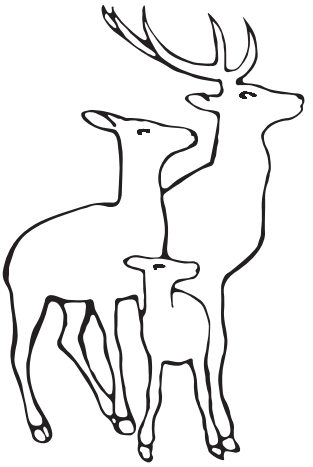
ISSN 0041-4301
www.turkishjournalpediatrics.org

THE TURKISH JOURNAL OF PEDIATRICS

ORIGINAL ARTICLES

volume 63
number 5
September-October 2021



THE TURKISH JOURNAL OF PEDIATRICS

www.turkishjournalpediatrics.org

Volume 63 ▪ Number 5
September-October 2021

ISSN: 0041-4301

THE TURKISH JOURNAL OF PEDIATRICS

ISSN 0041-4301
www.turkishjournalpediatrics.org

Cilt: 63 Sayı: 5, Eylül-Ekim 2021

KURUCU
İhsan DOĞRAMACI

EDİTÖR
Turgay COŞKUN

YARDIMCI EDİTÖR
Murat YURDAKÖK

EDİTÖR YARDIMCILARI
Ali DÜZOVA
Sinem AKGÜL

YÜRÜTME KURULU
Turgay COŞKUN
Murat YURDAKÖK
Ali DÜZOVA
Sinem AKGÜL

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.2021

ISSN 0041-4301
www.turkishjournalpediatrics.org

Vol: 63 Number: 5, September-October 2021

FOUNDER
İhsan DOĞRAMACI

CHIEF-EDITOR
Turgay COŞKUN

DEPUTY EDITOR
Murat YURDAKÖK

ASSOCIATE EDITORS
Ali DÜZOVA
Sinem AKGÜL

MANAGING EDITORS
Turgay COŞKUN
Murat YURDAKÖK
Ali DÜZOVA
Sinem AKGÜL

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.2021

THE TURKISH JOURNAL OF PEDIATRICS

FOUNDER
İhsan DOĞRAMACI

CHIEF-EDITOR
Turgay COŞKUN

DEPUTY EDITOR
Murat YURDAKÖK

ASSOCIATE EDITORS
Ali DÜZOVA
Sinem AKGÜL

MANAGING EDITORS
Turgay COŞKUN
Murat YURDAKÖK
Ali DÜZOVA
Sinem AKGÜL

LANGUAGE EDITOR
Deren RAMAN

SECTION EDITORS
Adolescent Medicine: Sinem AKGÜL
Allergy: Özge UYSAL SOYER
Cardiology: Dursun ALEHAN
Child and Adolescent Psychiatry: Halime Tuna ÇAK
Developmental Pediatrics & Social Pediatrics: Elif N. ÖZMERT
Endocrinology: E. Nazlı GÖNÇ
Gastroenterology: Hasan ÖZEN
Genetics: G. Eda UTINE
Hematology: Şule ÜNAL CANGÜL
Immunology: Deniz ÇAĞDAŞ AYVAZ
Infectious Diseases: Ateş KARA
Inherited Metabolic Diseases: Turgay COŞKUN
Neonatology: Murat YURDAKÖK
Nephrology: Ali DÜZOVA
Neurology: Dilek YALNIZOĞLU
Oncology: G. Burça AYDIN
Pediatric Emergency Medicine: Özlem TEKŞAM
Pediatric Surgery: Tutku SOYER
Pulmonary Medicine: Deniz DOĞRU ERSÖZ

EDITORIAL SECRETARY
Derya TOLUNAY

EDITORIAL BOARD
Errol ALDEN, USA
Didem ALİEFENDİOĞLU, Kırıkkale
Ayşe Engin ARISOY, Kocaeli
Sevcan BAKKALOĞLU EZGÜ, Ankara
Aysun BİDECİ, Ankara
Koray BODUROĞLU, Ankara
Yıldız CAMCIOĞLU, İstanbul
Yavuz COŞKUN, Gaziantep
Colleen CRAFT, USA
Asuman ÇOBAN, İstanbul
Ayhan DAĞDEMİR, Samsun
Orhan DERMAN, Ankara
Mesiha EKİM, Ankara
Vassilios FANOS, Italy
Mikko HALLMAN, Finland
Enver HASANOĞLU, Ankara
Figen Gülen İNCE, Lefkoşa
Berthold KOLETZKO, Germany
Andreas KONSTANTOPOULOS, Greece
Fatma Nirgül KÖKSAL, Bursa
Zafer KURUGÖL, İzmir
Tezer KUTLUK, Ankara
Ercan MIHÇI, Antalya
Leyla NAMAZOVA-BARANOVA, Russia
Nurullah OKUMUŞ, Ankara
Hasan ÖZEN, Ankara
Elif N. ÖZMERT, Ankara
M. Hakan POYRAZOĞLU, Kayseri
Ayşe SELİMOĞLU, İstanbul
Betül Berrin SEVİNİR, Bursa
Bülent Enis ŞEKEREL, Ankara
Naveen THACKER, India
Serap TURAN, İstanbul
Tomris TÜRMEN, Ankara
Tayfun UÇAR, Ankara
Betül ULUKOL, Ankara
Bilgin YÜKSEL, Adana

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: 63 NUMBER: 5 SEPTEMBER-OCTOBER 2021

ORIGINAL ARTICLES

CONTENTS

VOLUME: 63 NUMBER: 5 SEPTEMBER-OCTOBER 2021

CASE REPORTS

CONTENTS

VOLUME: 63 NUMBER: 5 SEPTEMBER-OCTOBER 2021

The Turkish Journal of Pediatrics 2021; 63: 735-742
https://doi.org/10.24953/turkjped.2021.05.001

Original Article

Ketogenic diet therapy map of Turkey

Ayşe Serdaroğlu, Ebru Petek Arhan, on behalf of the Ketogenic Diet Therapy Map of Turkey Study Group

Division of Pediatric Neurology, Department of Pediatrics, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Although the ketogenic diet (KD) is a well-established non-pharmacologic treatment for intractable epilepsy in pediatric patients, it is still perceived as theoretical information contained within textbooks rather than implementation in daily clinical practice. The aim of the present study was to primarily determine KD implementation frequency in daily clinical practice, the number of pediatric patients with intractable epilepsy, the conditions that hindered or facilitated KD implementation, and to provide a roadmap to improve patient outcomes.

Methods. A total of 27 pediatric neurologists, who were experienced in intractable epileptic pediatric patients and the implementation of KDs, responded to a 24-question survey. The survey was structured to outline patient selection criteria for KDs, prevalent treatment approaches in daily clinical practice for intractable epilepsy, level of physician awareness and impediments in KD implementation.

Results. Intractable epilepsy was diagnosed predominantly in children within the 7 to 12-year age group (44%). KD implementation was hindered mainly by lack of an adequate number of personnel (53.8%), lack of a dietitian (52%), inadequate training of patients (24%), and inadequate experience of healthcare professionals (23.1%). Lack of guidance in treatment, physician's hesitations due to probable problems, inadequate time spent for each patient, lack of awareness for KD therapy, and loss of appetite in these patients were also emphasized by the participants (each 16.7%).

Additional drawbacks were non-appealing taste (76.9%), need for continuous supervision (76.9%), and low patient motivation (73.1%). The treatment failure causes for KDs were ranked as imprecise cooking of recipes (94%), inadequate family support (92.3%), inadequate consumption of meals (73%), incorrect indication (53.9%), and inefficiency of KD despite correct application (42.3%).

Conclusion. The panoramic view of KDs in Turkey indicates that a National Guideline would increase both physician awareness level for KD, and the rate of structured therapy implementation in pediatric patients, who suffer from inadequate treatment.

Key words: ketogenic, diet, epilepsy, child.

The ketogenic diet (KD), first defined in 1921 by Dr. Wilder,[1] is a well-established non-pharmacologic treatment for intractable epilepsy in pediatric patients. Classical KD was defined initially and later on three more types were qualified according to clinical requirements: the modified Atkins diet (MAD), the medium chain triglyceride diet (MCT), and the low-glycemic index treatment (LGIT).[2] It is still unclear how KD therapy (KDT) improves drug resistant epilepsy, but it is considered that high fat and low carbohydrate content induces biochemical response to starvation, thus energy for the brain is supplied by ketone bodies.[3]

For decades, KDT has been included in books as supplementary information but it was rarely implemented on pediatric patients as a part of clinical practice.[2] Instead increasing number of antiepileptic drugs (AEDs) were commonly preferred. In the early 2000s, KDT was first

✉ Ayşe Serdaroğlu
ayseserdaroglu@gmail.com

Received 3rd November 2020, accepted 24th December 2020.

reported as an effective and safe approach in a small group of infants.[4] In the literature, it is reported that most of the pediatric patients with intractable epilepsy have been using at least two AEDs before they started KDT[2,5] and they typically discontinue one AED at a time during KDT.[2,6]

In December 2006, the Charlie Foundation invited 26 pediatric epileptologists and dietitians from nine countries with expertise in implementing the KDT for a panel, because KDT was used differently in various centers around the world, and standardized protocols were needed. After the consensus statement about patient selection, pre-KDT counseling and evaluation, specific dietary therapy selection, implementation, supplementation, follow-up management, adverse event monitoring, and eventual KDT discontinuation was published, it was supported by the Practice Committee of the Child Neurology Society.[7] In 2009, the first expert consensus guideline for the management of children on KDT was prepared for practical recommendations.[7] After nearly a decade, in 2018, the original committee members with more international experts gathered to evaluate new evidences in KDT.[2] The committee published key points about patient profiles for KDT, flexibility in initiation in this therapy, and recommendations for the ketogenic team.[2]

In the present survey study, we primarily aimed to determine KDT implementation frequency in daily clinical practice of pediatric neurologists who had expertise in this area; the number of pediatric patients with intractable epilepsy; the conditions that hindered or facilitated KD implementation; and also to discuss probable solutions, and to provide a roadmap so that KDT would become more prevalent for this patient group in Turkey.

Material and Methods

The present survey trial was performed on 27 pediatric neurologists who had expertise in KDT implementation, and were working in different affiliations in four main cities of Turkey; Adana (n= 3), Ankara (n= 12), Istanbul (n= 6) and Izmir (n= 6).

A 24-question survey was prepared to gather information from each center about the percentage of patients with intractable childhood epilepsy and their follow-up, alternative treatment options such as KDT, epileptic surgery and vagal nerve stimulation (VNS) applied in the center, physician approach to start the KD and to determine underlying reasons for the low rate of KDT. The survey contained 15 quantitative, 7 qualitative questions and 2 open ended discussion questions. Participants completed a Likert scale for qualitative questions related to KDT: 1= "I definitely do not agree", 2= "I do not agree", 3= "I am uncertain about it", 4= "I agree", 5= "I definitely agree".

Between December 2018 and February 2019, the survey was distributed electronically to all centers, and participants were invited to a meeting in their city for further discussion about the place of KDT among other treatment modalities in Turkey.

In the present descriptive study, statistical analysis of interrupted data was shown by using frequency (n) and percentage (%). It was performed by using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013) Program.

Results

In the first part of the survey, introductory characteristics of the participating centers were collected. More than half of the participants (51.9%; n= 14) examined 26-50 patients per day at the pediatric neurology outpatient clinic, and 16 out of 27 participants (59.3%) reported that %26-50 of patients applying daily were diagnosed with epilepsy. Of those diagnosed with epilepsy, <25% were diagnosed with intractable epilepsy by 63% of physicians (n= 17). Nearly half of pediatric neurologists

(n= 14) reported that they had >150 patients with intractable epilepsy in their systems. Of critical revision of the manuscript for important intellectual content; administrative support, study supervision recorded intractable epilepsy patients, 51-75% were followed regularly. The age range of patients with intractable epilepsy were ranked as 7-12 years (44%), 1-3 years (29.6%), and 4-6 years (25.9%). During daily clinical practice, 59.3% of physicians spent 11 to 20 minutes for each epilepsy patient. Of the participants 7.4% reported spending <10 minutes whereas 22.2% reported spending <30 minutes with each patient

The second part of the survey contained questions about intractable epilepsy treatment (Table I). Epileptic surgery and VNS were performed in 59.3% and 40.7% of participating centers, respectively.

The last part of the survey contained questions about KDT (Table II).

Physicians reported four leading reasons for KDT implementation in fewer patients as; lack of an adequate number of personnel (53.8%), lack of a dietitian (52%), inadequate training of patients (24%), and inadequate experience of healthcare professionals (23.1%). Physical environment specifications and low socio-cultural level (both 15.4%) were ranked as the fifth reason. Lack of guidance in treatment, physician's hesitations due to probable problems, inadequate time spent for each patient, physicians being mainly concentrated in the pharmacological treatment algorithms, and loss of appetite in these patients were also emphasized by the participants (each 16.7%).

For patient incompliance to the diet therapy, physicians reported that the taste of the diet would not be appealing for children due to the high fat content (76.9%); the patient would not have continuous supervision (76.9%), and that the physician or nurse or dietitian had inadequate time to motivate the patient (73.1%). Other causes were described as high cost (30.8%), frequent illness in children (23.1%), and low treatment success rate (7.7%). One fifth of participants (20%) agreed that parents would not be able to understand the significance of the diet, moods of parents would hinder therapy, disadvantage of taste, patients would not attend visits, and low parental education level.

Table I. Distribution of indications for treatment approaches in intractable epilepsy patients at the centers.

	Range	Number of physician (n)	Percentage (%)
Epileptic surgery indicated*	<10%	24	92.3
	11-20%	1	3.8
	21-30%	1	3.8
	>30%	0	0
VNS indicated	0-10%	19	70.4
	11-20%	5	18.5
	21-30%	1	3.7
	>30%	2	7.4
Mean number of concomitantly used antiepileptic agents	1	3	11.1
	2	2	7.4
	3	11	40.7
	>3	11	40.7
Maximum number of concomitantly used antiepileptics	1-3	3	11.1
	4-6	20	74.1
	>6	4	14.8

VNS: vagal nerve stimulation.
*: one physician did not answer this question

Table II. Data about attitudes and experiences of physicians about ketogenic diet therapy.

		Number of physician (n)	Percentage (%)
If had optimum conditions, what percentage of your patients would you start on ketogenic diet therapy?	<25%	2	7.4
	26-50%	10	37.0
	51-75%	10	37.0
	>75%	5	18.5
What percentage of patients, do you think, would comply to ketogenic diet therapy?	<20%	3	11.1
	21-40%	10	37.0
	41-60%	10	37.0
	>60%	4	14.8
What percentage of patients with intractable epilepsy have you started ketogenic diet therapy?	<25%	22	84.6
	26-50%	3	11.6
	51-75%	1	3.8
	>75%	0	0
What percentage of your patients have responded to the ketogenic diet therapy?	0-30%	10	37.0
	31-60%	12	44.4
	61-90%	3	11.1
	>90%	2	7.4

Parameters defined for treatment response according to physicians are given in Table III.

Reasons of treatment failure among physicians who agreed and completely agreed were imprecise cooking of recipes (94%), inadequate family support (92.3%), inadequate consumption of meals (73%), incorrect indication (53.9%), and inefficiency of KD despite correct application (42.3%). It was also mentioned that limited cooperation with families, disbelief of parents to KD, lack of well-trained personnel, and children not being able to follow their meal plan at school or due to imitation of their siblings were other causes for treatment failure (each 16.7%).

Physicians discussed facilitating factors to implement KDT. The highest ranked factors defined for implementation were defined as presence of trained dietitians and physicians (69.2%), distribution of contact information of a dietitian (57.7%) and well-running ketogenic diet outpatient clinic (57.7%). The complete list is presented in Table IV.

Discussion

In this first survey study on KDT implementation in our country, we obtained a panoramic view of the main barriers in the widespread use of KDT in pediatric patients with intractable epilepsy, and how they could be overcome. In the four meetings held in different cities, the committee members, who were all pediatric neurologists, shared their experiences as well as insights both for daily practice and the requirement of a national KDT guideline.

As the birthplace of KD, the USA has the best-established facilities including many KD centers, the Charlie Foundation is particularly interested in providing all necessary information in KDT, and has a ketocalculator program to support parents' needs. In many European countries, KDT has been used for many decades now. From the United Kingdom (UK), Neal et al.[8] published the first randomized clinical trial of the KDT. The UK has also a parent-support group (the Matthew's Friends), which has been dedicated to providing information,

Table III. Parameters to define treatment response, (*according to physicians' reports*).

	I completely disagree (n, %)	I disagree (n, %)	I am uncertain (n, %)	I agree (n, %)	I completely agree (n, %)
No seizure	0	0	0	20 (76.9)	6 (23.1)
Decreased number of seizures	0	0	0	17 (65.4)	9 (34.6)
Decreased number of drugs	0	0	0	19 (73.1)	7 (26.9)
Improvement in cognitive functions	0	3 (11.5)	0	15 (57.7)	8 (30.8)
Improvement in success in school	0	2 (7.7)	1 (3.8)	16 (61.5)	7 (26.9)
Increased QoL	0	0	1 (3.8)	17 (65.4)	8 (30.8)
Improvement in motor functions	0	2 (7.7)	3 (11.%)	15 (57.7)	6 (23.1)
Improvement in EEG	0	1 (3.8)	2 (7.7)	18 (69.2)	5 (19.2)
Decreased number of hospitalizations	0	1 (3.8)	1 (3.8)	19 (73.1)	5 (19.2)

EEG: electroencephalography, QoL: quality of life.

Table IV. Factors to improve implementation of ketogenic diet therapy, (*according to physicians' reports*).

	I completely disagree (n, %)	I do not agree (n, %)	I am uncertain (n, %)	I agree (n, %)	I completely agree (n, %)
Detailed training of the child and parents	0	1 (3.8)	1 (3.8)	13 (50)	11 (42.3)
Contact information of a dietitian that parents can easily reach	0	1 (3.8)	0	10 (38.5)	15 (57.7)
Well-running ketogenic diet outpatient clinic	0	1 (3.8)	2 (7.7)	8 (30.8)	1 (57.7)
Ketogenic treatment algorithm	0	2 (7.7)	1 (3.8)	12 (46.2)	11 (42.3)
Ketogenic diet treatment guideline	0	2 (7.7)	1 (3.8)	10 (38.5)	13 (50)
Trained dietitian and physician	0	2 (7.7)	0	6 (23.1)	18 (69.2)
Other factors		N		Percentage (%)	
Financial support for the family (providing baseline devices, and transportation compensation etc.)		1		25	
Establishment of a patient association for KDT		1		25	
Experience sharing with new KDT starters		1		25	
Presence of a metabolism specialist		1		25	

KDT: ketogenic diet therapy.

training, support and education on all aspects of dietary treatments for epilepsy.[9] The Charlie Foundation, the Matthew's Friends, results of clinical randomized trial and efforts of experts to prepare practical guidelines have raised awareness for KDT worldwide. Thus, adapting KD to the local culture, life-style and nutrition had been tried sometimes with limited resources.[9,10]

In the present survey study, pediatric neurologists reported a high number of intractable epilepsy patients in their databases, and a great workload in their daily outpatient clinics. The time for comprehensive evaluation of the patient for KDT initiation and selecting the most suitable diet type was very limited (11-20 minutes/patient). Similar to the literature, pediatric neurologists emphasized primarily

on limited resources such as inadequate staff and the lack of dietitians.[9,11] It is evident that if neurologists decide to implement KDT, then they should be involved not only in treatment and follow-up, but also in dealing with preparing a diet list, or hospitalizing patients in order to train mothers for recipe preparation. In some institutions, physicians sometimes receive help from dieticians from other departments. A majority of centers do not have a kitchen applied for training mothers. Lack of information about KD, and the loss of a patient's appetite are additional complicating issues at the initiation. Pediatric neurologists indicated that they hesitate in KD implementation, because of probable problems during the therapy, and the lack of consensus or an algorithm or guideline.

During discussions for solutions, it is described that health authorities, specialty associations and non-profit organizations should cover all aspects of problems related to KDT implementation. The Turkish Ministry of Health is expected to establish KDT centers with trained KD team members and required supplies; systematic extensive healthcare should be planned, and initiated in our country. This should include an increasing number of trained dietitians, complete reimbursement of ready-to-use keto-products, building a national database for children with intractable epilepsy, and financial support for the families traveling to the center for visits.

It is considered that specialty associations should prepare standard training programs and support participants with hard-copy material such as patient and/or parent leaflets, posters in KDT centers, and cook-books for parents. Moreover, specialty associations should be proactive in training pediatric neurologists in KDT by holding workshops at academic meetings. Training programs should contain practical recommendations rather than theoretical content. Consequently, trained physicians would approach their patients with more confidence, and KDT would reach more patients. Additionally, such training programs would increase communication between physicians with common interests. Physicians would be able to improve their practice, they may refer patients, and share experiences in specific situations readily. One of the most effective solutions for pediatric neurologists is to introduce a national guideline for KDT.

The establishment of non-profit organizations, such as the Charlie Foundation, would help patients and parents to feel that they are not alone, and reinforce effective interactions and experience-sharing among them. They may define common concerns of the Turkish patients and parents, which would provide more target-oriented training for all parties. Participants underlined that support from the pharmaceutical industry is necessary to cover some of the needs. Donations to organizations or associations would increase the quality of healthcare services provided for patients.

Parents and/or patients can be easily demotivated and break the diets due to meticulous efforts in preparation of meals, poor palatability due to high-fat content, pediatric age group, difficulty in understanding KDT recipes, and having limited supervision after the first training. Treatment success depends on a good interaction between the KD team, the patient, and the family. The patient and family should be encouraged to attend follow-up visits (current attendance rate is around 50%), whereas additionally parents must have a firm grasp on not only of the diet, but also how to identify and act quickly to minimize adverse events and complications.

In conclusion, in the first study concerning KDT in Turkish pediatric patients with intractable epilepsy, concrete decisions were made for the better implementation of KDT in Turkey. Priory, a consensus has been reached concerning the preparation of a National Guideline in KDT. Following that, continuous training programs will be planned at academic meetings to increase the knowledge level and KDT awareness among pediatric neurologists. The Turkish healthcare authorities will be informed about the challenges that these patients and

their parents are faced with. Participants of this study have decided to pool their databases to design and conduct clinical trials in the future.

List of the Study Group

Ayşe Serdaroğlu (Division of Pediatric Neurology, Department of Pediatrics, Gazi University Faculty of Medicine, Ankara), Ayşe Aksoy (Division of Pediatric Neurology, Department of Pediatrics, Ondokuz Mayıs University Faculty of Medicine, Samsun); Gül Aktan (Division of Pediatric Neurology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir), Ebru Petek Arhan (Division of Pediatric Neurology, Department of Pediatrics, Gazi University Faculty of Medicine, Ankara), Erhan Bayram (Division of Pediatric Neurology, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir), Gonca Bektaş (Pediatric Neurology Clinic, Pediatrics Clinic, the Ministry of Health İstanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital, İstanbul), Ömer Bektaş (Division of Pediatric Neurology, Department of Pediatrics, Ankara University Faculty of Medicine, Ankara), Kürşat Bora Çarman (Division of Pediatric Neurology, Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir), Aydan Değerliyurt (Pediatric Neurology Clinic, Pediatrics Clinic, the Ministry of Health Ankara City Hospital, Ankara), Sarenur Gökben (Division of Pediatric Neurology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir), Serhat Güler (Division of Pediatric Neurology, Department of Pediatrics, İstanbul University, Cerrahpaşa Faculty of Medicine, İstanbul), Özlem Hergüner (Division of Pediatric Neurology, Department of Pediatrics, Çukurova University Faculty of Medicine, Adana), Mustafa Kömür (Division of Pediatric Neurology, Department of Pediatrics, Mersin University Faculty of Medicine, Mersin), Gülten Öztürk (Division of Pediatric Neurology, Department of Pediatrics, Marmara University Faculty of Medicine, İstanbul), Sema Saltık (Division of Pediatric Neurology, Department of Pediatrics, İstanbul University, Cerrahpaşa Faculty of Medicine, İstanbul), Mine Serin (Division of Pediatric Neurology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir), Müjgan Sönmez (Private Practice Physician, Ankara), Nesrin Şenbil, (Private Practice Physician, Ankara) Serap Tıraş Teber, (Division of Pediatric Neurology, Department of Pediatrics, Ankara University Faculty of Medicine, Ankara), Leman Tekin Ongun (Division of Pediatric Neurology, Department of Pediatrics, Başkent University Faculty of Medicine, Adana), Meral Topçu (Division of Pediatric Neurology, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara), Aycan Ünalp (Pediatric Neurology Clinic, Pediatrics Clinic, the Ministry of Health İzmir Dr. Behçet Uz Child Diseases and Surgery Training and Research Hospital, İzmir), Tuğçe Uzunhan (Pediatric Neurology Clinic, Pediatrics Clinic, the Ministry of Health Prof. Dr. Cemil Taşçıoğlu City Hospital, İstanbul), Bülent Ünay (Division of Pediatric Neurology, Department of Pediatrics, Gülhane Military Hospital Faculty of Medicine, Ankara), Sanem Yılmaz (Division of Pediatric Neurology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir), Deniz Yüksel (Pediatric Neurology Clinic, Pediatrics Clinic, the Ministry of Health Dr. Sami Ulus Obstetrics, Child Health and Diseases Training and Research Hospital, Ankara)

Author contribution

All authors confirm their contribution with data input and interpretation, drafting the manuscript. EPA; contributed for survey planning, data acquisition, data analysis and interpretation, supervision for drafting the manuscript. AS; contributed for study concept and design, critical revision of the manuscript for important intellectual content; administrative support, study supervision. All authors reviewed and approved the final version of the manuscript.

Source of funding

Nutricia sponsored organization of scientific meetings.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Wilder RM. The effect of ketonemia on the course of epilepsy. Mayo Clin Bull 1921; 2: 307-308.
2. Kosoff EH, Zupec-Kania BA, Auvin S, et al; Charlie Foundation; Matthew's Friends; Practice Committee of the Child Neurology Society. Optimal clinical management of children receiving dietary therapies for epilepsy: updated recommendations of the International Ketogenic Diet Study Group. Epilepsia Open 2018; 3: 175-192.
3. Hartman AL, Gasior M, Vining EP, Rogawski MA. The neuropharmacology of the ketogenic diet. Pediatr Neurol 2007; 36: 281-292.
4. Nordli DR Jr, Kuroda MM, Carroll J, et al. Experience with the ketogenic diet in infants. Pediatrics 2001; 108: 129-133.
5. Lin A, Turner Z, Doerrer SC, Stanfield A, Kossoff EH. Complications during ketogenic diet initiation: prevalence, treatment, and influence on seizure outcomes. Pediatr Neurol 2017; 68: 35-39.
6. Kossoff EH, Pyzik PL, McGrogan JR, Rubenstein JE. The impact of early versus late anticonvulsant reduction after ketogenic diet initiation. Epilepsy Behav 2004; 5: 499-502.
7. Kossoff EH, Zupec-Kania BA, Amark PE, et al; Charlie Foundation, Practice Committee of the Child Neurology Society; Practice Committee of the Child Neurology Society; International Ketogenic Diet Study Group. Optimal clinical management of children receiving the ketogenic diet: recommendations of the International Ketogenic Diet Study Group. Epilepsia 2009; 50: 304-317.
8. Neal EG, Chaffe H, Schwartz RH, et al. The ketogenic diet for the treatment of childhood epilepsy: a randomized controlled trial. Lancet Neurol 2008; 7: 500-506.
9. Kossoff EH, Caraballo RH, du Toit T, et al. Dietary therapies: a worldwide phenomenon. Epilepsy Res 2012; 100: 205-209.
10. Gerges M, Selim L, Girgis M, El Ghannam A, Abdelghaffar H, El-Ayadi A. Implementation of ketogenic diet in children with drug-resistant epilepsy in a mediumresources setting: Egyptian experience. Epilepsy Behav Case Rep 2018; 11: 35-38.
11. Wibisono C, Rowe N, Beavis E, et al. Ten-year single-center experience of the ketogenic diet: factors influencing efficacy, tolerability, and compliance. J Pediatr 2015; 166: 1030-1036.e1.

The Turkish Journal of Pediatrics 2021; 63: 743-751
https://doi.org/10.24953/turkjped.2021.05.002

Original Article

Neuropsychiatric lupus in Malaysian children: clinical characteristics, imaging features and 12-month outcomes

Sern Chin Lim[1], Yusma Lyana Md Yusof[1], Bushra Johari[2], Roqiah Fatmawati Abdul Kadir[2], Swee Ping Tang[3]

Departments of [1]Paediatrics, [2]Radiology, Universiti Teknologi MARA (UiTM) Faculty of Medicine, Sg Buloh, Selangor; [3]Department of Paediatrics, Pediatric Rheumatology Unit, Selayang Hospital, Selangor, Malaysia.

ABSTRACT

Background. Neuropsychiatric lupus (NPSLE) serves as a marker of severe disease in children with juvenile onset systemic lupus erythematosus (JSLE). This study aims to characterise the clinical and imaging features at diagnosis; and outcomes after 12 months in Malaysian children with NPSLE.

Methods. A retrospective study of all NPSLE patients seen at the Pediatric Rheumatology Unit, Selayang Hospital from January 2004 to May 2017.

Results. Twenty-eight (19.8%) of 141 JSLE patients had NPSLE with a median presenting age of 10 years (IQR 9 – 12), median follow-up of 7 years (IQR 4 – 11) and female: male ratio of 3.7:1. Twenty-three patients had single episodes of NPSLE and five patients had two distinct episodes each. The mean disease activity score (SLEDAI-2K) was 24.9±11.8 at presentation with 81.8% having high disease activity (score >12). Majority (60.6%) present with NPSLE within the first year of SLE diagnosis whilst the remainder occurred at a median of five years (IQR 3-7) post-SLE diagnosis. Majority (75.8%) had central nervous system (CNS) involvement commonly presenting with seizures, delirium and visual complaints whilst 24.2% had peripheral nervous system (PNS) involvement. Frequent accompanying features included hypocomplementemia, acute cutaneous lupus and lupus nephritis. Autoantibodies were common; ANA (100%), anti-dsDNA (78.8%) anti-RNP (39.4%) and anti-Sm (39.4%). Abnormalities were seen in 85.7% of the magnetic resonance imaging (MRI) studies performed, predominantly supratentorial white matter hyperintensities on T2 images whilst cerebrospinal fluid examination was normal in the majority. All patients with CNS involvement received corticosteroids with immunosuppressive therapy: Cyclophosphamide (20), Rituximab (2). Treatment for PNS involvement included corticosteroids with Azathioprine (6) or Mycophenolate mofetil (2). At 12 months post-NPSLE, majority (85.7%) recovered without any neurological sequelae.

Conclusions. Juvenile-onset NPSLE presents with a myriad of clinical features. It is associated with high disease activity and non-specific MRI features. With early diagnosis and treatment, the majority had good prognosis.

Key words: systemic lupus erythematosus, neuropsychiatric, MRI.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder characterized by multisystem involvement, including the nervous system. It is defined as juvenile onset SLE (JSLE) when it presents before the age of 18 years which occurs in approximately 10–20 % of all SLE cases.[1]

✉ Sern Chin Lim
sernchin@gmail.com

Received 3rd December 2021, revised 27th January 2021, accepted 4th April 2021.

Neuropsychiatric SLE (NPSLE) is one of the more complex and poorly understood manifestation of JSLE.[2] Involvement of the central nervous system (CNS) is more frequent in JSLE and serves as a marker of severe disease in children.[2-4] The reported incidence of neuropsychiatric symptoms among JSLE patients is between 14-75%.[5]

The diagnosis of NPSLE is often challenging due to the myriad of symptomatology, many which are non-specific.[6] Documented NPSLE

manifestations include headache, seizures, strokes and demyelinating disorders with varying neuroimaging findings.[7,8] These manifestations are the result of complex pathophysiology affected by genetic, environmental and hormonal factors. It is hypothesized that ethnicity (Asian versus non-Asian) may be one of the potential predictors of neuropsychiatric manifestations and Asian JSLE have a higher risk of developing seizures than non-Asian population.[4,9,10] However, there is paucity of data of NPSLE in children with SLE in multi-ethnic Malaysia.

This study aims to explore and characterise the various clinical manifestations, laboratory features, imaging findings as well as the outcomes of Malaysian children with NPSLE. We aim to compare our findings with other cohorts of NPSLE children published in existing literature.

Material and Methods

This is a retrospective observational study of all children diagnosed with juvenile-onset NPSLE (according to ACR/SLICC 2012 NPSLE criteria[11]) and managed at the Paediatric Rheumatology Unit, Selayang Hospital, Malaysia from January 2004 till May 2017. This unit is the sole tertiary referral center for all pediatric rheumatology cases in Malaysia until mid-2016, after which an alternative private center was available, thus capturing most JSLE patients in the country during the study period. Subjects were identified from an existing database and data was collected from the hospital's electronic medical records. The diagnosis of NPSLE in the participants were further categorized in accordance with the American College of Rheumatology system for the neuropsychiatric syndromes of SLE.[12] Patients who did not have at least 12 months of follow-up post diagnosis of NPSLE were excluded from the study.

Data collected included patient demographics; age at onset and duration of SLE illness; clinical features, laboratory findings, treatment and radiological images during the neuropsychiatric episode; assessment of disease activity with Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K index)[13], and neurological outcomes at 12 months follow-up. Radiological (MRI, MRA, MRV) images were anonymised and reviewed by independent radiologists.

