary varicella Zoster Virus (VZV) infection may develop as a nonspecific syndrome or as acute or postinfectious CNS disease or with isolated symptoms such as acute cerebellar ataxia. In the absence of skin lesions, it is difficult to diagnose VZV encephalitis. In cases due to influenza-associated encephalitis, neurologic signs are observed several days after the onset of upper respiratory tract symptoms.[8]

Neuroradiological features of ANE include multifocal, symmetric brain lesions involving the thalami, brainstem, cerebral white matter, and cerebellum.[2] Bilateral thalamic nuclei are typically involved in all patients with ANE, which is known as a distinctive feature of ANE.[3,9] The pathophysiological changes in ANE include edema, petechial hemorrhage, and necrosis.[2] Generally in ANE, increased signal intensities are detected in the center of the lesions which are surrounded by the decreased signals on T1-weighted imaging (T1WI). T2-weighted imaging (T2WI) may reveal decreased signal intensities that are surrounded by increased or homogeneous increased signal intensities. The classical neuroimaging is "concentric/laminar structure" or "tricolor pattern" or target-like appearance.[3] In HSVE, neuroimaging studies with MRI reveal asymmetric hyperintense lesions on T2-weighted sequences at edematous areas in the mesiotemporal and orbitofrontal lobes and the insular cortex. Among early neuroradiologic menifestations, there is diffusion restriction on diffusion-weighted imaging (DWI) early in the course of HSVE.[10] It is reported that features of inflammation in EVE can be clearly determined particularly in the anterior horns of spinal cord, the dorsal pons, and the medulla on MRI.[11]

T1-weighted MRI of the present patient showed heterogeneous and decreased signal intensities, while T2WI revealed symmetric, heterogeneous, and hyperintense signal changes on the thalamic nuclei with decreased signal intensities in the center surrounded by increased signal intensities on the anterior thalamus. The SWI also showed multiple petechial hemorrhages, which were more prominent on the right thalamus.

The thalamus is a highly connected subcortical structure which integrates sensory and cortical information.[12] It is critical for increased awareness and cognition. Thalamic dysfunction may result from several viral infections or vascular pathologies, and can cause perception of pain, movement disorders, sleep disorders, and psychosis.[5] However, it is still unknown whether these findings present in different psychotic disorders and -regions, and how they relate to structural thalamic alterations.[12] The thalamocortical circuits are implicated in mood and psychotic disorders. Thalamo-cortical dysrhythmia (TCD) has attracted increasing attention as an underlying mechanism for psychiatric disorders. In TCD, persistent thalamic delta and/or theta range activity serves as the trigger for thalamo-cortical dysfunction. TCD has been proposed to be related to the dysfunction of low-voltage calcium channels.[13,14] Our case had psychotic symptoms including irritability, auditory and visual hallucinations, and intense agitation which might be compatible with the MRI findings with edema and multiple hemorrhagic areas in bilateral thalamic nuclei. We hypothesized that thalamic damage might lead to TCD and TCD possibly underpinned our patient's behavioral disturbances and personality changes.

The critical care follow-up and treatment are essential tools for management of all ANE patients as in other acute encephalopathies.[1] IVIG was recommended for the treatment of patients with encephalitis by the World Health Organization guide about hand-foot-mouth disease.[15] According to this guide, IVIG was recommended in treatment of patients with encephalitis. In the literature, treatment with antiviral drugs, methylprednisolone, dexamethasone, plasmapheresis, antithrombin III and therapeutic hypothermia were also tried

on ANE patients.[3,16] There is still a debate about the use of IV steroids in ANE. It is indicated in the literature that both steroid and IVIG may be given in ANE.[3,15,16] Most recent studies have suggested IV steroids for ANE.[1,17] The role of steroid therapy in ANE is controversial. Our patient was treated with supportive measures and IVIG in the acute phase. Steroid was also added after the exclusion of active viral infection with laboratory testing. Therefore, IVIG and pulse methyl prednisolone were administered simultaneously in our case. Although steroids may cause psychosis, psychiatric side effects with corticosteroids appear to be dose-dependent.[18] In the literature, it was reported that steroid-induced psychosis was more frequent in patients whom 40 mg/day prednisolone was administered, among women, and during the first two weeks of steroid treatment.[18]

Specific diagnosis of EV meningitis depends on EV isolation from CSF. The specificity of the method is only 65-75% due to low titers of EV serotypes in CSF.[19] EV isolation from oropharynx or feces is also valuable for diagnosis in patients with aseptic meningitis. After infection, EV may be present in oropharynx for one week, and in feces for weeks.[20] It is difficult to diagnose EV infection by using the immunoassay method, because there is no common antigen between different serotypes, and the virüs has low concentration in body fluids.[20] It is difficult to grow EV serotypes in cell culture. However, EV "reverse transcription PCR" is reported as a faster and sensitive diagnostic method in clinically critical time-frame. Therefore, PCR, with 94-100% diagnostic specificity, is the new gold standard for EV detection.[21] Moreover, presence of EV has been shown in healthy individuals during epidemics.[22] EV isolation from a source other than CSF or blood may indicate a previous infection. In the present case, it was concluded that ANE was due to EV because of the isolation of EV only from oropharyngeal swab.

The prognosis of ANE varies from complete recovery to death.[3] The mortality rate is about 30% and less than 10% of patients recover completely, although significant neurological problems are frequent in survivors.[2,23] In a large cohort, it was reported that prognosis of encephalitis in pediatric population was not promising; only one third of cases had incomplete recovery whereas 6.8% of patients died.[8] Prognosis depends mainly on the infectious agents. According to the literature, more than 35% of patients with HSVE recover with severe sequela or die. Early and late relapses were reported up to 26% of children who had HSV-1 encephalitis. In EBV encephalitis, persistent neurological deficits were observed less. In Japan and Taiwan, high mortality rates were reported in ANE associated with influenza infection.[8] Concrete data about the remaining viral etiologic agents is limited, because they were reported as case reports and small series.[8] In a large multicenter study conducted on Chinese children with viral encephalitis and meningitis, EVE is reported as the most common etiologic agent both in acute encephalitis and meningitis with the incidence of sequeala and fatality rate of 7.5% and 0.8%, respectively.[24] Our case recovered without any neurological sequelae. We, therefore, consider that early diagnosis and early steroid treatment played a critical role in our case in whom a favorable response was achieved.

In conclusion, early recognition and appropriate treatment after early diagnosis of ANE is of utmost important to obtain favorable outcomes. Although enterovirus is known as a rare pathogen for ANE, the laboratories should have tests for it in suspected cases. In addition, clinicians should be aware of this uncommon presentation of ANE, and patients with thalamic damage should be followed for neuropsychiatric manifestations.

REFERENCES

1. Tabarki B, Thabet F, Al Shafi S, Al Adwani N, Chehab M, Al Shahwan S. Acute necrotizing encephalopathy associated with enterovirus infection. Brain Dev 2013; 35: 454-457.
2. Mizuguchi M. Acute necrotizing encephalopathy of childhood: a novel form of acute encephalopathy prevalent in Japan and Taiwan. Brain Dev 1997; 19: 81-92.
3. Wu X, Wu W, Pan W, Wu L, Liu K, Zhang HL. Acute necrotizing encephalopathy: an underrecognized clinicoradiologic disorder. Mediators Inflamm 2015; 2015: 792578.
4. Voudris KA, Skaardoutsou A, Haronitis I, Vagiakou EA, Zeis PM. Brain MRI findings in influenza A-associated acute necrotizing encephalopathy of childhood. Eur J Paediatr Neurol 2001; 5: 199-202.
5. De Witte L, Brouns R, Kavadias D, Engelborghs S, De Deyn PP, Marien P. Cognitive, affective and behavioural disturbances following vascular thalamic lesions: a review. Cortex 2011; 47: 273-319.
6. Partlow GD, del Carpio-O'Donovan R, Melanson D, Peters TM. Bilateral thalamic glioma: review of eight cases with personality change and mental deterioration. AJNR Am J Neuroradiol 1992; 13: 1225-1230.
7. Okumura A, Mizuguchi M, Aiba H, Tanabe T, Tsuji T, Ohno A. Delirious behavior in children with acute necrotizing encephalopathy. Brain Dev 2009; 31: 594-599.
8. Glaser CA, Bloch KC. Encephalitis. In: Long SS, Prober CG, Fischer M (eds). Principles and Practice of Pediatric Infectious Diseases (5th ed). Philadelphia: Elsevier, 2018: 305-322.
9. Ravid S, Topper L, Eviatar L. Acute necrotizing encephalopathy presenting as a basal ganglia syndrome. J Child Neurol 2001; 16: 461-462.
10. Bradshaw MJ, Venkatesan A. Herpes simplex virus-1 encephalitis in adults: pathophysiology, diagnosis, and management. Neurotherapeutics 2016; 13: 493-508.
11. Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T. Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol 2010; 9: 1097-1105.
12. Fowlkes AL, Honarmand S, Glaser C, et al. Enterovirus-associated encephalitis in the California encephalitis project, 1998-2005. J Infect Dis 2008; 198: 1685-1691.
13. McGilchrist I, Goldstein LH, Jadresic D, Fenwick P. Thalamo-frontal psychosis. Br J Psychiatry 1993; 163: 113-115.
14. Schulman JJ, Cancro R, Lowe S, Lu F, Walton KD, Llinás RR. Imaging of thalamocortical dysrhythmia in neuropsychiatry. Front Hum Neurosci 2011; 5: 69.
15. Cardosa J, Farrar J, Zijian F, et al. A Guide to Clinical Management and Public Health Response for Hand, Foot and Mouth Disease (HFMD). Geneva, Switzerland: World Health Organization Western Pacific Region, 2011.
16. Yamamoto H, Okumura A, Natsume J, Kojima S, Mizuguchi M. A severity score for acute necrotizing encephalopathy. Brain Dev 2015; 37: 322-327.
17. Kim JH, Kim IO, Lim MK, et al. Acute necrotizing encephalopathy in Korean infants and children: imaging findings and diverse clinical outcome. Korean J Radiol 2004; 5: 171-177.
18. Skatun KC, Kaufmann T, Brandt CL, et al. Thalamo-cortical functional connectivity in schizophrenia and bipolar disorder. Brain Imaging Behav 2018; 12: 640-652.
19. Romero JR.Viral meningitis and the aseptic meningitis syndrome. In: Scheld WM, Whitley RJ, Marra CM, (eds). Infections of the Central Nervous System (4th ed). Philadelphia: Wolters Kluwer Health, 2014: 65-83.
20. Tunkel AR, Beek DV, Scheld WM. Acute meningitis. In: Bennett JE, Dolin R, Blaser MJ (eds). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (8th ed). Vol.1. Philadelphia: Churchill Livingstone Elsevier, 2015: 1097-1137.
21. Robinson CC, Willis M, Meagher A, Gieseker KE, Rotbart H, Glodé MP. Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. Pediatr Infect Dis J 2002; 21: 283-286.
22. Connoly KJ, Hammer SM. The acute aseptic meningitis syndrome. Infect Dis Clin North Am 1990; 4: 599-622.
23. Okumura A, Mizuguchi M, Aiba H, Tanabe T, Tsuji T, Ohno A. Delirious behavior in children with acute necrotizing encephalopathy. Brain Dev 2009; 31: 594-599.
24. Ai J, Xie Z, Liu G, et al. Etiology and prognosis of acute viral encephalitis and meningitis in Chinese children: a multicentre prospective study. BMC Infect Dis 2017; 17: 494.