Statistical analysis was done using SAS@ 9.4 software (SAS Institute, Cary NC). The demographic data, clinical, laboratory features and imaging features were analysed using descriptive statistics. Data were expressed as means for continuous variables if normally distributed and median with interquartile range (IQR) if distribution was skewed. Categorical variables are reported as frequencies or percentages. Chi-squared or Fisher exact test was employed for categorical variables and the independent-sample t-test for continuous variables. A p value of < 0.05 was considered as statistically significant.

This study was investigator initiated and approved by the Malaysian Research and Ethics Committee, National Institute of Health (Approval No: NMRR-19-603-46393) and conducted in accordance with the Declaration of Helsinki. Patient informed consent was waived by our institutional board as only anonymised secondary data was collected.

Results

Between January 2004 to May 2017, there were a total of 141 patients who fulfilled the criteria for Juvenile Systemic Lupus Erythematosus (JSLE) and had at least 12 months of follow-up. Of these, 28 patients (19.8%) had 33 distinct episodes which fulfilled criteria for the diagnosis of NPSLE. Twenty- three patients had a single NPSLE event whereas five patients presented with two separate and distinct episodes of NPSLE throughout their follow-up. The female to male ratio was 3.7 to 1. Most of the patients with NPSLE were of Malay ethnicity (79%) followed by Chinese (17%), Indian (6%)

and mixed ethnicity (3%). The median age of presentation was 10 years (IQR 9-12) with a range of three to 20 years and the median years of follow-up was seven years (IQR 4 – 11) with a range of one to 14 years.

Twelve (36.4%) of these 33 NPSLE episodes occurred at initial SLE diagnosis with the majority (60.6%) of patients presenting within the first year of diagnosis. The remainder of patients presented at a median of five years (IQR 3 - 7) after diagnosis of JSLE. Majority (75.8%) had CNS involvement with diffuse neuropsychiatric manifestations whilst 24.2% had PNS involvement.

The most common neurological manifestation was seizures whilst the predominant physical abnormalities were mental state dysfunction with soft neurological signs (Table I) . There was high anti-nuclear antibody (ANA) and anti-double stranded DNA (anti-dsDNA) antibody positivity (Table I). The most frequent NPSLE diagnoses in our patients was seizures followed by cerebrovascular disease, abnormal behavior and psychoses (Fig. 1). Most patients fulfilled more than one NPSLE categories.

Active lupus manifestations, both clinical and serological, were also commonly seen during the acute presentation of NPSLE (Table I). Majority of patients had high disease activity with a mean SLEDAI-2K score of 24.9 ± 11.8 (range 4.0 to 53.0) and those with major neurological symptoms (psychosis, delirium, seizures, abnormal behaviour) exhibited even higher disease activity with a mean SLEDAI-2K score of 31.7 (Table I).

Cerebrospinal fluid (CSF) findings were normal in two thirds of the patients despite the presence of dramatic clinical features. Of the remaining five, three had features of aseptic meningitis with high protein and low glucose whereas the remaining two had isolated elevations of CSF protein. Cerebrospinal fluid IgG screening was performed in three patients, of which only one was elevated and this was a patient with seizures and psychosis. Electroencephalogram (EEG) were performed in seven out of 16 patients who presented with seizures. Four were abnormal with diffuse slow and attenuated background waves reflective of their altered mental states.

The most common abnormality on brain MRI was non-specific white matter hyperintensities (WMH) in the supratentorial regions on T2 and FLAIR sequences (Table II). None of the presenting features were significantly associated

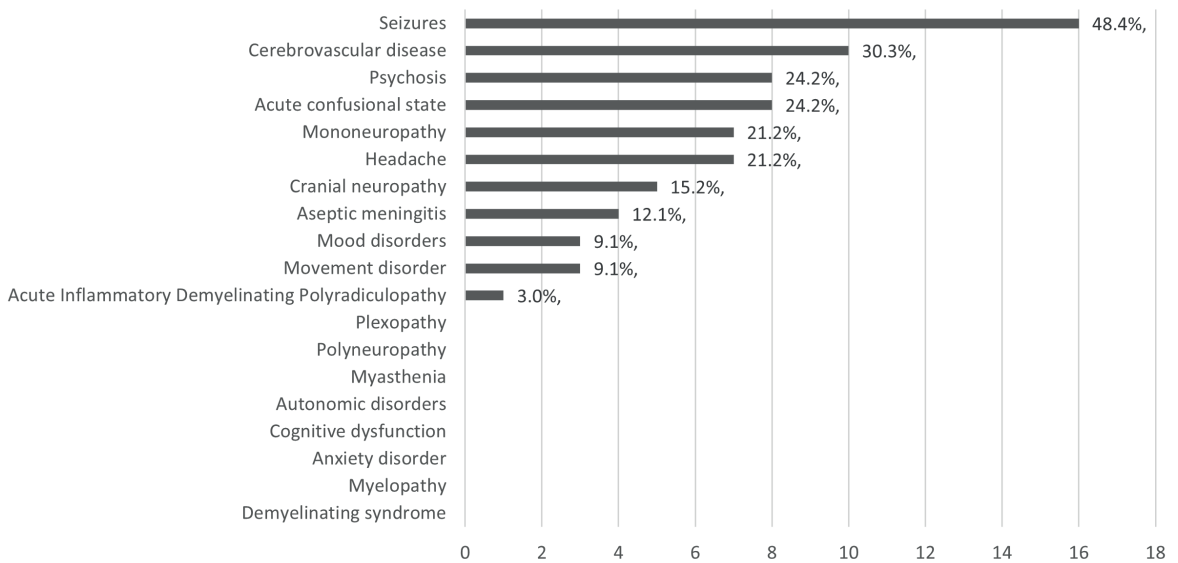


Fig. 1. Frequency of each neuropsychiatric lupus diagnostic category based on the American College of Rheumatology Nomenclature.[12]

Table I. Presenting features of patients with neuropsychiatric lupus.

Presenting features	Frequency (%) n=33	Presenting features	Frequency (%) n=33
Clinical features		Neurological abnormalities on examination	
Seizures	15(45.5)	Abnormal reflexes	13(39.4)
Delirium	10(30.3)	Mental state dysfunction	13(39.4)
Sensory deficit	9(27.3)	Reduced power	12(36.4)
Visual problems	9(27.3)	Abnormal tone	6(18.2)
Headache	8(24.2)	Fundoscopic abnormalities	6(18.2)
Psychosis	8(24.2)	Cranial nerve abnormalities	6(18.2)
Abnormal behavior	8(24.2)	Cerebellar signs	4(12.1)
Weakness of limbs	6(18.2)	Sensory deficit	4(12.1)
Mood disorder	4(12.1)		
Movement disorder	3(9.1)		
Others	3(9.1)	Disease activity (SLEDAI-2K scores)	
Photophobia	1(3.0)	High disease activity (> 12)	27(81.8)
Hearing problems	1(3.0)	Moderate disease activity (6-12)	5(15.2)
Developmental regression	0(0)	Low disease activity (<6)	1(3.0)
Lupus manifestations in other organs		Immunologic markers	
Low complement	28(84.8)	ANA	33(100.0)
Thrombocytopenia	15(45.5)	Anti dsDNA	26(78.80)
Acute cutaneous rash	15(45.5)	Anti Sm	13(39.4)
Renal	14(42.4)	Anti RNP	13(39.4)
Leukopenia	13(39.4)	Anti Ro	10(30.3)
Positive direct coombs	10(30.3)	Antiphospholipid	8(24.2)
Oral ulcer	9(27.3)	Anti La	7(21.2)
Alopecia	7(21.2)	Anti Jo1	2(6.1)
Chronic cutaneous rash	6(18.2)		
Serositis	5(15.2)		
AIHA	2(6.1)		
Synovitis	2(6.1)		

AIHA: Autoimmune hemolytic anemia
SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000
ANA: Anti-nuclear antibody
Anti dsDNA: Anti-double stranded DNA antibody
Anti Sm: Anti-Smith antibody
Anti RNP: Antinuclear ribonucleoprotein antibody
Anti Ro: Anti-Ro antibody
Anti La: Anti-La antibaody
Anti Jo1: Anti-Jo1 antibody

with MRI changes (p>0.05) but abnormal MRIs were commonly found in those with delirium, psychosis, seizures or abnormal behavior (Fig. 2). There was also no significant correlation between patients with cutaneous vasculitis or any of the NPSLE syndromes with the brain MRI changes (P>0.05). The abnormalities on magnetic resonance angiography (MRA)/ magnetic resonance venography (MRV) were venous infarction (1), sagittal sinus venous thrombosis (1) and irregularities of both middle cerebral arteries (2) (Table II).

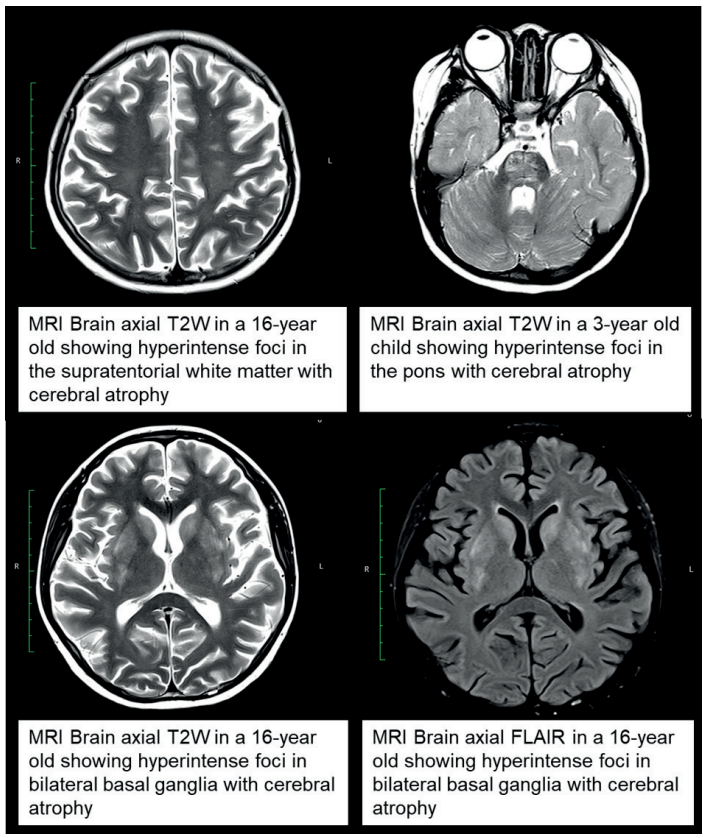


Fig. 2. Magnetic resonance images of the patients with neuropsychiatric lupus.

Table II. Magnetic resonance imaging and magnetic resonance angiography/venography findings in patients with neuropsychiatric lupus.

			Frequency		
MRI (n=28)					
Normal	4				
Abnormal	24				
Sites with abnormalities	Supratentorial white matter (n=17)	Brainstem (n=5)	Cortical grey matter (n=3)	Basal ganglia (n=4)	Cerebellum (n=2)
Types of abnormalities					
Signal change abnormalities					
T2 hyperintensities	17	5	2	3	2
FLAIR hyperintensities	16	5	3	4	2
Parenchymal defect	1				
Atrophy	11				
MRA/MRV (n=5)					
Normal	2				
Abnormal					
MRA abnormalities	2				
MRV abnormalities	1				

MRI: magnetic resonance imaging, MRA: magnetic resonance angiography, MRV: magnetic resonance venography

All patients received pulsed intravenous Methylprednisolone during their acute presentation. Of the 28 patients, all 20 who had CNS involvement were treated with intravenous Cyclophosphamide and 2 of these patients received two separate courses of 12 monthly intravenous Cyclophosphamide for each of their separate NPSLE episodes. Additionally, two patients on Cyclophosphamide received Rituximab as well. Of the eight patients with PNS involvement, two patients were treated with Mycophenolate mofetil while the other six received Azathioprine and one patient received additional intravenous Immunoglobulin (IVIG). All 16 patients with seizures required anti-epileptic drugs and three patients received anti-psychotic drugs. One patient had anticoagulant therapy for antiphospholipid syndrome with multiple cerebral sino-venous thrombosis.

At 12 months post diagnosis of NPSLE, 24 out of 28 patients (85.7%) had complete recovery with no neurological deficits despite initial high disease activity. Two patients (7.1%) had mood disorders, one patient (3.6%) developed epilepsy and one patient (3.6%) had both residual dystonic tremors and cortical blindness. In patients with two episodes of NPSLE, 60% (3/5) had complete recovery. There were two deaths (7.1%) in this cohort; both from infections but were unrelated to the treatment given for NPSLE.

Discussion

Our paper is the first to highlight characteristics of 28 children with NPSLE in Malaysia amongst a heterogenous South Asian population, contributing to global knowledge of pediatric NPSLE and helping to advance our understanding of the genetic influence on the lupus manifestations and response to therapy. Our findings are consistent with other studies which show a prevalence of NPSLE between 14-25% with the majority occurring in the first year of the disease.[7,9,14,15]

Neuropsychiatric lupus is a complex entity with broad range of categories under the American College of Rheumatology criteria.[12] This clinical diversity makes accurate assessments difficult in both adult and pediatric studies.[4,6,7,16] The most common reported clinical manifestations are diffuse neuropsychiatric syndromes and CNS involvement which is similarly seen in our cohort.[3,4,6,14,17]

Seizures featured predominantly in our cohort and this high prevalence of seizures was similarly seen in other studies involving Asian children.[3,4,9,10] On the contrary, non-Asian children tend to present more with headaches, cognitive dysfunction and psychosis.[6,7,16,18] These major neurological manifestations were also associated with higher overall disease activity which supports the current understanding that NPSLE is an immunologically and inflammatory active state although no single immunological pathway has yet to be identified.[2,19,20] The presence of cutaneous vasculitis did not correlate with major NPSLE syndromes (apart from cranial neuropathy) in our study and this lends further support to the growing body of evidence that vasculitis may not have a prominent role in the pathogenesis of NPSLE.[20,21]

Anti-phospholipid antibodies have been reported to be associated with distinct NSPLE syndromes such as movement disorders, seizures and cerebrovascular disease but these associations were not identified in our patients.[7] However, Anti-dsDNA, Anti-Smith (Sm) and Antinuclear ribonucleoprotein (antiRNP) antibodies which have been reported to be strongly associated with NPSLE syndromes, were similarly found in high frequencies in our patients.[19] Both Anti-dsDNA and Anti-RNP antibodies have been shown to have a possible correlation with seizure disorders. Studies have shown that these antibodies can cross the disrupted blood brain barrier in patients with SLE and demonstrate cross-reactivity with anti-N-methyl-D-aspartate receptor and neuronal surface antigens; causing neurotoxicity and inducing neuronal death.[2,22]

Interestingly, despite severe and often dramatic clinical symptoms, CSF examination was normal in two thirds of the 14 samples. Even when abnormal, the changes were mild with no specific correlation to clinical manifestations. Thus, CSF examination does not seem to provide any additional information to support the diagnosis of NPSLE; but is nevertheless important to exclude CNS infections.[20,22]

Magnetic resonance imaging abnormalities were seen in more than 80% of our cohort in contrast to prevailing studies which showed predominantly normal MRIs for both adult and pediatric NPSLE series (42-59%).[7,16,20,22-24] The most common abnormality in our study were white matter hyperintensities which is also reported in other studies.[20,22-25] These changes may suggest small vessel disease or vasculitis, but their specificity has yet to be established as these lesions have also been reported in patients with SLE and no neuropsychiatric findings.[23] We could not determine any correlation between NPSLE syndromes with specific MRI findings in our study due to the small number of subjects but no specific correlation has been found either in other published studies.[20,22,23] In our experience, even patients with severe clinical symptoms such as psychosis or seizures may not demonstrate any abnormalities on MRI.

The most common EEG change in our patients were diffuse slow and attenuated background waves, consistent with other studies and suggestive of a diffuse immunopathological process in NPSLE.[26] None showed any epileptiform discharges despite the occurrence of seizures but most of our EEG recordings were delayed due to resource constraints.

Corticosteroids is the mainstay of therapy for SLE and plays a crucial role in the acute management of NPSLE. While there is currently no true consensus on the optimal therapeutic regime for Juvenile onset NPSLE; clinical experience seems to point to the efficacy of corticosteroids in combination with Cyclophosphamide.[18,20,24,27] Other modalities such as Rituximab, IVIG and even plasmapheresis have been reportedly used when there is poor response.[20] However, the evidence for these treatments remain largely anecdotal with reports mainly from small clinical cohorts or case reports.[20,24] Mycophenolate mofetil and Azathioprine are frequently used in mild NPLSE and also recommended for maintenance therapy for all patients[20,24,27] Anticoagulation and antiplatelet therapy is recommended for any thrombotic process.[24,27] Patients may need other supportive therapies such as anti-epileptic drugs or psychiatric medications during the acute episode.[20,24,27] While there remains a scarcity of randomized controlled studies due to the rarity of this disease particularly in children coupled with the heterogeneity of disease presentations, there are international guidelines to assist with the management of this difficult condition.[27]

Our study is too small to offer any specific therapeutic approach but our cohort appears to have had good response to the combination of steroids and intravenous Cyclophosphamide or Rituximab. The overall survival rate and absence of permanent neuropsychiatric damage in our cohort is comparable to data from developed countries.[7,20,25,28]

The main limitation in our study was our small sample size and the retrospective nature of our study. There may also have been under-recognition of NPSLE in our cohort of JSLE patients, given that many of the features are non-specific. In addition, cognitive and subtle mood disorders may be missed in the absence of routine objective testing of all JSLE patients as similarly highlighted in other studies. The strength of our cohort is that we are the sole tertiary referral center for the whole country and are therefore able to give a fairly accurate reflection of this condition in Malaysia.

Neuropsychiatric lupus is rare in children but can be present in any patient with JSLE. It has a higher incidence within the first year of JSLE diagnosis and is associated with high disease activity. Diagnosis of NPSLE remains a challenge as a normal CSF finding does not exclude its presence, and MRI abnormalities

when present are often nonspecific. Although severe neurological manifestations are common, majority have good prognosis with complete neurological recovery following early recognition and intensive treatment.

Acknowledgements

The authors wish to thank the Director General of Health, Ministry of Health Malaysia for allowing their facilities to be used for this research.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SCL, SPT; data collection: SCL, YL, BJ, RFAK; analysis and interpretation of results: SCL, SPT, YL, BJ, RFAK; draft manuscript preparation: SCL, YL, SPT. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

This is an investigator-initiated research and received no external funding.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Smith PP, Gordon C. Systemic lupus erythematosus: clinical presentations. Autoimmun Rev 2010; 10: 43-45.
2. Duarte-Delgado NP, Vásquez G, Ortiz-Reyes BL. Blood-brain barrier disruption and neuroinflammation as pathophysiological mechanisms of the diffuse manifestations of neuropsychiatric systemic lupus erythematosus. Autoimmun Rev 2019; 18: 426-432.
3. Haji Muhammad Ismail Hussain I, Loh WF, Sofiah A. Childhood cerebral lupus in an Oriental population. Brain Dev 1999; 21: 229-235.
4. Yu HH, Lee JH, Wang LC, Yang YH, Chiang BL. Neuropsychiatric manifestations in pediatric systemic lupus erythematosus: a 20-year study. Lupus 2006; 15: 651-657.
5. Khajezadeh MA, Zamani G, Moazzami B, Nagahi Z, Mousavi-Torshizi M, Ziaee V. Neuropsychiatric involvement in juvenile-onset systemic lupus erythematosus. Neurol Res Int 2018; 2018: 2548142.
6. Kivity S, Agmon-Levin N, Zandman-Goddard G, Chapman J, Shoenfeld Y. Neuropsychiatric lupus: a mosaic of clinical presentations. BMC Med 2015; 13: 43.
7. Benseler SM, Silverman ED. Neuropsychiatric involvement in pediatric systemic lupus erythematosus. Lupus 2007; 16: 564-571.
8. Olfat MO, Al-Mayouf SM, Muzaffer MA. Pattern of neuropsychiatric manifestations and outcome in juvenile systemic lupus erythematosus. Clin Rheumatol 2004; 23: 395-399.
9. Sit JKK, Chan WKY. Risk factors for damage in childhood-onset systemic lupus erythematosus in Asians: a case control study. Pediatr Rheumatol Online J 2018; 16: 56.
10. Suwanpakdee P, Nabangchang C, Lumpaopong A. Neuropsychiatric manifestations in pediatric systemic lupus erythematosus. J Med Assoc Thai 2017; 100: 1196.
11. Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012; 64: 2677-2686.
12. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum 1999; 42: 599-608.
13. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002; 29: 288-291.
14. AlE'ed A, Vega-Fernandez P, Muscal E, et al; CARRA Neuropsychiatric Systemic Lupus Erythematosus Working Group. Challenges of diagnosing cognitive dysfunction with neuropsychiatric systemic lupus erythematosus in childhood. Arthritis CareRes (Hoboken) 2017; 69: 1449-1459.
15. Harel L, Sandborg C, Lee T, von Scheven E. Neuropsychiatric manifestations in pediatric systemic lupus erythematosus and association with antiphospholipid antibodies. J Rheumatol 2006; 33: 1873-1877.

16. de Amorim JC, Frittoli RB, Pereira D, et al. Epidemiology, characterization, and diagnosis of neuropsychiatric events in systemic lupus erythematosus. Expert Rev Clin Immunol 2019; 15: 407-416.

17. Demirkaya E, Bilginer Y, Aktay-Ayaz N, et al. Neuropsychiatric involvement in juvenile systemic lupus erythematosus. Turk J Pediatr 2008; 50: 126-131.

18. Faria R, Gonçalves J, Dias R. Neuropsychiatric systemic lupus erythematosus involvement: towards a tailored approach to our patients? Rambam Maimonides Med J 2017; 8: e0001.

19. Bertsias GK, Boumpas DT. Pathogenesis, diagnosis and management of neuropsychiatric SLE manifestations. Nat Rev Rheumatol 2010; 6: 358-367.

20. Magro-Checa C, Zirkzee EJ, Huizinga TW, Steup-Beekman GM. Management of neuropsychiatric systemic lupus erythematosus: current approaches and future perspectives. Drugs 2016; 76: 459-483.

21. Ho RC, Thiaghu C, Ong H, et al. A meta-analysis of serum and cerebrospinal fluid autoantibodies in neuropsychiatric systemic lupus erythematosus. Autoimmun Rev 2016; 15: 124-138.

22. Al-Obaidi M, Saunders D, Brown S, et al. Evaluation of magnetic resonance imaging abnormalities in juvenile onset neuropsychiatric systemic lupus erythematosus. Clin Rheumatol 2016; 35: 2449-2456.

23. Ainiala H, Dastidar P, Loukkola J, et al. Cerebral MRI abnormalities and their association with neuropsychiatric manifestations in SLE: a population-based study. Scand J Rheumatol 2005; 34: 376-382.

24. Govoni M, Hanly JG. The management of neuropsychiatric lupus in the 21st century: still so many unmet needs? Rheumatology (Oxford) 2020; 59(Suppl5): v52-v62.

25. Lopes SRM, Gormezano NWS, Gomes RC, et al; Brazilian Childhood-onset Systemic Lupus Erythematosus Group. Outcomes of 847 childhood-onset systemic lupus erythematosus patients in three age groups. Lupus 2017; 26: 996-1001.

26. Mansour HE, Habeeb RA, El-Azizi NO, et al. Electroencephalography in systemic lupus erythematosus patients with neuropsychiatric manifestations. Egypt J Int Med 2020; 32: 11.

27. Bertsias GK, Ioannidis JPA, Aringer M, et al. EULAR recommendations for the management of systemic lupus erythematosus with neuropsychiatric manifestations: report of a task force of the EULAR standing committee for clinical affairs. Ann Rheum Dis 2010; 69: 2074-2082.

28. Groot N, Shaikhani D, Teng YKO, et al. Long-term clinical outcomes in a cohort of adults with childhood-onset systemic lupus erythematosus. Arthritis Rheumatol 2019; 71: 290-301.

The Turkish Journal of Pediatrics 2021; 63: 752-757
https://doi.org/10.24953/turkjped.2021.05.003

Original Article

Tobacco use among working adolescents and high school students in Turkey: evaluating the effect of the national tobacco control policy

Erkan Çakır[1], Hakan Yazan[1], Nazlı Karacabey[2], Ahmet Hakan Gedik[1], Ali Özdemir[3], Nezih Varol[4], Fazilet Karakoç[5], Refika Ersu[5], Bülent Karadağ[5], Elif Dağlı[5]

[1]Division of Pediatric Pulmonology, [2]Department of Pediatrics, Bezmialem Vakif University Faculty of Medicine, İstanbul; [3]Division of Pediatric Pulmonology, Mersin Children's Hospital, Mersin; [4]Marmara University Faculty of Health Education, İstanbul; [5]Division of Pediatric Pulmonology, Marmara University Faculty of Medicine, İstanbul, Turkey.

ABSTRACT

Background. In our previous published study conducted in 2006 before the national tobacco control program (NTCP), we found that working adolescents (WA) more frequently consumed cigarettes than high school students (HSS). The objective of the present study was to compare the smoking status of WA and HSS before and after the NTCP.

Methods. A questionnaire including questions about the participant's socio-economic level and smoking status was administered.

Results. There were 668 subjects in the 2006 study and 869 subjects in the 2015 study. When we compared the 2015 results with the 2006 study, while there was a significant decline in the ever smokers ($p < 0.001$), there was no difference in current smokers in both the female and male WA groups. In the HSS group, there was a significant decline in ever smokers ($p < 0.01$), for both females and males. While there was a significant decline in current female smokers (p=0,002), no significant decrease was found in current male smokers ($p > 0.05$) in the HSS group.

Conclusions. After the initiation of the NTCP, we have not seen a reduction in the smoking rates of both female and male WA and male HSS. The NTCP should particularly focus on the adolescent group in Turkey.

Key words: tobacco, adolescents, tobacco control.

Tobacco smoking is the most important avoidable public health problem in the world. Smoking causes pulmonary and cardiovascular complications.[1] With public awareness of hazardous toxic effects of tobacco consumption, smoking has declined, especially in high income countries, but low and middle income countries remain the targets of the tobacco industry. Turkey is one of the leading tobacco consumer countries in the world.[2] In 2006, the Turkish Government implemented a smoke-free legislation (Law No: 4207/5727) and a comprehensive tobacco control plan that banned smoking in closed public areas, a ban on smoking advertising through any media and or communication platform, pictorial health warnings about smoking, and prohibition of the sale of tobacco products to individuals under 18 years of age. This campaign has been in force continuously since then. It is estimated that there has been a 13% decline in smoking consumption nationwide following the implementation of these regulations and the resulting heightened public awareness of the harmful health effects of tobacco smoking.[1]

✉ Erkan Çakır
erkancakir1@yahoo.com

Received 11th February 2021, revised 24th March 2021, accepted 25th March 2021.

It is well known that smoking addiction starts at an early age.[3] The young population are at risk for a longer exposure to the toxic effects of tobacco smoking. There is limited data, regarding both the smoking status of adolescents and the effect of the tobacco control policy on smoking consumption. In our previous published study conducted in 2006 before the national tobacco control program was instituted, we found that working adolescents (WA) more frequently consume cigarettes and were exposed to second-hand (cigarette) smoking at home more frequently than non-worker high school students (HSS).[4] The objective of the present study was to compare the smoking status of WA and HSS and to evaluate the effect of the national tobacco control policy on these groups.

Material and Methods

Both studies, in 2006 and 2015, were conducted in a district of Istanbul, which is populated with low to middle income people. The same target population and area were chosen from WA and HSS in both the earlier and later study. The WA group were students at a Vocational Training Center which offers apprenticeship training. The apprentices study at school once a week while they work during the rest of the week. Their training areas at the center include hairdressing, lathe-finishing, motor repair and textile manufacture. The HSS group was from a high school, which is located in the same district.

The study was approved by the local ethics committee of the Bezmialem Vakif University (Number: 36/12, date: 08.04.2013). Additionally, permissions were obtained from the Kartal Province National Educational Directorate, the management of the high school, and the parents of the participants. All study participants were informed about the study and its objectives and signed written consent forms before inclusion in the study. It was explained to the participants that the questionnaire results would be kept according to the principle of confidentiality. The questionnaires in the current study were designed in the same manner as the 2006 study.[4] A questionnaire included questions about the participant's socio-economic and smoking status. All questionnaires were carried out face-to-face by the same researcher. Participants who had 100 or more cigarettes in their lifetime and currently smoked cigarettes were considered current smokers. Adolescents who had tried at least one cigarette in their lifetime were considered ever smokers.[5] Smoking at home was defined as existence of an adult who was a current smoker at home.

In this study, we first compared the smoking status of the WA and HSS groups in the 2015 study. Then, we compared the 2006 and 2015 data for the WA and HSS groups with each other.

Statistical analysis

During the assessment of the study data, we described our numerical parameters with mean and standard deviation values while we investigated the distribution of the categorical measurements by frequency and percentages. An independent samples t-test was used for the evaluation of numerical parameters with normal distribution, and the Mann–Whitney U-test was used for the evaluation of parameters without normal distribution. A chi-square test was used in the univariate assessment of our parameters, also performed classifying numerical parameters. The Pearson chi-square, Fisher's exact chi-square, the Fisher Freeman Hatlon exact chi-square, and Yates correction chi-square tests were used for comparison of qualitative data. The results were evaluated at a 95% confidence interval and at a significance level of $p< 0.05$. The SPSS for Windows 13.0 and NCSS (Number Cruncher Statistical System) programs were used for the statistical analysis.

Results

There were 554 participants in the WA group and 244 subjects in the HSS group in the 2015 study versus 353 subjects in the WA group and 315 subjects in the HSS group in the 2006

study. Table I summarizes the demographic characteristics and smoking status of the participants of the 2015 study. Those who had ever smoked (ever smokers) (p <0.001), current smokers (p<0.001), and smoking exposure at home (p<0.005) were significantly higher in the WA group than HSS group.

Comparison of demographic characteristics and the smoking status of WA in 2006 and in 2015 is shown in Table II. There was no significant difference regarding the median age and gender between the WA groups. While there was a significant decline in the ever smokers (p <0.001), there was no difference in current smokers for both females and males.

Comparison of demographic characteristics and smoking status of HSS in 2006 and 2015 is shown in Table III. There was a significant decline in ever smokers (p <0.01), for both females and males. While there was a significant decline in current female smokers (p=0.002), no significant decrease was found in current male smokers (p >0.05).

There was no significant difference according to smoking exposure at home between 2006 and 2015 in both groups.

We also evaluated attitudes and behaviors of WA and HSS in 2015 about the smoking bans (Table IV). There were similar views in the two groups.

Table I. Demographic characteristics and smoking status of the participants in 2015.

		Working adolescents (n=554)		High school students (n=244)		p
Mean age		17.67±1.34		16.54±0.93		>0.05
		n	%	n	%	
Gender	Female	90	16.2	114	46.7	<0.001
	Male	464	83.8	130	53.7	
Ever smoker	Female	52	57.8	24	21.1	<0.001
	Male	282	60.8	43	33.1	<0.001
	Total	334	60.3	67	27.5	<0.001
Current smoker	Female	35	38.9	3	3.6	<0.001
	Male	203	43.8	17	13.1	<0.001
	Total	238	43.0	20	8.2	<0.001
Smoking at home		382	69.0	143	58.6	0.005

Table II. Comparison of demographic characteristics and the smoking status of the working adolescents in 2006 and 2015.

		Working adolescents, 2006 (n=353)		Working adolescents, 2015 (n=554)		p
Median age (year)		17 (14-20)		17 (14-19)		>0.05
		n	%	n	%	
Gender	Female	54	15.3	90	16.2	>0.05
	Male	299	84.7	464	83.8	
Ever smoker	Female	38	70.3	52	57.8	0.001
	Male	236	78.9	282	60.8	0.001
	Total	274	77.6	334	60.3	0.001
Current smoker	Female	19	35.1	35	38.9	>0.05
	Male	130	43.4	203	43.8	>0.05
	Total	149	42.2	238	43.0	>0.05
Smoking at home		266	75.4	382	69	>0.05

Table III. Comparison of demographic characteristics and the smoking status of the high school students in 2006 and 2015.

		High school students, 2006 (n=315)		High school students, 2015 (n=244)		p
Median age (year)		17 (14-19)		17 (14-19)		>0.05
		n	%	n	%	
Gender	Female	62	19.7	114	46.7	<0.001
	Male	253	80.3	130	53.3	
Ever smoker	Female	33	53.2	24	21.1	<0.001
	Male	136	53.8	43	33.1	<0.001
	Total	169	53.7	67	27.5	<0.001
Current smoker	Female	10	16.1	3	2.6	0.002
	Male	50	19.8	17	13.1	>0.05
	Total	60	19	20	8.2	<0.001
Smoking at home		195	61.9	143	58.6	>0.05

Table IV. Knowledge and behavior of students concerning the smoking ban.

		Working adolescents	High school students	p
		%	%	
Has anyone quitted smoking in your family after the ban?	Yes	23	30	>0.05
Do you support the ban	Yes	90	91	>0.05
Should the government take action against smoking?	Yes	89	88	>0.05
Is passive smoking harmful?	Yes	89	89	>0.05
Are smoking bans enough?	No	57	52	>0.05
Do you know that there are penalties for businesses that allow smoking?	Yes	99	93	>0.05
Do you know that if a person sells cigarettes to someone under 18 they can be imprisoned?	Yes	56	54	>0.05
Do you know that tobacco companies cannot advertise?	Yes	80	80	>0.05
Do you know that waste such as cigarette butts cannot be thrown?	Yes	54	47	>0.05

Discussion

In this study, the smoking behavior of WA and HSS were evaluated after the national tobacco control policy went into effect. There was no decline in the current smoker ratios of the WA before and after the national smoking policy was initiated. In the HSS, the current smoker ratio declined in girls but not in boys. As in 2006, the WA smoking ratio was higher than that for HSS in 2015.

Smoking most often starts in adolescence; thus, to prevent smoking, interventions should be done in this period. It is estimated that smoking varies between 13.8% and 28% for the HSS group in different parts of the world. According to the Global Youth Tobacco Survey (GYTS) study that was conducted between 2013-2014 and included forty-five countries, the ratio of current smokers of high school age varies between 1.7% to 28.9% (Kazakhstan 1.7% and Timor-Leste 28.9%).[6]

In 2006 in Turkey, a school-based survey of 15,957 students between the ages of 13 and 15 years was conducted, and it was established

that around one third of students had already tried smoking, and 10% were current smokers.[7] In most studies, current smokers were predominantly male. However, a study from the Turkish city of Izmir showed that females were slightly higher current smokers, but that was not statistically significant (F/M: 23.7% & 22.7%).[8]

There are a few studies regarding the smoking status of WA in the literature. The frequency of smoking of WA was found to be significantly higher than that of HSS in Turkey (ranging between 21.7% and 50.5%) as in our study.[4,9,10,11] Similar ratios were seen in a few studies in other countries.[12,13]

The National Tobacco Law is the leading regulation along with other comprehensive smoke-free environmental campaigns in Turkey. There are not enough studies that compare the smoking status before and after National Tobacco Policy was instituted. In our study, we compared the smoking status of both WA students and HSS students before and after the National Tobacco Law was initiated. While there was a significant reduction in the ever and current smoker ratios in the high school girl group, there was no reduction in current smoker ratios in high school boys and both female and male working adolescents. Our study is the only study in Turkey that compares the smoking ratios in the same population before and after the smoking ban. In the literature, we found two studies that evaluated the smoking ratios before and after the smoking ban in Turkey, but the study design and the groups were different from our study. The study population of these two studies were 10th grade high school students but not in the same area. Ever smoker ratios were evaluated in these studies. There was a significant decrease in the ever smoker ratio (the 2005 study, 37% and the 2013 study, 24.4%). Current smokers were not evaluated in these studies.[14,15] A study of adults that evaluated tobacco control activities in Turkey found a 13% reduction in current smokers from 2006 to 2013 (31.7% and 27.1%).[16]

There are a few studies in the literature from around the world that compare the smoking ratio in HSS. In a study from Argentina, after the implementation of anti-smoking regulations in 2011, the overall proportion of youths aged approximately 13-15 years who reported ever smoking a cigarette declined from 52% in 2007 to 41.9% in 2012.[17]

To the best of our knowledge, there is no study comparing WA before and after smoking bans either in Turkey and the world at large; thus, our study may be the first one.

We also evaluated smoking exposure in both groups, comparing 2006 and 2015. Smoking exposure at home was reduced from 2006 to 2015, but it was not statistically significant. Bilir et al.[16] assessed second hand smoke in various places before and after the ban. In Bilir's study, after the National Tobacco Policy was instituted, exposure to second hand smoke reduced in restaurants (55.9% and 12.9%), homes (56.3% and 38.3%), workplaces (37.3% and 15.6%), public transportation (16.5% and 10.4%), and government buildings (11.3% and 6.5%).

In this study, we also evaluated the knowledge and behavior of adolescents in terms of the smoking ban in 2015. Both the WA and HSS groups had similar views about the smoking ban, and most of the adolescents supported the ban.

In conclusion, after the National Tobacco Control Program was instituted, we did not see a reduction in the smoking ratios of working adolescents and high school boys. Therefore, the National Tobacco Control program was found to be less effective than expected, especially in the adolescent group. New studies encompassing a large area evaluating smoking status after the ban are needed. New policies against smoking are also needed to reduce the smoking ratios, especially in adolescents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EÇ,

FK, RE, BK, ED; data collection: BNK, AHG; analysis interpetation of results: EÇ, AÖ, HY, NV; draft manuscript preperation: EÇ, AÖ, HY. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the local ethics committee of the Bezmialem Vakif University (Number: 36/12, date: 08.04.2013).

Source of funding

The authors declared that this study has received no financial support.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Bozkurt AI, Sahinoz S, Ozcirpici B, et al. Patterns of active and passive smoking and associated factors, in the South-east Anatolian Project (SEAP) region in Turkey. BMC Public Health 2006; 6: 15.
2. World Health Organization. Enforcing bans on tobacco advertising, promotion and sponsorship. Report on the global tobacco epidemic -2013. Available at: https://www.who.int/publications/i/item/9789241505871 2013 (Accesssed on November 2, 2019).
3. Arabi Z. An epidemic that deserves more attention: epidemiology, prevention, and treatment of smokeless tobacco. South Med J 2007; 100: 890-894.
4. Cakir E, Karakoc F, Ersu R, Karadag B, Varol N, Dagli E. Comparing the smoking status of working adolescents with adolescents enrolled in high school in Turkey. Pediatr Allergy Immunol Pulmonol 2013; 26: 32-34
5. National Center for Health Statistics. Available at: https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm (Accessed on May 16, 2021).
6. D'Angelo D, Ahluwalia IB, Pun E, Yin S, Palipudi K, Mbulo L. Current cigarette smokers, access, and purchases from retail outlets among students aged 13-15 years global youth tobacco survey, 45 countries, 2013 and 2014. MMWR Morb Mortal Wkly Rep 2016; 65: 898-901.
7. Erguder T, Soydal T, Ugurlu M, Cakir B, Warren CW. Tobacco use among youth and related characteristics, Turkey. Soz Praventivmed 2006; 51: 91-98.
8. Goksel T, Cirit M, Bayındır Ü. İzmir ili lise öğrencilerinin sigara alışkanlığını etkileyen faktörler. Toraks Dergisi 2001; 3: 49-53.
9. Erbaydar T, Lawrence S, Dagli E, Hayran O, Collishaw NE. Influence of social environment in smoking among adolescents in Turkey. Eur J Public Health 2005; 15: 404-410.
10. Alikasifoglu M, Erginoz E, Ercan O, Uysal O, Kaymak-Albayrak D, Ilter O. Cigarette smoking among Turkish high school students. J Adolesc Health 2002; 30: 7-8.
11. Kutlu R. Meram çıraklık okulu öğrencilerinde sigara kullanma sıklığı. TSK Koruyucu Hekimlik Bülteni 2006; 5: 424-433.
12. Warren CW, Jones NR, Peruga A, et al; Centers for Disease Control and Prevention (CDC) Global youth tobacco surveillance, 2000-2007. MMWR Surveill Summ 2008; 57: 1-28.
13. Fagan P, Stoddard AM, Hunt MK, Frazier L, Girod K, Sorensen G. The feasibility of evaluating a tobacco control intervention for working youth. Tob Control 2003; 12(Suppl 4): IV34-IV39.
14. Ögel K, Taner S, Eke CY. Onuncu sınıf öğrencileri arasında tütün, alkol ve madde kullanım yaygınlığı: İstanbul örneklemi. Bağımlılık Dergisi 2006; 7: 18-23.
15. Evren C, Ogel K, Demirci A, Evren B, Yavuz BG, Bozkurt M. Prevalence of lifetime tobacco, alcohol and drug use among 10th grade students in İstanbul. Bull Clin Psychopharmacol 2014; 24: 201-210.
16. Bilir N, Ozcebe H. Tobacco control activities in Turkey. Turk J Public Health 2013; 11: 96-103.
17. Konfino J, Ferrante D, Goldberg L, Caixeta R, Palipudi KM, Centers for Disease Control and Prevention Tobacco use among youths-Argentina, 2007 and 2012. MMVR Morb Mortal Wkly Rep 2014; 63: 588-590.

The Turkish Journal of Pediatrics 2021; 63: 758-766
https://doi.org/10.24953/turkjped.2021.05.004

Original Article

Assessment of PD-L1 expression in patients with neuroblastoma and renal tumors

Seher Şener[1], Aylar Poyraz[2], Arzu Okur[1], Faruk Güçlü Pınarlı[1], Ceyda Karadeniz[1]

Departments of [1]Pediatric Oncology and [2]Pathology, Gazi University Faculty of Medicine Hospital, Ankara, Turkey.

ABSTRACT

Background. Programmed death 1 (PD-1) is a co-receptor which is located at the surface of cells like natural killer, monocytes, T and B cells. It has two ligands including programmed death ligand-1 (PD-L1) and ligand-2 (PD-L2). T cell functions are inhibited by activation of PD-1/PD-L1 pathway and this pathway is used by viruses and some tumor cells in order to escape from immune eradication. In our study we evaluated PD-L1 expression in the tissue specimens of patients with Wilms tumor, neuroblastoma and other renal tumors.

Methods. Totally 60 patients who were followed up at Gazi University Hospital with the diagnosis of neuroblastoma, Wilms tumor and other renal tumors were included. PD-L1 expression was examined in tumor samples of the patients.

Results. Positive staining with PD-L1 was detected only in two male patients. Both of them had neuroblastoma and advanced stage disease. None of the patients with Wilms tumor and other renal tumors had positive PD-L1 staining.

Conclusions. Unlike adult tumors, PD-L1 expression is not common in childhood tumors due to differences in immune system between children and adults. Further studies are needed to establish the importance and effects of PD-1/PD-L1 pathway in pediatric tumors.

Key words: programmed death-1, programmed death ligand-1, programmed death ligand-2, neuroblastoma, childhood renal tumors.

The Programmed death 1 (PD-1) receptor is located on the surface of natural killer (NK) cells, activated monocytes and some subgroups of dendritic cells as well as T cells and B cells.[1] The PD-1 receptor has two ligands; "programmed death ligand 1 (PD-L1)" and "programmed death ligand 2 (PD-L2)".[2,3] Although PD-L1 expression is easily inducible in many different cell types, PD-L2 expression is limited to only antigen presenting cells. This finding suggests that PD-L1 may play a more general and more specific role in inhibiting T cell activation than PD-L2. The PD-1/PD-L1 pathway is used by some tumors and viruses to escape from immune eradication. Activation of PD-1/PD-L1 pathway leads inhibition of T cell functions in secondary lymphoid tissues.[4]

PD-L1 is a transmembrane surface glycoprotein expressed in many solid tumors. There are many studies about the role of PD-1/PD-L1 pathway in adult malignancies and PD-1 and PD-L1 inhibiting agents have an important place in the treatment of adult cancers. However, the number of studies on the expression of PD-L1 in childhood solid tumors like neuroblastoma (NB), Wilms tumor (WT) and other primary renal tumors are not enough and results of these studies are conflicting. PD-1 and PD-L1 inhibiting agents have also begun to be used in the treatment of some childhood cancers.[5,6] Although PD-L1 blockade may be an appropriate

✉ Seher Şener
kzl_seher@hotmail.com

Received 16th October 2020, revised 9th February 2021, accepted 7th March 2021.

treatment option especially in patients with unfavorable prognosis and refractory disease despite treatment with current therapies, there is insufficient data about the effects of PD-L1 blockade on immune functions in pediatric patients.[7] Therefore, we aimed to investigate whether PD-L1 expression is increased in tumor tissues of NB, WT and other primary renal tumors [congenital mesoblastic nephroma (CMN), rhabdoid tumor of the kidney (RTK), renal cell carcinoma (RCC), clear cell sarcoma (CCS)] and whether there is a relationship between the degree of PD-L1 expression and the stage and prognosis of diseases.

Material and Methods

A total of 60 patients under 18 years of age who were followed between 2006 and 2017 with the diagnoses of NB (n=34), WT (n=17) and other renal tumors (n=9) at Department of Pediatric Oncology in Gazi University Faculty of Medicine were included in the study. We evaluated NB patients' characteristics including age, Turkish Pediatric Oncology Group (TPOG) risk group of tumor, Shimada histological classification, metastasis status, mitosis karyorrhexis index (MKI), neuron-specific enolase (NSE), vanillylmandelic acid (VMA)/homovanillic acid (HVA) ratio, ferritin and lactate dehydrogenase (LDH) level, maximum standardized uptake value (SUVmax) on PET/CT, MYCN gene amplification and other mutations.[8,9] We also assessed prognostic factors in patients with WT such as stage, tumor weight, tumor histology (presence of anaplasia) and metastasis status. In addition, stage and metastasis status in patients with other primary renal tumors were evaluated. Overall survival (OS) and event-free survival (EFS) were estimated in all patients. The time from diagnosis to death was defined as OS, and the time from diagnosis to relapse or treatment failure was defined as EFS.

PD-L1 expression was evaluated in tumor tissues of all cases. Hematoxylin & eosin stained slides of each case were examined and two separate slides of each case with dense tumor and the least necrotic ones were selected. Immunohistochemical staining of PD-L1 was performed on slides prepared by modified tissue microarray method. Formalin fixed sections (4 μm thickness) were exposed to anti-PD-L1 (clone SP142 rabbit monoclonal primary antibody) antibody and they were stained via OptiView DAB IHC Detection Set and OptiView Amplification kit on VENTANA BenchMark ultra instrument. Tonsil tissue was used as positive control. PD-L1 stained slides were evaluated by two different observers who were unaware of the clinical features of the cases. PD-L1 expression was evaluated in detail, assuming significant membranous staining in tumor cells, similar to previous studies. Staining of the tumor cells were scored according to percentage of stained cells as shown in Table I.[6,10] Tumor cells scored +1, +2 and +3 were considered positive for PD-L1 expression.

The study protocol was approved by the Institutional Ethics Committee of Gazi University Faculty of Medicine with the decision number 11/2017-546 of December 01, 2017. This study was supported by Gazi University Scientific Research Projects Unit. Informed consent was taken from all families.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL). Continuous variables were summarized as mean ± standard deviation (SD) while discrete data were presented as median and minimum-maximum values. Survival analysis was performed using Kaplan-Meier curves and log-rank tests.

Table I. Assessment of staining score and percentage of stained tumor cells.

Staining score in tumor cells	Percentage of stained tumor cells (%)
0	<1
+1	≥1 and <5
+2	≥5 and <50
+3	≥50

Results

A total of 60 cases enrolled in the study. Mean age was 41.6 ± 38.8 months and 53.3% (n=32) of the patients were male. Most of the patients were diagnosed NB (n=34) while other patients were diagnosed WT (n=17) and other renal tumors (n=9). The median follow-up was 79 (6-143) months.

Patients with neuroblastoma

Thirty-four patients with NB were included in the study. Mean age was 30.7 ± 27.9 months and 20 patients (58.8%) were male. The demographic characteristics of the cases are showed in Table II. Mean OS was 110.1 ± 10.1 months and EFS was 92.8 ± 11.6 months (Fig. 1).

Patients with Wilms Tumor

Seventeen patients with WT were included in the study. Mean age was 42.5 ± 39.7 months and 6 (35.2%) patients were male. The demographic characteristics of the cases are discussed in detail in Table III. Mean OS was 104.2 ± 9.3 months and EFS was 103.0 ± 8.8 months (Fig. 2).

Patients with Other Renal Tumors

Four patients with RCC were included in the study. The mean age of the patients with RCC was 138.6 ± 59.4 months and three of them were male. Two patients of them had stage II and others had stage IV disease. Tumor tissues of three patients with RCC were obtained before chemotherapy. Two patients of them had metastatic disease and they died at 4th and 21th months of treatment. Another two patients with RCC are still alive with complete remission for 103 months.

There were two patients with CMN. Ages of the patients with CMN were 10 and 20 months and one of them was female. Tumor tissues were obtained before chemotherapy in both of the patients with CMN and they did not have metastatic disease and no relapse or death was observed during the follow-up period. They are still alive with remission for 56 months.

Table II. Characteristics of patients with neuroblastoma.

Number of patients (n)	34
Age (months), mean (± SD)	30.7 ± 27.9
<18 months	15 (44%)
≥18 months	19 (56%)
Sex, male, n (%)	20 (58.8%)
Stage	
I	3 (%8.8)
II	1 (%2.9)
III	9 (%26.5)
IV	21 (%61.8)
Biopsy	
Before chemotherapy	32 (94%)
After chemotherapy	2 (6%)
SUVmax	
<2.5	6 (23.1%)
≥2.5	20 (76.9%)
Metastasis	20 (59%)
Metastasis site	
Bone marrow	13 (28.9%)
Bone	10 (22.3%)
Liver	6 (13.4%)
Lymph node	5 (11.1%)
Others	11 (32.3%)
TPOG risk group	
Low risk	5 (14.7)
Intermediate risk	13 (38.2%)
High risk	16 (47.1%)
Shimada classification	
Favorable	26 (76.5%)
Unfavorable	8 (23.5%)
MKI	
<2%	21 (61.8%)
2-4%	7 (20.6%)
>4%	6 (17.6%)
Primary region	
Abdomen	26 (76.5%)
Posterior mediastinum	6 (17.6%)
Pelvic	2 (5.8%)
NSE >100 ng/mL	30 (88%)
Ferritin >150 ng/mL	11 (32.4%)
VMA/HVA ratio >1	27 (79.4%)
LDH >1500U/L	12 (35.3%)
Mutations	
MYCN gene amplification	8 (23.5%)
1p deletion	18 (75%)
11q deletion	9 (37.5%)
Gain of 17q	17 (70.8%)
Hyperdiploid	8 (33.3%)
Relapse/resistant disease	9 (26.4%)
Death	7 (20.6%)

SUVmax: maximum standardized uptake value, TPOG: Turkish Pediatric Oncology Group, MKI: mitosis karyorrhexis index, NSE: neuron-specific enolase, HVA: homovanillic acid, VMA: vanillylmandelic acid, LDH: lactate dehydrogenase.

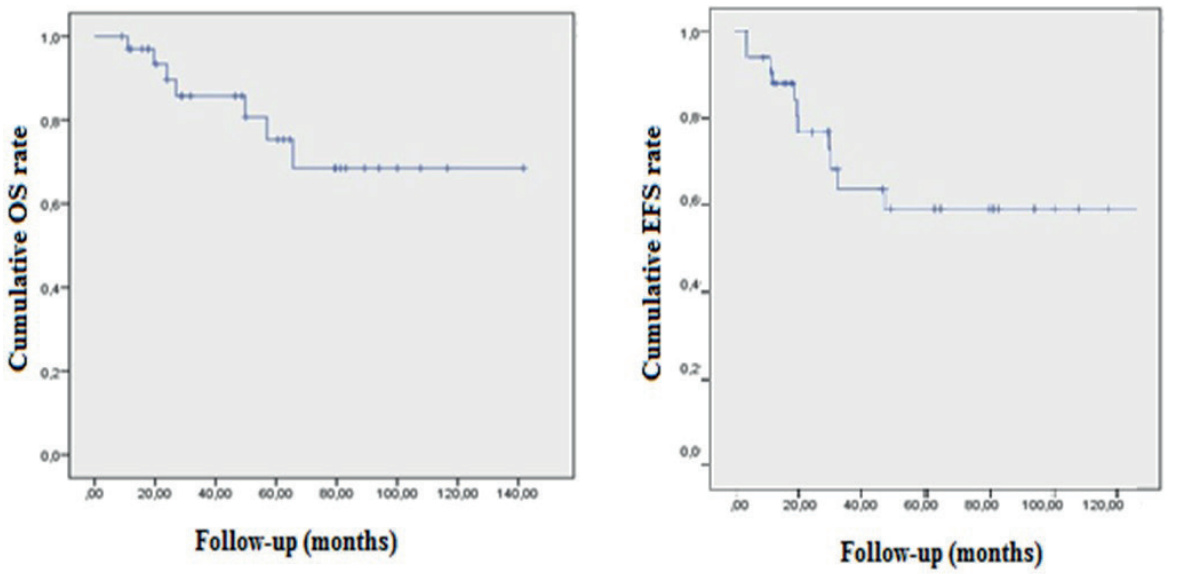


Fig. 1. OS and EFS curve of patients with neuroblastoma.

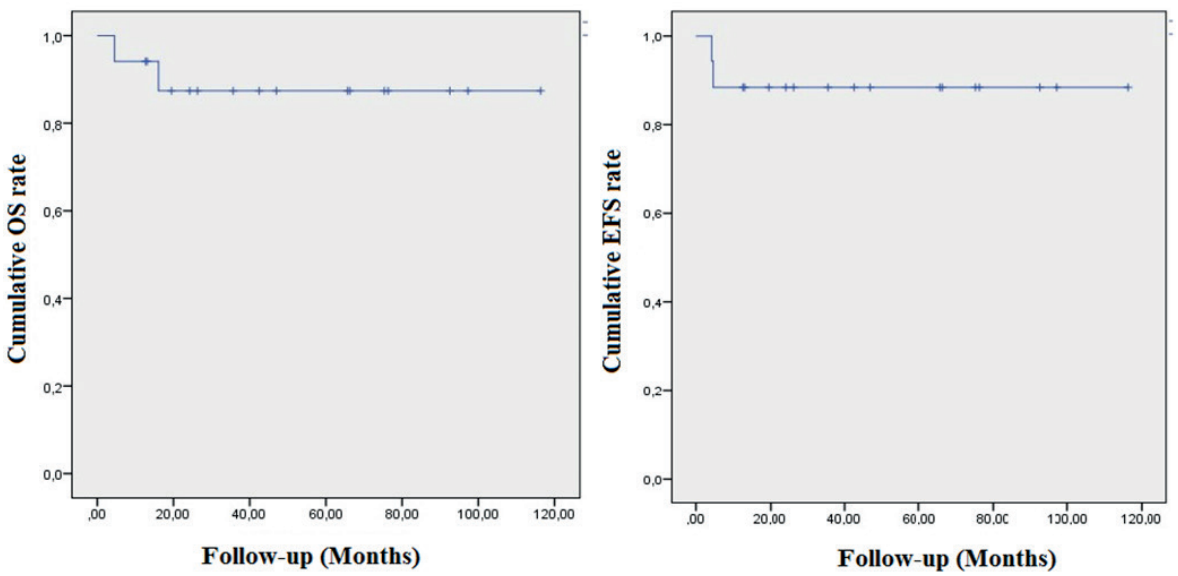


Fig. 2. OS and EFS curve of patients with Wilms tumor.

There were two patients with RTK. Ages of the patients with RTK were 10 and 102 months and one of them was male. Tumor specimen was obtained before chemotherapy in one patient and after chemotherapy in the other case. Both of the patients with RTK had metastatic disease and they died at 7th and 11th months of treatment.

The only patient with CCS diagnosis was male and he was 30 months of age. His tumor tissue was obtained after chemotherapy and he did not have metastatic disease. No relapse occurred and he is alive with remission for 83 months.

PD-L1 Expression in Patients with neuroblastoma

PD-L1 positive staining (membranous staining of 1% or more) (Fig. 3) was detected in two of 34 patients with NB (Table IV). In one patient with NB, PD-L1 staining was observed below 1% and

it was accepted negative for PD-L1 expression. Table V shows detailed clinical features of PD-L1 positive staining cases.

Table III. Characteristics of patients with Wilms tumor.

Number of patients (n)	17
Age (months), mean (± SD)	42.5 ± 39.7
Sex, male, n (%)	6 (35.2%)
Stage	
I	3 (17.6%)
II	3 (17.6%)
III	5 (29.4%)
IV	6 (35.4%)
Biopsy	
Before chemotherapy	6 (35.3%)
After chemotherapy	11 (64.7%)
Metastasis	5 (29.4%)
Metastasis site	
Lung	5 (100%)
Pathology	
Favorable	15 (88.2%)
Unfavorable/Anaplasia (+)	2 (11.8%)
Tumor weight	
<550 gr	11 (64.7%)
≥550 gr	6 (35.3%)
Relapse/resistant disease	1 (5.9%)
Death	2 (11.8%)

PD-L1 Expression in Renal Tumors

None of the patients with WT, CMN, RTK, RCC and CCS had positive staining for PD-L1. Therefore, the relationship between PD-L1 expression and prognostic factors, stages and prognosis of patients could not be evaluated in these group of patients.

Discussion

Significant advances occurred in targeted therapy thanks to the definition of various genetic mutations in childhood cancers. Immunotherapy which detects tumor cells as foreign bodies and activates the host immune response has become prominent in the treatment of some cancers, due to the strong relationship between tumor microenvironment and host immune system. Antibodies targeting the PD-1/PD-L1 pathway which was developed to induce immune system against tumor cells might also increase patients' survival with less toxicity than conventional chemotherapeutic regimens.[11] Therefore; the clinical importance of PD-L1 expression and its relationship with prognosis and treatment response should be highlighted in childhood cancers. So we evaluated PD-L1 expression in NB and childhood renal tumors.

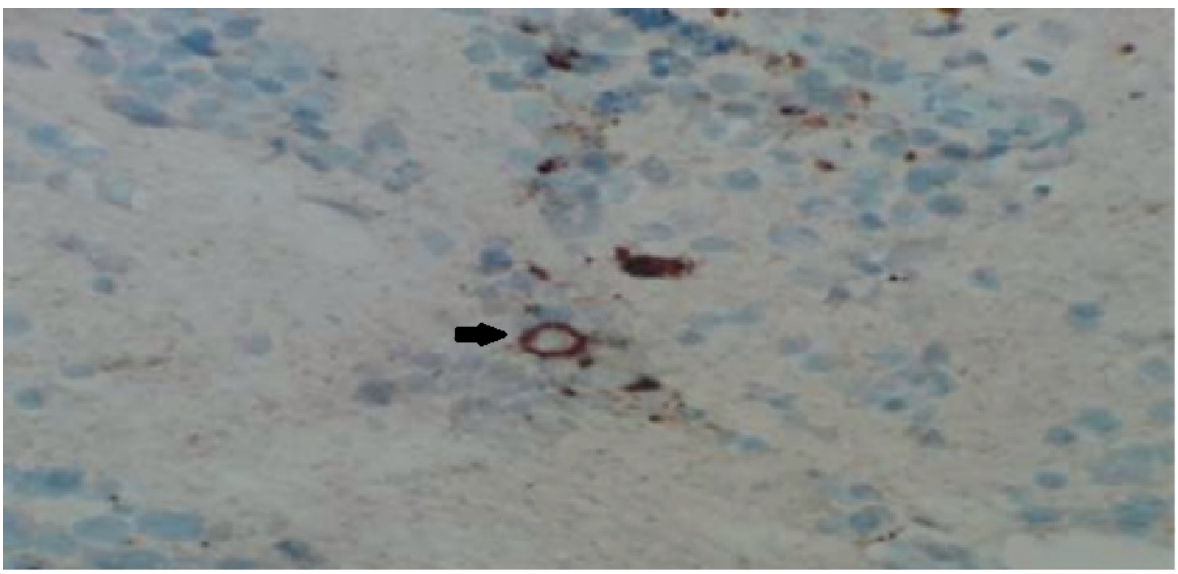


Fig. 3. PD-L1 staining between 1% and 5% of tumor cells in neuroblastoma (X100).

Table IV. PD-L1 expression and clinicopathologic features in patients with neuroblastoma.

	PD-L1 (-)	PD-L1 (+)
Sex (n=34)		
Female	14 (100%)	0
Male	18 (90%)	2 (10%)
Age (months)		
<18	14 (93.3%)	1 (6.7%)
≥18	18 (94.7%)	1 (5.3%)
Stage		
I, II, III, IVS	13 (100%)	0
IV	19 (90.5%)	2 (9.5%)
Biopsy		
Before chemotherapy	30 (93.7%)	2 (6.3%)
After chemotherapy	2 (100%)	0
TPOG risk group		
Low risk	5 (100%)	0
Medium risk	13 (100%)	0
High risk	14 (87.5%)	2 (12.5%)
Shimada classification		
Favorable	26 (100%)	0
Unfavorable	6 (75%)	2 (25%)
MKI		
<2%	20 (95.2%)	1 (4.8%)
2-4%	7 (100%)	0
>4%	5 (83.3%)	1 (16.7%)
MYCN gene amplification		
Negative	24 (92.3%)	2 (7.7%)
Positive	8 (100%)	0

TPOG: Turkish Pediatric Oncology Group, MKI: mitosis karyorrhexis index.

Table V. Demographic characteristics of two PD-L1 positive patients with neuroblastoma.

	Case 1	Case 2
Age (months)	24	14
Sex	Male	Male
Stage	IV	IV
Biopsy	Before chemotherapy	Before chemotherapy
SUVmax	4.9 (↑)	5.3 (↑)
Primary region	Posterior mediastinum	Abdomen
Metastasis	Yes	Yes
Metastasis site	Bone marrow	Bone, bone marrow, lymph node
TPOG risk group	High risk	High risk
Shimada classification	Unfavorable	Unfavorable
MKI	<2% (↓)	>4% (↑)
NSE	159 ng/mL (↑)	187 ng/mL (↑)
VMA/HVA ratio	1.2 (↑)	0.8
Ferritin	54 ng/Ml	197 ng/Ml (↑)
LDH	2300 U/L (↑)	1873 U/L (↑)
MYCN gene amplification	Negative	Negative
Hyperdiploid	Not assessed	No
1p deletion	Not assessed	Positive
11q deletion	Not assessed	Negative
Gain of 17q	Not assessed	Negative
Relapse/resistant disease	No	No
Death	No	Yes
Outcome	Exitus (28^{th} months)	Exitus (11^{th} months)

SUVmax: maximum standardized uptake value, TPOG: Turkish Pediatric Oncology Group, MKI: mitosis karyorrhexis index, NSE: neuron-specific enolase, HVA: homovanillic acid, VMA: vanillylmandelic acid, LDH: lactate dehydrogenase.

In our study, positive PD-L1 expression was observed in only two of 34 cases with NB. Due to the small number of PD-L1 positive cases (n=2), it was not suitable to perform statistical analysis and evaluate the relationship between PD-L1 expression and prognostic factors. Both of the PD-L1 positive cases were stage IV NB and high risk NB. Therefore; PD-L1 positivity was detected in 9.5% of the stage IV NB patients and in 12.5% of the high-risk NB cases. Both of the patients were within 8 cases with unfavorable prognosis according to Shimada classification (25%) and they were within 26 cases with negative MYCN gene amplification (7.7%). The two cases with positive PD-L1 expression died at 11^{th} and 28^{th} months of follow-up. Although there are many studies in the literature about PD-L1 expression in adult cancers, there are limited and small studies about PD-L1 expression in pediatric malignant tumors.[11-13] Moreover, contradictory results have been obtained in the current studies.[14-19] It

is reported that PD-L1 expression is increased in patients with unfavorable prognosis according to Shimada classification and advanced stage tumors in NB. PD-L1 expression correlates with poor overall survival, but there are also studies showing no relationship between PD-L1 expression and OS rate, tumor stage and histologic type.[14-16,20] In the study published by Uehara et al.[15] that includes 41 pediatric patients with NB high PD-L1 expression was detected in 5 patients (12%) with advanced stage tumors, and PD-L1 expression was associated with poor OS. In another study including 43 patients with NB, positive PD-L1 expression was shown in 31 cases (72%), and tumors with high PD-L1 expression were found to have a better OS rates than those without expression.[16] Majzner et al.[20] expressed that positive PD-L1 expression was observed in 17 (14%) of 118 patients with NB and no significant relationship was found between positive PD-L1 expression and OS rates at any stage or in any risk group. Dondero et al.[21] showed positive PD-L1 expression in 3 (15.7%) of 19 patients with metastatic NB. There is only one study which compares the prognostic factors and PD-L1 expression in patients with NB in the literature. In this study by Saletta et al.[19], positive PD-L1 expression was found in 48 (19%) of 254 patients with NB. Positive PD-L1 expression was detected in 19 (20.9%) of 91 cases with advanced stage NB and in 13 (17.6%) of 74 cases with high risk NB. Positive PD-L1 expression was reported in 38 (31.9%) of 119 cases with negative MYCN gene amplification and only two out of 34 patients with positive MYCN gene amplification (5.9%). Positive PD-L1 expression was significantly higher in patients with negative MYCN gene amplification than counterparts with positive MYCN gene amplification, and there was no statistically significant relationship between PD-L1 expression and other prognostic factors. Additionally, unlike other studies, it has been observed that NB patients with positive PD-L1 expression have better OS rates.[19]

PD-L1 positive staining was not detected in any of the patients with WT, CMN, RTK, RCC and CSS in our study. In the literature; Routh et al.[14] found that positive PD-L1 expression was detected in 11 (14%) of 81 patients with WT and PD-L1 expression has been reported to be a prognostic marker. In another study by Pinto et al.[18], no positive PD-L1 expression was detected in any cases with WT as in our study.

In our study, chemotherapy was not administered to 44 cases (73.3%) before biopsy. Thirty-two patients with NB (94%) and 6 patients with WT (35.3%) did not receive chemotherapy before biopsy, including the two patients with positive PD-L1 expression. Although biopsy samples of our patients were taken before chemotherapy (73.3%), PD-L1 expression rate was found to be low. Other studies in the literature also included patients with biopsies taken both before and after chemotherapy as similar with our study.[15,16,19,20] In the study by Saletta et al.[19] reported that 64 patients with NB (34.4%) received chemotherapy while 122 patients with NB (65.6%) did not receive chemotherapy before biopsy. In the same study, PD-L1 expression positivity was found to be higher in patients who did not receive chemotherapy prior to biopsy [27.9% (34/122)] than those who received chemotherapy [15.6% (10/64)].