The Turkish Journal of Pediatrics 2020; 62: 326-331
https://doi.org/10.24953/turkjped.2020.02.022

Case Report

A rare case of syndromic severe congenital neutropenia: JAGN1 mutation

Funda Erol Çipe[1], Çiğdem Aydoğmuş[1], Kübra Baskın[1], Gonca Keskindemirci[1,2], Wojciech Garncarz[3], Kaan Boztuğ[3]

[1]Department of Pediatric Allergy and Immunology, Kanuni Sultan Suleyman Training and Research Hospital, İstanbul; [2]Department of Social Pediatrics, İstanbul University Institute of Child Health, İstanbul, Turkey; [3]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, and Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria.

ABSTRACT

Background. Neutrophils are essential innate cells to fight bacterial and fungal pathogens. Jagunal homolog 1 (JAGN1) mutations were recently defined as rare genetic defects causing severe congenital neutropenia. JAGN1 participates in the secretory pathway and is required for granulocyte colony-stimulating factor receptor-mediated signalling. This gene is required for normal ultrastructure and granulation of endoplasmic reticulum of myeloid progenitor cells. Its defect is related to increased predisposition to apoptosis. In the literature, a few cases have been reported with congenital anomalies such as cardiac and renal anomalies.

Case. Here we report a patient in which JAGN1 deficiency was found after several years. Apart from syndromic facial appearance we were unable to detect any other systemic malformations.

Conclusion. The causes of multisystemic features of mutations in JAGN1 gene remain unknown. JAGN1 mutations must be considered in patients with severe congenital neutropenia especially with facial dismorphism even in the absence of systemic manifestations.

Key words: severe congenital neutropenia, JAGN1 mutation.

Severe congenital neutropenia is characterized by susceptibility to recurrent life threatening bacterial infections due to maturation arrest of neutrophils. Different studies have shown mutations in ELA 2, HAX 1, G6PC3, WAS, GF11 and VPS45 genes.[1-7] In 2014, Boztug et al.[8] described mutations in Jagunal homolog 1 (JAGN1) gene that play a role in neutrophils differentiation and maintenance. JAGN1 is an endoplasmic reticulum (ER) resident protein responsible for normal ultrastructure of the granules in neutrophils, and also contributes to N-glycosylation of multiple proteins. Mutations of this gene cause absent granules in the neutrophils and increased apoptosis of the neutrophils.[8] Here we report a patient with severe congenital neutropenia that showed homozygous JAGN1 mutation.

Case Report

Our patient is a 10-year-old male born to first cousin parents. He was the first child of the family and was born 1900 grams at 37 weeks of gestation. He was admitted due to neonatal sepsis for a month on the fourth day after birth. He was re-hospitalized because of ulcers and abscesses on the bilateral lower extremities which did not regress with antibiotics at 6 months of age. After these 2 episodes of hospitalizations, severe neutropenia was noticed on the complete blood count analysis.

✉ Funda Erol Çipe
ferol76tr@hotmail.com

Received 13th December 2017, revised 8th January 2018, 2nd March 2018, 24th May 2018, accepted 30th November 2019.

This study was presented at the 4th The Friends of Children Congress, İstanbul, Turkey, 24-26th March 2016.

In initial laboratory tests leukocyte count was 5120/mm^3, while absolute neutrophil count (ANC) was 100/mm^3, hemoglobin was 10.9 g/dl and thrombocyte count was 357000/mm^3. Deep neutropenia was seen in repeated complete blood counts (ANC: 0-200/mm^3). Bone marrow aspiration revealed maturation arrest of the neutrophils. Granulocyte – Colony Stimulating Factor (G-CSF) (5 μg/kg) treatment was started with the presumed diagnosis of Kostmann syndrome. All ANC were 0-200 / mm^3 without G-CSF. After G-CSF, ANCs increased up to just maximum 700 / mm^3. Physical examination was normal except triangular face and ears. His growth and mental development were within normal percentiles. Cardiac and abdominal investigations did not show any accompanying congenital anomalies.

He was treated for recurrent skin ulcers and abscesses until 1.5 years of age. He had recurrent infections including pneumonia, otitis media, sinusitis and skin abscess. At 3 years of age, he was hospitalized due to severe pneumonia and cavernous lesions were seen on thorax computed tomography. As anti-bacterial treatments did not cause any regression, anti-fungal and anti- tuberculosis treatments were given empirically. None of the fungal agents or *M. Tuberculosis* bacillus were revealed by culture. After 6 months of treatment, cavernous lesions regressed and treatment was stopped.

Serum levels of immunoglobulins and lymphocyte subtypes were within normal ranges. Because he had complaints that were suggestive of asthma and allergic conjunctivitis, skin prick test was performed and positive result was found against house dust mites. A summary of laboratory evaluation is shown in Table I.

In the investigation of genetic causes of severe congenital neutropenia, mutation analysis for ELENA, HAX1, G6PC3 and GCSF receptor mutations were found to be negative in 2012. After identification of JAGN1 deficiency in 2014, the relevant gene was sequenced and a homozygous missense mutation was detected in exon 2 of JAGN1 gene (c 130 c>T , p. His 44 Tyr) (Fig. 1).

Table I. Laboratory evaluation of the patient.

	Patient's result	Normal values
Hemoglobin	10.9 g/dl	12-14 g/dl
Leukocytes	5120 / mm^3	4000-15000 / mm^3
Absolute neutrophil count (ANC)	0-200 / mm^3	1500-6000 / mm^3
Absolute lymphocyte count (ALC)	4200 / mm^3	1500-4000 / mm^3
Absolute eosinophil count (AEC)	500-1800 / mm^3	0-500 / mm^3
Absolute monocyte count (AMC)	300-4000 / mm^3	100-1000 / mm^3
Plateletes	357000/ mm^3	150000-450000 / mm^3
IgG level	898 mg/dl	842-1943 mg/dl
IgA level	67.2 mg/dl	62-390 mg/dl
IgM level	97 mg/dl	54-392 mg/dl
IgE level	17.6 KU/L	<161.3 KU/L
Lymphocyte subtypes (%)	CD3: 72,	55 – 78
	CD4: 38,	27 – 53
	CD8: 18,	19 – 34
	CD16+56: 12,	4 – 26
	CD19: 22,	10 – 31
	CD20:24,	10 – 30
	HLA-DR: 18	2-12
Genetic analysis	JAGN1 gene (c 130 c>T, p. His 44 Tyr	

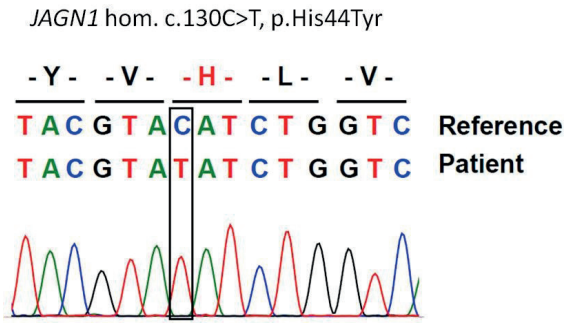


Fig. 1. Mutation analysis of the patient by sanger sequencing.

The patient is still being followed in our immunology department and receiving high doses of G-CSF (10 μg /kg). He requires hospitalization 2-3 times every year due to pneumonias and higher doses of G-CSF during infections, nevertheless, neutrophil counts have not increased adequately. Matched unrelated donor screening continues for bone marrow transplantation because of a lack of a family matched donor.

Informed consent was received from the parents before preperation of manuscript.

Discussion

In an animal study Wirnsberger et al.[9], showed, that JAGN1 deficient mice do not show neutropenia, they are characterized by increased susceptibility to fungal infections due to defective killing capacity of neutrophil granulocytes. In this report, we describe a patient with JAGN 1 mutation with different features which is not defined in the original report[8] and the report of Baris et al.[10] The original report including 14 patients described recurrent respiratory tract infections, sepsis, skin abscess and pancolitis. Multisystemic manifestations such as short stature, convulsion, bone abnormalities (hip dysplasia, amelogenesis imperfecta, osteoporosis, scoliosis), pyloric stenosis, pancreatic insufficiency and coarctation of aorta were also reported in this cohort.[8] Differently, our patient did not show any multisystem abnormalities except facial dysmorphism. Baris et al.[10] described urogenital abnormalities, short stature, learning disorders, hypothyroidism and hypogammaglobulinemia. In our patient no immunologic abnormalities were seen besides neutropenia. Now his main symptoms include cough, rhinorrhea and conjunctivitis, after recovering from serious infections which were frequent during his younger childhood years. They were considered due to a house dust mite allergy. An allergic condition has not been reported before in these patients. We think that it may be seen co-incidentally and needs further investigation.

Like the other patients in the original cohort, our patient did not respond to high dose G-CSF treatment.[8] Interestingly, in the mice study, the authors stated that JAGN1 knock out mice's neutrophils showed increased killing capacity with GM-CSF.[9] We could not try this type of CSF in our patient during his infections.

Homozygous missense mutation in the exon 2 detected in our patient was the same as the Turkish patients in the study of Baris et al.[10] and the original cohort.[8] In the series containing 14 patients, there were 2 Turkish patients carrying the same mutation as our patient. Skull bone thickness due to extramedullary hematopoesis were reported as a different clinical finding. We think that this mutation may be a common mutation in the Turkish population and may be used to screen for the etiology of severe congenital neutropenia in Turkish patients. Because of lack of hypogammaglobulinemia and multisystemic manifestations in our patient as in previous reported Turkish patients, we think that this type of mutation does not cause the same phenotype in all patients. The cause of multisystemic features of mutations in JAGN 1 gene remains unknown. All cases presented previously are summarized in Table II.

In conclusion, 3 patients were reported with the same JAGN 1 mutations and all were Turkish worldwide, all of them manifested with multisystemic congenital anomalies and neutropenia. We suggest that JAGN 1 gene mutation must be considered in patients with

Table II. The summary of all reported patients.[8,10]

	Gender	Country	Mutation	Beginning symptoms	Extrahematopoetic manifastations	Treatment and Clinical status
Patient 1	F	Algeria	c.3G>A; p.Met1Ile	ENT infections, aphtosis, perianal cellulitis, skin abscesses	None	Alive without treatment
Patient 2	F	Algeria	c.3G>A; p.Met1Ile	ENT infections,	Short stature (height of 1.46 m)	Alive without treatment
Patient 3	M	Algeria	c.3G>A; p.Met1Ile	Aphtosis, skin abscesses, balanitis, pneumonitis, lung abscess, osteitis perianal cellulitis	Pyloric stenosis	Alive without treatment
Patient 4	F	Algeria	c.3G>A; p.Met1Ile	Otitis, paraodontopathy	Scoliosis, dental malformations	Alive without treatment
Patient 5	M	Algeria	c.3G>A; p.Met1Ile	ENT infections, aphtosis, skin abscesses, pneumonitis, lung abscess, perianal cellulitis	None	Alive without treatment
Patient 6	F	Iran	c.59G>A; p.Arg20Glu	Upper respiratory tract infections, pneumonia, skin abscesses	Febrile convulsion, focal epilepsy	Alive without treatment
Patient 7	M	Turkey	c.130C>T; p.His44Tyr	Upper respiratory tract infections, pneumonia, skin and perianal abscesses, sepsis (Haemophilus influenza)	Extramedullary hematopoiesis with thickening of skull bones	Alive without treatment
Patient 8	F	Turkey	c.130C>T; p.His44Tyr	Upper respiratory tract infections, skin abscess	Bilateral hip dysplasia, extramedullary hematopoiesis with thickening of skull bones	Alive without treatment
Patient 9	F	Iran	c.40G>A; p.Gly14Ser	Skin abscesses, onycholysis	None	Alive without treatment
Patient 10	M	Israel	c.297C>G; p.Tyr99	Aspergillosis (none after HSCT)	Severe osteoporosis and repeated bone fractures (continuing after HSCT)	Alive with HSCT
Patient 11	F	Morocco	c.485A>G; p.Gln162Arg	Skin abscesses, omphalitis, pancolitis	Lipomatosis, pancreatic insufficiency, bone abnormalities, dental malformations	Died at age 5 years owing to pancolitis and septicemia
Patient 12	F	Albenia	c.63G>T; p.Glu21Asp	Upper respiratory tract infections, pneumonia, skin abscess	Short stature (5 cm below third percentile), amelogenesis imperfecta, neurodevelopmental delay	Alive without treatment

Table II. Continue.