Variations in PD-L1 expression rates in studies may also be associated with heterogeneity of the patient population and differences in the use of different scoring systems, antibody staining kits, staining procedures and antigen uptake techniques. For example, while evaluating PD-L1 staining positivity in tumor tissue, the median H-score was used as a cut-off value in some studies; while the median value of the staining percentage of 5% and above was considered as the cut-off value in other studies.[14-17] In our study, positive PD-L1 expression was defined as the presence of ≥1% staining in tumor cells. In previous studies, staining in ≥1% of tumor cells were also accepted as positive for expression of cytokines, IFN-ϒ and other immune markers.[6,19,22-24] We preferred the most commonly used scoring system; however, different results might have been attained with separate scoring systems.

The use of different antibodies (recombinant/polyclonal antibodies) in the immunohistochemical evaluation of PD-L1 expression in the studies may also play an important role in conflicting results. Different clone anti PD-L1 antibodies may lead to different results in the same tumor.[25] Majzner et al.[20] used clone 28-8 as PD-L1 antibody in their study while clone 5H1 was used as the PD-L1 antibody in another study published by Routh et al.[14] in patients with WT. The anti-PD-L1 antibody in these studies is different from the clone (SP 142) which we used in our study. However, a lower rate of response to tumor-associated antigens may occur in children, since burden of mutations is low but immunogenicity is higher in childhood cancers than adult counterparts. Therefore, PD-L1 expression in childhood tumors is lower than in adult cases in the literature.[26]

Modified tissue microarray method has been used the most in the literature for evaluating PD-L1 expression in childhood tumors as in our study.[19,20] When PD-L1 expression is evaluated in sections using modified tissue microarray method, it is observed that PD-L1 shows a very heterogeneous distribution within the tumor. Hence, immunohistochemical examination with modified tissue microarray method may cause false negativity in terms of PD-L1 expression. Therefore, it is more appropriate to use whole tissue sections in the evaluation of PD-L1 expression. However; complete tissue sections can be obtained in prospective studies.

The first limitation of our study is the small number of cases and retrospective design. Some cases could not be included in the study because we couldn't reach the tissue specimens of the patients diagnosed before 2006 and some of the samples were very small and insufficient to evaluate PD-L1 expression. In the beginning, we had aimed to evaluate the relationship between PD-L1 expression and prognostic factors but due to the small number of PD-L1 positive cases, statistical analysis could not be performed.

To conclude, although PD-L1 positivity rate is higher in adult tumors and is often associated with advanced stage disease with poor prognosis, it is very low in childhood tumors. This situation may be due to differences in the pathogenesis of childhood and adult tumors. However, prospective studies with larger populations are needed to clarify this issue.

Acknowledgment

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

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SŞ, AP, AO, FGP, CK; data collection: SŞ; analysis and interpretation of results: SŞ, AP, CK; draft manuscript preparation: SŞ, CK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the Institutional Ethics Committee of Gazi University Faculty of Medicine with the decision number 11/2017-546 of December 01, 2017.

Source of funding

No financial relationship.

Conflict of interest

All the authors declare no conflict of interest.

REFERENCES

1. Keir M, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Ann Rev Immunol 2008; 26: 677-704.
2. Latchman Y, Wood C, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2001; 2: 261-268.

3. Tseng S, Otsuji M, Gorski K, et al. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. J Exp Med 2001; 193: 839-845.

4. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA 2002; 99: 12293-12297.

5. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443-2454.

6. Herbst RS, Soria JS, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014; 515: 563-567.

7. van Dam LS, de Zwart VM, Meyer-Wentrup FA. The role of programmed cell death-1 (PD-1) and its ligands in pediatric cancer. Pediatr Blood Cancer 2014; 62: 190-197.

8. Aksoylar S, Varan A, Vergin C, et al. Treatment of high-risk neuroblastoma: National protocol results of the Turkish Pediatric Oncology Group. J Can Res Ther 2017; 13: 284-290.

9. Shimada H, Ambros IM, Dehner LP, et al. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer 1999; 86: 364-372.

10. Bertolini G, Bergamaschi L, Ferrari A, et al. PD-L1 assessment in pediatric rhabdomyosarcoma: a pilot study. BMC Cancer 2018; 18: 652.

11. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res 2013; 19: 1021-1034.

12. Afreen S, Dermine S. The immunoinhibitory B7-H1 molecule as a potential target in cancer: killing many birds with one stone. Hematol Oncol Stem Cell Ther 2014; 7: 1-17.

13. Ohigashi Y, Sho M, Yamada Y, et al. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res 2005; 11: 2947-2953.

14. Routh JC, Ashley RA, Sebo TJ, et al. B7-H1 expression in Wilms tumor: correlation with tumor biology and diseaser currence. J Urol 2008; 179: 1954-1960.

15. Uehara S, Nakahata K, Kawatsu M, et al. The PD-L1 expression increases after consecutive multimodal therapeies in neuroblastoma. Pediatr Blood Cancer 2015; 62: S334.

16. Chowdhury F, Dunn S, Mitchell S, Mellows T, Ashton-Key M, Gray JC. PD-L1 and $CD8^+$ $PD1^+$ lymphocytes exist as targets in the pediatric tumor microenvironment for immunomodulatory therapy. Oncoimmunology 2015; 4: e1029701.

17. Aoki T, Hino M, Koh K, et al. Low frequency of programmed death ligand 1 expression in pediatric cancers. Pediatr Blood Cancer 2016; 63: 1461-1464.

18. Pinto N, Park JR, Murphy E, et al. Patterns of PD-1,PD-L1 and PD-L2 expression in pediatric solid tumors. Pediatr Blood Cancer 2017; 64: e26613.

19. Saletta F, Vilain RE, Gupta AK, et al. Programmed Death-Ligand 1 expression in a large cohort of pediatric patients with solid tumor and association with clinicopathologic features in neuroblastoma. JCO Precis Oncol 2017; 1: 1-12.

20. Majzner RG, Simon JS, Grosso JF, et al. Assessment of programmed death-ligand 1 expression and tumor-associated immune cells in pediatric cancer tissues. Cancer 2017; 123: 3807-3815.

21. Dondero A, Pastorino F, Della Chiesa M, et al. PD-L1 expression in metastatic neuroblastoma as an additional mechanism for limiting immune surveillance. Oncoimmunology 2015; 5: e1064578.

22. Altungoz O, Aygun N, Tumer S, Ozer E, Olgun N, Sakizli M. Correlation of modified Shimada classification with MYCN and 1p36 status detected by fluorescence in situ hybridization in neuroblastoma. Cancer Genet Cytogenet 2007; 172: 113-119.

23. Madore J, Vilain RE, Menzies AM, et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res 2015; 28: 245-253.

24. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti PD-1 therapy. Clin Cancer Res 2014; 20: 5064-5074.

25. McLaughlin J, Han G, Schalper KA, et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. JAMA Oncol 2016; 2: 46-54.

26. Watson IR, Takahashi K, Futreal PA, Chin L. Emerging patterns of somatic mutations in cancer. Nat Rev Genet 2013; 14: 703-718.

The Turkish Journal of Pediatrics 2021; 63: 767-779
https://doi.org/10.24953/turkjped.2021.05.005

Original Article

Autism spectrum disorder in patients with inherited metabolic disorders-a large sample from a tertiary center

Tuba Çelen Yoldaş[1], Berrak Bilginer Gürbüz[2], Halil Tuna Akar[2], Elif Nursel Özmert[1], Turgay Coşkun[2]

Divisions of [1]Developmental Pediatrics, [2]Pediatric Metabolism, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. There is increased awareness regarding the co-occurrence of autism spectrum disorder (ASD) and inherited metabolic disorders (IMD), and this is crucial for the management of both diagnoses in clinical practice. We aimed firstly to report twenty-two patients with a dual diagnosis of IMD and ASD who are still being followed up in the child metabolism outpatient clinic; secondly to evaluate the time of both IMD and ASD diagnosis and the clinical progress of their metabolic disorders to underline treatable conditions.

Methods. Among the patients admitted to the Pediatric Metabolism outpatient clinic because of IMD, twenty-two of them who had a diagnosis of ASD were included in the study. Data of the patients were collected from their medical records. The most recent progress of the patients concerning their metabolic disorder was obtained from the patients' files.

Results. Six cases with Phenylketonuria, 2 cases with partial Biotinidase Deficiency, 3 cases with Cerebral Creatine Deficiency Syndrome (CCDS), 5 cases with Mucopolysaccharidosis (MPS) Type-3b, 2 cases with MPS Type-3a, 1 case with MPS Type 4, 2 cases with Hypervalinemia and 1 case with Maple Syrup Urine Disease were all diagnosed as also having ASD. The diagnoses of CCDS and MPS Type 3 were after the diagnosis of ASD. Phenylketonuria and Mucopolysaccharidosis were the most common diagnoses in our study. In addition, rare entities such as MPS Type 3b and Type 4 and Hypervalinemia were also reported to co-occur with autism.

Conclusions. Considering the co-occurrence of both disorders and implementing intervention strategies accordingly will certainly be beneficial in clinical practice and particularly in countries with a high rate of consanguinity.

Key words: autism spectrum disorder, inherited metabolic disorders, developmental delay.

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder and the prevalence of ASD tends to be an important problem as the rate (1 in every 68 children) has increased.[1] The heritability of ASD is quite heterogeneous and the co-occurrence of ASD with certain inherited metabolic disorders (IMD) is not rare. Furthermore the co-occurrence of both disorders is critical because the diagnosis of one may mask the other. This comorbidity is crucial for the management and progress of both diagnoses in clinical practice.[2]

The disruption of metabolic processes can give rise to a number of deviant effects in the central nervous system and lead to several neurodevelopmental disorders.[2-4] The altered neurodevelopmental process can cause psychiatric symptoms and sometimes these symptoms can occur before irreversible neurological lesions. To prevent or decrease any disabilities associated with IMD, it is important to detect the metabolic disease as early as possible.[3] As most metabolic disorders are autosomal recessive and common in countries where the rate of consanguinity is high, it is

✉ Tuba Çelen Yoldaş
tuba.celen@hacettepe.edu.tr

Received 19th October 2020, revised 5th March 2021, 12th April 2021, accepted 3rd May 2021.

particularly important for those communities to decrease the degree of mortality and morbidity with early detection.[5,6] When ASD is diagnosed in children with IMD, intervention strategies including social, behavioral, cognitive therapies can be planned in addition to the treatment of such metabolic disorders.[2,3] Conversely IMD should be considered in children with symptoms of ASD. Although metabolic disorders are usually recognizable with clinical signs such as dysmorphic features, ataxia, microcephaly, seizures, coma, hepatosplenomegaly and intellectual disability some of them can present by symptoms of ASD.[7] A metabolic work-up should be reserved for patients bearing any clinical indicator of a metabolic disorder.[3] If an IMD can be confirmed, a specific treatment may be accessible to avoid metabolic decompensation and therefore ASD may be preventable by the early treatment of IMD.[8] Furthermore many of these conditions are important for implications of genetic counselling.[3]

As a result of the scarce data relating to the co-occurrence of the two conditions, we firstly aimed to report twenty-two patients with a dual diagnosis of IMD and ASD who are still being followed up in the child metabolism outpatient clinic. Secondly we aimed to address the time of both IMD and ASD diagnosis and the clinical progress of their metabolic disorders to underline the treatable conditions which we did not come across in the literature.

Material and Methods

The study was conducted by the Pediatric Metabolism Division and Developmental Pediatrics Division, Department of Pediatrics. The study protocol was approved by the Ethics Committee of Hacettepe University Medical Faculty (GO 18/407) and the participation involved informed consent. Among the patients admitted to the Pediatric Metabolism outpatient clinic because of IMD between March 2015 and April 2018, twenty-five of them who had a diagnosis of ASD were included in the study. The inclusion criteria of the study was having a definitive diagnosis of both IMD and ASD and exclusion criteria was to be undergoing a process of diagnosis. Each patient was examined by a child metabolism subspecialist and a developmental pediatrician and was screened for the eligibility for the study. For the final evaluation 22 patients remained who had a definitive diagnosis of both IMD and ASD. The age at diagnosis of IMD and ASD was accepted as the first time a physician defined the diagnosis. Metabolic data of the patients were collected from their medical records. Data relating to the diagnosis of ASD was acquired from the patients' files and medical records. A definite diagnosis of ASD was accepted if the patient had an official special education therapy record. Diagnosis of ASD had been given at several different child and adolescent psychiatry departments, however the treatment and follow-up of all the patients for their metabolic disorders were being continued in the same pediatric metabolism department. The clinical progress of the patients concerning their metabolic disorder was obtained from the patients' files.

Data analysis

Most of the data was defined by numbers and percentages in the study. Numerical variables were evaluated for normality and parametric tests were used for data with normal distributions whereas non-parametric tests were used otherwise. Descriptive analysis and bivariate comparisons were used to assess group differences. Chi-square was used to examine the difference in gender across the subgroups of IMD. Differences between the median diagnostic ages of the two disorders were analyzed by Wilcoxon Signed Rank. Statistical tests were considered to be significant when $p<0.05$. Statistical analyses were performed using Statistical Package for the Social Sciences 23.0.

Results

Among the 25 patients, three did not meet the inclusion criteria of the study and finally 22

patients suffering from both disorders were enrolled in the study. In the total sample, 13 (59.1%) were male, 9 (40.9%) were female. The median ages of the patients at the time of diagnosis of IMD and ASD were 31.5 (range 0.13-108) months and 45 (range 18-100) months, respectively. There was no difference between the median diagnostic ages of the two disorders (p=0.20). Across the subgroups of IMD there was no difference in gender (p=0.761).

The diagnoses of Cerebral Creatine Deficiency Syndrome (CCDS) and MPS Type 3a-3b were made after the diagnosis of ASD with a 52 month and 18-24 month delay, respectively. On the contrary, the diagnoses of PKU, Biotinidase Deficiency, MSUD, and MPS 4 were all diagnosed before the diagnosis of ASD. The diagnostic age of ASD and IMD were similar in patients with hypervalinemia. Detailed clinical characteristics of the patients across the subgroups are given in Table I.

Case 1 was diagnosed with PKU at 6 months of age when investigating the reason for development delay in 2005. Nationwide neonatal screening program for PKU was started in 2006 in Turkey. Her blood phenylalanine level was measured as 1615.2 μmol/L (26.92 mg/dl) and she was diagnosed with classical PKU. Sequencing of the *PAH* gene (OMİM*612349) in the patient revealed homozygote mutation: IVS2+5G>C. The patient received a phenylalanine restricted diet. It was observed that the patient's compliance with diet therapy was not good. The mean serum phenylalanine value was 445.8 μmol/L (7.43 mg/dl). She was diagnosed with ASD at the age of 61 months due to her social communication deficits and self-harming behavior. At the time of diagnosis of ASD, her phenylalanine level was 840.6 μmol/L (14.01 mg/dl). She was urged to comply more strictly to her diet. Seizures appeared during her clinical follow-up.

Case 2 was diagnosed with PKU at the age of 6 years because he had missed the newborn screening and he was living in institutional care. He was referred to the Pediatric Metabolism outpatient clinic because of his learning disabilities and short stature. His blood phenylalanine level was measured in our clinic and found to be 1740 μmol/L (29 mg/dl), also consistent with classical PKU. He had nonverbal communication deficits and restrictive, repetitive behaviors and, serious intellectual disability. He had received an ASD diagnosis at the age of 100 months. On his physical examination he had short stature because of growth hormone deficiency. Although phenylalanine- restricted diet therapy was implemented his behavioral difficulties did not improve.

Case 3 was referred for his developmental delay and received the diagnosis of PKU at

Table I. Characteristics of patients across subtypes of metabolic diseases.

Metabolic Diagnosis Total sample, N=22 (%)	Median age of IMD diagnosis (month) (min-max)	Median age of ASD diagnosis (month) (min-max)	Gender Male N(%)	Consanguinity Yes N(%)
Phenylketonuria, N=6 (27.3)	5 (0.16-72)	45 (36-100)	3(50)	1(16.7)
Biotinidase deficiency, N=2 (9.0)	0.56 (0.13-1)	24 (18-30)	2 (100)	0
Cerebral creatine deficiency, N=3 (13.6)	73 (39-108)	21 (18-48)	2(66.7)	1(33.3)
Mucopolysaccharidosis Type 3a, N=2 (9.0)	72 (60-84)	54 (48-60)	1(50)	0
Mucopolysaccharidosis Type 3b, N=5 (22.8)	72 (18-97)	48 (36-90)	2(40)	2(40.0)
Mucopolysaccharidosis Type 4, N=1 (4.6)	32	68	0	1(100)
Hypervalinemia, N=2 (9.0)	34.5 (31-38)	30 (24-36)	2(100)	0
Maple syrup urine disease, N=1 (4.6)	0.26	84	1(100)	0

IMD: inherited metabolic disorders, ASD: autism spectrum disorder

the age of 20 months. His blood phenylalanine level was measured as 2419.2 μmol/L (40.32 mg/dl) and he was diagnosed with classical PKU. The patient was diagnosed in 2006 while investigating his developmental delay. The neonatal screening program was not active in the year when the patient was born. Phenylalanine-restricted diet therapy was implemented. The mean phenylalanine level in the follow-up of the patient was 307.2 μmol/L (5.12 mg/dl). He received the diagnosis of ASD at the age of 42 months due to his deficits in social communication and restricted behavior patterns. He had also intellectual disability, seizures, and hyperactivity on his follow-up.

Case 4 was referred to the Pediatric Metabolism outpatient clinic following newborn screening on postnatal day 5. Her blood phenylalanine level was measured in our clinic and found to be 925.2 μmol/L (15.42 mg/dl). The patient could not be followed regularly. While she was receiving restricted diet therapy, her mean phenylalanine level was 409.2 μmol/L (6.82 mg/dl). ASD was diagnosed at the age of 39 months because of her limited eye contact, stereotypical behavior, and not responding to her name. She also received special education and, her behavioral difficulties improved partially.

Case 5 was referred to the Pediatric Metabolism outpatient clinic because of jaundice and received the diagnosis of PKU at the age of 1 month. His quantitative plasma amino acid analysis was significantly high for phenylalanine at a level of 1782 μmol/L (29.7 mg/dl). Sequencing of the *PAH* gene (OMİM*612349) in the patient revealed homozygote mutation IVS4+ lG>A. The patient's follow-up was irregular. The mean phenylalanine level at follow-up of the patient was 333 μmol/L (5.55 mg/dl). Social communication difficulties appeared at the age of 36 months and received an ASD diagnosis. After implementing diet therapy and special education behavioral symptoms improved however, his learning difficulties continued.

Case 6 was referred to the Pediatric Metabolism outpatient clinic because of suspected seizure history and developmental delay and was diagnosed with PKU at the age of 4 months in 2001.Her blood phenylalanine level was measured in our clinic and found to be 780 μmol/L (13 mg/dl). The patient's compliance with diet therapy was not good. She didn't have seizures on her follow up. Because of behavioral problems and social communication difficulties she received an ASD diagnosis at the age of 48 months. Her behavioral symptoms did not improve following diet and special education therapy. Furthermore, she received a diagnosis of the Systemic Lupus Erythematosus on her follow up.

Case 7 received the diagnosis of partial biotinidase deficiency at the age of 1 month. He was referred to the Pediatric Metabolism outpatient clinic following newborn screening. His biotinidase activity was 1.77 U/L (24.9%) and 1.60 U/L (22.5%). He had c.235C> T; p.Arg79Cys heterozygous mutation. Although the mutation in the patient was heterozygous, the biotinidase activity of the patient was found to be low (checked twice), consistent with partial deficiency. His parents did not mention either hair loss or skin manifestations. Oral biotin replacement was started at a dose of 5 mg per day. At the age of 18 months he had an ASD diagnosis due to deficits in socioemotional reciprocity such as poorly integrated verbal and nonverbal communication. He also had seizures at follow up.

Case 8 received the diagnosis of partial biotinidase deficiency at his postnatal 4 day newborn screening. His first biotinidase activity was 3.07U/L (43.2%), the second was 2.00 U/L (28.16%) and, there was homozygote c.1330G>C mutation on *BTD* gene. His parents did not mention either hair loss or skin manifestations. The patient's hearing examination and audiogram were reported to be normal. Oral biotin replacement was started at a dose of 5 mg per day. Because of his impaired reciprocal communication and stereotypical movements, she had an ASD diagnosis at the age of 30 months. Despite continued biotin therapy and special education therapy, complete response was not achieved.

Case 9 had an ASD diagnosis at the age of 12 months due to stereotypical hand movements and communication problems. She was referred to the metabolic outpatient clinic because of seizures, intellectual disability, and tonus hyperactivity. Serum creatinine level was 0.11 mg / dl. The level of her urine Guanidoasetat was 1414 μmol/L. The creatinine level in spot urine was 93.91 mg / dl. The cranial magnetic resonance imaging (MRI) examination of the patient was normal. In the magnetic resonance spectroscopy (MRS) examination, the creatine peak value was seen to have lost its significant level at a level close to 0 in the parietooccipital cortex. This finding was compatible with creatine deficiency syndrome. The guanidino acetate (GAA) peak was not found. Genetic test revealed c.261_269del (p.W87fsX) homozygote mutation found on guanidinoacetate methyltransferase (*GAMT*) gene. She received a CCDS diagnosis at the age of 108 months. She was receiving special education therapy and, despite adding the metabolic therapy with creatine monohydrate, L-ornithine and an Arginine-protein restricted diet a limited improvement was observed in her clinical findings.

Case 10 had received a diagnosis of ASD at the age of 15 months due to social communication problems. He was referred to the Pediatric Metabolism outpatient clinic because of seizures and social communication problems. His blood creatine level was 0.09g/dl. Genetic test results were homozygous frameshift mutation, hemizygous (X linked) on SLC6A8 *gene*. He received the diagnosis of creatine transporter deficiency at the age of 73 months. He was receiving special education therapy. Despite adding metabolic therapy with creatine monohydrate, L-arginine, L-glycine, S-adenosyl, methionine, risperidone, and aripiprazole, a complete response was not achieved.

Case 11 was referred for global developmental delay and received a CCDS diagnosis at the age of 39 months. The level of blood creatinine was 0.28 mg/dl and blood guanidinoacetate was 0 μmol/L. *GAMT* gene analysis showed a homozygous c.327G>A mutation on Exon 2. Symmetrical T2 signal increase in bilateral globus pallidus was noted on the cranial MRI of the patient. In multivoxel short and intermediate echo examinations made from deep gray matter and single voxel examination involving left parietal gray-white matter composition, creatine was found to be close to 0 or 0 in all voxels. He had seizures, behavioral problems, limited self-care, and social communication difficulties. The diagnostic age of ASD was 48 months. Despite special education therapy and drug therapy such as creatine monohydrate, pregabalin, no improvement was observed in his clinical findings.

Case 12 had an ASD diagnosis at the age of 48 months. She was referred for gross motor delay and verbal and nonverbal communication and behavioral problems. On her physical examination, short stature, pectus carinatum and hepatosplenomegaly were observed. Her urine mucopolysaccharides was 15.23 mg/dl and sulphamidase activity in leucocytes was 0.13 nmol/mg/17 hr (normal range: 3.2-20.4). She received a diagnosis of MPS Type3a at the age of 60 months. She received multidisciplinary supportive therapy and special education however no improvement was observed in her clinical findings.

Case 13 was diagnosed with an ASD due to delayed speech, difficulty with socialization, and restricted interest in his surroundings at the age of 60 months. He was referred to the Pediatric Metabolism outpatient clinic because of skeletal problems. His urine mucopolysaccharides was 18.12 mg/dl and he had a diagnosis of MPS Type 3a at the age of 84 months. He received multidisciplinary supportive therapy and special education however no improvement was observed in his clinical findings.

Case 14 was referred for seizures, global developmental delay, and physical appearance. He had a diagnosis of MPS Type3b at the age of 18 months. He had ASD diagnosis at the age of 48 months with complaints of impaired reciprocal communication and a lack of eye contact, and echolalia. He received multidisciplinary

supportive therapy and special education however, no improvement was observed in his clinical findings.

Case 15 was referred for global developmental delay, behavioral problems and, skeletal problems, and she had a diagnosis of MPS Type 3b at the age of 25 months. Plasma alpha N acetylglucosamidase activity was found to be severely reduced. In the genetic analysis, c.235G> T (p.G79C) homozygous mutation was detected in exon 1 in the *NAGLU* gene. She was diagnosed with ASD at the age of 39 months due to delayed speech and difficulty with socialization, a lack of interest in her surroundings, as well as stereotypical movements. She did not continue her follow-up regularly.

Case 16 had an ASD diagnosis at the age of 60 months because of his impaired reciprocal communication and a lack of eye contact, and echolalia. He was referred to the Pediatric Metabolism outpatient clinic because of his developmental delay and coarse facial features. A diagnosis of MPS was suspected and the leukocyte acetyl alpha glucosaminidase enzyme activity was found to be 0 nmol/hour/mg. He was diagnosed with MPS Type 3b at the age of 72 months. Seizures were controlled by an antiepileptic. He received multidisciplinary supportive therapy and special education however no improvement was observed in his clinical findings.

Case 17 was diagnosed with an ASD due to socialization problems and a lack of interest in her surroundings, as well as stereotypical movements at the age of 90 months. She was consulted to the pediatric metabolism outpatient clinic due to atypical behavior patterns, coarse facial features, adenoid vegetation, tonsillar hypertrophy, and intellectual disability. She had a diagnosis of MPS Type 3b at the age of 97 months with enzyme analyses. Despite multidisciplinary therapy, no improvement was observed in his clinical findings.

Case 18 had a diagnosis of MPS Type 3b at the age of 84 months when she was referred to the metabolic outpatient clinic for short stature and intellectual disability. Acetyl-alpha-glucosaminidase specific enzyme activity in plasma was found to be 0 nmol/hour/ml. The reference range was 5-58 nmol/hour/ml. Urinary glycosaminoglycan electrophoresis showed pronounced heparan sulfate band. Total urinary glucosaminoglycan level was found to be 209.3 mg/g creatinine. Healthy control values between the ages of 2-13 years were 66-371 mg/g creatinine. She had a diagnosis of ASD at the age of 36 months because of her social communication deficits and stereotypical behavior. She did not continue her follow-up regularly.

Case 19 was admitted to the general pediatric outpatient clinic with the complaint of increasing protrusion of her rib cage. After the skeletal survey, radiographical findings revealed signs of mucopolysaccharidosis and she was referred to the child metabolism department. Diagnosis of Morquio A Syndrome was made at the age of 32 months by physical examination, skeletal radiographs and keratan sulfate band in the electrophoresis of urinary GAG and non-enzymatic activity of N- acetylgalactosamine -6 sulfate-sulfatase. She had global developmental delay when she received the diagnosis of MPS Type 4. Enzyme therapy was started. However, she received the diagnosis of ASD at the age of 68 months because of her social communication deficits and, restricted joint attention and eye contact, echolalia, and a stereotypical head movements. Although special education therapy was implemented, her behavioral difficulties improved very little.

Case 20 was referred to the Pediatric Metabolism outpatient clinic for delayed speech, dysmorphic features, and echolalia. His combined amino acid analysis was significant for valine of 480.9 nmol/L (<250 nmol/L) and received a diagnosis of Hypervalinemia (valine transaminase deficiency) at the age of 31 months. Peripheral

chromosome and FISH analyses were observed as normal. He had an ASD diagnosis at the age of 24 months because of his social communication deficits and repetitive behaviors. Following special education therapy, improvements in his behaviors were observed.

Case 21 was referred to the Pediatric Metabolism outpatient clinic for verbal and nonverbal communication problems. His combined amino acid analysis was significant for valine of 294.7 nmol/L (<250 nmol/L) and received a diagnosis of hypervalinemia (valine transaminase deficiency) at the age of 38 months. Further genetic investigation was not performed due to family issues. No findings were found to explain the clinical picture, except for hypervalinemia. At the age of 36 months, he was diagnosed with ASD at a similar time when IMD was detected. He had social communication deficits, restricted interests and stereotypical behaviors such as lining up toys or flipping objects. Following special education therapy, improvements in his behaviors were observed.

Case 22 was referred with restlessness, contractions in the hands and feet, decreased feeding, decreased activity and diagnosed with MSUD at his postnatal 8 days. The patient was found to have deficient leucine decarboxylation. He had 13 pmol/hr/mg 1-^{14}C leucine decarboxylation level whereas the normal value was 784 pmol/hr/mg. He had verbal and nonverbal communication and self-care problems and stereotypical behaviors. He was diagnosed with ASD at the age of 84 months. Restricted diet from leucine and protein was implemented however, complete response was not achieved.

Description of the clinical findings and diagnosis of the patients are presented in Table II.

Discussion

We identified twenty-two cases with inherited metabolic disorders who also had ASD. There was no difference between the median diagnostic ages of the two disorders however, the median age of ASD diagnosis was 45 months of age. The variability in the average age of ASD diagnosis might be related to the heterogeneity of symptoms.[9] Symptomatic presentation and the developmental course of ASD may differ according to whether the child has a co-occurring condition.[10,11]

Phenylketonuria was one of the most common diagnoses with 6 cases in the study. Consistent with previous literature, PKU is the most common genetic metabolic disorder associated with ASD.[12,13] Among well-documented potential pathophysiological mechanisms associated with neurodevelopmental impairment, functional and molecular alterations in the prefrontal cortex including excitation/inhibition (E/I) imbalance is noticeable in the etiology.[12] Frontal and subcortical white matter changes were also reported in patients with phenylketonuria which had been treated early.[14] Similarly in autism there are frontal dysfunction and connectivity problems in the cerebral white matter. Considering the prefrontal dysfunction and connectivity problems in both disorders, it is not surprising to see autism phenotype in PKU patients.[15] Phenylketonuria has a prevalence of approximately 1/4,500 in Turkey. Although there is a national newborn screening program using the Guthrie test, one patient in our study was diagnosed as late as 72 months of age and his metabolic prognosis was badly controlled. The possibility of PKU should be considered in any child presenting symptoms of autism and developmental delay. This is because even late diagnosed patients may benefit from a restricted diet.[13] Further, it should be considered that having both diagnoses could make following dietary restrictions more challenging for families and patients.

Mucopolysaccharidosis type 3 (MPS 3, Sanfilippo syndrome) was another common diagnosis in the present study, 2 cases with Type-A and 5 cases with Type-B. MPS 3 is

Table II. Description of clinical findings and diagnosis of patients.

Patients	Diagnosis	Current age (years)	Diagnostic age of IMD/ASD (months)	Gender	Consanguinity	Physical examination*	Cranial neuroimaging**	Mental retardation or global developmental delay
Patient 1	PKU	15,16	6/61	female	yes	-	-	yes
Patient 2	PKU	16	72/100	male	no	Short stature	-	yes
Patient 3	PKU	16,5	20/42	male	no	-	-	yes
Patient 4	PKU	8,08	0.16/39	female	no	-	-	no
Patient 5	PKU	21	1/36	male	no	-	-	no
Patient 6	PKU	20	4/48	female	no	-	-	yes
Patient 7	BD	6,66	1/18	male	no	Hepatomegaly	-	yes
Patient 8	BD	5,66	0.13/30	male	no	Strabismus	-	yes
Patient 9	CCDS	20	108/12	female	yes	-	Decreased creatine peak	yes
Patient 10	CCDS	9,5	73/15	male	no	Walking with a wide step	Decreased creatine peak, PVL	yes
Patient 11	CCDS	16,58	39/48	male	no	-	PPV hyperintense gliotic lesions in white matter	yes
Patient 12	MPS3a	15,33	60/48	female	no	Short stature, pectus carinatum, hepatosplenomegaly		yes
Patient 13	MPS3a	17,58	84/60	male	no	Short stature	-	yes
Patient 14	MPS3b	18	18/48	male	yes	Pectus carinatum, mild macrocephaly, macroglossi, periodontal soft tissue increase.	Signal increase in subcortical white matter, cerebral atrophy secondary to volume loss.	yes
Patient 15	MPS3b	10,5	25/39	female	no	Hepatomegaly	-	yes
Patient 16	MPS3b	18	72/60	male	yes	Hirsutism, pectus carinatum, coarse facial features, hepatomegaly	Slight signal increase in supratentorial periventricular deep white matter	yes
Patient 17	MPS3b	14,5	97/90	female	no	Short stature, hepatomegaly-splenomegaly	-	yes

*-: means that physicalexamination demonstrated no abnormalities, **-:means that brain imaging was not performed or unavailable.
PKU: phenylketonuria, BD: biotinidase deficiency, CCDS: cerebral creatine deficiency syndrome, MPS: mucopolysaccharidosis, valin: hypervalinemia, MSUD: maple syrup urine disease, IMD: inherited metabolic disorders, ASD: autism spectrum disorder, PPV: posterior periventricular, PVL: periventricular leukomalacia

Table II. Continued.