	Gender	Country	Mutation	Beginning symptoms	Extrahematopoetic manifastations	Treatment and Clinical status
Patient 13	F	Pakistan	c.485A>G; p.Gln162Arg	ENT infections, upper respiratory tract infections, pneumonia, sepsis (Escherichia coli)	Failure to thrive (height 5 cm below third percentile, weight 3.8 kg below third percentile), coarctation of the aorta, mild developmental delay	Alive, awaiting HSCT
Patient 14	F	Germany	c.35_43del CCGACGGCA; p.Thr12_ Gly14del	Pneumonia (none after HSCT), bronchiectasis	None	Alive with HSCT
Patient 15	M	Turkey	c.130C>T, p. His44Tyr	Gluteal abscess, cervical lymphadenopathies, pneumonia, bronchiectasis, diarrhea, otitis and gingivitis	Failure to thrive, dysmorphic face, hypothyroidism, hypospadias and left undescended testis, hypogammaglobulinemia	Alive without treatment
Patient 16	F	Turkey	c.130C>T, p. His44Tyr	Recurrent skin abscesses, otitis and pneumonia	Learning disability, for triangular face, amelogenesis imperfecta, gingival hypertrophy and short stature, hypogammaglobulinemia	Alive without treatment
Our Patient	M	Turkey	c.130C>T, p. His44Tyr	Neonatal sepsis, ulcers and abscesses on lower extremities, recurrent pneumonia, otitis media and sinusitis	Triangular face, extrovert ears, allergic rhinoconjuntivitis with sensitization against house dust mites	Alive without treatment

ENT: Ear nose throat, HSCT: Hemopoetic stem cell transplantation.

severe congenital neutropenia especially those with facial dysmorphism even in the absence of multisystemic manifestations like our patient.

Acknowledgement

All Turkish authors are primary clinicians of the patient and wrote the manuscript. The authors from Austria performed genetic analysis of the patient. We thank all contributors and the patient's family as well as the patient.

REFERENCES

1. Klein C. Genetic defects in severe congenital neutropenia: emerging insights into life and death of human neutrophil granulocytes. Annu Rev Immunol 2011; 29: 399-413.
2. Grenda DS, Murakami M, Ghatax J, et al. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. Blood 2007; 110: 4179-4187.
3. Klein C, Grudzien M, Appaswamy G, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet 2007; 39: 86-92.
4. Boztug K, Appaswamy G, Ashikov A, et al. A novel syndrome with congenital neutropenia caused by mutations in G6PC3. N Engl J Med 2009; 360: 32-43.
5. Devriendt K, Kim AS, Mathijs G, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet 2007; 27: 313-317.
6. Person RE, Li FQ, Duan Z, et al. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA_2. Nat Genet 2003; 34: 308-312.
7. Stepensky P, Saada A, Cowan M, et al. The Thr224Asn mutation in the VPS45 gene is associated with congenital neutropenia and primary myelofibrosis of infancy. Blood 2013; 121: 5078-5087.
8. Boztug K, Järvinen PM, Salzer E, et al. JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nat Genet 2014; 46: 1021-1027.
9. Wirnsberger G, Zwolanek F, Stadlmann J, et al. Jagunal homolog 1 is a critical regular of neutrophil function in fungal host defense. Nat Genet 2014; 46: 1028-1033.
10. Baris S, Karakoc-Aydıner E, Ozen A, et al. JAGN1 deficient severe congenital neutropenia: two cases from the same family. J Clin Immunol 2015; 35: 339-343.

The Turkish Journal of Pediatrics 2020; 62: 332-337
https://doi.org/10.24953/turkjped.2020.02.023

Case Report

Maternal and fetal tuberous sclerosis complex: a case report questioning clinical approach

Özge Sürmeli Onay[1], Adviye Çakıl Sağlık[1], Pelin Köşger[2], Zeynep Nurhan Saraçoğlu[3], Uğur Toprak[4], Birsen Uçar[2], Ayşe Neslihan Tekin[1]

Divisions of [1]Neonatology and [2]Pediatric Cardiology, Department of Pediatrics, Departments of [3]Dermatology and [4]Radiology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey.

ABSTRACT

Background. Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disease with multisystem involvement.

Case. Here, a mother and infant couple was presented with maternal and fetal TSC including demonstrative clinical findings and genetic analysis. The interesting point of this case report is that maternal and fetal TSC was identified after the mother gave birth to a child with a cardiac rhabdomyoma. The genetic analysis revealed a novel mutation which was the same in both the mother and her infant.

Conclusion. We would like to bring to the attention of clinicians this entity and to emphasize that maternal and fetal TSC can adversely affect maternal and fetal health, and deserves close follow up. Our recommendation is that if cardiac rhabdomyoma/cortical tuber/renal angiomyolipoma are present in prenatal ultrasonography, the parents should be evaluated for TSC.

Key words: maternal, fetal, tuberous sclerosis complex, rhabdomyoma, newborn.

Tuberous sclerosis complex (TSC, OMIM #191100, #613254) is an autosomal dominant disease characterized by benign tumor growth in many organs including the brain, heart, skin, eyes, kidneys, and lungs which presents with seizures, developmental delay, behavioral problems, skin abnormalities, and kidney disease.[1] According to global statistics 1/6,000-10,000 people are born with TSC.[2] Since molecular testing has become widely performed, the genetic examination of TSC was included in the Tuberous Sclerosis Complex Diagnostic Criteria Update: Recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference.[3] To date, many case reports about TSC have been published but reports dealing with maternal and fetal TSC are limited.[4-7] The interesting point of this case report is that maternal and fetal TSC was identified after the mother gave birth to a child with cardiac rhabdomyoma. Also, the genetic analysis revealed a novel mutation which was the same in both the mother and her infant. We would like to bring to the attention of clinicians this entity and to emphasize that maternal and fetal TSC can adversely affect maternal and fetal health.

Case Report

A female infant was born to a 28-year-old mother by emergency cesarean section due to severe preeclampsia at 25[6] weeks of gestation with a birth weight of 860 g (50 percentile). The mother suffered from chronic hypertension and polycystic kidney disease. Her first pregnancy ended at 25[4] weeks of gestation because of severe preeclampsia and fetal

✉ Özge Sürmeli Onay
ozgeonay79@gmail.com

Received 7th March 2019, revised 6th May 2019, accepted 21st June 2019.

This case report was presented as poster presentation in UNEKO 27, 3-7 April 2019, Antalya, Turkey.

demise. There was no consanguinity between the parents. Apgar scores were 4/7/8 at 1th, 5th and 10th minutes, respectively. The initial physical examination revealed respiratory distress. Surfactant replacement therapy was required and antibiotics (ampicillin, amicasin) were initiated. There was no cardiac murmur, hypotension or no necessity of inotropic support at follow up. At postnatal 48 hours, routinely performed transthoracic echocardiogram revealed multiple nonobstructive homogenous echogenic masses in interventricular septum and the papillary muscles of both ventricles protruding to the ventricular cavities and in the posterior wall of the left ventricle invading toward posterior atrioventricular groove consistent with rhabdomyomas (Fig. 1). Isolated premature supraventricular beats up to 2000 per day were observed during clinical follow-up. Cardiac rhabdomyomas in our patient were nonobstructive, hemodynamically not significant and did not cause any drug-resistant arrhythmia, so no medical or surgical treatment was initiated. We followed her on cardiac monitorization and spontaneous regresssion was detected on recurrent echocardiographic examinations. The diameters of the rhabdomyomas decreased 50 percent in eight months. The dermatological examination with Wood lamp, transfontanelle and abdominal ultrasound revealed no abnormalities associated with TSC. The infant remained hemodynamically stable throughout the hospitalisation, she was extubated at postnatal 9th day, required no O_2 support at postnatal 71th day and discharged at postnatal 85th day.

To evaluate maternal and fetal TSC, the mother of the infant was examined extensively. Maternal physical examination revealed facial angiofibromas on bilateral malar eminences and nasolabial folds, ungual fibromas, shagreen patch and hypomelanotic macule (Fig. 2) (Written informed consent was obtained from the mother). Cortical tubers and subependymal nodules were clearly identified on the cranial magnetic resonance images (MRI) (Fig. 3).

Although clinical diagnostic criteria for TSC was met in the mother, genetic testing was performed and the diagnosis was confirmed. The results of TSC 1 gene of the mother and infant revealed a heterozygous deletion mutation in TSC1 gene: NM-000368.4, c.2252-2253delAG (p.Lys751Serfs*2). To our knowledge, this mutation associated with TSC has not been reported previously.

Discussion

Tuberous sclerosis complex is an autosomal dominant neurocutaneous disease with

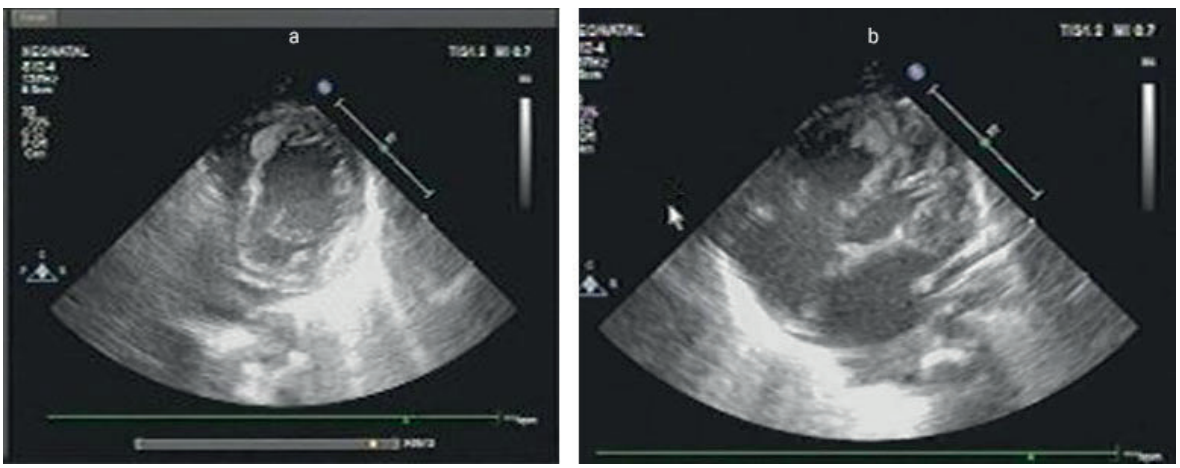


Fig. 1. Transthoracic echocardiography: (A) parasternal short axis view showing the mass inserted on the right side of the interventricular septum; and (B) apical four-chamber view of the mass inserted on the moderator band.

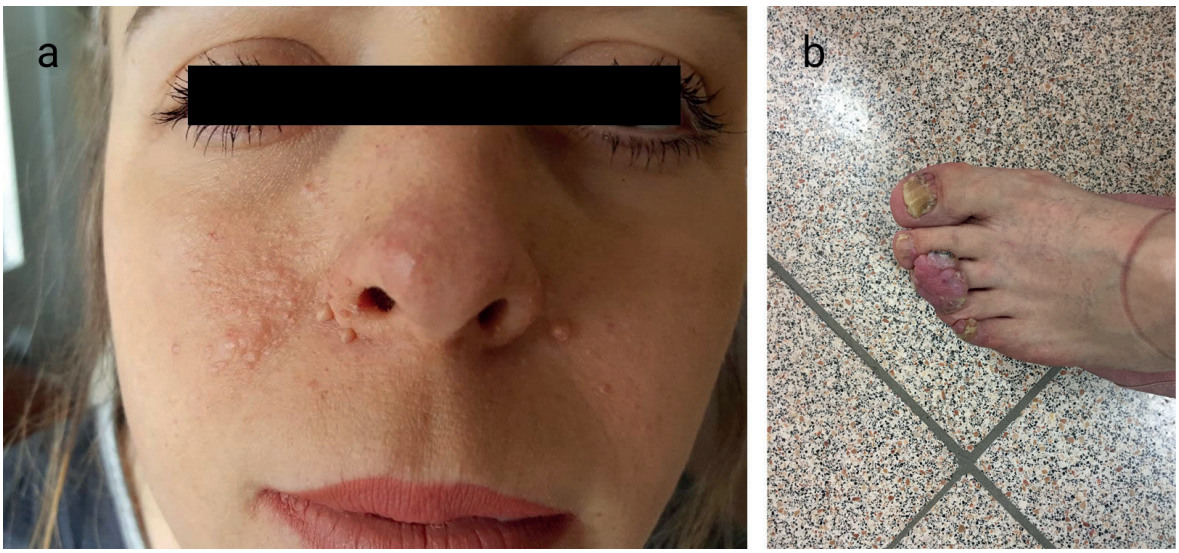


Fig. 2. (A).Facial angiofibromas on bilateral malar eminences and nasolabial folds (B). Subungual fibromas in the left foot.

multisystem involvement. Two tumor suppressor genes associated with TSC were demonstrated as follows; TSC1 is situated in region q34 of chromosome 9 and encodes the protein Hamartin, and TSC2 is situated in region p13 of chromosome 16 and encodes the protein Tuberin.[8] In the absence of clinical manifestations, the identification of a pathogenic mutation of TSC1 or TSC2 is adequate for the diagnosis of TSC.[3] Tuberous sclerosis can come to exist by genetic transition from one parent with TSC or can result from a de novo genetic mutation. The possibility of inheritance for a child, if one of the parents has TSC is 50%.[5] Among TSC patients, pathogenic TSC2 gene mutations were reported in two-thirds of all while pathogenic variants in TSC1 were determined in the remaining one-third.[8] In addition, there are TSC patients who have no mutation identified or have a variant mutation with unknown significance. Similarly, our cases have a new heterozygous deletion mutation which has not been reported to date. There has been no genotype–phenotype correlations including specific pathogenic variants of TSC1 reported yet. However, the presentation of TSC can be different among the family members with the same mutation because of the wide intrafamilial clinical variability.[9]

Cardiac rhabdomyoma is the most common cardiac tumor in infancy and childhood. Approximately 40% of patients with cardiac rhabdomyomas are diagnosed with TSC. Cardiac rhabdomyomas are present in 50–70% of patients with TSC.[4] Renal manifestations of TSC commonly includes renal angiomyolipoma (AML) and renal cysts, rarely polycystic kidney disease or renal cell carcinoma.[10] The prevalence of renal AML among TSC patients is 34 to 80%. The clinical presentation of renal AML can be as variable as palpable mass, flank pain, urinary tract infections, spontaneous hemorrhage, arterial hypertension or kidney failure.[11] However, the increase in circulating blood volume and renal blood flow during pregnancy, makes renal AML grow faster and leads to life threatening complications like rupture and retroperitoneal hemorrhage.[12]

This entity can adversely affect fetal and maternal morbidity and mortality. The complications of pregnant patients with TSC are preeclampsia, intrauterine growth retardation, preterm labor, preterm premature rupture of membrane, oligohydramnios, polyhydramnios, hydrops, abruption, hemorrhage from rupture of renal tumor, renal failure and fetal demise.[6,7] In the literature, there are limited reports and

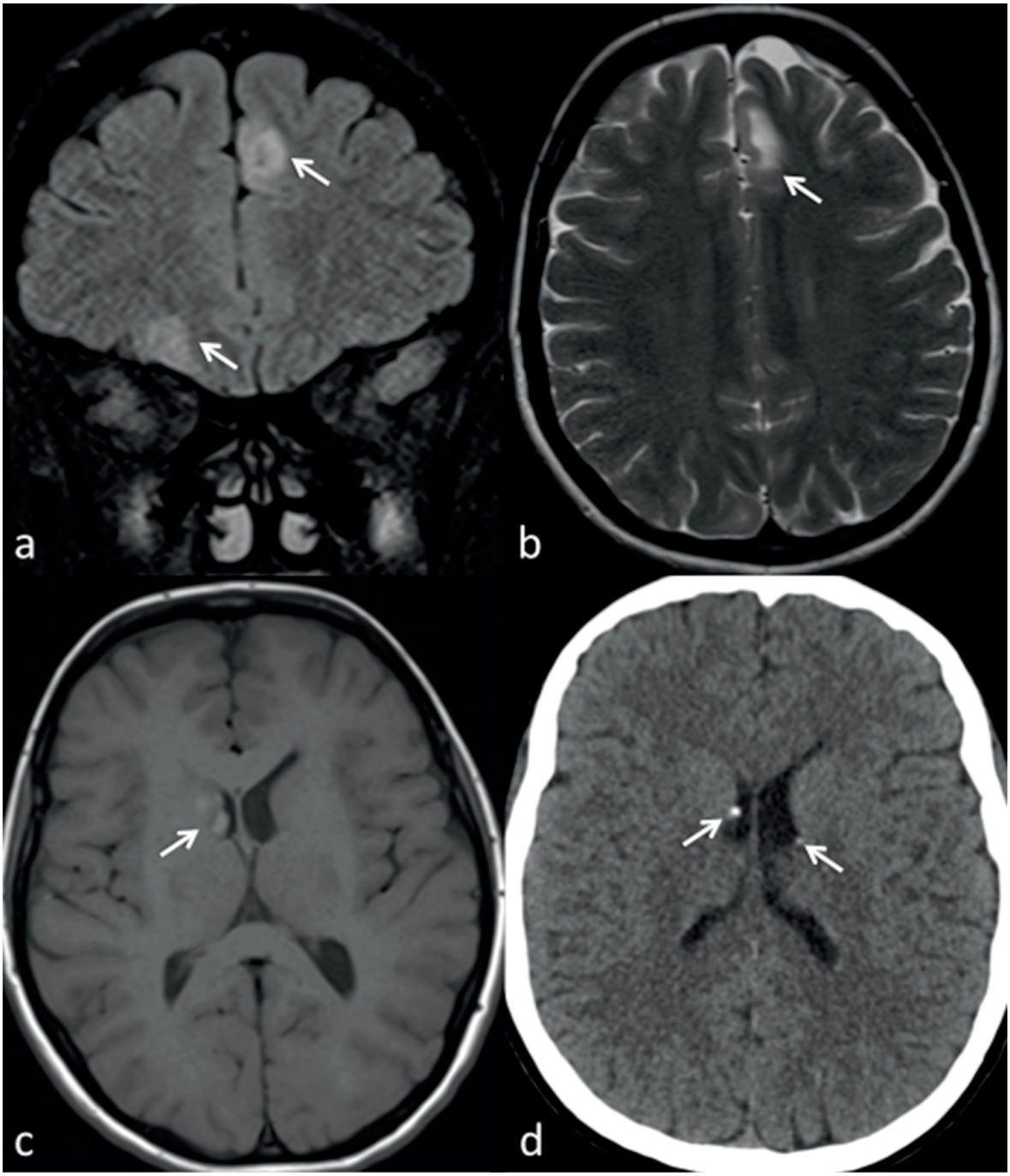


Fig. 3. (a) T2-weighted coronal FLAIR and (b) T2W axial MRI sequences demonstrate hyperintense cortical tubers (arrows) (c) T1W axial MRI image shows the subcortical, hyperintense subependymal nodule adjacent to the foramen Monro (arrow) (d) Axial computed tomography section shows subependymal calcified nodules (arrows).

inadequate awareness on maternal and fetal TSC. King et al.[7] reported a pregnant patient with TSC who experienced the complications as preeclampsia, preterm labor, and fetal demise. Cardiac rhabdomyoma, intracranial tubers and hydrops fetalis were detected in her fetus. Similarly, kidney disease and chronic hypertension were the components of TSC

in the mother of our case. So her pregnancy was complicated with preeclampsia, preterm labor and previous fetal demise. In another case report, cardiac rhabdomyomas were determined in both mother and fetus. Also, the left radical nephrectomy of the mother after delivery revealed renal AML and her infant died due to cardiac failure after delivery.[4] Beyond these reports, there is a report presenting no intrapartum or peripartum complications in a couple of mother and infant having maternal and fetal TSC.[6] However, Sharma et al.[5] focused their attention to maternal and fetal TSC by questioning if obstetricians know enough about maternal and fetal TSC. The authors presented a pregnancy which ended with fetal demise due to fetal cardiac rhabdomyoma and emphasized genetic counseling for couples having a family history of TSC. Our experience taught us that early diagnosis of the mother can prevent complications of TSC and give a chance for genetic counselling before bearing a child.

With the advances in perinatal medicine, cardiac rhabdomyoma, cerebral lesions and renal AML can be identified in fetal ultrasonography or MRI. In the presence of these findings in prenatal ultrasonography, obstetricians should be aware of maternal and fetal TSC and carry out investigations for TSC. Maternal and fetal TSC deserves close follow up; if maternal TSC is known, the fetus should be evaluated with fetal Doppler echocardiography and if possible an MRI including brain and renal parenchyma.[5] Reversely, our recommendation is that if cardiac rhabdomyoma/cortical tuber/renal AML are present in prenatal ultrasonography, the parents should be evaluated for TSC. Genetic counseling is necessary for parents with a family history of TSC because today, prenatal/preimplantation diagnosis is possible. We believe that increasing awareness of maternal and fetal TSC will decrease the morbidity and mortality and increase the quality of life for patients with TSC.

In conclusion, it is noteworthy that the physical examination findings of the mother were overlooked despite many medical examinations for hypertension and pregnancy up until the age of 28 years. The delayed diagnosis of the mother shows the importance of performing a complete physical examination whatever the patient's complaint.

Acknowledgements

We thank to Associate Professor Kürşat Bora Çarman for his suggestions.