Patients	Diagnosis	Current age (years)	Diagnostic age of IMD/ASD (months)	Gender	Consanguinity	Physical examination*	Cranial neuroimaging**	Mental retardation or global developmental delay
Patient 18	MPS3b	13,25	84/36	female	no	Short stature	Hyperintensity in white matter in PPV and mild dilatation in the lateral ventricles, thin corpus callosum, frontoparietal cerebral volume loss	yes
Patient 19	MPS4	9,5	32/68	female	yes	Coarse facial features, pectus carinatum	Nonspecific findings	yes
Patient 20	Valin	7,66	31/24	male	no	Epicanthus, prominent forehead, short nose bridge, high palate	-	yes
Patient 21	Valin	6,83	38/36	male	no	-	-	no
Patient 22	MSUD	27	0.26/84	male	no	-	-	yes

*-: means that physicalexamination demonstrated no abnormalities, **-:means that brain imaging was not performed or unavailable.
PKU: phenylketonuria, BD: biotinidase deficiency, CCDS: cerebral creatine deficiency syndrome, MPS: mucopolysaccharidosis, valin: hypervalinemia, MSUD: maple syrup urine disease, IMD: inherited metabolic disorders, ASD: autism spectrum disorder, PPV: posterior periventricular, PVL: periventricular leukomalacia

the most common of the seven MPS with a heterogeneous presentation and a progressive clinical course. It is characterized by progressive neurocognitive decline, behavioral difficulties and relatively mild somatic manifestations.[16] Although the incidence of the subtypes varies according to geographic distribution Type-A and Type-B are more common than Type-C and Type-D.[16,17] Since MPS 3 is characterized by early-onset developmental delay and subtle somatic features in early years, the important point here is that young children with MPS 3 are easily misdiagnosed with idiopathic developmental delay, attention deficit hyperactivity disorder or ASD as were the cases in our study. The diagnoses of MPS Type 3a and Type 3b were given after the diagnosis of ASD with an 18 and 24 months delay, respectively. Therefore, children with any developmental delay, especially with a characteristic somatic feature or behavioral abnormality, should be screened for MPS 3.[16] Otherwise these children who are misdiagnosed may be referred to more invasive testing, dietary restrictions or even unproven alternative therapies.[18]

We also had one case with MPS-4 (Morquio A Syndrome) and ASD. To the best of our knowledge, the association between Morquio A Syndrome and autism has never been published before. It is caused by impaired catabolism of specific glycosaminoglycans (GAGs) keratan sulfate (KS) and chondroitin -6- sulfate (C6S). The accumulation of these GAGs becomes evident primarily in the cartilage and causes multi-systemic impairments.[19-21] It is widely accepted that the syndrome is not directly related to the central nervous system (CNS). However a recent study has speculated that the biochemical mechanism, a kind of interplay between mitochondrial, calcium and lysosomal trafficking dysfunction may contribute to potential CNS involvement. Behavioral problems have been previously reported in MPS 4 and only one patient with ASD has been briefly mentioned in the same study. Although the evidence is still insufficient, the physiological role to coordinate the formation of neuroaxonal connections of KS and C6S in CNS is suggested in the early phases of brain development.[22] Therefore it is plausible that we have defined a case with MPS 4 and a definite diagnosis of ASD. Nevertheless, MPS 4A and ASD coexistence may be coincidental.

We had two cases with partial Biotinidase Deficiency and ASD diagnosis. Biotinidase Deficiency is a metabolic disorder with homozygous or compound heterozygous variants.[23] In the literature there has been only one case reported with partial biotinidase deficiency and ASD. The case was almost 4 years of age when he received the diagnosis and after beginning cofactor biotin treatment his autistic behavioral patterns were not resolved. It has been suggested that serious neurological problems such as autistic behaviors could be irreversible even with biotin supplementation.[24] There should be further studies to explore the underlying mechanism relating to neurodevelopmental impairment in these cases because our two cases received their diagnosis in their first months and began biotin therapy early, however, they showed autistic features later at approximately two years of age. Coexistence of partial biotinidase deficiency, ASD and seizures is very likely coincidental in early diagnosed patients.

We had three cases with CCDS and ASD diagnosis. Loss-of-function mutations in the creatine transporter which transports creatine at the blood–brain barrier and into the neurons causes CCDS. Patients with these syndromes show various neurodevelopmental disorders, including developmental delay, seizures and intellectual disability; many of which are also evident in children with ASD.[25] The observation of autistic symptoms in patients with CCDS implies that impairment in creatine metabolism may play a role in the neurobiology of ASD. Therefore, it may represent a treatable cause of ASD.[26] Patients with all forms of CCDS have been reported to have ASD symptoms.[26,27] Although Schulze et al.[26] has revealed a very low prevalence of CCDS in children with non-syndromic ASD and no obvious association

between creatine metabolites and autism, we had three cases with this syndrome and, two of them received the diagnoses of CCDS after the diagnosis of ASD.

We had one case with a diagnosis of MSUD and ASD. Branched-chain α-ketoacid dehydrogenase catalyzes the critical step in the branched-chain amino acid catabolic pathway and mutations in the complex disrupt many fundamental metabolic pathways and cause multiple human diseases including MSUD, autism, and other related neurological disorders.[28] Maple syrup urine disease is a disorder of branched-chain keto acid metabolism. In previous literature three children have been reported with an intermediate form of MSUD characterized by intellectual disability, seizures, autistic features, and movement disorder.[29] The patient in the study demonstrated intellectual disability and autistic features with no abnormalities on his physical examination.

There are two cases with hypervalinemia in our study. They were identified during the evaluation of their developmental delay and autistic features. Very few cases of hypervalinemia have been previously reported in literature. Swarna et al.[30] have identified two cases of hypervalinemia characterized by distinct physical and intellectual disability and muscular atrophy. However, our two patients had language, social-emotional delay and mild cognitive delay with normal physical growth.

The limitation of this study was that data relating to the diagnosis of ASD was obtained from their medical records and there was no standard documentation relating to the progress of ASD. This is because they were being followed up for ASD at different health centers around Turkey. Additionally, other well-known metabolic disorders with autistic features such as classical homocystinuria, urea cycle disorders, and purine metabolic pathway disorders were not presented in the study as possible cases with these disorders did not have a definitive diagnosis of ASD during the study period.

The main strength of the study is its' unique ability to address the co-occurrence of ASD in a large sample including rare metabolic diagnoses such as MPS Type 3b and Type 4 and Hypervalinemia. Furthermore, the time of both IMD and ASD diagnosis and the clinical progress of their metabolic disorders were addressed to underline the treatable conditions with the aim of contributing to the literature.

In conclusion, we have presented some inherited metabolic disorders associated with ASD. Phenylketonuria and Mucopolysaccharidosis were the most common diagnoses in our study. The diagnoses of CCDS and MPS Type 3 were later than the diagnosis of ASD. In addition, rare entities such as MPS Type 3b and Type 4 and Hypervalinemia were also reported to be associated with autism. Viewing the whole picture with this awareness and implementing intervention strategies considering the co-occurrence of both disorders should be an essential component of any treatment.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TÇY, TC; data collection: TÇY, BBG, HTA; analysis and interpretation of results: TÇY, BBG, ENÖ, TC; draft manuscript preparation: TÇY, BBG, HTA, ENÖ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the Ethics Committee of Hacettepe University Medical Faculty (GO 18/407) and the participation involved informed consent.

Source of funding

There is no any financial source.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Lappe M, Lau L, Dudovitz RN, Nelson BB, Karp EA, Kuo AA. The diagnostic odyssey of autism spectrum disorder. Pediatrics 2018; 141(Suppl 4): S272-S279.
2. Ghaziuddin M, Al-Owain M. Autism spectrum disorders and inborn errors of metabolism: an update. Pediatr Neurol 2013; 49: 232-236.
3. Simons A, Eyskens F, Glazemakers I, van West D. Can psychiatric childhood disorders be due to inborn errors of metabolism? Eur Child Adolesc Psychiatry 2017; 26: 143-154.
4. Sedel F, Baumann N, Turpin JC, Lyon-Caen O, Saudubray JM, Cohen D. Psychiatric manifestations revealing inborn errors of metabolism in adolescents and adults. J Inherit Metab Dis 2007; 30: 631-641.
5. Wertheim-Tysarowska K, Gos M, Sykut-Cegielska J, Bal J. Genetic analysis in inherited metabolic disorders--from diagnosis to treatment. Own experience, current state of knowledge and perspectives. Dev Period Med 2015; 19: 413-431.
6. Al-Owain M, Al-Zaidan H, Al-Hassnan Z. Map of autosomal recessive genetic disorders in Saudi Arabia: concepts and future directions. Am J Med Genet A 2012; 158A: 2629-2640.
7. Schiff M, Benoist JF, Aissaoui S, et al. Should metabolic diseases be systematically screened in nonsyndromic autism spectrum disorders? PLoS One 2011; 6: e21932.
8. Kiykim E, Zeybek CA, Zubarioglu T, et al. Inherited metabolic disorders in Turkish patients with autism spectrum disorders. Autism Res 2016; 9: 217-223.
9. Zablotsky B, Colpe LJ, Pringle BA, Kogan MD, Rice C, Blumberg SJ. Age of parental concern, diagnosis, and service initiation among children with autism spectrum disorder. Am J Intellect Dev Disabil 2017; 122: 49-61.
10. Maenner MJ, Schieve LA, Rice CE, et al. Frequency and pattern of documented diagnostic features and the age of autism identification. J Am Acad Child Adolesc Psychiatry 2013; 52: 401-413.e8.
11. Shattuck PT, Durkin M, Maenner M, et al. Timing of identification among children with an autism spectrum disorder: findings from a population-based surveillance study. J Am Acad Child Adolesc Psychiatry 2009; 48: 474-483.
12. De Jaco A, Mango D, De Angelis F, et al. Unbalance between excitation and inhibition in phenylketonuria, a genetic metabolic disease associated with autism. Int J Mol Sci 2017; 18: 941-951.
13. Mazlum B, Anlar B, Kalkanoglu-Sivri HS, Karli-Oguz K, Ozusta S, Unal F. A late-diagnosed phenylketonuria case presenting with autism spectrum disorder in early childhood. Turk J Pediatr 2016; 58: 318-322.
14. Anderson PJ, Wood SJ, Francis DE, et al. Neuropsychological functioning in children with early-treated phenylketonuria: impact of white matter abnormalities. Dev Med Child Neurol 2004; 46: 230-238.
15. Christ SE, Moffitt AJ, Peck D. Disruption of prefrontal function and connectivity in individuals with phenylketonuria. Mol Genet Metab 2010; 99(Suppl 1): S33-S40.
16. Wijburg FA, Wegrzyn G, Burton BK, Tylki-Szymanska A. Mucopolysaccharidosis type III (Sanfilippo syndrome) and misdiagnosis of idiopathic developmental delay, attention deficit/hyperactivity disorder or autism spectrum disorder. Acta Paediatr 2013; 102: 462-470.
17. Valstar MJ, Ruijter GJ, van Diggelen OP, Poorthuis BJ, Wijburg FA. Sanfilippo syndrome: a mini-review. J Inherit Metab Dis 2008; 31: 240-252.
18. de Ruijter J, Valstar MJ, Wijburg FA. Mucopolysaccharidosis type III (Sanfilippo syndrome): emerging treatment strategies. Curr Pharm Biotechnol 2011; 12: 923-930.
19. Khan S, Almeciga-Diaz CJ, Sawamoto K, et al. Mucopolysaccharidosis IVA and glycosaminoglycans. Mol Genet Metab 2017; 120: 78-95.
20. Tomatsu S, Yasuda E, Patel P, et al. Morquio A syndrome: diagnosis and current and future therapies. Pediatr Endocrinol Rev 2014; 12(Suppl 1): 141-151.
21. Harmatz P, Mengel KE, Giugliani R, et al. The Morquio A Clinical Assessment Program: baseline results illustrating progressive, multisystemic clinical impairments in Morquio A subjects. Mol Genet Metab 2013; 109: 54-61.
22. Davison JE, Kearney S, Horton J, Foster K, Peet AC, Hendriksz CJ. Intellectual and neurological functioning in Morquio syndrome (MPS IVa). J Inherit Metab Dis 2013; 36: 323-328.
23. Kury S, Ramaekers V, Bezieau S, Wolf B. Clinical utility gene card for: biotinidase deficiency-update 2015. Eur J Hum Genet 2016; 24. doi: 10.1038/ejhg.2015.246.
24. Zaffanello M, Zamboni G, Fontana E, Zoccante L, Tato L. A case of partial biotinidase deficiency associated with autism. Child Neuropsychol 2003; 9: 184-188.

25. Uemura T, Ito S, Ohta Y, et al. Abnormal N-glycosylation of a novel missense creatine transporter mutant, G561R, associated with cerebral creatine deficiency syndromes alters transporter activity and localization. Biol Pharm Bull 2017; 40: 49-55.

26. Schulze A, Bauman M, Tsai AC, et al. Prevalence of creatine deficiency syndromes in children with nonsyndromic autism. Pediatrics 2016; 137: e20152672.

27. Schulze A. Creatine deficiency syndromes. Handb Clin Neurol 2013; 113: 1837-1843.

28. Jia F, Cui M, Than MT, Han M. Developmental defects of caenorhabditis elegans lacking branched-chain α-ketoacid dehydrogenase are mainly caused by monomethyl branched-chain fatty acid deficiency. J Biol Chem 2016; 291: 2967-2973.

29. Bindu PS, Shehanaz KE, Christopher R, Pal PK, Ravishankar S. Intermediate maple syrup urine disease: neuroimaging observations in 3 patients from South India. J Child Neurol 2007; 22: 911-913.

30. Swarna M, Jyothy A, Usha Rani P, Reddy PP. Amino acid disorders in mental retardation: a two-decade study from Andhra Pradesh. Biochem Genet 2004; 42: 85-98.

The Turkish Journal of Pediatrics 2021; 63: 780-789
https://doi.org/10.24953/turkjped.2021.05.006

Original Article

Comparison of serum anti-neuronal antibody levels in patients having autism spectrum disorder with and without regression

Cihan Aslan[1], Bahadır Konuşkan[2], Burçin Şener[3], Fatih Ünal[1]

Departments of [1]Child and Adolescent Psychiatry, [2]Pediatric Neurology and [3]Medical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Autism Spectrum Disorders (ASD) may present with a delay in social and communication development, or less frequently, with regression in social and language skills. The reasons for this difference in clinical presentation are unknown, and the regressive symptoms in the second group suggest an acquired process.

Methods. We investigated serum autoantibodies in these two types of ASD in a cross-sectional design in a total of 50 children, 24 with autistic regression and 26 with classical ASD according to the DSM-5 criteria. Clinical assessment by the Childhood Autism Rating Scale (CARS) and Ankara Developmental Screening Test (ADST), parental questionnaires consisting of the Aberrant Behavior Checklist (ABC) and Autism Behavior Checklist (AuBC) were completed. Serum samples were tested for anti-neuronal antibodies including anti-N-methyl-D-aspartate receptor (NMDAR), anti-contactin-associated protein (CASPR2), anti-leucine rich glioma inactivated 1 (LG1), anti-glutamate type 2-amino-3-propionic Acid (AMPA) 1-2, anti-gamma amino butyric acid (GABA) B, anti-dipeptidyl aminopeptidase-like protein 6 (DPPX) and anti-glutamic acid decarboxylase 65(GAD).

Results. Serum anti-GAD antibodies were at detectable levels in five (20.8%) patients with autistic regression, of whom three had 2 to 4-fold increased titers, and in none of the patients with classical ASD. The age of the father at the patient's birth and the duration of autistic regression correlated with anti-GAD IgG levels (P: 0,045, P: 0.855 respectively) in the ASD-regression group. No other antibodies were detected in either group.

Conclusions. Our results do not suggest a causative role of anti-neuronal antibodies, but the possibility of an autoimmune process accompanying regressive symptoms in a small subgroup of ASD.

Key words: autism, regression, autoimmunity, antibody mediated, encephalitis.

Autism spectrum disorder (ASD) is a neurodevelopmental condition involving deficiencies in interaction, communication, and social interest. Its etiopathogenesis, phenotype and course are heterogeneous. About one third of ASD cases show sudden or gradual loss of previously attained skills at 12-24 months of age.[1] Some authors consider regression as a subtype of ASD with a particular etiology and course and also poorer outcome, while others suggest classical and regressive ASD cases have similar behavioral and adaptive outcomes.[2]

ASD can be associated with immune dysfunction, as supported by studies on cell profiles of the immune system, plasma cytokine levels, serum immunoglobulins, and autoantibodies against neuronal proteins.[3] The various targets of the antibodies detected in ASD cases include multiple brain regions and somatic tissues: neuronal membranes, folate receptors, mitochondria, gastrointestinal epithelial cells,

✉ Cihan Aslan
cihanaslan@yahoo.com

Received 12th November 2020, revised 31st January 2021, accepted 23rd February 2021.

10th International Congress on Psychopharmacology & 6th International Symposium on Child and Adolescent Psychopharmacology, April 25-29, 2018, Antalya, Turkey.

endothelial cells, ribosome P, or antinuclear antibody.[3,4] The heterogeneity of targets and lack of consistency among results of the studies warrant further specific studies in order to clarify the role of antibodies in ASD. Uncertainty exists about the autoimmune process involving a causative relationship or an immune activation in response to an unknown etiological agent. As ASD is a broad and heterogeneous condition, the role of inflammatory pathways may be more prominent in certain subgroups, such as ASD with regressive course. Febrile diseases, family history of autoimmune disorders, elevated serum inflammatory biomarkers have been observed more frequently in this group.[5-7] HLA polymorphisms detected in autoimmune disorders were also described in ASD with regression.[8] Microglial activation leading to neuronal and synaptic loss was shown in the central nervous system (CNS) in up to 69% of ASD cases, mostly in those with regressive course.[9] Recently auto-antibodies against CNS antigens have been implicated in the etiology of ASD; a systematic review underlined the need for studies on autoimmune encephalitis and HLA haplotypes.[4] Indeed cases and experimental animal models of autoimmune encephalitis can exhibit autistic behavior.[10,11] Another clinical example is the Landau-Kleffner Syndrome, a steroid-responsive epileptic syndrome with autoimmune or inflammatory pathogenesis and autistic features.[12]

Regression remains an intriguing feature adding to the clinical variability of ASD. However, the etiology and pathogenesis underlying this particular clinical characteristic are unclear. In this study, we investigated serum autoantibodies in ASD with and without regression.

Material and Methods

This cross-sectional case-control study was performed in Hacettepe University Faculty of Medicine following the approval of Hacettepe University Non-Interventional Clinical Studies Ethics Committee (#GO 16/73-09).

Patient Groups

Children 2-6 years old followed-up in the Child and Adolescent Psychiatry Department for ASD based on DSM-5 diagnostic criteria were prospectively recruited after parental consent. Patients diagnosed with childhood disintegrative disorder, children with regression starting after age 36 months, those who lost motor skills or toilet training, those with associated epilepsy or electroencephalogram (EEG) abnormality were not included. Subjects with hearing loss, genetic or metabolic syndromes, and any concomitant immunotherapy were excluded from the study.

Definition of Regression

Symptoms of regression were assessed through semi-structured interviews with parents, from hospital records and, whenever available, video recordings. Acquisition of early developmental milestones and criteria for regression were identified as defined and used in the literature[13]:

- Significant loss in communication skills (loss of at least 5 words in a child who could speak earlier, or loss of mimics and / or ability to understand words, sentences or interpretations in a child whose maximum vocabulary was 4 meaningful words when the regression started) before 36 months of age for more than 3 months, and
- Significant loss in social skills (ASD related symptoms in at least 1/3 of social skills or behavioral patterns defined as social smile, eye contact, joint attention, responding to name, waving hand, pointing, interest in playing with others, imaginary play, functional use of objects and toys, repetitive / stereotypic behavior or limited interests) before 36 months of age for more than 3 months.

Regression was identified as acute when significant loss occurred over 4-6 weeks and gradual (or subacute/chronic) when it occurred over >6 weeks. Physical factors (high fever, infection, seizure, trauma, vaccination etc.) and environmental changes associated with onset

of regression such as birth of a sibling, loss of a family member, moving, change of caregiver, decreased quality of stimuli were recorded.

Psychiatric Evaluation

Psychiatric evaluation included the Childhood Autism Rating Scale (CARS) to confirm the diagnosis and assess disease severity and Ankara Developmental Screening Test (ADST) for global development. Parents were asked to fill the Autism Behavior Checklist (AuBC) and the Aberrant Behavior Checklist (ABC).[14-17] Sociodemographic and clinical information was recorded by a standard form prepared for this study. Medical problems of the patient and family members such as recurrent infections, asthma/allergies, sleep problems, recurrent constipation/diarrhea, psychiatric symptoms or neurodevelopmental delay were recorded.

Measuring Serum Antibodies

Custom-made panel of antibodies have been designed based on literature data: anti-N-methyl-D-aspartate receptor (NMDAR), anti-contactin-associated protein (CASPR2), anti-leucine rich glioma inactivated 1 (LG1), anti-glutamate type 2-amino-3-propionic Acid (AMPA) 1-2, anti-gamma amino butyric acid (GABA) B, anti-dipeptidyl aminopeptidase-like protein 6 (DPPX) were tested in serum samples stored at -80^{0}C. An indirect immunofluorescent antibody assay on transfected cells (Mosaic 6, Euroimmun, Perkin Elmer, Lubeck, Germany) was applied in accordance with the manufacturer's recommendations.[18] Antibodies against glutamic acid decarboxylase isoform 65 (anti-GAD 65 IgG) were tested using the radioimmune assay (RIA) method (Beckman Coulter, Czech Republic).[19] Positive controls for the investigated autoantibodies were included in the test kits used in the study.

Statistical Analysis

Quantitative data was assessed for normal distribution using Kolmogorov-Smirnov test. Comparisons of numeric data between 2 groups was done with independent sample t-test for variables with normal distribution, and Mann Whitney-U test for those with non-normal distribution. Categorical (qualitative) variables were expressed as number and percentage. Pearson Chi-Square and Fisher's Exact Chi-Square tests were used depending on the status of the expected frequencies. The power of the correlation between variables was evaluated by Pearson or Spearman Rho correlation coefficients. All analyses were performed through the Statistical Program for Social Sciences (SPSS 21.0) in a two-tailed pattern with <0.05 as margin of limit for statistical significance.

Results

Demographic and Clinical Characteristics

The ASD series (n=50) comprised two groups based on the presence of a history of regression: the "regression" (n=24) and "classical course" (n=26) groups. The two groups were similar in distribution of mean age (50.7 ± 13.3 and 50.1 ± 12.0 months respectively) and gender (79.2% male, 20.8% female and 84.6% male, 15.4% female) as well as the prevalence of accompanying medical conditions in patients and their first- and second-degree relatives. None had any systemic disorders or chronic medication, and all patients had been treated with behavioral interventions only.

Early developmental milestones: language, toilet training, walking had been acquired earlier in the regression group (Table I). The two groups showed similar CARS, ADST, AuBC and ABC scores ($t = 0.35/p>0.05$, $t = -1.4/p>0.05$, $t = 1.1/p>0.05$, $t = 1.6/p>0.05$).

Clinical Characteristics of the Regression Group

The mean age of onset of regression was 24± 8.6 (13-36) months; blood samples were collected at mean 50.7 ± 13.3 months, the interval between onset of symptoms and blood sampling was 26.7 ± 11.6 (6-42) months. The pattern of regression

Table I. Clinical data.

	ASD-regression		ASD-classical		
Clinical variables	N	%	N	%	statistics
Age developmental milestones reached					
Single word(s)					
< 18 months	16	66.7	3	11.5	
18-36 months	7	29.2	8	30.8	χ^2=21.16**
>36 months	1	4.2	15	57.7	
median	12		36		U=67**
(min-max)	(9-48)		(12-70)		
Sentences					
<30 months	12	50	2	7.7	χ^2=11.08**
>30 months	12	50	24	92.3	
Toilet training					
<36 months	11	45.8	3	11.5	χ^2=7.28**
>36 months	13	54.2	23	88.5	
Walking (months) median	12		16		U=184,5*
(min-max)	(10-20)		(10-20)		
Accompanying medical conditions in patients					
Asthma / Allergy	2	8.3	1	3.8	χ^2=0.44^{NS}
Recurrent infection	2	8.3	2	7.7	χ^2=0.00^{NS}
Gastrointestinal symptoms	10	41.7	13	50	χ^2=0.34^{NS}
Constipation	7	29.2	11	42.3	χ^2=0.93^{NS}
Diarrhea	2	8.3	2	7.7	χ^2=0.00^{NS}
Sleep problems					
Resistance to sleep	13	54.2	9	34.6	χ^2=1.93^{NS}
Delay in falling asleep	9	37.5	8	30.8	χ^2=0.25^{NS}
Difficulty maintaining sleep	7	29.2	12	46.2	χ^2=1.52^{NS}
Medical conditions in first or second degree relatives					
Autoimmunity	11	45.8	13	50	χ^2=0.08^{NS}
Asthma / Allergy	4	16.7	9	34.6	χ^2=2.09^{NS}
Cardiovascular	10	41.7	15	57.7	χ^2=1.28^{NS}
Psychiatric	4	11	5	19.2	χ^2=0.05^{NS}
Neurodevelopmental	12	50	14	53.8	χ^2=0.07^{NS}
Psychiatric tool variables	Mean ± SD		Mean ± SD		
CARS scores	42.5 ± 4.4		43.1 ± 6.4		t = 0.353^{NS}
ADST scores	97.2 ± 10.8		94.3 ± 18.2		t =-1.487^{NS}
AuBC scores					
Total	57.2 ± 15.2		64.9 ± 27		t = 1.137^{NS}
Sensory	7.8 ± 4.9		9.9 ± 7.2		t = 1.167^{NS}
Relating (Social skills)	15.2 ± 6.7		18.7 ± 8.1		t = 1.623^{NS}
Body-object use	11.7 ± 6.1		13.8 ± 9.2		t = 0.960^{NS}
Language	11.7 ± 5.4		11.1 ± 4.2		t =-0.384^{NS}
Social-self help	11.2 ± 4		11.2 ± 4.8		t = 0.00^{NS}
ABC scores					
Total	40.8 ± 14.9		49.6 ± 22.3		t = 1.603^{NS}
Irritability-agitation	6.9 ± 4.1		9.13 ± 5.9		t = 1.464^{NS}
Lethargy-social withdrawal	11.2 ± 7.9		13.5 ± 8.9		t = 0.938^{NS}
Stereotypic behavior	4.6 ± 2.7		6.04 ± 4.1		t = 0.821^{NS}
Hyperactivity	17 ± 7.1		18.4 ± 8.9		t = 0.568^{NS}
Inappropriate speech	2.17 ± 1.8		2.5 ± 2.2		t = 0.704^{NS}

n: number, U: Mann Whitney U test, min: minimum, max: maximum, χ^2: chi square test, t test, SD: standard deviation, NS: not significant, ASD: autism spectrum disorder, CARS: childhood autism rating scale, ADST: Ankara Developmental screening test, AuBC: autism behavior checklist, ABC: aberrant behavior checklist, * p<0.05, **p<0.01

documented: acute loss of language and social skills (n=4 children, 16.7%), gradual loss of language and social skills (n=13, 54.2%), gradual loss of language skills (n=3, 12.4%) and gradual loss in language but sudden loss in social skills (n=4, 16.7%). Regression was associated with a physical illness or condition including febrile illness, febrile convulsion, vaccination, or trauma in 12 (50 %) and a change in the child's environment in 7 (29%) children respectively. Similar rates were reported in the "classical course" group: physical illness/disturbance in 11 patients (42%), environmental changes in 8 patients (30%) were recognized at the time of the first symptoms. These associations were verified by parental reports.

The younger the child made sentences and the more words he/she pronounced before regression, the more words he regained after intervention ('spearman correlation coefficient' P: -0.559, p: 0.005 and P: 0.421, p: 0.041). Cases with gradual loss in social skills had higher mean scores in AuBC total (62.8 ± 14.1 vs. 47.5 ± 12.4, t= -2.5/ p<0.05) and AuBC social-self-help subscales (8.5 ± 2.6 vs. 12.6 ± 4, t= -2.6/p<0.05), but fewer words before regression (mean 42.5, range 5-300 vs. mean 10, range 0-400 words, U= 27/p<0.05) compared to those with sudden loss.

Autoantibodies in Regression and Classical Course Groups

Anti-NMDAR, anti-AMPA 1-2, anti-CASPR2, anti-LGI1, anti-GABAB, anti-DPPX antibodies were not detected in either group. The detection range of anti-GAD titers was 0.09-4.33 U/ml. Titers above the upper limit (1 U/mL) were measured in five (20.8%) patients of the regression group and none of the "classical course" group (χ^2 = 6.019, p<0.05). Regressive cases positive and negative for anti-GAD antibody were the same in terms of sociodemographic characteristics, early developmental milestones, medical and family history, test results, or interval between onset of symptoms and collection of the serum sample (Table II).

Three patients whose anti-GAD titer was more than twice the normal titer included two boys and a girl with developmental arrest predominantly in the language area. The highest titer, 4.33 u/mL, was documented in a 5-year-old girl having an acute language arrest followed by regression over 2 years after an upper respiratory infection. She had 15-20 words before regression and had regained 50-60 words at the time of the study. A 5-year-old boy with anti-GAD titer of 3.01 u/mL, a family history of developmental problems and ASD in distant relatives demonstrated subacute arrest and subsequent regression after familial and environmental changes at age 2 years. The third patient, aged 3,5 years and anti-GAD titer 2.18 u/mL at the time of study, had a family history of autoimmune disease in grandparents and schizophrenia in maternal uncle. He showed acute language arrest at age 1.5 years after otitis and the birth of a sibling, followed by subacute regression. His hearing test was normal. Currently they have been under therapy and follow-up. Three of the anti-GAD positive patients were re-evaluated after the study and screened for autoimmune diseases: no significant clinical or laboratory findings were detected.

Correlation analysis between the clinical scores and anti-GAD autoantibody levels of those five patients using Spearman test showed no correlation. Analysis within the entire ASD-regression group showed the age of father at birth and the duration of regression correlated positively with the level of anti-GAD antibody (pearson correlation, P: 0,045 p: 0,025 and P:0,855 p:0.00)

Discussion

This study aimed to compare ASD with and without regression according to clinical and certain laboratory features. Demographics, medical history, developmental characteristics, clinical and environmental variables were not found to differ significantly between these two groups. Early developmental milestones (i.e., language skills, toilet training, walking)

Table II. Clinical characteristics of the regression group.

	Anti-GAD-positive (n=5, 20%)	Anti-GAD-negative (n=19, 80%)
Age range at onset (months)	18-25	13-36
Age range at study (years)	3.5-5	2-6
Gender (M/F)	4/1	16/3
Time (months) between onset of symptoms-collection of sample, mean± SD*	33.4 ± 7.6	25 ± 12
CARS scores, mean± SD*	41.8 ± 4.1	42.7 ± 4.6
ADST scores, mean± SD*	98.6 ± 13	96.9 ± 9
AuBC scores, mean± SD*		
Total	54.8 ± 15.3	58.4 ± 15.5
Sensory	7.2 ±5.6	8±4.8
Relating (Social skills)	15.8 ±11	15±5.3
Body-object use	10±3.8	12.1 ±6.6
Language	11.4 ±3.9	11.7 ±5.8
Social-self help	10.4 ±3	11.4 ±4.3
ABC scores, mean± SD*		
Total	36.6 ± 9.3	42± 16.1
Irritability-agitation	6.4 ± 4.3	7.1 ±4.2
Lethargy-social withdrawal	10± 8.9	11.5 ±7.8
Stereotypic behavior	1.8 ± 2.1	3.8 ±2.7
Hyperactivity	16.4 ± 4	17.2 ±7.8
Inappropriate speech	2± 2.9	2.2 ±1.6
Onset patterns: loss of skills		
Sudden social & Language	1 (20%)	3 (15%)
Gradual social & Language	3 (60%)	10 (50%)
Sudden social & Gradual Language	-	4 (21%)
Gradual social & Sudden Language	1 (20%)	2 (10%)

n: number, SD: standard deviation, M: male, F: female, Anti-GAD: anti-glutamic acid decarboxylase, CARS: childhood autism rating scale, ADST: Ankara Developmental screening test, AuBC: autism behavior checklist, ABC: aberrant behavior checklist, * $p<0.05$, t test, not significant

were attained earlier in the regressive group. In fact, some of the ASD-regression cases had a developmental pattern very similar to typically developing children. Overall, these clinical findings were similar to those in the literature.[8]

The immune system has been implicated in the etiopathogenesis of ASD although it is unclear whether this is a causative relationship or an indirect association. The hypothesis of immune dysfunction might be more relevant to a particular subgroup within ASD, namely, the group ASD-regression in this study. Autoimmunity, neuroinflammation, and microglial activity resulting in neuronal death and synaptic loss have been considered in the pathogenesis of autistic regression. An association of ASD with autoimmune and allergic diseases has been shown in a large nationwide cohort.[20] The frequency of febrile illnesses in the 6 months prior to initial manifestations of ASD, and the higher prevalence of autoimmune disorders including type 1 diabetes and thyroiditis in the family also may point towards autoimmune pathogenesis in ASD with regression.[21] Recent literature observed developmental milestones being reached significantly earlier in ASD cases with regression than in classical ASD: this

may also imply the role of an acquired process compatible with autoimmunity.[22] Rather than being the primary cause, the immune response might act as a "biological hit" in a child with genetic or metabolic predisposition to ASD.[23] For this reason we studied antineuronal antibodies described in the literature as associated with behavioral and autistic symptoms, and became part of the diagnostic work-up of acute or subacute encephalopathies and epilepsies.[24] The commercial kits include antineuronal antibodies demonstrated in previous laboratory and clinical studies on autoimmune CNS disorders as well as in the limited number of prior studies in autism. The most common anti-neuronal antibody found in children is anti-NMDAR. It causes an encephalitis manifesting with behavioral changes, loss of social, speech, and communication skills, all symptoms evocative of the autistic regression in young children.[25] In fact, two children demonstrating autistic regression when 2 years old were reported with anti-NMDAR antibody positivity in their serum and cerebrospinal fluid (CSF).[26] Neuronal AMPA receptors, GABA B receptors and VGKC complex have a role in ASD as shown in laboratory and clinical studies.[27-29] The absence of these antibodies in our series argues against these receptors being affected by autoimmunity in young children with ASD. Alternatively, by testing the serum but not the CSF and antibodies of the IgG type only, we may have overlooked some antibodies: serum can be negative in as many as 14% of anti-NMDAR encephalitis cases, and certain anti-NMDAR antibodies are of the IgA and IgM types, undetected in routine assays. Also, antibody titers can decrease spontaneously within months regardless of the clinical course.[30] Serum samples can be useful in antibody-mediated CNS disorders where the blood-brain barrier is impaired, but this is not the case in the majority of childhood autism cases.