REFERENCES

1. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. N Engl J Med 2006; 355: 1345-1356.
2. Hallett L, Foster T, Liu Z, Blieden M, Valentim J. Burden of disease and unmet needs in tuberous sclerosis complex with neurological manifestations: systematic review. Curr Med Res Opin 2011; 27: 1571-1583.
3. Northrup H, Krueger DA; International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: Recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatr Neurol 2013; 49: 243-254.
4. Zungsontiporn N, Tantrachoti P, Puwanant S.Foetal and maternal cardiac rhabdomyomas associated with tuberous sclerosis. Eur Heart J 2016; 37: 2036.
5. Sharma N, Sharma S, Thiek JL, Ahanthem SS, Kalita A, Lynser D. Maternal and fetal tuberous sclerosis: do we know enough as an obstetrician? J Reprod Infertil 2017; 18: 257-260.
6. Gupta N, Singh N, Sarangi S, Dalmia S, Mittal S. Fetal cardiac rhabdomyoma with maternal tuberous sclerosis complicating pregnancy. Arch Gynecol Obstet 2008; 278: 169-170.
7. King JA, Stamilio DM. Maternal and fetal tuberous sclerosis complicating pregnancy: a case report and overview of the literature. Am J Perinatol 2005; 22: 103-108.
8. Peron A, Au KS, Northrup H. Genetics, genomics, and genotype-phenotype correlations of TSC: insights for clinical practice. Am J Med Genet C Semin Med Genet 2018; 178: 281-290.
9. Yang G, Shi ZN, Meng Y, et al. Phenotypic and genotypic characterization of Chinese children diagnosed with tuberous sclerosis complex. Clin Genet 2017; 91: 764-768.

10. Koo KC, Kim WT, Ham WS, Lee JS, Ju HJ, Choi YD. Trends of presentation and clinical outcome of treated renal angiomyolipoma. Yonsei Med J 2010; 51: 728-734.

11. Bissler JJ, Kingswood JC. Renal angiomyolipomata. Kidney Int 2004; 66: 924-934.

12. Zapardiel I, Delafuente-Valero J, Bajo-Arenas JM. Renal angiomyolipoma during pregnancy: review of the literature. Gynecol Obstet Invest 2011; 72: 217-219.

The Turkish Journal of Pediatrics 2020; 62: 338-342
https://doi.org/10.24953/turkjped.2020.02.024

Case Report

Persistence of right umbilical vein: a singular case

Giovanni Pinna[1,*], Maria Pia De Carolis[1,*], Antonio Lanzone[1], Gelsomina Del Sordo[1], Angela Botta[2], Sara De Carolis[2]

Departments of [2]Obstetrics, Gynaecology and [1]Pediatrics, Fondazione Policlinico Gemelli IRCCS, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy.

ABSTRACT

Background. Persistent right umbilical vein (PRUV) is one of the most common prenatally detected venous anomaly. In the intrahepatic variant (I-PRUV) the right umbilical vein fuses with right portal vein and through the ductus venous drains into inferior vena cava, while in the uncommon extrahepatic variant (E-PRUV), the vein bypasses the liver completely. E-PRUV has a worse prognosis compared to I-PRUV, due either to severe hemodynamic effects or to the frequent association with other severe fetal malformations.

Case. Here we report a case of E-PRUV with good outcome. Prenatal fetal ultrasonography (US) performed at 33 weeks of gestation in 28-year old woman, highlights the presence of E-PRUV with right UV draining in inferior vena cava. In the male neonate born at 35 weeks of gestation by C-section, the Apgar Score was $9^{5'}$- 10^{10} and no other associated malformations and hemodynamic decompensation were found. Postnatal abdominal US showed the presence of enlarged paraumbilical veins.

Conclusions. The association of E-PRUV draining into the inferior vena cava with shunt through paraumbilical veins, could have preserved offspring by severe cardiac overload, positively affecting prognosis.

Key words: persistent right umbilical vein, fetus, neonate.

Persistent right umbilical vein (PRUV) is one of the most common prenatally detected venous anomaly, with an estimated wide range prevalence between 1 in 250 and 1 in 1250.[1] In a recent retrospective review of 20,452 fetuses of consecutive pregnancies PRUV was identified in 23 cases, yielding an incidence of 1 in 889 total births (0.11%).[3]

Two main presentations are described: Intra-hepatic PRUV (I-PRUV) and Extra-hepatic PRUV (E-PRUV). The former is the most prevalent, since it is reported in about 90-95% of cases.[2,3] It may coexist with the left umbilical vein (UV) as an intrahepatic supernumerary structure or may present as unique vein joining the portal system at the level of the sinus venosus and giving rise to the ductus venosus (DV). It is associated with normal DV development and its prognosis is good.[4]

E-PRUV is characterized by the persistence of right umbilical vein bypassing the liver and draining directly into right atrium or in intracardiac portion of inferior vena cava (IVC), or into iliac veins, subsequently these porto-systemic anastomosis can cause cardiac overload and consequently fetal congestive heart failure. E-PRUV has a worse prognosis compared to I-PRUV due either to severe hemodynamic effects or to the frequent association with DV agenesis and other severe fetal malformations.[2]

We report a singular case of E-PRUV without DV and with the presence of enlarged paraumbilical veins (PUVs), with good prognosis.

Case Report

A 24-years-old Chinese woman, gravida 1, para 0, was referred to our hospital at 33 weeks of

✉ Sara De Carolis
sara.decarolis@unicatt.it

*Giovanni Pinna and Maria Pia De Carolis are co-authors.

Received 24th October 2018, revised 26th January 2019, accepted 8th April 2019.

gestation for vaginal bleeding due to central placenta praevia. The patients underwent prenatal diagnosis by amniocentesis that excluded chromosomal abnormalities.

Fetal Echocardiography, performed at admission in our hospital, showed moderate cardiomegaly with cardio-toracic circumference ratio equal to 0.62 (normal value < 0.50) and overload of right-sided heart, associated with ectasia of IVC and undetectable DV. For this reason, the scanning of the venous system, including the imaging of target vessels with two-dimensional colour Doppler mapping, was performed and showed that the UV ran parallel to the stomach and its intrahepatic course resulted medial with respect to the gallbladder. E-PRUV draining directly into IVC was diagnosed, due to this sonographic configuration and the initial signs of cardiac overload (Fig. 1).

Fetal ultrasonography (US) weekly performed confirmed this diagnosis, ruling out heart failure. At 36 weeks of gestation C-section was necessary due to further vaginal bleeding. At birth the neonate had a normal heart rate and the Apgar Score was of $9^{5'}$, $10^{10'}$. The male neonate (weight 2500 g, cranic circumference 32.5 cm, length 48 cm) was admitted to *Neonatal Intensive Care Unit*, and heart rate, O_2 Saturation, blood pressure monitoring was started.

Echocardiographic evaluation, performed at one hour of life, showed normal cardiac structure and normal functional parameters. However, a mild dilatation of the right sections associated with moderate right ventricle hypertrophy and isosystemic pulmonary pressure were observed.

On the 2nd day of life (DOL), the abdominal and cerebral US did not show any coexisting abnormalities, while Doppler US highlighted

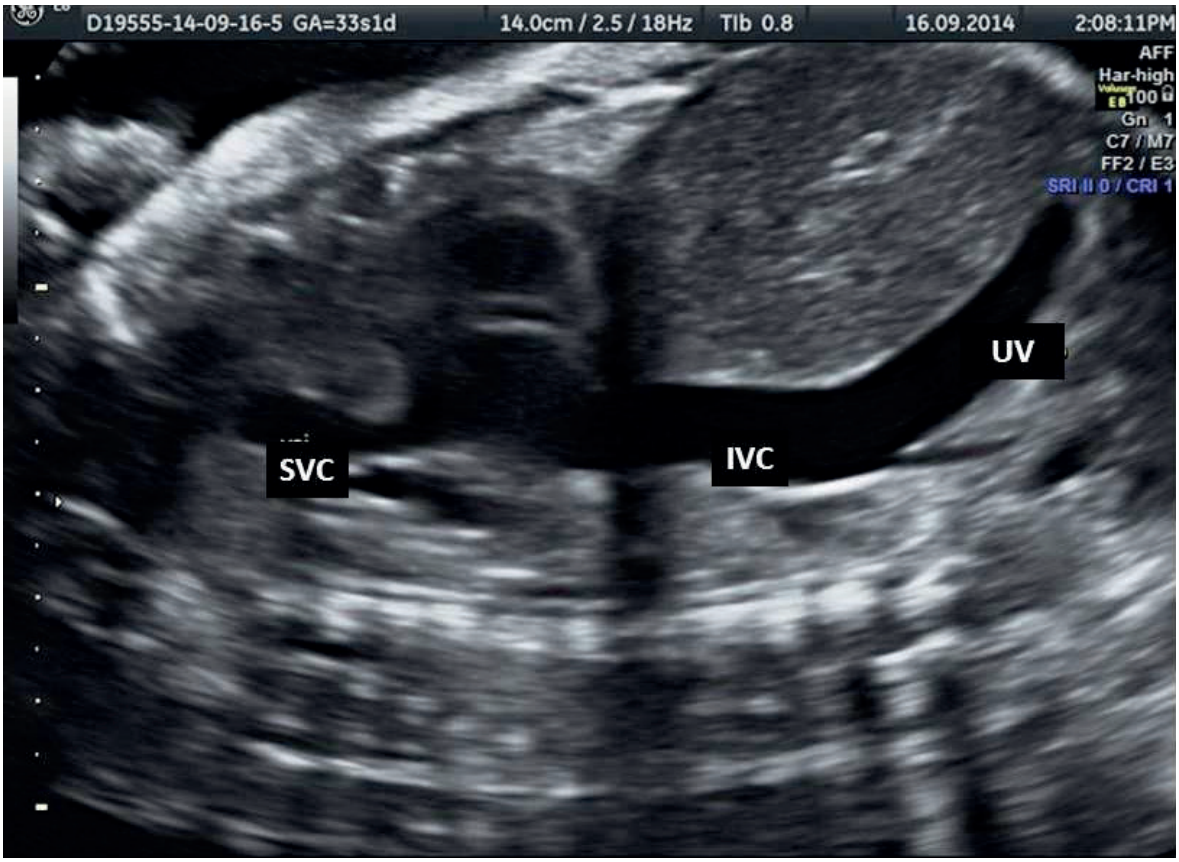


Fig. 1. Prenatal ultrasound examination shows the right umbilical vein (UV) draining directly in enlarged inferior vena cava (IVC).

the portal vein joining to a patent venous paraumbilical circulation (Fig. 2). On the third DOL, an additional Echocardiographic evaluation confirmed the mild dilatation of the right-side sections while the pulmonary pressure was physiologically decreased. No indicative findings of liver dysfunction were observed, therefore on the sixth DOL the neonate was discharged.

At one month of life, the communication between portal vein and venous paraumbilical circulation was still present, but it was not detectable at 3 months of life. During this follow-up period no emerging clinical complications were detected.

Consent written informed consent was obtained from the patient's legal guardians for publication of this case report and any accompanying images.

Discussion

The incidence of umbilical vessels abnormalities is quite rare, but, in the last years, with the introduction of color Doppler and 3D scans, the diagnosis *in utero* of these conditions became more feasible and so more frequent. Anomalies of the umbilical and portal veins constitute the largest group of congenital venous anomalies detected *in utero*. They include three main entities: 1. agenesis of the DV with extrahepatic umbilico-systemic shunt or with intrahepatic umbilico-hepatic shunt; 2. PRUV with or without intact DV; 3. UV varix.[5] Among these, PRUV is the most frequently detected fetal venous system anomaly. In normal conditions, by the 4th week of pregnancy the right UV begins to obliterate and on the 7th week of gestation the process is completed. Failure of right UV regression results in the PRUV anomaly.

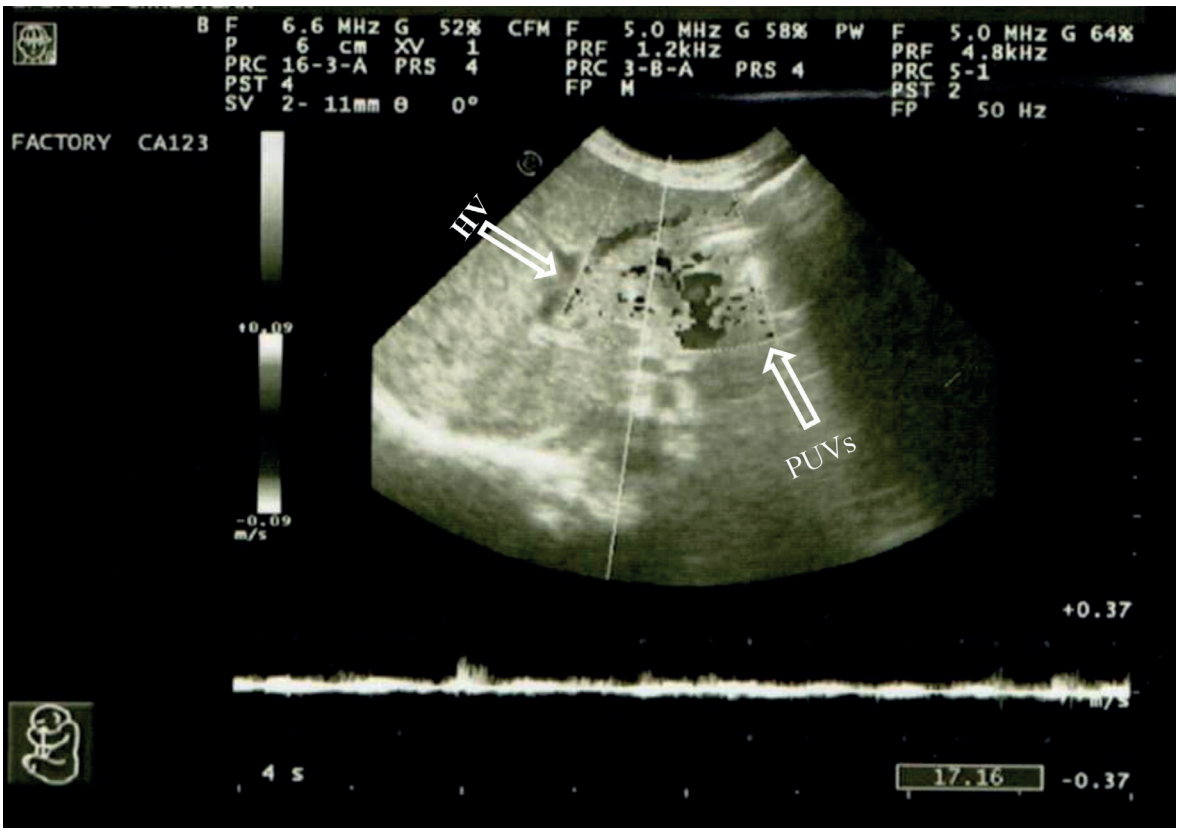


Fig. 2. Postnatal Doppler ultrasonography examination showing paraumbilical veins (PUVs) and hepatic vein (HV).

The underlying pathogenetic mechanism causing PRUV is not completely clear.[2] Teratogenic agents, such as retinoic acid or deficient folate[6], as well as early obstruction of the left UV, caused by external pressure or thrombus occlusion, induced the PRUV anomaly.[1] In fact, it has been suggested that primary or secondary occlusion by thromboembolic events, arising from the placenta, may lead to early streaming of blood through the right UV, therefore causing this anomaly.[7]

In our case, the images of prenatal and postnatal Doppler US suggest a possible role of an obstruction of umbilical vein flow at the level of the umbilical-portal junction, which is the area of "critical anastomosis" between left UV and omphalomesenteric veins.[8] In prenatal Doppler US both left UV and DV were not detected. Since DV is the structure connecting left UV to VCI, an occlusion of left UV can reduce or interrupt the flow in DV, therefore DV can result undetectable. On the other hand, the postnatal US showed the presence of dilated paraumbilical veins (PUVs). The PUVs are thick-walled with a similar structure to the umbilical vein. Together they constitute an accessory portal system which is confined between the layers of the falciform ligament and is in communication with the veins of the ventral abdominal wall. The constituents form an ascending series, namely Burow's veins, and Sappey's inferior and superior veins. The main channel of Sappey's inferior veins may be the remnant of the right umbilical vein since it communicates with the vessels of right rectus sheath and often communicates directly with the portal system within the right lobe of the liver. This channel communicates with the portal system in a variable manner: a) with the extra-hepatic part of the portal system at, or near, the recessus umbilicalis, b) directly with the intra-hepatic part by a branch to the free margin of the quadrate lobe, so forming an accessory portal system.[9]

Occlusion of left UV, due to thromboembolic event or to failure to delineate vascular connections[10] before the right UV vanished, can determine a persistence of right UV, but also leads to shunting of umbilical blood through other vessels such as PUVs.

In our case the aforementioned pathogenetic hypothesis is also supported by normal karyotype and by the absence of other anomalies. In E-PRUV anomaly it is reported a frequent association with other congenital anomalies and chromosomal abnormality. It is generally recommended during prenatal US scan to evaluate the presence of other congenital abnormalities, such as genitourinary, gastrointestinal, cardiac and skeletal malformations.[11,12]

In our case, PRUV diagnosis was made by US and color Doppler according to criteria described by Jeanty[7]: the UV ran right laterally respect to the gallbladder with an aberrant course toward the stomach (instead of being roughly parallel), and UV was not connected to the left portal vein. The extrahepatic variant was suspected in prenatal life because of the presence of PRUV draining directly into the IVC.

In previous reports, a high incidence of congestive heart failure, due to increased hemodynamic burden is clearly demonstrated, in case of extrahepatic drainage. In fact, the blood from the umbilical vein bypass the hepatic venous system flowing directly into the IVC and determining an overload of right-sided heart and heart failure.[13]

As underlined by Martinez[1] and Hajdu[14], E-PRUV requires prenatal careful monitoring to determine the timing of delivery in order to prevent congestive heart failure and hydrops. In our case, the diagnosis of E-PRUV occurred at 33 weeks of gestation, later than usual.[1] After this time the fetus was weekly monitored to evaluate the impact of this condition on the hemodynamic system.

However, we did not observe a hemodynamic impairment during the time the mother was hospitalized probably due to the short interval between diagnosis and delivery. The C-section

performed only two weeks after diagnosis could have influenced the hemodynamic outcomes, as well as the type of collateral channels opened. The presence of enlarged PUVs in postnatal Doppler US study can reflect the shunt by right UV through PUVs to the portal system and hence into the hepatic sinusoid. This umbilicoportal-hepatic shunt decreases the cardiovascular overload determined by the direct connection of E-PRUV with IVC.

In conclusion, we report for the first time the association of E-PRUV with enlarged PUVs without severe hemodynamic effects, showing the possible association of E-PRUV with the intra-hepatic shunt.

In our opinion, in case of E-PRUV, it is important to define how blood drainage is established. The visualization of the connection of right UV to the systemic circulation as well as the presence of associated anomalies are the most important prognostic factors. In case of E-PRUV draining directly into the IVC or in the right atrium, there is an elevated risk of cardiac overload and hemodynamic stresses. Instead, when E-PRUV is associated with intrahepatic shunt, this risk could be attenuated. The accurate evaluation of this complex venous system during fetal life is necessary, even if difficult, to provide adequate antenatal counseling, timing of delivery and appropriate neonatal care.

Acknowledgements

We would like to thank the guardians for giving permission to submit this case report, which will undoubtedly develop our understanding about this rare condition.

REFERENCES

1. Martinez R, Gamez F, Bravo C, et al. Perinatal outcome after ultrasound prenatal diagnosis of persistent right umbilical vein. Eur J Obstet Gynecol Reprod Biol 2013; 168: 36-39.
2. Weichert J, Hartge D, Germer U, Axt-Fliedner R, Gembruch U. Persistent right umbilical vein: a prenatal condition worth mentioning? Ultrasound Obstet Gynecol 2011; 37: 543-548.
3. KumarSV, Chandra V, Balakrishnan B, Batra M, Kuriakose R, Kannoly G. A retrospective single centre review of the incidence and prognostic significance of persistent foetal right umbilical vein. J Obstet Gynaecol 2016; 36: 1050-1055.
4. Lide B, Lindsley W, Foster MJ, Hale R, Haeri S. Intrahepatic persistent right umbilical vein and associated outcomes: a systematic review of the literature. J Ultrasound Med 2016; 35: 1-5.
5. Yagel S, Kivilevitch Z, Cohen SM, et al. The fetal venous system, Part II: ultrasound evaluation of the fetus with congenital venous system malformation or developing circulatory compromise. Ultrasound Obstet Gynecol 2010; 36: 93-111.
6. Monie IW, Nelson MM, Evans HM. Persistent right umbilical vein as a result of vitamin deficiency during gestation. Circ Res 1957; 5: 187-190.
7. Jeanty P. Persistent right umbilical vein: an ominous prenatal finding? Radiology 1990; 177: 735-738.
8. White JJ, Brenner H, Avery ME. Umbilical vein collateral circulation: the caput medusae in a newborn infant. Pediatrics 1969; 43: 391-395.
9. Martin BF, Tudor RG. The umbilical and paraumbilical veins of man. J Anat 1980; 130(Pt 2): 305-322.
10. Jaiman S, Nalluri HB. Abnormal continuation of umbilical vein into extra-hepatic portal vein: Report of three cases. Congenit Anom (Kyoto) 2013; 53: 170-175.
11. Hoehn T, Lueder M, Schmidt KG, Schaper J, Mayatepek E. Persistent right umbilical vein associated with complex congenital cardiac malformation. Am J Perinatol 2006; 23: 181-182.
12. Blazer S, Zimmer EZ, Bronshtein M. Persistent intrahepatic right umbilical vein in the fetus: a benign anatomic variant. Obstet Gynecol 2000; 95: 433-436.
13. Bell AD, Gerlis LM, Variend S. Persistent right umbilical vein case report and review of literature. Int J Cardiol 1986; 10: 167-175.
14. Hajdú J, Marton T, Kozsurek M, et al. Prenatal diagnosis of abnormal course of umbilical vein and absent ductus venosus--report of three cases. Fetal Diagn Ther 2008; 23: 136-139.

The Turkish Journal of Pediatrics 2020; 62: 343-346
https://doi.org/10.24953/turkjped.2020.02.025

Case Report

Pediatric case of persistent hiccups associated with hypertrophic olivary degeneration

Pınar Arıcan[1], Özgür Öztekin[2], Dilek Çavuşoğlu[3], Sema Bozkaya Yılmaz[1], Atilla Ersen[1], Nihal Olgaç Dündar[4], Pınar Gençpınar[4]

Departments of [1]Pediatric Neurology and [2]Radiology, İzmir Tepecik Education and Research Hospital, İzmir; [3]Department of Pediatric Neurology, Afyon Kocatepe University, Afyon; [4]Department of Pediatric Neurology, İzmir Katip Çelebi University, İzmir, Turkey.

ABSTRACT

Background. Hypertrophic olivary degeneration (HOD) is a rare degenerative disorder that is thought to occur subsequent to a disruption of the dentate-rubro-olivary pathway.