The only antibodies found in our series were anti-GAD65 IgG. These are detected in diabetes but also in various autoimmune conditions. The prevalence of anti-GAD positivity in healthy children is consistently reported at around 2%.[31] Neurological phenotypes associated with anti-GAD antibody are the stiff person syndrome, temporal lobe epilepsy, cerebellar ataxia and limbic encephalitis.[32] These antibodies affect cerebellar Purkinje cells which have been implied in the pathogenesis of ASD: for instance, maternal anti-GAD antibodies may cause loss of fetal Purkinje cells.[33] Anti-GAD seropositivity at low titers in our study may suggest a nonspecific response against undefined, possibly neural antigenic targets rather than a direct etiological finding.[34] Follow-up of the titers may be envisaged in future studies in order to support or refute an ongoing secondary immune response. In our study, serum was tested: although the concordance rate of CSF and serum samples is high in anti-GAD antibody-related neurological syndromes, fluctuations in anti-GAD65 levels and false negativity are possible and CSF analysis might be more specific, as some anti-GAD65 antibody is synthesized intrathecally.[35-37] The GAD enzyme has two isoforms, GAD 65 and 67, which are both reduced in the cerebellum in autism.[38] Our tests did not comprise anti-GAD67 antibodies; although they are rare in children, some cases might have been missed for this reason.[38] Another possible factor is the interval between our testing for antibodies and the onset of symptoms. This may constitute more of a drawback for other antineuronal antibodies, as anti-GAD titers tend to persist even after treatment.[39] Among the few previous studies on ASD and anti-GAD antibody, one showed 3/20 (15%) were positive while others reported all samples as seronegative.[36,40-42] These studies did not include clinical assessment. To the best of our knowledge, this is the first report investigating anti-GAD in ASD comparing classical and regressive patterns.

The question of immunomodulatory medication arises especially in the current era of personalized treatments. Successful outcomes obtained with steroids in the Landau-Kleffner Syndrome and in autism secondary to Autoimmune Lymphoproliferative Syndrome

(ALPS) suggest their use in the treatment of regressive symptoms of autism.[43,44] In regressive ASD, a recent retrospective study reported more clinical improvement in steroid-treated than non-steroid groups and a 12-week, randomized, single-blinded, placebo controlled trial showed a significant effect in CARS and ABC.[45,46] Intravenous immunoglobulin treatment evaluated in an open-label study demonstrated some improvement in ABC and/or Social Responsiveness Scale (SRS) in the majority of children of children with autoimmune encephalitis and ASD.[47] A recent review concluded that some subsets of ASD where immune-mediated mechanisms play a role may benefit from immunomodulatory treatment.[48]

Immune mechanisms' role in ASD has been subjected to numerous studies in the last decade; however, as it is not possible to examine the immune system comprehensively in a single study, hypotheses drawn from observational reports or published cases need to be investigated longitudinally in a multicentric fashion. Our results comparing two subtypes of autism can only be attributed to a certain group of patients. On the other hand, the strengths of this study include the comparability of groups in terms of symptom severity, developmental level and age range: these reduce any possible bias in the reporting of regression. The single center design allows homogeneity of patient groups and methodology. The main limitations of the study are its cross-sectional rather than longitudinal design, small sample size precluding generalization of our findings, and variations in the interval between the onset of regression and sample collection. Some information obtained from caregivers might introduce a recall bias, as under-reporting of the age when regression started is common.[49] On the other hand, parents of older children tend to report that regression begun later. This effect is described as "telescoping effect" and confirmed with a recent meta-analysis.[1] To alleviate this, we applied strict definitions of early developmental stages, symptoms, duration, triggering factors at onset, losses and regains in language. Whereas retrospective design is an inevitable limitation, prospective studies have their own limitations in distinguishing loss of skills from developmental delay.[50] In our study, antibodies were not tested in CSF which is more sensitive for most antibodies, but limited by ethical issues. Testing different age groups, re-testing after clinical remission and clinical-laboratory correlation could contribute to such studies because neurological manifestations associated with an antibody can vary depending on the developmental stage and age-dependent vulnerability of the nervous system. Thus, future studies should adopt longitudinal observation of children at risk according to the age of onset and using standard assessment methods.

Acknowledgments

This study was supported by the Hacettepe University Scientific Research Projects Coordination Unit (project no: THD-2016-10593).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: CA, BK, FÜ; data collection: CA, BK, FÜ; analysis and interpretation of results: CA, BŞ. FÜ; draft manuscript preparation: CA, BK, BŞ, FÜ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

This cross-sectional case-control study was performed in Hacettepe University Faculty of Medicine following the approval of Hacettepe University Non-Interventional Clinical Studies Ethics Committee (#GO 16/73-09).

Conflict of interest

The authors declare that they have no conflicts of interest.

REFERENCES

1. Barger BD, Campbell JM, McDonough JD. Prevalence and onset of regression within autism spectrum disorders: a meta-analytic review. J Autism Dev Disord 2013; 43: 817-828.
2. Jones LA, Campbell JM. Clinical characteristics associated with language regression for children with autism spectrum disorders. J Autism Dev Disord 2010; 40: 54-62.
3. Hughes HK, Mills Ko E, Rose D, Ashwood P. Immune dysfunction and autoimmunity as pathological mechanisms in autism spectrum disorders. Front Cell Neurosci 2018; 12: 405.
4. Zou T, Liu J, Zhang X, Tang H, Song Y, Kong X. Autoantibody and autism spectrum disorder: a systematic review. Res Autism Spectr Disord 2020; 75: 101568.
5. Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: effects of age, gender, and latitude. J Psychiatr Res 2019; 115: 90-102.
6. Prosperi M, Guiducci L, Peroni DG, et al. Inflammatory biomarkers are correlated with some forms of regressive autism spectrum disorder. Brain Sci 2019; 9: 366.
7. Han YM, Chan AS, Sze SL, et al. Altered immune function associated with disordered neural connectivity and executive dysfunctions: a neurophysiological study on children with autism spectrum disorders. Res Autism Spectr Disord 2013; 7: 662-674.
8. Boterberg S, Charman T, Marschik PB, Bölte S, Roeyers H. Regression in autism spectrum disorder: a critical overview of retrospective findings and recommendations for future research. Neurosci Biobehav Rev 2019; 102: 24-55.
9. Morgan JT, Chana G, Pardo CA, et al. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 2010; 68: 368-376.
10. Won H, Lee HR, Gee HY, et al. Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. Nature 2012; 486: 261-265.
11. Hacohen Y, Wright S, Gadian J, et al. N-methyl-d-aspartate (NMDA) receptor antibodies encephalitis mimicking an autistic regression. Dev Med Child Neurol 2016; 58: 1092-1094.
12. Caraballo RH, Cejas N, Chamorro N, Kaltenmeier MC, Fortini S, Soprano AM. Landau–Kleffner syndrome: a study of 29 patients. Seizure 2014; 23: 98-104.
13. Ozonoff S, Williams BJ, Landa R. Parental report of the early development of children with regressive autism: the delays-plus-regression phenotype. Autism 2005; 9: 461-486.
14. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Autism Dev Disord 1980; 10: 91-103.
15. Savasır I, Sezgin N, Erol N. Ankara Gelişim Tarama Envanteri. (3. Basım) Ankara: Türk Psikologlar Derneği Yayınları, 1998.
16. Krug DA, Arick J, Almond P. Behavior checklist for identifying severely handicapped individuals with high levels of autistic behavior. J Child Psychol Psychiatry 1980; 21: 221-229.
17. Aman MG, Singh NN, Stewart AW, Field CJ. The aberrant behavior checklist: a behavior rating scale for the assessment of treatment effects. Am J Ment Defic 1985; 89: 485-491.
18. Ricken G, Schwaiger C, De Simoni D, et al. Detection methods for autoantibodies in suspected autoimmune encephalitis. Front Neurol 2018; 9: 841.
19. Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG. Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. Ann Neurol 2010; 67: 470-478.
20. Chen MH, Su TP, Chen YS, et al. Comorbidity of allergic and autoimmune diseases in patients with autism spectrum disorder: a nationwide population-based study. Res Autism Spectr Disord 2013; 7: 205-212.
21. Scott O, Shi D, Andriashek D, Clark B, Goez HR. Clinical clues for autoimmunity and neuroinflammation in patients with autistic regression. Dev Med Child Neurol 2017; 59: 947-951.
22. Ozonoff S, Iosif AM, Young GS, et al. Onset patterns in autism: correspondence between home video and parent report. J Am Acad Child Adolesc Psychiatry 2011; 50: 796-806.e1.
23. Young AM, Chakrabarti B, Roberts D, Lai MC, Suckling J, Baron-Cohen S. From molecules to neural morphology: understanding neuroinflammation in autism spectrum condition. Mol Autism 2016; 7: 9.
24. Brenton JN, Goodkin HP. Antibody-mediated autoimmune encephalitis in childhood. Pediatr Neurol 2016; 60: 13-23.
25. Scott O, Richer L, Forbes K, et al. Anti–N-methyl-D-aspartate (NMDA) receptor encephalitis: an unusual cause of autistic regression in a toddler. J Child Neurol 2014; 29: 691-694.
26. Hacohen Y, Wright S, Gadian J, et al. N-methyl-d-aspartate (NMDA) receptor antibodies encephalitis mimicking an autistic regression. Dev Med Child Neurol 2016; 58: 1092-1094.

27. Peñagarikano O, Geschwind DH. What does CNTNAP2 reveal about autism spectrum disorder? Trends Mol Med 2012; 18: 156-163.

28. Oblak AL, Gibbs TT, Blatt GJ. Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. J Neurochem 2010; 114: 1414-1423.

29. O'Connor EC, Bariselli S, Bellone C. Synaptic basis of social dysfunction: a focus on postsynaptic proteins linking group-I mGluRs with AMPARs and NMDARs. Eur J Neurosci 2014; 39: 1114-1129.

30. Gresa-Arribas N, Titulaer MJ, Torrents A, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. Lancet Neurol 2014; 13: 167-177.

31. Cambuli VM, Incani M, Cossu E, et al. Prevalence of type 1 diabetes autoantibodies (GADA, IA2, and IAA) in overweight and obese children. Diabetes Care 2010; 33: 820-822.

32. Menon D, Menon RN, Kumar H, et al. The expanding spectrum of pediatric anti-glutamic acid decarboxylase antibody mediated CNS disease-a chance association? Neuroimmunol Neuroinflamm 2016; 3: 219-224.

33. Rout UK, Dhossche DM. A pathogenetic model of autism involving Purkinje cell loss through anti-GAD antibodies. Med Hypotheses 2008; 71: 218-221.

34. Najjar S, Pearlman D, Devinsky O, et al. Neuropsychiatric autoimmune encephalitis without VGKC-complex, NMDAR, and GAD autoantibodies: case report and literature review. Cogn Behav Neuro 2013; 26: 36-49.

35. Gresa-Arribas N, Ariño H, Martínez-Hernández E, et al. Antibodies to inhibitory synaptic proteins in neurological syndromes associated with glutamic acid decarboxylase autoimmunity. PLoS One. 2015; 10: e0121364.

36. Rout UK, Mungan NK, Dhossche DM. Presence of GAD65 autoantibodies in the serum of children with autism or ADHD. Eur ChildAdolesc Psychiatry 2012; 21: 141-147.

37. Jarius S, Stich O, Speck J, et al. Qualitative and quantitative evidence of anti-glutamic acid decarboxylase-specific intrathecal antibody synthesis in patients with stiff person syndrome. J Neuroimmunol 2010; 229: 219-224.

38. Fatemi SH. Cerebellar pathology in autism. In: Gruol DL, Koibuchi N, Manto M, Molinari M, Schmahmann JD, Shen Y (eds). Essentials of Cerebellum and Cerebellar Disorders. Switzerland: Springer, 2016: 539-543.

39. Haberlandt E, Bast T, Ebner A, et al. Limbic encephalitis in children and adolescents. Arch Dis Child 2011; 96: 186-191.

40. Kalra S, Burbelo PD, Bayat A, et al. No evidence of antibodies against GAD65 and other specific antigens in children with autism. BBA Clin 2015; 4: 81-84.

41. Bayram AK, Kardas F, Demirci EO, et al. Lack of serum antineuronal antibodies in children with autism. Bratisl Lek Listy 2016; 117: 77-79.

42. Hamed NO, Al-Ayadhi L, Osman MA, et al. Understanding the roles of glutamine synthetase, glutaminase, and glutamate decarboxylase autoantibodies in imbalanced excitatory/inhibitory neurotransmission as etiological mechanisms of autism. Psychiatry Clin Neurosci 2018; 72: 362-373.

43. Stefanatos G. Changing perspectives on Landau-Kleffner syndrome. Clin Neuropsychol 2011; 25: 963-988.

44. Shenoy S, Arnold S, Chatila T. Response to steroid therapy in autism secondary to autoimmune lymphoproliferative syndrome. J Pediatr 2000; 136: 682-687.

45. Duffy FH, Shankardass A, McAnulty GB, et al. Corticosteroid therapy in regressive autism: a retrospective study of effects on the Frequency Modulated Auditory Evoked Response (FMAER), language, and behavior. BMC Neurol 2014; 14: 70.

46. Malek M, Ashraf-Ganjouei A, Moradi K, Bagheri S, Mohammadi MR, Akhondzadeh S. Prednisolone as adjunctive treatment to risperidone in children with regressive type of autism spectrum disorder: a randomized, placebo-controlled trial. Clin Neuropharmacol 2020; 43: 39-45.

47. Connery K, Tippett M, Delhey LM, et al. Intravenous immunoglobulin for the treatment of autoimmune encephalopathy in children with autism. Transl Psychiatry 2018; 8: 148.

48. Hafizi S, Tabatabaei D, Lai MC. Review of clinical studies targeting inflammatory pathways for individuals with autism. Front Psychiatry 2019; 10: 849.

49. Ozonoff S, Li D, Deprey L, Hanzel EP, Iosif AM. Reliability of parent recall of symptom onset and timing in autism spectrum disorder. Autism 2018; 22: 891-896.

50. Pearson N, Charman T, Happé F, Bolton PF, McEwen FS. Regression in autism spectrum disorder: reconciling findings from retrospective and prospective research. Autism Res 2018; 11: 1602-1620.

The Turkish Journal of Pediatrics 2021; 63: 790-800
https://doi.org/10.24953/turkjped.2021.05.007

Original Article

COVID-19-related anxiety in phenylketonuria patients

Halil Tuna Akar[1], Yamaç Karaboncuk[2], Kısmet Çıkı[1], Ayça Burcu Kahraman[1], İzzet Erdal[1], Turgay Coşkun[1], Ayşegül Tokatlı[1], Ali Dursun[1], Yılmaz Yıldız[1], H. Serap Sivri[1]

[1]Department of Pediatric Metabolism and Nutrition Unit, Hacettepe University Faculty of Medicine, Ankara; [2]Ferhunde Öktem Mental Health Center, Ankara, Turkey.

ABSTRACT

Background. Phenylketonuria (PKU) is an inherited disorder of amino acid metabolism, the treatment of which often requires a special diet to prevent adverse neuropsychiatric outcomes. In the COVID-19 pandemic, which has had a substantial effect on the whole world since the beginning of 2020, PKU patients represent a vulnerable population because they may be dependent on special nutritional products, have limited access to routine care and display increased levels of anxiety.

Methods. For this reason, an online questionnaire assessing the anxiety levels and various personal opinions and practices regarding the pandemic was sent to the PKU patients managed at our clinic, who were 12 years of age or older. Ninety-eight patients responded to the questionnaire. Median age of the participants was 19 years.

Results. Most patients were compliant with the hygiene and social distancing recommendations regarding the spread of COVID-19. Of the patients, 61.2% felt more anxious since the pandemic. The most common concern was the possibility of not being able to obtain special nutritional products (58.2%). Anxiety level was significantly higher in females.

Conclusions. These data suggest that food security is an important issue of concern in PKU patients. In line with the changing world after the pandemic, different strategies should be considered in the management of patients with inborn errors of metabolism, including PKU.

Key words: COVID-19, phenylketonuria, anxiety.

Phenylketonuria (PKU) is an inherited disorder of the breakdown pathyway of the amino acid phenylalanine.[1] The resulting accumulation of phenylalanine causes intellectual disability and many other neuropsychiatric symptoms.[2] Treatment must be initiated soon after birth and maintained lifelong, usually utilizing a phenylalanine-restricted diet to keep the blood phenylalanine level within a target range.[3] Turkey has the highest incidence of PKU, at least partly due to the high rate of consanguinity.[4] The newborn PKU screening program in Turkey, initiated by our department as a pilot study in 1986, became a national screening program in 2006.[5-7]

It is widely observed that adherence to dietary treatment is very good in childhood, but decreases thereafter, especially after adolescence, with phenylalanine levels exceeding the target levels. In fact, 31.1% of the PKU patients in our clinic are poorly compliant to their dietary treatment.[7-9] The diet is extremely challenging for the patients and their families, and often creates significant psychosocial and financial problems.[10-13] Patients have difficulties in maintaining their diet at school and work, and experience anxiety and low self-esteem.[14,15] Patients with PKU have a higher risk of psychiatric illness and cognitive impairment, even if they are treated early and

✉ H. Serap Sivri
ssivri@hacettepe.edu.tr

Received 8th March 2021, revised 31st March 2021, accepted 2nd April 2021.

effectively.[16] Prevalence of neuropsychiatric symptoms such as carelessness, hyperactivity, depression and anxiety in PKU are above the general population estimates. Neuropsychiatric symptoms and executive dysfunction have been demonstrated to correlate with phenylalanine levels.[17]

COVID-19, the disease caused by SARS-CoV-2 was declared a pandemic by the World Health Organization on March 11, 2020. As of March 30, 2021, 3,277,880 confirmed cases and 31,385 deaths have been reported in our country. The elderly seem to develop more serious diseases and hypertension, cardiopulmonary diseases, cancer and diabetes are additional risk factors.[18-20] In order to prevent the spread, staying at home and social isolation is encouraged, social activities have been canceled or postponed, and commercial activities were temporarily suspended or restricted in many countries.[21] It is not surprising that such devastating alterations to social and economic life have had significant psychological impact on people all over the world. For example, in a general population study on the COVID-19 pandemic in India, the prevalence of anxiety and depression has been shown to increase.[22] In the United Kingdom, pandemic-related anxiety, depression and trauma were shown to be associated with an underlying disease.[22] There are not many studies on how patients with rare diseases are affected by this pandemic. However, in a study conducted in Italy in April 2020, 49% of patients receiving enzyme replacement therapy were reported to have pandemic-related disruptions in their treatment.[23] National initiatives such as the French Rare Diseases Health Care Network for Neuromuscular Diseaeses (FILNEMUS) have sought to draw a path for how to track and manage patients with rare diseases.[24] Similarly, patients with PKU may be affected by humanitarian, social and economic fluctuations due to their special needs. From this point of view, one may wonder how patients with PKU, a rare disease that may predispose them to neuropsychiatric findings such as anxiety, are coping with this pandemic. Therefore, we aimed to evaluate how they were affected by the COVID-19 pandemic, and assess their anxiety levels.

Material and Methods

This cross-sectional, observational study was carried out at the Pediatric Metabolism Unit of Hacettepe University İhsan Doğramacı Children's Hospital, which is one of the largest metabolic centers in the region, and follows patients from all around Turkey, predominantly from Central Anatolia. Classical PKU patients 12 years of age and older who were actively followed in our clinic (came to at least one routine outpatient follow-up visit within the past year) were included in the study. In order to adhere to the social distancing efforts, patients in our center were contacted via telephone or social media, and questionnaire forms were delivered via Google Forms to the eligible patients followed at our center, and data was collected from the forms filled and submitted back by the patients anonymously. Written informed consent was obtained from the patients before participating in the online study.

As there were no validated self-report anxiety scales developed in relation to the COVID-19 pandemic, we sought to adapt a validated tool to the task at hand in order to formulate a questionnaire. The commonly used Beck Anxiety Scale consists of 21 questions and is fit for face-to-face application.[25] Since face-to-face interviews were not applicable in the pandemic conditions, the scale needed to be condensed and simplified, so that it could be well understodd by an average older PKU patient with short attention span and possibly mildly impaired executive functions. The questionnaire was developed by physicians specialisng in metabolic disorders and clinical psychologists with years of experience with PKU patients. In summary, the questionnaire included structured questions adapted from Beck Anxiety Scale, encompassing demographic data, medical information, and self-reported

anxiety, using short text answers, multiple choice and open-ended questions. Except for questions that inquired about demographic data, all questions were explicitly worded to inquire thoughts, feelings and practices in the last 15 days. The online questionnaire was sent out on 31 March 2020, and data collection was terminated on 1 June 2020.

The data obtained in the study were evaluated with SPSS (Statistical Package for the Social Sciences) version 21. Descriptive statistics, categorical variables were shown with numbers and percentages, and numerical variables with mean, standard deviation, median, range and interquartile range (IQR). Kolmogorov-Smirnov Test was used to assess normality of distributions. Mann Whitney U and Kruskal Wallis tests were used in comparing two and more independent groups, respectively. Chi-square and Fisher exact tests were used in the analysis of categorical variables. Spearman correlation test was used in the correlation analysis. Instances where the type-1 error level is below 5% ($p < 0.05$) were considered statistically significant. Hacettepe University Ethics Board for Non-interventional Clinical Studies approved the study (Approval Date: 31 March 2020 Issue: 2020/07-20).

Results

The questionnaire forms were sent out to 347 patients, 98 of whom (28.2% of all eligible patients) participated in the study by returning the filled out forms. The demographic information of the patients is summarized in Table I. Of the patients, 50% (n = 49) were female. The median age of the participants was 19 years (range:12-51 years). A median of four people resided in the household (IQR=2). Fifteen patients had comorbidities (three had hypothyroidism, and one each had anxiety disorder, osteoporosis, heart failure, ulcerative colitis, hereditary fructose intolerance, epilepsy, attention deficit and hyperactivity disorder). Of the patients 86.6% were being treated with phenylalanine restricted diet. All 41 patients

Table I. Clinical and demographic data of the study group (N=98).

	n	%
Gender		
Male	49	50
Female	49	50
Civil status		
Lives with spouse/partner	21	21.4
Single	74	75.4
Divorced	3	3.1
Working status of patients		
Working constantly	28	28.6
Not working	70	70.4
Modality of treatment		
Phenylalanine restricted diet	84	86.6
Other	8	8.2
Does not comply with diet / treatment	5	5.2
Presence of additional chronic disease	15	15.3
Working status of mother		
Working constantly	15	15.3
Not working	82	83.7
Not specified	1	1
Working status of father		
Working constantly	71	72.4
Not working	25	25.5
Not specified	2	2
Living with an individual over the age of 65	11	11.2
Living with another patient with phenylketonuria	13	13.3
Media for social contact with acquaintances during pandemic		
Face to face contact	16	16.10
By phone	52	53.10
By video call	33	33.7
Internet based systems	38	38.8
Via social media	33	33.7
Distribution of the sources from which patients received information during the COVID-19 pandemic		
Television broadcasts	80	81.6
Physician's opinion	17	17.3
Internet broadcasts	51	52
Family members	47	48
Friends	12	12.2
Other	3	3.10

under the age of 18 attended school. Among the remaining 57 patients older than 18 years, 25 (43.8%) had a steady job.

Of the patients 76.5% (n = 75) stated that they had social contact with friends during the pandemic. Most of the patients (53.1%) contacted their friends over the phone.

Patients were asked if their knowledge level about the COVID-19 pandemic was sufficient. Of the patients 67.3% (n = 66) stated that they had sufficient information about the pandemic. The patients were also asked what their sources of information were. The majority of patients (81.6%) stated that they learned about the pandemic via television, followed by internet broadcasts (52%).

The self-reported anxiety levels of the patients are given in Table II. Of the participants , 61.2% reported that they felt more anxious since the pandemic. The biggest concern of the patients was the possibility of not being able to obtain special nutrition products (58.2%). Of the patients 79.6% (n=78) described at least one sign of anxiety. Of the participants , 21.4% stated that they had difficulty in dealing with anxiety. Female participants significantly more commonly reported that they had difficulty coping with anxiety since the onset of the pandemic (37.2% vs. 10.4%, p=0.010), felt sad or unhappy (59.6% vs. 36.2%, p=0.023), worthless (19.1% vs. 4.3%, p=0.025), scared without reason (75.6% vs. 46.9%, p=0.005), and had palpitations unrelated to physical activity (21.7% vs. 6.1%, p=0.027).

The precautionary measures taken by patients against the COVID-19 pandemic were also inquired. The patients' responses are summarized in Table II. Patients mostly paid attention to hygiene recommendations (66.3%). Of the participants 55.1% fully complied with the rule of staying at home.

The patients were asked to give their anxiety levels a score from 1 to 5. Self-reported anxiety levels and age, gender, source of information, level of knowledge, and treatment modality were compared (Table III). No statistically significant results were obtained, except for gender. Anxiety level was higher in females than in males. Age was not significantly correlated with anxiety levels (Spearman's correlation coefficient ϱ=0.085, p=0.413). No statistically significant relationship was found between the source of the information about pandemic and the level of anxiety.

Before the participants participated in the survey, their consent was obtained online.

Discussion

The COVID-19 pandemic affected the world as well as our country, and our patients. We wanted to investigate the concerns of a vulnerable and rare patient population in this difficult process and the factors that may influence this anxiety, using a web-based study, befitting the new norms of communication during this unprecedented pandemic.

The time frame of this study coincides with the initial stages of the pandemic response in Turkey. On 11 March 2020, the first cases of SARS-CoV-2 detected in Turkey was announced by the Ministry of Health. The first COVID-19-related death occurred on March 15, 2020. The Ministry of Health announced on April 1, 2020 that coronavirus cases were seen in all regions of the country. As of March 13, 2020, restrictions were gradually imposed on foreign entries and exits. As of March 16, 2020, restrictions have been placed on social-cultural-religious meetings within the country. On April 3, 2020, a curfew was imposed across the country for those born later than December 31, 1999, masks were imposed in public areas such as markets, and entrances and exits to 30 metropolitan cities were halted for 15 days. Curfews of different days and lengths were declared as of April 10, 2020. The easing of the initial restrictions did not ocur until June 1, 2020, which coincides with the end of data collection for this study. In other words, the data was collected at a time when the scientific information was premature,

Table II. Anxiety level assesments of patients and evaluation of the measures taken by patients during COVID-19 pandemic.

	n	%
How did your anxiety change during the pandemic?		
Decreased	3	3.1
Did not change	34	34.7
Increased	60	61.2
What are your concerns about the pandemic?		
I feel more at risk than the general population	55	56.1
I think I am at risk of having problems accessing healthcare services	41	41.8
I think I may have difficulties in accessing special nutrition products	57	58.2
I think I may have difficulty in accessing medicinal products	41	41.8
I think I'm more likely to get infected	53	54.1
I think that if I get infected, I will be seriously ill	49	50
I think one of my family members will get sick	28	28.6
Other	14	14.3
How was your last 15 days since the pandemic started?		
Partially coped	27	27.6
Neutral	43	43.9
Difficult to deal with	21	21.4
Not specified	7	7.1
I felt unhappy and sad		
Yes	45	45.9
No	49	50
Not specified	4	4.1
I felt worthless		
Yes	11	11.2
No	83	84.7
Not specified	4	4.1
I had a hard time taking the first step to do a job		
Yes	27	27.6
No	64	65.3
Not specified	7	7.1
Sometimes I feel scared even though it doesn't have a logical explanation		
Yes	57	58.2
No	37	37.8
Not specified	4	4.1
I had difficulty breathing even though I did not do a physical activity		
Yes	9	9.2
No	85	86.7
Not specified	4	4.1
I felt my heart beating fast even though I was not doing any physical activity		
Yes	13	13.3
No	82	83.7
Not specified	3	3.1

Table II. Continued.

	n	%
I feel that I am more sensitive		
Yes	13	13.3
No	82	83.7
Not specified	3	3.1
I tend to overreact to events		
Yes	35	35.7
No	59	60.2
Not specified	4	4.1
What would your score be if you scored 1 to 5 on the level of anxiety the pandemic created in you?		
1-I'm not worried at all	9	9.2
2- I'm less concerned	21	21.4
3-I'm concerned	41	41.8
4-I'm very worried	22	22.4
5-I can't deal with my anxiety	3	3.1
Have you experienced an event or events today that caused your anxiety to increase?		
Yes	33	33.7
No	31	31.6
Not specified	34	34.7
What COVID-19 precaution measures are you taking?		
I pay attention to hygiene.	65	66.3
I try to follow social isolation rules.	59	60.2
I am trying to strengthen my immune system.	9	9.2
Do you follow the rule of staying at home?		
I comply fully	54	55.1
I rarely go out (eg only for the market)	41	41.8
I don't follow the rule of staying at home	2	2
Not specified	1	1

daily news were concerned primarily with the pandemic and even the short-term future was full of uncertainties. The stressful time in which the study was conducted might have contributed to the high anxiety levels reported in the study questionnaire.

The majority of the participants reported at least some adherence to their diet, and many of them managed to maintain social contact with peers, mostly over the phone. Of participants 81.6% received information about the pandemic via television and 52% via the internet. Although new media platforms come to the fore, it is worthy to note that, at least in this patient population, television broadcasting still has a very important effect. Only 17.3% of the patients received information from a physician. This low rate may have resulted from the decrease in routine physician or hospital visits during the pandemic. It may be difficult to achieve the classical patient-physician relationship in the pandemic, when face-to-face interactions are avoided unless absolutely necessary. Telemedicine practices in many different disciplines were strongly discussed in the pandemic process.[26-29] It is necessary to mention the critical role of social media and mass media in the acquisition of information in this process. However, this has some drawbacks. In a study

Table III. Comparison of pandemic-related anxiety levels of patients in terms of gender, knowledge level, measures taken and treatment modalities.

	COVID-19 self-reported anxiety state					p
	1+	2+	3+	4+	5+	
Gender						0.002*
Male	6(12.5%)	15(31.3%)	20(41.7%)	7(14.6%)	0(0%)	
Female	3(6.3%)	6 (12.5%)	21(43.8%)	15(31.3%)	3(6.3%)	
Age						0.602
< 18 years	4(10%)	8(20%)	20(50%)	8(20%)	0(0%)	
≥ 18 years	5(9.1%)	13(23.6%)	21(38.2%)	13(23.6%)	3(5.5%)	
Pandemic subjective knowledge level						0.0990
Enough	6(9.1%)	11(16.7%)	35(53%)	13(19.7%)	1(1.5%)	
Not enough	3(10%)	10(33.3%)	6(20%)	9(30%)	2(6.7%)	
Compliance with lockdown						0.487
Fully compliant	8(14.8%)	11(20.4%)	21(38.9%)	12(22.2%)	2(3.7%)	
Rarely goes out	1(2.5%)	9(22.5%)	19(47.5%)	10(25%)	1(2.5%)	
Non-compliant	0(0%)	1(50%)	1(50%)	0(0%)	0(0%)	
Measure taken						0.616
Paying attention to hygiene	7(10.9%)	16(25%)	24(37.5%)	14(21.9%)	3(4.7%)	
Following social isolation	4(6.9%)	14(24.1%)	27(46.6%)	13(22.4)	0(0%)	
Trying to strengthen immune system	1(11.1%)	2(22.2%)	2(22.2%)	4(44.4%)	0(0%)	
Treatment modality						0.769
Phenylalanine restricted diet	8(9.5%)	21(25%)	34(40.5%)	18(21.4%)	3(3.6%)	
Other	0(0%)	0(0%)	6(75%)	2(25%)	0(0%)	
Does not comply with diet / treatment	1(25%)	0(0%)	1(25%)	2(50%)	0	
Source of information about COVID-19 pandemic						
Television broadcasts	6(7.6%)	16(20.3%)	34(43.0%)	20(25.3%)	3(3.8%)	0.425
Physician's opinion	1(5.9%)	5(29.4%)	9(52.9%)	2(11.8%)	0(0%)	0.651
Internet broadcasts	2(4%)	11(22%)	21(42%)	15(30%)	1(%2)	0.198
Family members	3(6.5%)	11(23.9%)	17(37%)	14(30.4%)	1(2.2%)	0.406
Friends	0(0%)	5(41.7%)	4(33.3%)	3(25%)	0(0%)	0.425

* $p<0.05$

+ 1-I'm not worried at all
2- I'm less concerned
3-I'm concerned
4-I'm very worried
5-I can't deal with my anxiety

conducted in Turkey, Youtube videos related to the COVID-19 pandemic were examined. Video contents were audited and only 37.5% were found useful. Of those evaluated 15.8% of Turkish and 10.4% of English videos were found to have false/misleading information.[30] It may be possible to use social and new media tools to our advantage to alleviate anxiety during an epidemic. It is important to use and produce accurate, reliable and reputable sources of information.[31,32]

Of the patients in the study group 61.2% stated that their anxiety increased during the pandemic, 45.9% were unhappy and upset, 11.2% felt unworthy and 35.7% stated that they

overreacted to events. It is noteworthy that these and similar symptoms of depression are present in the study group. In the COVID-19 pandemic, many health-care workers showed signs of anxiety and depression.[33] Similarly, anxiety levels in the general population have also increased.[15,22,34,35] However, such studies addressing patients with rare diseases have been lacking, which has motivated us to perform this study.