Case. We report a pediatric case of unilateral HOD presented with persistent hiccups and palatal tremor. Radiological examination of diaphragm was normal considering ultrasound and chest x-ray. On T2WI (weighted images) and Fluid Attenuated Inversion Recovery (FLAIR) images, hyperintense enlargement of the right inferior olivary nucleus was seen. No abnormal enhancement was detected on post-contrast scans and no evidence of restricted diffusion was seen. Susceptibility weighted imaging (SWI) sequences revealed a chronic hemorrhage involving the medulla oblongata and cerebellum. Cranial magnetic resonance imaging (MRI) findings were consistent with unilateral HOD. Palatal tremor and dentate-rubral tremor are frequent presentation of HOD, however to our knowledge persistent hiccups had not yet been reported in children with HOD.

Conclusion. We highlight a pediatric case of unilateral HOD, which presented with persistent hiccups. Awareness of clinical and radiological findings of HOD is important to avoid misinterpretation as a mass lesion, an ischemic event, or a demyelinating disease and provide adequate management.

Key words: children, hiccups, hypertrophic olivary degeneration.

Hypertrophic olivary degeneration (HOD) is a transsynaptic degeneration, occurring subsequent to a lesion involving dentato-rubro-olivary pathway, also called the Guillain-Mollaret triangle.[1] Any lesions involved in any components of this pathway from dentate nucleus to inferior olivary nucleus can lead to the loss of afferent signals into inferior olivary nucleus and cause HOD.[2]

The clinical manifestations of HOD are variable. Palatal tremor, dentate-rubral tremor, ocular myoclonus and cerebellar symptoms including ataxia are classically associated clinical findings of HOD.[3] Patients also may represent no clinical manifestations associated with HOD and diagnosis of HOD can be made with routine surveillance magnetic resonance imaging (MRI).[4]

Rhythmic involuntary jerking movements are frequent presentation of HOD, however persistent hiccups had not yet been reported in children with HOD. We report a pediatric case of persistent hiccups associated with unilateral HOD.

Case Report

A 17-year-old male patient admitted with complaints of persistent hiccups over a period of 5 days. Two years ago, he presented with postural tremor and was diagnosed with

✉ Pınar Gençpınar
pinargencpinar@yahoo.com.tr

Received 19th November 2018, revised 28th December 2018, accepted 31st January 2019.

unilateral HOD. He did not develop any additional clinical features of HOD such as ocular myoclonus or ataxia within two years. He was the fourth child of consanguineous parents with no relevant medical familial history. He was born full term after a normal pregnancy. His history revealed that he had another episode of persistent hiccups for 3 days two months ago. On physical examination, his weight, height, and head circumference were normal for his age. There were no organomegaly or dysmorphic features. Neurologic examination was normal, except for postural tremor and palatal tremor.

Hemoglobin level, serum electrolytes, renal function, liver function and thyroid function tests were normal. Radiological examination of diaphragm was normal considering ultrasound and chest x-ray. Right inferior olivary nucleus was enlarged and hyperintense on both T2WI (weighted images) and Fluid Attenuated Inversion Recovery (FLAIR) images, no enhancement detected after contrast administration. No evidence of restricted diffusion was seen. Susceptibility weighted imaging (SWI) sequences revealed a chronic hemorrhage involving the medulla oblongata and cerebellum. Cranial MRI findings were consistent with unilateral HOD (Fig 1). Hematologic and coagulation parameters were normal. Magnetic resonance angiography demonstrated no evidence of vascular malformation.

For further genetic investigation of possible metabolic disease, TruSight Inherited Disease Sequencing Panel (Illumina Inc.) was used. This gene panel covered 552 genes including POLG and SURF1 genes associated with HOD. Next generation sequencing analysis of TruSight Inherited Disease Sequencing Panel did not reveal a pathogenic mutation associated with the patient's phenotype. Serum ceruloplasmin level was 292 mg/L (200-600mg/L). Serum autoantibodies including antinuclear antibody, anti-double stranded DNA, HLA-B51 for systemic vasculitis were negative. A written informed consent was obtained from the parents of the patient.

Discussion

We identified a pediatric case of unilateral HOD present with persistent hiccups and palatal tremor. Hypertrophic olivary degeneration can be associated with synchronous movements of the larynx, pharynx, diaphragm, and facial muscles.[5] Palatal tremor and dentato-rubral tremor are the most specific rhythmic involuntary movements attributable to HOD. The exact mechanism underlying the synchronous movements remains unclear but the accepted hypothesis involves the loss of GABAergic inhibitory cerebellar afferents of the dentate-olivary tract. Olivary neurons are believed to have intrinsic oscillator property and decreased GABAergic descending inhibition leads to hypersynchronous discharge.[6,7]

Histologically, there is vacuolar cytoplasmic degeneration and an increase in the number of astrocytes resulting in hypertrophy of the affected inferior olivary nucleus neurons which most frequently develops weeks or months secondary to disruption of the dentate-rubro-olivary tract. Which is followed by subsequent atrophy and gliosis within 3-4 years.[3,8]

MRI findings of HOD is classical and problem solving, in which inferior olivary nucleus has both an increased signal intensity in T2WI and FLAIR images and hypertrophic. The absence of contrast enhancement is important as it differentiates HOD from malignant tumors and inflammation and the absence of restricted diffusion differentiates from ischemic event.[9]

The most common causes of HOD are hemorrhage, tumor, trauma and vascular malformations. Hypertrophic olivary degeneration has been also reported in metabolic, infectious, neurodegenerative disease and metronidazole intoxication. Prognosis depends on the underlying etiology.[10-12] In one in every five patients with HOD no cause can be identified.[9] Resection of posterior cranial fossa tumors and cavernous angioma leading to hemorrhage which subsequently developed HOD have been reported.[6,8] In our patient,

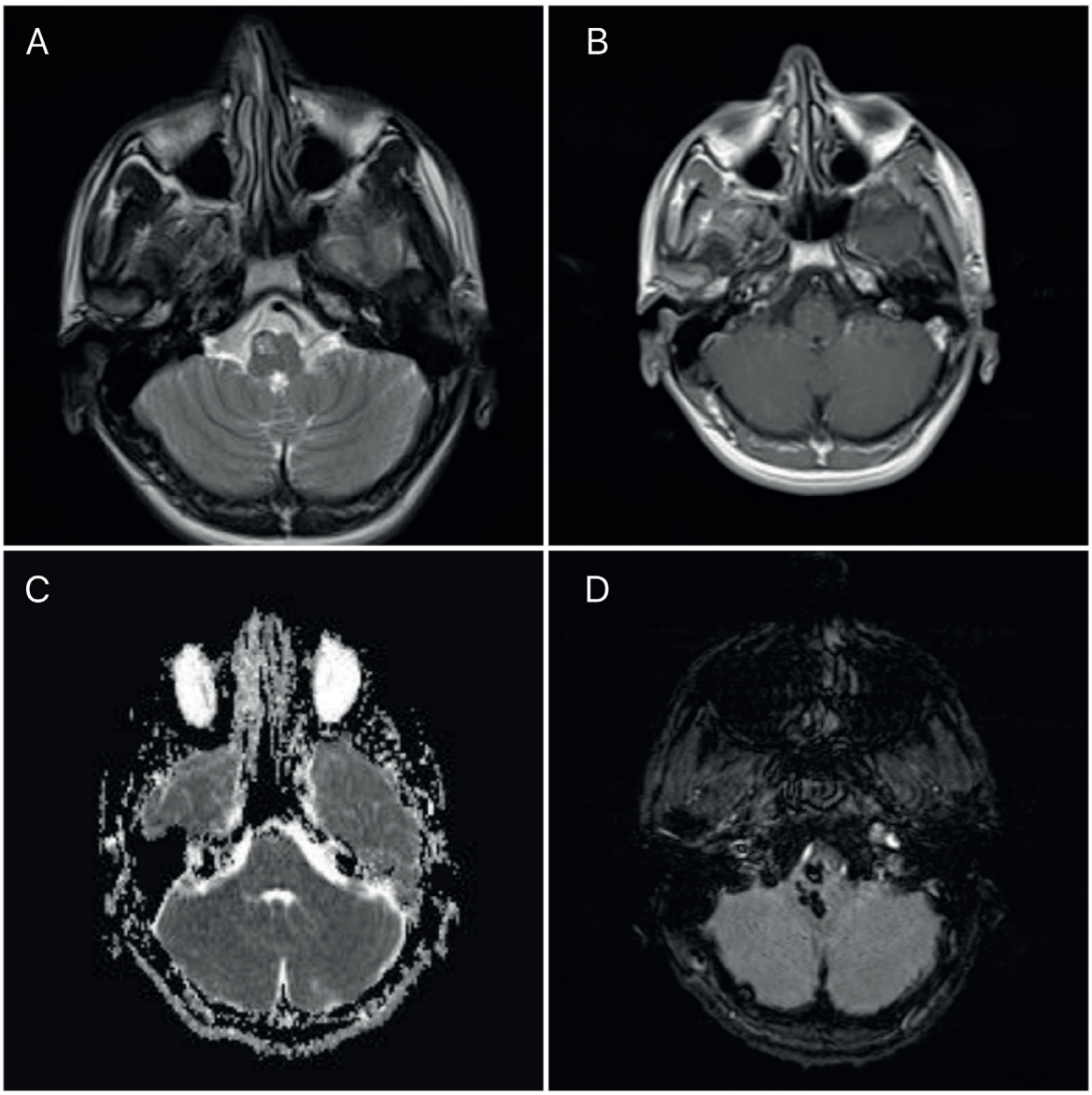


Fig. 1. a. Axial T2WI shows increased signal and enlargement of right inferior olivary nuclei. **b.** Axial T1WI shows hypointensity in right inferior olivary nuclei with no enhancement on contrast enhanced MRI. **c.** Axial apparent diffusion coefficient (ADC) image shows shows enlarged hyperintense inferior olivary nucleus with no diffusion restriction. **d.** SWI sequences shows a chronic hemorrhage involving the medulla oblongata and cerebellum.

chronic hemorrhage involving the medulla oblongata and cerebellum caused the ipsilateral HOD however the etiology of hemorrhage couldn't be found.

Persistent hiccups can indicate a number of different neurological diseases such as cerebral injury, encephalitis/meningitis, neuromyelitis optica, multiple sclerosis, or cerebrovascular diseases. Cranial MRI should be performed to determine the underlying neurological disease.[13,14]

In conclusion, HOD is a rare degenerative disorder that is thought to occur subsequent to a disruption of the dentate-rubro-olivary

pathway. There is limited data in the literature about the clinical and radiological features of this disease in pediatric populations. We highlight a pediatric case of unilateral HOD, which presented with persistent hiccups. Hiccups was described in neuro-Behcet related to HOD[15] however to our knowledge it has not been described in children with unilateral HOD in the literature. Awareness of clinical and radiological findings of HOD is important to avoid misinterpretation as a mass lesion, an ischemic event, or a demyelinating disease and provide adequate management.

REFERENCES

1. Gautier JG, Blackwood W. Enlargement of the inferior olivary nucleus in association with lesions of the central tegmental tract or dentate nucleus. Brain 1961; 84: 341-361.
2. Goyal M, Versnick E, Tuite P, et al. Hypertrophic olivary degeneration: metaanalysis of the temporal evolution of MR findings, AJNR Am J Neuroradiol 2000; 21: 1073-1077.
3. Ballestero MFM, Viana DC, Teixeira TL, Santos MV, de Oliveira RS. Hypertrophic olivary degeneration in children after posterior fossa surgery. An underdiagnosed condition. Childs Nerv Syst 2018; 34: 409-415.
4. Tartaglione T, Izzo G, Alexandre A, et al. MRI findings of olivary degeneration after surgery for posterior fossa tumours in children: incidence, time course and correlation with tumour grading. Radiol Med 2015; 120: 474-482.
5. Tilikete C, Desestret V. Hypertrophic olivary degeneration and palatal or oculopalatal tremor. Front Neurol 2017; 8: 302.
6. Walker G, Limaye K, Jankowitz BT, Jadhav AP. Hypertrophic olivary degeneration after cerebellar hemorrhage. J Clin Neurosci 2017; 43: 162-164.
7. Nishie M, Yoshida Y, Hirata Y, Matsunaga M. Generation of symptomatic palatal tremor is not correlated with inferior olivary hypertrophy. Brain 2002; 125(Pt 6): 1348-1357.
8. Sanverdi SE, Oguz KK, Haliloglu G. Hypertrophic olivary degeneration in children: four new cases and a review of the literature with an emphasis on the MRI findings. Br J Radiol 2012; 85: 511-516.
9. Konno T, Broderick DF, Tacik P, Caviness JN, Wszolek ZK. Hypertrophic olivary degeneration: A clinico-radiologic study. Parkinsonism Relat Disord 2016; 28: 36-40.
10. Kinghorn KJ, Kaliakatsos M, Blakely EL, et al. Hypertrophic olivary degeneration on magnetic resonance imaging in mitochondrial syndromes associated with POLG and SURF1 mutations. J Neurol 2013; 260: 3-9.
11. Oh SI. Hypertrophic olivary degeneration following listerial rhombencephalitis. Can J Neurol Sci 2018; 45: 593-595.
12. JI Seok, Yi H, Song YM, Lee WY. Metronidazole-induced encephalopathy and inferior olivary hypertrophy: lesion analysis with diffusion-weighted imaging and apparent diffusion coefficient maps. Arch Neurol 2003; 60: 1796-1800.
13. Eisenächer A, Spiske J. Persistent hiccups (singultus) as the presenting symptom of medullary cavernoma. Dtsch Arztebl Int 2011; 108: 822-826.
14. Sweeney J, Bodman A, Hall WA. Brain abscess of basal ganglia presenting with persistent hiccups. World Neurosurg 2018; 112: 182-185.
15. Franco-Macías E, Roldán-Lora F, Martínez-Agregado P, Cerdá-Fuertes N, Moniche F. Neuro-Behçet: pons involvement with longitudinal extension to midbrain and hypertrophic olivary degeneration. Case Rep Neurol 2015; 7: 148-151.

NOTICE TO AUTHORS

Instruction for authors

General Information:

The Turkish Journal of Pediatrics publishes the following peer-reviewed material oriented in the field of clinical pediatrics: original articles, case reports, review of the literature, short communications, clinicopathological exercises and letter to the editor. The Journal does not publish animal studies.

All papers are subject to editorial revision for the purpose of conformity to the style adopted by The Turkish Journal of Pediatrics, and authors may be requested to shorten or revise their papers. Statements and opinions expressed in the articles are those of the authors and not necessarily those of the Editor or publisher; the Editor and publisher disclaim any responsibility or liability for such material. Neither the Editor nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication; neither do they guarantee any claim made by the manufacturer of such product or service.

Preparation of Manuscript: Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts. Manuscript should be uploaded in a blinded manner; any affiliation must be covered.

Format: Manuscripts should be typed in 12pt (Times New Roman), double-spaced, with wide margins, and pages must be numbered on the right upper corner.

Covering Letter: The covering letter is important to help the Editor in his/her preliminary evaluation, please indicate why you think the paper is suitable for publication. A statement that each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript; if more than 6 authors, an explanation of the contributions of each author must be provided. The covering letter will be uploaded as a separate file.

Title Page: A separate title page should include authors' names and academic degrees; departmental and institutional affiliations of each author, and sources of financial assistance, if any. Designate one author as the correspondent, and provide an address as well as business and home telephone numbers. Galley proofs and order forms for reprints will be sent to the corresponding author, at a later date. On the title page please add a short running title, word count (excluding abstract and references), number of tables and figures.

Types of Articles:

Original Article: Original articles should report scientific findings within the pediatric field of interest. They must include a unstructured abstract of less than 300 words, to appear after the title page. The manuscript should be structured with the following headings: introduction, material and methods and Results. The introduction should put the study in context with the current literature and reflect the purpose of the study. The material and method should include the study methodology, the setting for the study (although affiliation must be covered), the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The Results section should include the outcome of the study and statistical significance, if appropriate. The Conclusion(s) states the significance of the results and limitations of the study.

Case Reports: Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Case Reports should consist of an unstructured abstract that summarizes the case(s), a brief introduction (recommended length, 1-2 paragraphs), a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process.

Review Articles: Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission.

Structured manuscript for systematic reviews are recommended. Headings should include: Unstructured abstract, Introduction, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions. The general instructions regarding submission (including cover letter, title page requirements, journal style guidance, and conflict of interest statements) also apply to Review Articles.

Short communication: Short communications are opinion pieces consisting of a main point and supporting discussion. They may address general issues or controversies in the field of pediatrics. No abstract is needed but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to short communications.

Clinicopathological exercises: Authors can submit for consideration an clinicopathological exercises that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to clinicopathological exercises.

Letters to the Editor: Letters to the Editor should pertain to papers published in The Turkish Journal of Pediatrics within the past year. Provide a unique title for the Letter on the title page with complete contact information for the author(s). Double-space the text of the Letter. References, including reference to the pertinent article(s) in The Journal, should conform to style for manuscripts. No abstract is needed for a letter to editor. Letters to the Editor may be sent to the authors of the original article to which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same issue as the Letter to the Editor.

Summary / Abstract and key words: An informative summary not exceeding 300 words must accompany each manuscript, Unexplained abbreviations and references should not be given. The abstract should state the objective (the purpose of the study and hypothesis) and summarize the rationale for the study. Following the material and methods it is critically important that all significant findings should be presented in the abstract. The abstracts should be unstructured.

The authors should list three to five key words or phrases taken from Index Medicus

Medical Subject Headings (http://www.nlm.nih.gov/mesh/MBrowser.html).

Abbreviations: Abbreviations must conform to accepted standards. Laboratory slang, clinical jargon and colloquialisms must be avoided. The full term for an abbreviation should precede its first use in the text. Generally, abbreviations should be limited to those defined in the AMA Manual of Style, current edition

Informed Consent: The Materials and Methods section should include a statement that the research was reviewed and approved by an institutional review board, and that participation involved informed consent. Please give information concerning where and when the study was approved, with the report number. The ethics committee report should also be uploaded to the online system.

Written consent from the family is required before a case report can be published. On submission, authors must attest that they have written consent from the family. Instances where there are extenuating circumstances in which family consent may be problematic will be handled on a case-by-case basis. A sentence stating that informed consent was received from the family should be added to the case section of the main document.

Conflicts of Interest: A conflict of interest is defined as any financial, personal, academically competitive or intellectual relationship between the author, referee or editor that involves article submission, referee evaluation, editorial decision and author-referee-editor correspondence and that could lead to a prejudiced and unfair action during the evaluation stage.

The authors must declare any financial relationship or conflict of interest areas that need to be known regarding the study. All financial contributions and sponsorships for the study and all financial relationships and conflict of interest areas should be included in the copyright transfer form that accompanies the article. The authors must explain the study sponsor's role in collecting, evaluating and analyzing the data, writing the article and submitting it for publication. Declaration of conflict of interest should be given at the end of the manuscript. If there is no conflict of interest, this section must include a "The authors declare no conflict of interest" statement at the end of the manuscript. All sources of funding should be mentioned in the manuscript.

The evaluation process for the article is not started if a conflict of interest statement is not present at the time the article is sent. If a conflict of interest becomes apparent after the article is published, the journal may make an official correction or withdraw the entire article.

The referees also need to declare all their interests that they feel may represent a potential conflict of interest. The referees must declare to the editor any conflicts of interest that may prejudice their evaluation of the article and must avoid such evaluation of certain articles if a potential prejudice is present.

The editors who will make the final decision on the articles cannot have any personal, professional or financial relationship with any of the authors of the articles they will decide on. The editors will also try to avoid any referees who have a possible conflict of interest, for example those working in the same department or facility as any of the authors.

Tables: Tables should be numbered with Roman numerals according to the order of mention in the text, each table must be typed double-spaced. A title for each table must be given. The first letter of each word should be capitalized. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, â footnote must give full credit to the original source. Tables should be added as a separate file.

Figures: Figures and illustrations should be of direct interest to the subject of the paper and numbered with Arabic numerals according to order of reference in the text. Art should be created / scanned and saved as either TIFF or JPEG format, submitted as a separate file, and not embedded in the text file. All lettering must be professional and in proportion to the drawing, graph, or photograph. A list of legends for the figures and illustrations must also be added as a separate file.

References: References to the literature should be limited to those cited by the author, arranged and numbered according to order they appear in the text; do not alphabetize. In text, tables, and legends, identify references with superscript Arabic numerals at the end of the sentence (after the full stop). List all authors up to 6; if more than 6, list the first 3 followed by "et al."

If reference to an author is made in the text the reference must be given after the name of the author rather than at the end of a sentence.

Example: In a study by Bier DM et al.23 the prevelance of…….

Names of journals are to be abbreviated in accordance with the style of Index Medicus.

References to journal articles:

Example: Kardelen F, Çeliker A, Özer S, et al. Sinus node dysfunction in children and adolescents: treatment by implantation of a permanent pacemaker in 26 patients. Turk J Pediatr 2002; 44: 312-316.

References to books:

Example: 2. Praat RTC, The Genetics of Neurological Disorders. London: Oxford University Press, 1967: 173-174.

References to chapters in books:

Example: 3, Kissane M. Development of the kidney and congenital malformations. In: heptinstall RH (ed). Pathology of the Kidney (2nd ed) Vol. 1. Boston: Little, Brown and Co, 1974: 69-109.

Acknowledgement: An "acknowledgement(s)" section may be added following these sections to thank those who helped the study or preparation of the article, if any. The acknowledgements are placed at the end of the article, before the references. This section contains statements of gratitude for personal, technical or material help, etc. If a grant has been received for the study this should also be given in the acknowledgments.

Submission of article: All manuscripts must be submitted on-line through the web site http://www.turkishjournalpediatrics.org. The corresponding author should upload the "Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form" with the main manuscript. The copyright transfer form can be downloaded during the online submission process.

Announcements: Announcements of scheduled meetings, symposia, or postgraduate courses may be sent to the publisher for consideration at least 5 months in advance of the meeting date.

Footnote: If the study has been presented at a scientific meeting or is part of a thesis this can be added to the end of the manuscript as a footnote.

No manuscript will be considered for review until all authors have completed an uploaded the copyright form.

Checklist

- Please check your article as described below before sending it to the Editor.
- Covering letter
- Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form.
- Title page
  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
  - Word count and number of tables and figures
- Article
  - Original articles: Summary, Introduction, Material and Methods, Results, Discussion,
  - Case Reports: Summary, Introduction, Case Report, Discussion,
  - Key words
- Manuscript should be uploaded in a blinded manner; any affiliation must be covered.
- Declaration of informed consent in the material methods section
- Declaration of conflict of interest at the end of the manuscript.
- References on a separate page
- Tables should be uploaded separately
- Figures should be uploaded separately

CASE REPORTS

Printed by METEKSAN in Ankara, Turkey