The most common concern of the patients (58.2%) was the possibility of not being able to obtain special nutrition products. It is known that many people around the world stock food with the concern that they may not be able to find basic food items.[36,37] It is not surprising that PKU patients experience a higher anxiety regarding access to food because they acquire their medical foods from pharmacies, or they already have financial or logistic difficulties in obtaining their special low-protein food products.[38,39] This additional burden is unique to patients with PKU and with other rare inborn errors of metabolism requiring special diets. It is important to advocate for policies that ensure food and medicinal product security in this vulnerable patient population suffering from rare, orphan diseases. It is also concerning that Turkey is dependent on import of medical foods and drugs, especially in the rare diseases group. In particular, the possibility that the closure of the borders would disrupt the global logistics chain and/or cause a decrease in production capacities probably contributed to the anxiety of the participants.

In our study, pandemic-related anxiety levels were more common in females. This may be significant in terms of gender preference of anxiety symptoms in the general population.[40] In the literature, it has been shown that clinical findings of anxiety appear during adolescence and early adulthood.[41] However, when the distribution of anxiety levels in terms of age was examined in our study, no statistical difference was found. This may be related to the fact that the participants were all older than 12 years.

Our study has its limitations. Since the questionnaires were not completed via a face-to-face interview and the patients are asked to complete the questionnaire themselves, it is not known whether the patients understood the questions completely. In addition, the patients self-expressed their anxiety levels and knowledge levels. Performing face-to-face mental state examinations to assess anxiety more objectively was not feasible. As the filled questionnaires were returned anonymously, researchers were blinded to the identities of the participants, making it impossible to correlate the answers with clinical data.

Since the COVID-19 pandemic is a process full of unknowns, and was especially so in the initial stages, it cannot be denied that there were many questions raised in the inborn errors of metabolism patient community. We had the opportunity to observe this both during the study and while providing patient service. In the beginning, the questions mainly concerned whether they would be sick more easily than the society and what their prognosis would be if they were sick. So far, there has been no indication that people with PKU may be under higher risk of infection, or of more severe disease. The results of this study show that the level of COVID-19 pandemic-related anxiety was high in patients with PKU, and significantly higher in females. Food insecurity regarding medical and low-protein foods was a major concern, which was not an issue previously addressed in the literature. Our study suggests that different support strategies should be brought into the spotlight by taking into consideration the changing world and medical practices in the post-COVID-19 era. Especially, in new communication channels such as social media and the internet, it may be useful to open areas where health care providers can provide patients with reliable information, and share their concerns and problems. In addition, guaranteeing the supply chain of food and medicinal products in cooperation with the local authorities may reduce the anxiety levels.

This may also applicable to other rare metabolic disorders requiring special diets. In this respect, monitoring of inborn errors of metabolism patients with telehealth applications should be discussed in the community. At this point, the contribution of patients, families and non-governmental organizations cannot be overemphasized.

Acknowledgment

We would like to thank our patients, their families, patient and family associations. Their support has always been with us.

Ethical approval

Hacettepe University Ethics Board for Non-interventional Clinical Studies approved the study (Approval Date: 31 March 2020 Issue: 2020 / 07-20).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HTA, YK, AD, AT, TC SS; data collection: HTA, KÇ, ABK, İE; data analysis and interpretation: HTA, YK, YY, SS; drafting of manuscript: HTA, YK, YY; critical review of manuscript: YY, AD, AT, TC, SS. All authors approve and take responsibility for the final version of the manuscript.

Source of funding

The study did not received funding.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. Lancet 2010; 376: 1417-1427.
2. van Spronsen F, Huijbregts SC, Bosch AM, Leuzzi V. Cognitive, neurophysiological, neurological and psychosocial outcomes in early-treated PKU-patients: a start toward standardized outcome measurement across development. Mol Genet Metab 2011; 104(Suppl): S45-S51.
3. Ney DM, Gleason ST, van Calcar SC, et al. Nutritional management of PKU with glycomacropeptide from cheese whey. J Inherit Metab Dis 2009; 32: 32-39.
4. Özalp I, Coşkun T, Ceyhan M, et al. Incidence of phenylketonuria and hyperphenylalaninaemia in a sample of the Turkish newborn population. In: Addison GM, Harkness RA, Isherwood DM, Pollit RJ (eds). Practical Developments in Inherited Metabolic Disease: DNA Analysis, Phenylketonuria and Screening for Congenital Adrenal Hyperplasia. Dordrecht: Springer, 1986: 237-239.
5. Özalp I, Coşkun T, Tokatli A, et al. Neonatal PKU screening in Turkey: 7 years experience in a developing country. Screening 1995; 4: 139-147.
6. Tezel B, Dilli D, Bolat H, et al. The development and organization of newborn screening programs in Turkey. J Clin Lab Anal 2014; 28: 63-69.
7. Ozalp I, Coşkun T, Tokatli A, et al. Newborn PKU screening in Turkey: at present and organization for future. Turk J Pediatr 2001; 43: 97-101.
8. Yilmaz Ö. Fenilketonürili bireylerde diyet fenilalanin toleransının değerlendirilmesi. Ankara: Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, 2017.
9. Abstracts presented at the 13th International Congress of Inborn Errors of Metabolism - ICIEM 2017. J Inborn Errors Metab Screen 2017; 5: 1-413.
10. Wang L, Zou H, Ye F, et al. Household financial burden of phenylketonuria and its impact on treatment in China: a cross-sectional study. J Inherit Metab Dis 2017; 40: 369-376.
11. Eijgelshoven I, Demirdas S, Smith TA, van Loon JM, Latour S, Bosch AM. The time consuming nature of phenylketonuria: a cross-sectional study investigating time burden and costs of phenylketonuria in the Netherlands. Mol Genet Metab 2013; 109: 237-242.

12. Gramer G, Haege G, Glahn EM, Hoffmann GF, Lindner M, Burgard P. Living with an inborn error of metabolism detected by newborn screening—parents' perspectives on child development and impact on family life. J Inherit Metab Dis 2014; 37: 189-195.

13. MacDonald A, Smith TA, de Silva S, Alam V, van Loon JM. The personal burden for caregivers of children with phenylketonuria: a cross-sectional study investigating time burden and costs in the UK. Mol Genet Metab Rep 2016; 9: 1-5.

14. Ford S, O'Driscoll M, MacDonald A. Living with phenylketonuria: lessons from the PKU community. Mol Genet Metab Rep 2018; 17: 57-63.

15. Gunduz M, Arslan N, Unal O, Cakar S, Kuyum P, Bulbul SF. Depression and anxiety among parents of phenylketonuria children. Neurosciences (Riyadh) 2015; 20: 350-356.

16. Ashe K, Kelso W, Farrand S, et al. Psychiatric and cognitive aspects of phenylketonuria: the limitations of diet and promise of new treatments. Front Psychiatry 2019; 10: 561.

17. Bilder DA, Noel JK, Baker ER, et al. Systematic review and meta-analysis of neuropsychiatric symptoms and executive functioning in adults with phenylketonuria. Dev Neuropsychol 2016; 41: 245-260.

18. Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: a comparison with young and middle-aged patients. J Infect 2020; 80: e14-e18.

19. Jordan RE, Adab P, Cheng K. Covid-19: risk factors for severe disease and death. Br Med J 2020; 368: m1 198.

20. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395: 1054-1062.

21. Wilder-Smith A, Freedman D. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. J Travel Med 2020; 27: taaa020.

22. Roy D, Tripathy S, Kar SK, Sharma N, Verma SK, Kaushal V. Study of knowledge, attitude, anxiety & perceived mental healthcare need in Indian population during COVID-19 pandemic. Asian J Psychiatr 2020; 51: 102083.

23. Sechi A, Macor D, Valent S, et al. Impact of COVID-19 related healthcare crisis on treatments for patients with lysosomal storage disorders, the first Italian experience. Mol Genet Metab 2020; 130: 170-171.

24. Solé G, Salort-Campana E, Pereon Y, et al; FILNEMUS COVID-19 study group Guidance for the care of neuromuscular patients during the COVID-19 pandemic outbreak from the French Rare Health Care for Neuromuscular Diseases Network. Rev Neurol (Paris) 2020; 176: 507-515.

25. Julian LJ. Measures of anxiety: State-Trait Anxiety Inventory (STAI). Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A) 2011; 63(Suppl 11): S467-S472.

26. Bini SA, Schilling PL, Patel SP, et al. Digital orthopaedics: a glimpse into the future in the midst of a pandemic. J Arthroplasty 2020; 35: S68-S73.

27. Chung CJ, Nazif TM, Wolbinski M, et al. Restructuring of structural heart disease practice during the Covid-19 pandemic. J Am Coll Cardiol 2020; 75: 2974-2983.

28. Schrag D, Hershman DL, Basch E. Oncology practice during the COVID-19 pandemic. JAMA 2020; 323: 2005-2006.

29. Vaccaro AR, Getz CL, Cohen BE, Cole BJ, Donnally CJ 3rd. Practice management during the COVID-19 pandemic. J Am Acad Orthop Surg 2020; 28: 464-470.

30. Atac O, Ozalp Y, Kurnaz R, Guler OM, Inamlik M, Hayran O. YouTube as an information source during the Coronavirus disease (COVID-19) pandemic: evaluation of the Turkish and English content. medRxiv doi: https://doi.org/10.1101/2020.05.06.20093468.

31. Wiederhold BK. Using social media to our advantage: alleviating anxiety during a pandemic. Cyberpsychol Behav Soc Netw 2020; 23: 197-198.

32. Rosenberg H, Syed S, Rezaie S. The Twitter pandemic: the critical role of Twitter in the dissemination of medical information and misinformation during the COVID-19 pandemic. CJEM 2020; 22: 418-421.

33. Pappa S, Ntella V, Giannakas T, Giannakoulis VG, Papoutsi E, Katsaounou P. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: a systematic review and meta-analysis. Brain Behav Immun 2020; 88: 901-907.

34. Limcaoco RSG, Mateos EM, Fernandez JM, Roncero C. Anxiety, worry and perceived stress in the world due to the COVID-19 pandemic, March 2020. Preliminary results. medRxiv doi: https://doi.org/10.1101/2020.04.03.20043992.

35. Zhang J, Lu H, Zeng H, et al. The differential psychological distress of populations affected by the COVID-19 pandemic. Brain Behav Immun 2020; 87: 49-50.

36. Nicola M, Alsafi Z, Sohrabi C, et al. The socio-economic implications of the coronavirus pandemic (COVID-19): a review. Int J Surg 2020; 78: 185-193.

37. Manderson L, Levine S. COVID-19, risk, fear, and fall-out. Med Anthropol 2020; 39: 367-370.

38. Berry SA, Brown CS, Greene C, Camp KM, McDonough S, Bocchini JA Jr; Follow-up and Treatment (FUTR) Workgroup for the Advisory Committee on Heritable Disorders in Newborns and Children. Medical foods for inborn errors of metabolism: history, current status, and critical need. Pediatrics 2020; 145: e20192261.

39. Coakley KE, Porter-Bolton S, Salvatore ML, Blair RB, Singh RH. Food insecurity in females with phenylketonuria. JIMD Rep 2020; 53: 103-110.

40. Li SH, Graham BM. Why are women so vulnerable to anxiety, trauma-related and stress-related disorders? The potential role of sex hormones. Lancet Psychiatry 2017; 4: 73-82.

41. de Lijster JM, Dierckx B, Utens EM, et al. The age of onset of anxiety disorders: a meta-analysis. Can J Psychiatry 2017; 62: 237-246.

The Turkish Journal of Pediatrics 2021; 63: 801-810
https://doi.org/10.24953/turkjped.2021.05.008

Original Article

Anxiety among the parents of pediatric patients receiving IVIG therapy during the Covid-19 pandemic

Özge Yılmaz Topal[1], Ayşe Metin[1], Esra Çöp[2], Gülser Şenses Dinç[2], Özden Şükran Üneri[3]

Divisions of [1]Pediatrics Allergy and Immunology and [2]Child and Adolescent Psychiatry University of Health Sciences, Ankara City Hospital, Ankara; [3]Department of Psychology, İstanbul Gelişim University Faculty of Economics, Administrative and Social Sciences, İstanbul, Turkey.

ABSTRACT

Background. Symptomatic COVID-19 cases in children occur mostly in those with primary immunodeficiency (PID), chronic lung diseases, and heart disease. Guidelines recommend that patients with PID continue to use their regular medication during the pandemic.

Objectives. This study aimed to evaluate anxiety related to COVID-19 in the parents of patients receiving intravenous immunoglobulin (IVIG) treatment in our hospital and to evaluate the effect of their anxiety on the continuity of treatment.

Methods. The parents of the patients who underwent IVIG therapy in our clinic during the pandemic (between May 15, 2020 and July 1, 2020) were included in our study.

Results. Twenty-seven patients with PID whose IVIG therapy was initiated before the pandemic and 29 non-PID control subjects were included in the study. All patients received IVIG treatment in our clinic continued treatment during the pandemic at the same dose intervals. Parents in the IVIG group had significantly higher state (p=0.003) and trait (p=0.003) anxiety scores compared to control parents. IVIG group showed statistically significant higher scores in Beck depression inventory, than the control group (p=0.002).

Conclusions. The parents of PID patients who needed to come to the hospital for IVIG therapy had higher anxiety levels than the parents of similar aged children who presented to our clinic for different complaints between the same dates. Despite their concerns, the parents of all patients under IVIG therapy maintained treatment continuity at the recommended treatment intervals. None of our immunodeficient patients who presented for treatment during the pandemic contracted COVID-19 infection during our study.

Key words: intravenous immunoglobulin treatment, anxiety, COVID-19, pandemic.

Primary immunodeficiencies (PIDs) are a congenital and heterogeneous group of disorders that can affect one or more systems. More than half of patients with PID have impaired antibody production. Affected individuals are more likely to have more severe and frequent infectious disease such as upper and lower respiratory tract infections, sinusitis, and opportunistic microorganism infections. Patients with predominant antibody deficiency need immunoglobulin therapy to reduce the risk of infection.[1-3]

Coronavirus disease 2019 (COVID-19, caused by the novel coronavirus SARS-CoV-2) has a spectrum ranging from asymptomatic to fatal infection disease. Among children, symptomatic COVID-19 cases have occurred mostly in those who have PID, chronic lung diseases, and heart disease.[4] The European Academy of Allergy and Clinical Immunology (EAACI) recommends that patients with PID continue using their regular medication to avoid

✉ Özge Yılmaz Topal
ozgeyilmaztopal@gmail.com

Received 27th January 2021, revised 3rd March 2021, 4th April 2021, accepted 9 April 2021.

clinical exacerbations, underlying diseases, and complications, including autoimmune or other symptoms.[5]

Yuan et al.[6] reported that the anxiety levels of parents of pediatric patients hospitalized during the COVID-19 pandemic were higher than in the non-epidemic period. Being in the hospital can increase the anxiety of patients and their parents due to the risk of nosocomial coronavirus transmission.[6] Our study aimed to evaluate the anxiety that may occur due to COVID-19 in the parents of our patients who received intravenous immunoglobulin (IVIG) treatment in our hospital and to evaluate the effect of this anxiety on the continuity of treatment.

Material and Methods

The parents of patients who underwent IVIG therapy in our pediatric allergy and immunology clinic during the pandemic between May 15, 2020 and July 1, 2020 were included in our study. The control group included patients who had allergic rhinitis and no active symptoms and presented to the outpatient clinic for routine follow-up. The patients' age, sex, type of PID, follow-up times, and age at initiation of IVIG therapy were recorded in a standard form. In addition, any history of COVID-19 infection in the patients, their relatives, and the medical staff working in our clinic were recorded.

The parents' age and gender were recorded upon presentation to our clinic and they completed a questionnaire asking their opinions about coronavirus transmission. The contents of the questionnaire forms for the parents of patients receiving IVIG are given in Table I.

The study was approved by the institutional ethics committee of Ankara City Hospital (No: E1-20-648/ Date:2020) and the study protocol was approved by the Turkish Ministry of Health. Written informed consent was obtained from the parents or legal guardians of the patients.

COVID-19 precautions in our center

After the pandemic started, the body temperature of all patients and their parents were measured at the entrance of our hospital. They were also asked about any history of travel abroad, COVID-19 symptoms such as fever and cough, and contact with patients with known COVID-19 infection. Furthermore, everyone entering the hospital was required to wear a mask and observe social distancing rules.

Patients with fever and history suggesting possible COVID-19 infection were evaluated in separate pandemic outpatient clinics.

Management of IVIG therapy

Patients who had no symptoms of COVID-19 and no history of risky contact when they presented for treatment were admitted to the infusion room to receive IVIG therapy. Patients received IVIG therapy every 3 to 4 weeks. Dosage was 300 to 800 mg/kg at an infusion rate of 0.01 to 0.08 ml/kg/min, as tolerated by the patient.[2,7,8]

IVIG therapy was performed in a dedicated room separate from other patients. Social distance was observed and the number of people in the clinic was reduced by scheduling a certain number of patients for treatment per day and staggering appointments. Patients, their parents, and all health workers wore surgical masks during all procedures. The patients and their parents spent at least 4 hours in this room.

Assessment of parental anxiety levels

The State-Trait Anxiety Inventory (STAI) was used to assess the current and general anxiety levels of the patients' parents. The test-retest reliability coefficients in the initial development ranged from 0.31 to 0.86 with intervals ranging from 1 hour to 104 days.[9] The validity and reliability study of the Turkish version was performed by Oner and Le Compte.[10] The state anxiety subscale consists of 20 items regarding

Table I. The questionnaire form for the parents of patients receiving IVIG therapy.

Q1: Your age:	Q8: How often does your child receive IVIG treatment? □ Once every 3 weeks □ Once every 4 weeks				
Q2: Gender: □ Female □ Male	Q9: Does your child have any chronic and/or psychiatric diseases? If so, please state...				
Q3: Level of education □ Primary school □ Secondary school □ University	Q10: Do you have any chronic and/or psychiatric disease? If so, please state...				
Q4: Place of residence □ In Ankara □ Outside of Ankara	Q11: Have any of your relatives or friends developed symptoms of COVID-19? □ Yes □ No If yes, please write who developed symptoms of corona virus infection?				
Q5: How many years has your child been followed with a diagnosis of immunodeficiency?	Q12: Have you developed symptoms of COVID-19? □ Yes □ No				
Q6: What are the treatments your child is receiving due to immunodeficiency?	Q13: Have any of your relatives or friends had a confirmed COVID-19 infection? □ Yes □ No If yes, please write whom:				
Q7: How long has your child been receiving IVIG treatment?	Q14: Have you had a confirmed COVID-19 infection? □ Yes □ No				
Q15: What do you think the likelihood is of you contracting COVID-19?	□ Never	□ 25%	□ 50%	□ 75%	□ Absolutely
Q16: What do you think the likelihood is of your relatives contracting COVID-19?	□ Never	□ 25%	□ 50%	□ 75%	□ Absolutely
Q17: What do you think the likelihood is of your child contracting COVID-19?	□ Never	□ 25%	□ 50%	□ 75%	□ Absolutely
Q18: To what extent does the likelihood of contracting COVID-19 increase when you come to hospital for your child's immunotherapy?	□ Never	□ 25%	□ 50%	□ 75%	□ 100%
Q19: What mode of transport are you coming to the hospital with?			□ Public transport	□ Private car	
Q20: To what extent does the likelihood of contracting COVID-19 increase during transit to the hospital for IVIG therapy?	□ Never	□ 25%	□ 50%	□ 75%	□ 100%
Q21: What do you think the likelihood of your child contracting COVID-19 from healthcare personnel who administerd the IVIG is?	□ Never	□ 25%	□ 50%	□ 75%	□ 100%
Q22: What do you think the likelihood of your child contracting COVID-19 while waiting at the hospital during IVIG therapy (in the waiting and treatment rooms) is?	□ Never	□ 25%	□ 50%	□ 75%	□ 100%
Q23: What do you think the likelihood of your child contracting COVID-19 from the doctor who examines your child before and after the IVIG therapy is?	□ Never	□ 25%	□ 50%	□ 75%	□ 100%
Q24: What do you think the likelihood of your child contracting COVID-19 from the other patients who have come for IVIG therapy is?	□ Never	□ 25%	□ 50%	□ 75%	□ 100%

the current state of anxiety. Feelings of subjective tension, nervousness, anxiety, and activation/arousal "right now" are rated by selecting the response of "not at all", "somewhat", "moderately so", and "very much so". The trait anxiety subscale also consists of 20 items regarding anxiety tendencies independent of the current situation. The items evaluate the "general" frequency of emotions with options of "almost never", "sometimes", "often", and "almost always".[9,10] Higher scores indicate a higher level of anxiety.

The Beck Depression Inventory (BDI) was used to measure the presence and severity of various manifestations of depression. It is a 21-item self-report instrument and total score ranges from 0 to 63.[11] The validity and reliability study of the Turkish version was performed by Hisli et al.[12]

Statistical Analyses

Statistical analyses were performed using SPSS Statistics version 22.0 for Windows (IBM, Armonk, NY, USA) statistical software package. Continuous variables were expressed as mean and standard deviation for data with a normal distribution and as median and interquartile range (IQR) for non-normally distributed data. The chi-square test was used to compare nonparametric data; the Mann–Whitney U test was used for comparisons among non-normally distributed continuous variables and independent samples t-test for normally distributed continuous variables. A value of $p<0.05$ was considered statistically significant

Results

Twenty-seven patients who received IVIG treatment due to PID before the pandemic and 29 control subjects were included in the study. The characteristics of the patients in study group are given in Table II.

The patients in the IVIG group had a median age of 12 (IQR: 8-15) years; median age in the control group was 11 (IQR: 6-14) years. The mean ages of the parents were 40.07±5.7 (min-max: 31-51) years in the IVIG group and 37.48±6.17 (min-max: 22-48) years in the control group. There was no statistical difference between the groups in terms of the age of the patients (p=0.300) or their parents (p=0.109).

All patients who were receiving IVIG treatment in our clinic continued treatment during the pandemic at the same dosing intervals. No patient exhibited an adverse reaction associated with IVIG therapy during the study period. In addition, none of the patients developed COVID-19 symptoms or had confirmed COVID-19 infection during treatment, nor

Table II. Demographic characteristics of patients receiving IVIG therapy.

Gender n,%	
Male	13 (48.1)
Age (years)	
Mean (IQR)	12 (8-15)
PID of the patients n,%	
CVID	13 (48.15)
CID	
ICF	4 (14.8)
Ataxia-telangiectasia	3 (11.1)
STAT1 deficiency	1 (3.7)
IL-21R deficiency	1 (3.7)
SCID	
Jak-3 deficiency	1 (3.7)
ALPS	2 (7.4)
X-linked agammaglobulinemia	1 (3.7)
RAS-associated autoimmune leukoproliferative disease	1 (3.7)
Time interval of IVIG dosage	
Once in 3 weeks	3(11.1)
Once in 4 weeks	24(88.9)
Duration of IVIG therapy	
<1 years	7 (25.9)
1-2 years	4 (14.8)
3-5 years	3 (11.1)
>5 years	13 (48.1)

*PID: primary immune deficiency, CVID: common variable immune deficiency, CID: combined immunodeficiency, ICF: immunodeficiency with centromeric instability and facial anomalies, SCID: severe combined immune deficiency, ALPS: autoimmune lymphoproliferative syndrome, n: number, IQR: interquartile range

did the examining physician, the nurse who administered IVIG therapy, or any of the other health workers.

According to the parents' responses on the COVID-19 questionnaire, 4 patients had relatives or friends diagnosed with COVID-19 in the control group. None of the parents in the IVIG group reported confirmed COVID-19 in their family or immediate social circle. In both groups, about half of the parents estimated their chance of contracting COVID-19 as about 25% (IVIG group: 15/27, 55.6%; control group: 14/29, 48.3%). Similar proportions in each group believed their children also had a 25% chance of contracting COVID-19 (IVIG group: 15/27, 55.6%; control group: 17/29, 58.6%). The responses of the parents on the COVID-19 questionnaire are given in Tables III and IV. There was no difference between the two groups in the percentage of parents who believed their child's chance of contracting COVID-19 was 50% or higher (p=0.672).

When the percentage of the risk coming to the hospital was questioned among parents, 48.3% (14/29) of the parents in the control group answered as 50% and another 24.1% (7/29) of the parents replied as 25%. In the IVIG group, 40.7% (11/27) of the parents thought that coming to the hospital increased the risk of infection to 25%, while 37% (10/27) of the parents thought it increased to 50%. There was no statistical difference between the groups in the proportion of parents who stated that coming to the hospital increased the risk of infection to 25% or higher (p=0.44).

In the IVIG group, 40.7% (11/27) of the parents thought that there was almost no possibility of transmission from healthcare personnel other than physicians. Similarly, most parents in the same group thought that physicians did not increase the risk at all (18/27; 66.7%). In the control group, a similar proportion of parents thought that there was almost no possibility of transmission from health personnel other than physicians (12/29; 41.4%), while relatively fewer did not consider the physicians as increasing the risk of disease at all (14/29; 48.3%). There were no significant differences between the groups in the proportions of parents who believed that there was almost no possibility of transmission from health personnel other than physicians (p=0.961), that there was no risk of transmission from physicians (p=0.165), and that 50% or more of the transmission risk was in the waiting and

Table III. Answers to questionnaire form for parents about parental COVID-19 history.

	IVIG group n (%)	Control group n (%)
Have any of your relatives or friends developed symptoms of COVID-19?		
Yes	1 (3.7)	11 (37.9)
No	26 (96.3)	18 (62.1)
Have you developed symptoms of COVID-19?		
Yes	0	2 (6.9)
No	27 (100)	27 (93.1)
Have any of your relatives or friends had a confirmed COVID-19 infection?		
Yes	0	4 (13.8)
No	27 (100)	25 (86.2)
Have you had a confirmed COVID-19 infection?		
Yes	0	0
No	27 (100)	29 (100)

n: number
Percentage values are calculated by columns

Table IV. Answers to questionnaire form for parents about parental perceptions about COVID-19 risk.

	Never n (%)	25% n (%)	50% n (%)	75% n (%)	Absolutely n (%)
What do you think the likelihood is of you contracting COVID-19?					
IVIG group	6(22.2)	15(55.6)	0	4(14.8)	2(7.4)
Control group	4(13.8)	14(48.3)	7(24.1)	4(13.8)	0
What do you think the likelihood is of your relatives contracting COVID-19?					
IVIG group	5(18.5)	13(48.1)	6(22.2)	3(11.1)	0
Control group	1(3.45)	12(41.4)	12(41.4)	3(10.3)	1(3.45)
What do you think the likelihood is of your child contracting COVID-19?					
IVIG group	5(18.5)	15(55.6)	5(18.5)	2(7.4)	0
Control group	3(10.3)	17(58.6)	8(27.6)	1(3.45)	0
To what extent does the likelihood of contracting COVID-19 increase when you come to hospital?					
IVIG group	2(7.4)	11(40.7)	10(37)	2(7.4)	2(7.4)
Control group	4(13.8)	7(24.1)	14(48.3)	3(10.3)	1(3.45)
To what extent does the likelihood of contracting COVID-19 increase during transit to the hospital?					
IVIG group	11(40.7)	5(18.5)	9(33.3)	2(7.4)	0
Control group	16(55.2)	6(20.7)	4(13.8)	3(10.3)	0
What do you think the likelihood is of your child contracting COVID-19 from the healthcare personnel?					
IVIG group	11(40.7)	9(33.3)	5(18.5)	2(7.4)	0
Control group	12(41.4)	8(27.6)	7(24.1)	2(6.9)	0
What do you think the likelihood is of your child contracting COVID-19 while waiting at the hospital (in the waiting and treatment rooms)?					
IVIG group	8(29.6)	13(48.1)	5(18.5)	1(3.7)	0
Control group	4(13.8)	12(41.4)	11(37.9)	1(3.45)	1(3.45)
What do you think the likelihood is of your child contracting COVID-19 from the doctor who examines your child?					
IVIG group	18(66.7)	8(29.6)	0	1(3.7)	0
Control group	14(48.3)	9(31)	5(17.2)	1(3.45)	0
What do you think the likelihood is of your child contracting COVID-19 from the other patients who have come to hospital?					
IVIG group	5(18.5)	15(55.6)	0	7(25.9)	0
Control group	3(10.3)	7(24.1)	14(48.3)	5(17.2)	0

n: number

treatment rooms (6/27 patients [22.2%] in the IVIG group; 13/29 patients [44.8%] in control group; p=0.074).

Of the total 32 parents from both groups who stated that coming to the hospital increased their child's risk of COVID infection to 50% or higher, 18 (56.25%) believed that 50% or more of the transmission risk was in the waiting and treatment rooms and 22 (68.75%) thought the greatest risk was from other patients and their relatives.

When the STAI results were evaluated, the mean state anxiety score was 44.22 (SD: 11.18, min-max: 25-70) and the mean trait anxiety score was 44.37 (SD: 7.9, min-max: 33-63) in the IVIG group. In the control group, the mean state and trait anxiety scores were 36.07 (SD: 8.4, min-max: 24-53) and 37.9 (SD: 7.6, min-max: 26-53), respectively. Comparisons between the groups showed that the IVIG group had significantly higher scores for both state (p=0.003) and trait (p=0.003) anxiety (Table V).

The median BDI score was 10 (IQR: 7-13) in the IVIG group and 3 (IQR: 1-9.5) in the control group. IVIG group was found to have statistically significantly higher scores than the control group (p=0.002).

In the IVIG group, 15 patients were between 0 and 13 years old and 12 patients were 14 or older; in the control group, 18 patients were between 0 and 13 years old and 11 patients were 14 or older. There were no significant differences among parents in the IVIG and control groups when trait anxiety scores were compared according to the children's age group (p>0.05). However, the parents of children over 14 years of age in the IVIG group had higher mean state anxiety score (p=0.023).

When IVIG and control group were evaluated together, the median STAI state anxiety score of the whole population was 41. Using this as the cut-off value, there was a statistically significant difference between the IVIG and control groups in terms of the number of parents above this cut-off value. While the number of parents scoring 41 and above was 18 (66.7%) in the IVIG group, it was 11 (37.9%) in the control group (p=0.032). Also in the IVIG group, the median age of the children of the parents with state anxiety scores of 41 and above were higher than those of parents with lower state anxiety scores (median age of the patients: 13.7&8 years; p=0.004).

Discussion

In this study of patients who needed to present to hospitals for regular IVIG therapy, it was observed that all patients whose treatment was initiated before the pandemic continued their treatment during the pandemic. The anxiety level of the parents of patients receiving

Table V. Demographic characteristics and depression and anxiety scores of parents.

	Parents in the IVIG group (n=27)	Parents in the control group (n=29)	P
Gender n,%			
Female	20 (74.1%)	22 (75.9%)	0.87
Age (years)			
Mean	40.07 ± 5.7	37.48 ± 6.17	0.109
STAI state anxiety score			
Mean	44.22 ± 11.18	36.07 ± 8.4	0.003
STAI trait anxiety score			
Mean	44.37 ± 7.9	37.9 ± 7.6	0.003
Beck Depression Inventory score			
Median (IQR)	10 (7-13)	3 (1-9.5)	0.002

*n: number, IQR: interquartile range

IVIG therapy was found to be higher than in the control group. In both the control group and the IVIG group, most of the patients' parents believed that there was nearly no risk of transmission from physicians and other healthcare personnel. Due to preventive measures in the hospital and clinic, there was no transmission associated with the patients' hospital visits or treatment.

Currently available IVIG products do not contain specific antibodies against SARS-CoV-2, but they may include cross-reactive antibodies for SARS-CoV, Middle East respiratory syndrome coronavirus, or other human coronaviruses. Therefore, IVIG preparations could potentially provide some protection from COVID-19.[13,14] The EAACI guideline, which addresses the management of immunodeficiencies during the SARS-CoV-2 pandemic, recommends that patients to continue the treatment they were receiving.[5] The patients in our study had 100% treatment adherence and all patients were admitted to the hospital without delaying the time needed for treatment. We attribute this to having informed the patients and their families in detail about the disease and the fact that IVIG therapy would protect these patients against most infections. Despite high anxiety levels, all patients under IVIG treatment were fully compliant and continued at the same dose intervals as before the pandemic. This indicated that families consider protecting their children from illnesses more important than their own anxiety.

In our study, state anxiety scores assessed at hospital presentation were higher in the IVIG group than in the control group. There are no cut-off values or ranges to identify high anxiety levels for the Turkish version of the STAI. When we used the overall median state anxiety score as the cut-off value, we detected a statistical difference between the patient and control groups in terms of the proportions of parents above this threshold.

We also found that parents in the IVIG group had higher trait anxiety scores and higher median BDI scores compared to the control group. Although none of the parents exceeded the cut-off score of 17 for BDI, we believe this difference may be attributable to the BDI items that evaluate anxiety. It may also be related to awareness among parents in the IVIG group that their children's immunodeficiency put them at risk for many diseases and that COVID-19 infection could lead to more serious consequences in their children than in immunocompetent children.

The patients' IVIG infusions required them to spend several hours in the hospital. During this time, patients and their parents were advised to observe social distancing rules. In a study done in China, anxiety was reported to be more pronounced in the parents of children hospitalized during the epidemic.[6] In both the IVIG and control group in our study, the majority of parents considered the likelihood of contracting COVID-19 to be 25% at most and believed that coming to the hospital increased this risk to 25% or 50%. Although the IVIG group having to spend a longer time in the hospital compared to the control group, there was no significant difference between the two groups in terms of the perceived increase in infection risk due to coming to the hospital and the perceived sources of potential transmission in the hospital.

The fact that parents did not think health workers, especially doctors, increased the risk of transmission is an important finding. These data can be interpreted as an indicator of the trust patients' families have in the protective measures taken by the health care professionals during the COVID-19 period. Although not evaluated in our study, possible factors contributing to this perception are media reports of health workers taking various measures to reduce the spread of the epidemic, reduce viral load, and reduce the burden on the health care system to ensure continuing accessibility to everyone, both in our country and worldwide.[15] In addition, in our allergy and immunology clinic where the study was conducted, we continued regularly scheduled

follow-up of all patients with severe and poorly controlled disease and explained to the families of patients who needed to come to the hospital that measures were taken to observe social distancing guidelines and create a suitable examination and treatment setting.

Our patients continued to receive treatment at the same doses and intervals in our hospital during the pandemic and none of them developed COVID-19 infection during the study period. In addition to the measures taken in our outpatient clinic, another contributing factor may be that patients requiring IVIG therapy underwent treatment in a room separate from other outpatients with social distancing and masks due to their status as a COVID risk group. All of these measures may explain the absence of nosocomial COVID-19 transmission among our patients during the study period. Our findings support the effectiveness of complying with mask and social distance guidelines and segregating risk groups in designated areas to reduce transmission among patients who must visit the hospital.

When the parents in the IVIG group were evaluated among themselves, it was determined that the parents of children aged 14 and over had higher state anxiety scores at presentation than the parents of children under 14 years old. Furthermore, in the IVIG group, the median age of the children of parents with state anxiety scores of 41 and above were higher than those of parents with lower state anxiety scores. Higher anxiety among the parents of adolescent children may be due to age-related characteristics such as impulsivity and increased independent behavior.

The main limitation of this cross-sectional study is that the parents' pre-pandemic anxiety levels could not be evaluated. However, the difference observed between state and trait anxiety may indicate that their anxiety level increased with the pandemic. Another limitation of the study is that the anxiety levels of the pediatric patients in the IVIG group could be not determined. In fact, psychological distress in exposed children may lead to adverse events during the pandemic.[16] The initial study plan included using age-appropriate anxiety scales to evaluate the patients as well, but the presence of comorbid mental retardation diagnosis in a substantial proportion of the patients in the IVIG group precluded this assessment. Another limiting factor was the different lengths of time spent in the hospital by the control and patient groups.

In conclusion, the treatment of pediatric patients with PID continued in our clinic during the COVID-19 pandemic. The parents of patients who needed to come to the hospital for this treatment were found to have higher anxiety levels than the parents of similar aged children who presented to our clinic for different complaints between the same dates. Despite their concerns, the parents of all patients under IVIG therapy ensured the necessary treatment continuity and maintained treatment adherence. None of our immunodeficient patients who required continued treatment during the pandemic contracted COVID-19 infection during our study, again highlighting the importance of measures such as masks, social distance, and the use of designated areas in limiting transmission.

Ethical approval

The study was approved by the institutional ethics committee of Ankara City Hospital (No: E1-20-648/ Date: 2020) and the study protocol was approved by the Turkish Ministry of Health.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: OYT, AM, OSU; data collection: OYT, AM; analysis and interpretation of results: OYT, AM, OSU, EC, GSD; draft manuscript preparation: OYT, AM, OSU, EC, GSD. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

There is no financial support.

Conflict of interest

None to declare.

REFERENCES

1. Seidel MG, Kindle G, Gathmann B, et al; ESID Registry Working Party and collaborators. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J Allergy Clin Immunol Pract 2019; 7: 1763-1770.
2. Cunningham-Rundles C. Key aspects for successful immunoglobulin therapy of primary immunodeficiencies. Clin Exp Immunol 2011; 164(Suppl 2): 16-19.
3. Slatter MA, Gennery AR. Clinical immunology review series: an approach to the patient with recurrent infections in childhood. Clin Exp Immunol 2008; 152: 389-396.
4. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 among children in China. Pediatrics 2020; 145: e20200702.
5. Brough HA, Kalayci O, Sediva A, et al. Managing childhood allergies and immunodeficiencies during respiratory virus epidemics - The 2020 COVID-19 pandemic: a statement from the EAACI-section on pediatrics. Pediatr Allergy Immunol 2020; 31: 442-448.
6. Yuan R, Xu QH, Xia CC, et al. Psychological status of parents of hospitalized children during the COVID-19 epidemic in China. Psychiatry Res 2020; 288: 112953.
7. Bonilla FA, Khan DA, Ballas ZK, et al; Joint Task Force on Practice Parameters, representing the American Academy of Allergy, Asthma & Immunology; the American College of Allergy, Asthma & Immunology; and the Joint Council of Allergy, Asthma & Immunology. Practice parameter for the diagnosis and management of primary immunodeficiency. J Allergy Clin Immunol 2015; 136: 1186-1205.e1-78.
8. Aghamohammadi A, Farhoudi A, Nikzad M, et al. Adverse reactions of prophylactic intravenous immunoglobulin infusions in Iranian patients with primary immunodeficiency. Ann Allergy Asthma Immunol 2004; 92: 60-64.
9. Julian LJ. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). Arthritis Care Res (Hoboken) 2011; 63(Suppl 11): S467-S472.
10. Öner N, Le Compte WA. Durumluk-Sürekli Kaygı Envanteri El Kitabı. İstanbul: Boğaziçi Üniversitesi Yayınları, 1983: 333.
11. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961; 4: 561-571.
12. Hisli N. Beck depresyon envanterinin üniversite öğrencileri için geçerliği, güvenirliği. Psikoloji Dergisi 1989; 7: 3-13.
13. Diez JM, Romero C, Gajardo R. Currently available intravenous immunoglobulin contains antibodies reacting against severe acute respiratory syndrome coronavirus 2 antigens. Immunotherapy 2020; 12: 571-576.
14. Gao X, Zhou H, Wu C, et al. Antibody against nucleocapsid protein predicts susceptibility to human coronavirus infection. J Infect 2015; 71: 599-602.
15. Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. Science 2020; 368: 860-868.
16. Jiao WY, Wang LN, Liu J, et al. Behavioral and emotional disorders in children during the Covid-19 epidemic. J Pediatr 2020; 221: 264-266.e1.

The Turkish Journal of Pediatrics 2021; 63: 811-817
https://doi.org/10.24953/turkjped.2021.05.009

Original Article

Mesenteric tissue oxygenation status on the development of necrotizing enterocolitis

Hilal Özkan[1], Merih Çetinkaya[2], Bayram Ali Dorum[3], Nilgün Köksal[1]

[1]Department of Neonatology, Uludağ University Faculty of Medicine, Bursa; [2]Department of Neonatology, Başakşehir Çam and Sakura City Hospital, İstanbul; [3]Department of Neonatology, Bursa City Hospital, Bursa, Turkey.

ABSTRACT

Background. Necrotizing enterocolitis (NEC) is an important cause of morbidity and mortality in preterm infants. There is limited data about the role of mesenteric oxygenation status during the first enteral feeding. Therefore, the aim of this study was to determine the mesenteric tissue oxygen saturation values before, during and after the first enteral feeding and to evaluate the effect of these values on the development of NEC in preterm infants.

Methods. A total of 105 preterm babies with ≤ 32 gestational weeks were included in this prospective study. The continuous monitoring of the mesenteric tissue oxygenation status was performed before, during and 3 hours after the first feeding by near-infrared spectroscopy (NIRS).

Results. The mean gestational week and birth weight of the study group were 28.8±2.1 weeks, and 1215±387 g, respectively. The first enteral feeding was started at 2.4±1.4 days with breast milk in 85% of infants. A total of 12 infants (11.4%) developed NEC (66% stage II, 34% stage III). The mean mesenteric tissue oxygen saturation levels of the infants that developed NEC were significantly lower both before and one hour after feeding (56.1±3.4 vs. 34±8.8, and 47.4±3.3 vs 37.8±10.9, respectively) compared with infants that did not develop NEC.

Conclusions. Lower mesenteric tissue oxygenation values measured before, and one hour after enteral feeding was associated with NEC development. We suggest that lower mesenteric tissue oxygenation during continuous monitoring of first enteral feeding may be used to predict NEC development during follow-up.

Key words: enteral feeding, near-infrared spectroscopy, necrotizing enterocolitis, NICU, premature.

Necrotizing enterocolitis (NEC) is the most frequent and lethal gastrointestinal tract emergency in preterm newborns.[1] Though more than 50 years have passed since its definition, its pathophysiology has not been elucidated completely.[1] Prematurity, bacterial colonization, formula feeding, and intestinal ischemia were reported as the main risk factors that contribute to the complex pathogenesis.[2] The most accepted NEC hypothesis includes enteral feeding in the presence of intestinal hypoxia-ischemia-reperfusion, and abnormal intestinal colonization with pathogens that provoke an inappropriate inflammatory response in intestinal epithelial cells of premature infants.[3,4]

Therefore, it seems reasonable to establish the intestinal oxygenation status before and during the first enteral feeding attempts in premature infants and determine the high-risk infants for the development of NEC.

Near-infrared spectroscopy (NIRS) has been increasingly used to provide continuous monitoring of tissue oxygen saturation (StO_2) in neonates, especially for cerebral, renal and mesenteric oxygenation.[5] Limited number of studies investigated the effect of intestinal oxygenation status during enteral feeding as a

✉ Hilal Özkan
hiozkan@hotmail.com

Received 23rd November 2020, revised 12th March 2021, 1st April 2021, accepted 21st April 2021.

This study was presented as an oral presentation at the 25th National Neonatology Congress held in Antalya in April 2017.

biomarker of mesenteric perfusion on feeding intolerance and intestinal complications in preterm infants.[6-9] In addition, lower mesenteric tissue oxygenation and increased fractional tissue oxygen extraction (FTOE) was found in preterm infants during the early course of NEC.[10] Also, low mesenteric/cerebral oxygen saturation values and high mesenteric/cerebral FTOE levels were reported to be associated with bowel perforation or death in premature infants with NEC.[11]

However, to our best of knowledge, no study evaluated the possible association between mesenteric tissue oxygenation during the first enteral feeding and subsequent development of NEC in preterm infants. The aim of this prospective study was to determine the continuous mesenteric tissue oxygenation during the first enteral feeding and also to explore the possible association between these values and subsequent NEC development during the follow-up period.

Material and Methods

Study population

This prospective observational study was performed at two tertiary Neonatal Intensive Care Units. Preterm infants ≤32 gestational weeks, and hospitalized in these two centers between December 2015 and December 2017 were included. Infants with gastrointestinal/major congenital and/or genetic anomalies, hemodynamically significant patent ductus arteriosus (diameter ≥2.0 mm or left atrium to aortic root ratio ≥1.4 or retrograde flow in descending aorta), severe intraventricular hemorrhage (grade III, IV) and hemodynamically unstable patients, need of volume or inotrope treatment, babies who have anemia (hemoglobin <12 g/dl) and need red blood cell transfusion, infants died within the first 3 days of life were all excluded.

Nasal continuous positive airway pressure (NCPAP) (6 cmH_2O) was applied to all babies after birth. They were transferred to the neonatal intensive care unit (NICU) with nasal CPAP. In nasal CPAP failure, firstly non-synchronized nasal intermittent positive pressure ventilation was applied. However, babies whose respiratory support was insufficient were intubated and received ventilation support in volume-guaranteed mode. Target oxygen saturation was targeted between 90-94%. Intratracheal surfactant were administered to infants who needed FiO_2 more than 0.30 for target saturation.

Uludag University Faculty of Medicine Clinical Research Ethics Committee approved the study (2012-26/11) and the signed approved parental consent was obtained from all families.

Study design

All of the infants were started total parenteral nutrition during the admission and enteral feeding was started with breast milk as soon as possible and if not available with preterm formula at amounts of 10-20 ml/kg given at 3-hour intervals as a bolus. With the decision of enteral feeding, mesenteric, renal, and cerebral StO_2 were measured by NIRS for a 4 hours period starting from 1 hour before the first feeding, and continued during 3 hours of enteral feeding. The systemic oxygen saturation (SaO_2) of the infants were monitored simultaneously using a pulse oximetry device (Nellcor, Covidien-Medtronic, Minneapolis, US). The patients were followed up for the development of NEC. During follow-up, modified Bell criteria were used for the diagnosis, and staging of infants with NEC.[12] Demographical, prenatal and natal characteristics, and neonatal morbidities were all recorded. The infants with stage I NEC were excluded.

Near-infrared spectroscopy

INVOS 5100 near-infrared spectroscopy (Covidien, Mansfield, US) was used. NIRS data were recorded at 6-second intervals. Cerebral and renal sensors were placed on the anterior frontal region and on the left lumbar region, respectively. For mesenteric StO_2 measurement, sensors were placed on the infraumbilical region at the center of the abdominal wall.

Mean cerebral, renal, and mesenteric NIRS data obtained 1 hour before, during and 1, 2, and 3 hours after first enteral feeding were calculated. To minimize erroneous measurements stemming from movements, and malposition of the sensors, mean values of all measurements performed within +/- 15 minutes were taken into consideration. FTOE of the patients were calculated using the following formula: FTOE=SaO_2-StO_2 /SaO_2.

Statistical analysis

Data were analyzed using the IBM Statistical Package for Social Sciences v23 (SPSS Inc., Chicago, IL, USA). Continuous data were presented as mean ± standard deviation or median (minimum-maximum), as appropriate. All differences associated with a chance probability of 0.05 or less were considered statistically significant. Receiver-operating characteristics (ROC) analysis was performed by MedCalc version 18.2.1 statistical program. Values of $p<0.05$ were considered significant.

Results

During a period of two years, a total of 147 babies born at a gestational age of ≤ 32 weeks were hospitalized in the NICU. When the infants with exclusion criteria and without parental consent were excluded, 105 preterm babies were included in the study (Fig. 1).

The mean gestational age, and birth weights of the study group were 28.8±2.1 weeks, and 1215±387 g, respectively. The mean first enteral feeding time was 2.4 ± 1.4 days and 85% of the patients were breastfed. A total of 12 infants (11,4%) developed NEC; 8 (7,6%) had stage 2 and 4 (3.8 %) had stage 3. Although there were no significant differences in infants with and without NEC in terms of demographical features, the time of first enteral feeding was statistically significantly later in babies with NEC ($p<0.001$) (Table I).

No significant differences were detected between infants with and without NEC in terms of mean cerebral and renal StO_2. The mean mesenteric StO_2 were significantly lower before feeding (56.1±3.4 vs. 34±8.8) and one hour after feeding (47.4±3.3 vs 37.8±10.9) in cases that subsequently developed NEC compared with those who did not develop NEC (Table II). In the NEC group, mesenteric tissue oxygen saturations at the 2nd and 3rd hours after the first enteral feeding were found to be lower, but this difference was not significant (Table II). Similarly, FTOE levels before and 1 hour after feeding were significantly higher in cases that developed NEC (Fig. 2). The ROC analysis showed a cut-off of 42, 43, 47, 45 % for before the first feeding, the first hour after first feeding, the second hour after first feeding and, the third hour after the first feeding respectively for prediction of NEC (Table III).

Discussion

To the best of our knowledge, this is the first study that evaluated the possible role

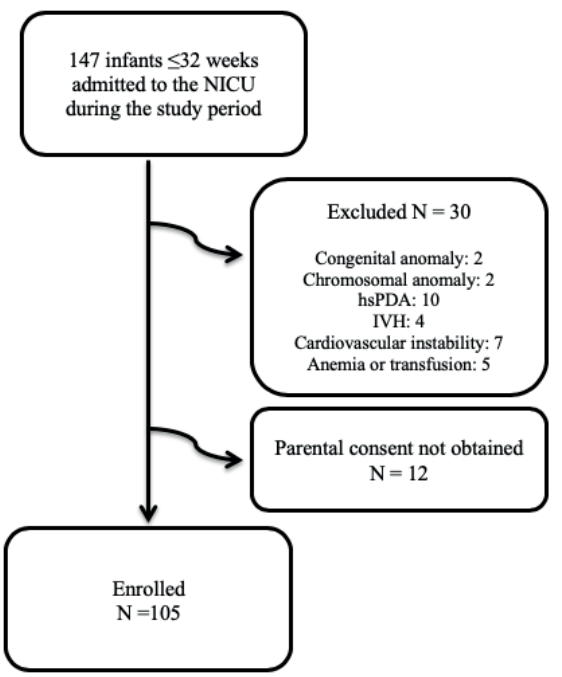


Fig. 1. Flow chart of the study enrollment (NICU: Neonatal intensive care unit, hsPDA: Hemodynamically Significant Patent Ductus Arteriosus, IVH: Intraventricular hemorrhage).

Table I. Demographics and characteristics of all infants enrolled in the study.

	NEC group n=12	Non-NEC group n=93
Maternal features		
Preeclampsia, n (%)	7 (58.3)	35 (37.6)
Premature rupture of membranes, n (%)	1 (8.3)	7 (7.5)
Antenatal steroid, n (%)	9 (75)	66 (70.9)
Caesarian section, n (%)	10 (83.3)	77 (82.7)
Neonatal features		
Birth weight (g), mean±std	1068±335	1237±358
Gestational age, mean±std	28.1±1.3	28.9±2.2
Small gestational age (<10 percentile), n (%)	4 (33.3)	20 (21.5)
Male gender, n (%)	7 (58.3)	46 (49.4)
Apgar score-1, mean±std	5.7±2.0	4.8±2.1
Apgar score-5, mean±std	7.0±1.7	6.8±1.7
Respiratory distress syndrome, n (%)	9 (75)	60 (64)
Mechanical ventilation, n (%)	8 (66.6)	50 (53.7)
Human milk feeding, n (%)	10 (83.3)	80 (86)
First day of enteral feeding, mean±std*	4.0±2.6	2.2±0.9
Development of NEC, day	12.3±5.3	-
Probiotic supplementation, n (%)	4 (33.3)	20 (21.5)
CRIB-II Score, mean±std	7.5±2.6	5.7±3.3

*p=0.0001

Table II. Cerebral, renal, and mesenteric tissue oxygen saturation (StO_2) values of infants before and after first feeding.

	NEC group n=12	Non-NEC group n=93
Before first feeding (%)		
• Cerebral StO_2, mean±std	69±7.3	70±13.2
• Renal StO_2, mean±std	64.6±17.2	66.2±20
• Mesenteric StO_2, mean±std*	34.8±10.9	56.1±3.4
1st hour after feeding (%)		
• Cerebral StO_2, mean±std	64.5±8.3	69.7±13
• Renal StO_2, mean±std	66.1±16.4	68±19.2
• Mesenteric StO_2, mean±std*	37.8±10.9	47.4±3.3
2nd hour after feeding (%)		
• Cerebral StO_2, mean±std	69±7.8	68.1±15.8
• Renal StO_2, mean±std	72±18.8	70±18.7
• Mesenteric StO_2, mean±std	40±23	52±24
3rd hour after feeding (%)		
• Cerebral StO_2, mean±std	67±8.1	67.8±17.4
• Renal StO_2, mean±std	71±17.5	65.4±20.5
• Mesenteric StO_2, mean±std	41.7±23	49.5±25

NEC: necrotizing enterocolitis, StO_2: tissue oxygen saturation
*p=0.0001

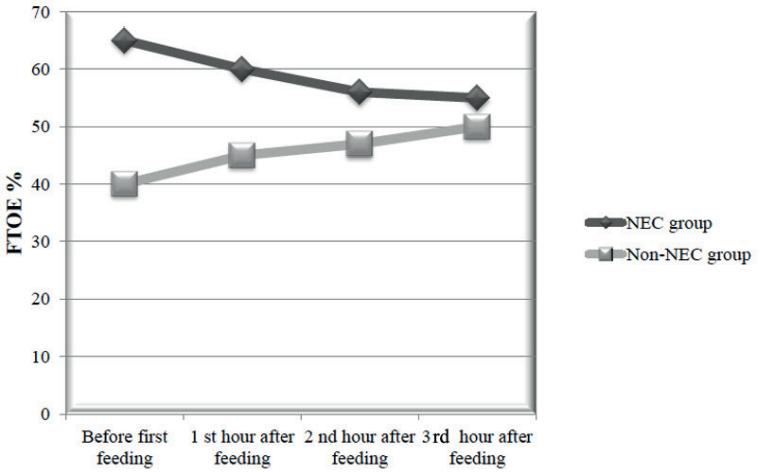


Fig. 2. Fractional tissue oxygen extraction (FTOE) values of infants with necrotizing enterocolitis (NEC) and non-NEC infants before and after first feeding.

Table III. Criterion values and coordinates of the ROC curve.

Variable Mesenteric StO_2, (%)	Cut off	Sensitivity	Specificity	AUC	95% Cl	P
Before first feeding	42	83	100	0.987	0.890 to 1.000	<0.001
1st hour after feeding	43	83	92	0.924	0.797 to 0.983	<0.001
2nd hour after feeding	47	75	93	0.813	0.661 to 0.918	<0.001
3rd hour after feeding	45	83	87	0.833	0.684 to 0.931	0.005

ROC: receiver operating characteristic, StO_2: tissue oxygen saturation, AUC: area under the ROC curve

of mesenteric tissue oxygenation during the introduction of first enteral feeding and subsequent development of NEC in preterm infants. This study suggested that lower mesenteric StO_2 and higher FTOE values before, and during the first hour of the enteral feeding were associated with the development of NEC during the follow-up period.

The incidence of NEC in very-low birth weight (VLBW) babies was reported up to 13% according to the large multicenter studies and neonatal networks.[13] The incidence of severe NEC (11.4%) in our study was similar to the literature. Despite major recent developments in the care of preterm infants, a highly sensitive and specific test for the early diagnosis of NEC is still lacking.

As prematurity, ischemia, feeding and abnormal colonization were reported as the main risk factors, a non-invasive diagnostic approach for these risk factors may provide early and accurate NEC diagnosis in preterm infants. NIRS has been increasingly used in the last years for the assessment of cerebral perfusion in both term and preterm infants. In addition, the non-invasive measurement of StO_2 and FTOE may be also calculated by the SaO_2 measurements.[11]

As the ischemic necrosis of intestinal mucosa is a stable sign in the histopathological examination of advanced stage NEC, detection of the decrease in the abdominal StO_2 related to mesenteric perfusion alterations before the development of NEC may offer a very reasonable diagnostic approach. Indeed, animal studies also yielded that lower NIRS measurements might be used for the early diagnosis of NEC.[14]

After these experimental data, abdominal StO_2 were found to be significantly lower in infants that developed NEC in a two-centered clinical

study and abdominal StO_2<56% were stated as an independent risk factor for the development of NEC in preterm infants.[15] The authors also reported significantly more variations both during and after feeding in the first two weeks of life. Our results were in accordance with this study as the mean mesenteric saturation levels were always lower than 56% in infants before, during and after first enteral feeding in premature infants. Therefore, we may speculate that lower mesenteric oxygen saturation, especially lower than 42% may predict subsequent NEC development during the hospitalization period.

A strong association between mesenteric FTOE and intestinal fatty acid binding protein levels were reported during the first 16 hours after NEC onset that suggested the simultaneous occurrence of decreased splanchnic perfusion and intestinal damage.[10] The authors suggested that mesenteric FTOE might offer valuable information about the degree of intestinal injury. NIRS monitoring was reported to be useful in preterm infants with definite NEC to differentiate the infants who would develop complicated NEC.[11] The lower mesenteric and cerebral oxygenation values and increased FTOE were also found to be associated with adverse outcomes including bowel perforation and death.[11] Similarly, increased mesenteric FTOE during the first enteral feeding was detected in our study. Therefore, we suggest that increased mesenteric FTOE levels may help neonatologists to identify the high-risk infants for NEC development. However, we could not find any differences in both cerebral and renal oxygenation levels in association with first enteral feedings to predict subsequent NEC development.

There are conflicting data about the correlation between mesenteric tissue oxygenation and feeding intolerance during the first introduction of enteral feeding in preterm infants. In a clinical study, lower abdominal saturations and mesenteric-cerebral oxygenation ratio were detected in infants that developed feeding intolerance.[7] Similarly, lower mesenteric oxygenation and increased FTOE were reported in response to both initial and full enteral feedings in infants with absent/reversed antenatal end diastolic flow (AREDF).[12] As both feeding intolerance and AREDF are important risk factors for NEC, these results should be interpreted in this manner. In contrast, abdominal StO_2 recorded during the first postnatal days was found to not provide helpful information about nutritional tolerance in the follow-up period.[6] This difference may be explained by the cerebral autoregulation mechanisms that keep tissue oxygenation stable.

Although the superior mesenteric artery flow rates after feeding show an increase in healthy preterm infants, this finding was not detected by Doppler US in infants who later developed feeding intolerance or NEC.[16] Contrary to Doppler US, NIRS provides continuous data about mesenteric oxygenation without the need of trained personnel. In our study, persistently lower mesenteric oxygenation levels were detected in infants that subsequently developed NEC. Therefore, we may suggest using NIRS alone or in combination with Doppler US for the prediction of NEC earlier in high-risk preterm infants.

In conclusion, NIRS may provide valuable data about the intestinal oxygenation status during the first enteral feeding in preterm infants. Lower mesenteric StO_2 and increased FTOE levels in preterm infants with normal cerebral and renal StO_2 during the introduction of first enteral feeding may predict subsequent NEC development. NIRS findings may be used to determine, characteristics of enteral feeding in high-risk infants for NEC development. However, prospective studies including a larger number of infants with prolonged NIRS monitorization periods are required to elucidate the exact role of intestinal tissue oxygenation during the early enteral feeding on subsequent NEC development in preterm infants.

Acknowledgments

The project was supported by the funds from scientific research project council of our university (ID: OUAP(T) - 2013/24).

Author contribution

The contributions of all authors must be described in the following manner: The authors confirm contribution to the paper as follows: study conception and design: HO, MÇ, NK; data collection: BAD, MÇ; analysis and interpretation of results: HO, MÇ, NK; draft manuscript preparation: BAD, HO. All authors reviewed the results and approved the final version of the manuscript

Ethical approval

Uludag University Faculty of Medicine Clinical Research Ethics Committee approved the study (2012-26/11) and the signed approved parental consent was obtained from all families.

Conflict of interest

Authors state no conflict of interest.

REFERENCES

1. Schat TE, van der Laan ME, Schurink M, et al. Abdominal near-infrared spectroscopy in preterm infants: a comparison of splanchnic oxygen saturation measurements at two abdominal locations. Early Hum Dev 2014; 90: 371-375.
2. Frost BL, Modi BP, Jaksic T, Caplan MS. New medical and surgical insights into neonatal necrotizing enterocolitis: a review. JAMA Pediatr 2017; 171: 83-88.
3. Wu SF, Caplan M, Lin HC. Necrotizing enterocolitis: old problem with new hope. Pediatr Neonatol 2012; 53: 158-163.
4. Chen AC, Chung MY, Chang JH, Lin HC. Pathogenesis implication for necrotizing enterocolitis prevention in preterm very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 2014; 58: 7-11.
5. Bozzetti V, Paterlini G, Bel FV, et al. Cerebral and somatic NIRS- determined oxygenation in IUGR preterm infants during transition. J Matern Fetal Neonatal Med 2016; 29: 443-446.
6. Dani C, Corsini I, Generoso M, Gozzini E, Bianconi T, Pratesi S. Splanchnic tissue oxygenation for predicting feeding tolerance in preterm infants. JPEN J Parenter Enter Nutr 2015; 39: 935-940.
7. Corvaglia L, Martini S, Battistini B, Rucci P, Faldella G, Aceti A. Splanchnic oxygenation at first enteral feeding in preterm infants: correlation with feeding intolerance. J Pediatr Gastroenterol Nutr 2017; 64: 550-554.
8. Martini S, Aceti A, Beghetti I, Faldella G, Corvaglia L. Feed-related splanchnic oxygenation in preterm infants with abnormal antenatal Doppler developing gut complications. J Pediatr Gastroenterol Nutr 2018; 66: 755-759.
9. Cortez J, Gupta M, Araram A, Pizzino J, Sawhney M, Sood BG. Noninvasive evaluation of splanchnic tissue oxygenation using near-infrared spectroscopy in preterm infants. J Matern Fetal Neonatal Med 2011; 24: 578-582.
10. Schat TE, Heida FH, Schurink, et al. The relation between splanchic ischemia and intestinal damage in necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed 2016; 101: F533-F539.
11. Schat TE, Schurink M, van der Laan ME, et al. Near-infrared spectroscopy to predict the course of necrotizing enterocolitis. PLoS One 2016; 11: e0154710.
12. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986; 33: 179-201.
13. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol 2016; 13: 590-600.
14. Gay AN, Lazar DA, Stoll B, et al. Near infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. J Pediatr Surg 2011; 46: 1034-1040.
15. Patel AK, Lazar DA, Burrin DG, et al. Abdominal near-infrared spectroscopy measurements are lower in preterm infants at risk for necrotizing enterocolitis. Pediatr Crit Care Med 2014; 15: 735-741.
16. Bora R, Mukhopadhyay K, Saxena AK, Jain V, Narang A. Prediction of feed intolerance and necrotizing enterocolitis in neonates with absent end diastolic flow in umbilical artery and the correlation of feed intolerance with postnatal superior mesenteric artery flow. J Matern Neonatal Med 2009; 22: 1092-1096.

The Turkish Journal of Pediatrics 2021; 63: 818-831
https://doi.org/10.24953/turkjped.2021.05.010

Original Article

Screen media exposure in pre-school children in Turkey: the relation with temperament and the role of parental attitudes

Burcu Akın Sarı[1], Hande Ayraler Taner[1], Zulal Törenli Kaya[2]

Departments of [1]Child and Adolescent Psychiatry and [2]Psychiatry, Başkent University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Electronic media have become an important element in the lives of modern children. Devices like televisions, smartphones and tablets are widely used by some parents in order to manage hyperactive, stubborn and impulsive children who need high-intensity stimuli. Consequently, a child's temperament and parental attitudes affect the duration and frequency of 3-7-year-old children's screen (television-smartphone-internet) use. Based on this information, the objective of the present study was to evaluate the relation between screen media exposure, the child's temperament and parental attitudes in 3-7-year-old children.

Methods. The participants of this study were 210 children of 3 to 7 years of age. Rothbart's Child Behavior List was used to assess temperament; the Parenting Attitude Research Instrument was used to determine the parental attitudes. Screen media exposure assessment questionnaire, which included questions about the age the child started using the TV, smartphone and/or internet, and the duration of their daily usage, were filled in with the children's parents.

Results. It was found that the increase in activity level, approach and discomfort was negatively correlated to the age the child started watching television, while shyness was positively related to the same phenomenon. The scores of the discomfort temperament subscale had a direct relation to the duration of watching television. Background television is negatively related to attention, inhibitory control, and perceptual sensitivity. Dependency, marital conflict and strictness and authoritarianism parameters were found to be positively related to the duration of playing with a smartphone. Also, in this study we found that negative temperament characteristics adversely affected screen media exposure and poor parenting styles worsen this relationship.

Conclusions. Both temperament and parenting styles affect screen media exposure. In addition, it was understood that parenting styles also affect the relation between temperament and screen exposure. Parental information programs on this subject can eliminate the lack of information related to early screen media exposure in preschool children.

Key words: temperament, parenting, screen, toddler.

Temperament, which represents the child's responses to certain situations, exists from birth and becomes relatively stable over time. Temperament has a biological basis and this can provide individual differences about personal reactions to life events.[1,2] The genetic, biological and environmental factors have an intricate influence on temperament traits.[3] Genetically based temperament features of the child and the environmental factors like parental attitudes interact with each other, and "goodness of fit" facilitates healthy development.[4] Parenting is defined as the actions aimed to guarantee the survival and development of the child.[5]

In the literature, it was found that the temperament of the child is associated with parental attitudes, and both temperament and

✉ Hande Ayraler Taner
h_ayraler@hotmail.com

Received 13th August 2020, revised 13th February 2021, accepted 26th April 2021.

This study was presented at 9th International Congress on Psychopharmacology & 5th International Symposium on Child and Adolescent Psychopharmacology, 26-30 April 2017 Antalya and abstract of preliminary reports of this study were published in the journal called "Psychiatry and Clinical Psychopharmacology".

parenting have an impact on the characteristics of each other.[6] As the temperament of the child becomes difficult to manage, so does parenting. Also, incompetent parenting that is demonstrated by means of more authoritarian and insensitive parental attitudes are associated with concomitant stable patterns of difficult temperamental traits and problem behaviors.[6] It seems that temperament and parental attitudes have a bi-directional relationship.

The American Academy of Pediatrics recommends avoiding digital media use for children younger than 18-24 months old. They also suggested that screen time should be limited to 1 hour or less when the child is 24-month or older; however, screen time should be shared with a parent.[7] Literature findings emphasize that watching television may impair cognitive development and restrain children from social interaction. Even if the child does not watch television, background television may also be harmful for them.[8]

When studies concerning computer, console, tablet and smartphone games and internet usage are examined, it is apparent that screen media exposure is gradually increasing among children of pre-school age; it is reported for children between the ages of 0 to 8 that, 71% of them have smart phones while 58% have tablets. In the literature, studies related to computer and internet usage were mostly conducted with adolescents and in these studies it was determined that problems such as sleeplessness, academic failure, addiction, contact with malicious people, and stealing money from the family in order to spend on online games were highly common for this group.[9]

It was also reported that temperament has a role in the attitude of watching television and children with difficult temperament traits watch more television.[10] In a study conducted with two-year-olds, it was found that there was a positive relationship between more authoritarian and permissive parenting styles and the duration of watching television. However, in the same study, it was found that the baby's temperament had no effect on the duration of watching television.[11] In addition, the content of the program is very important when watching television. The shows that children watch with their parents and informative programs such as "Sesame Street" could be more beneficial for children. However, families with poor parental attitudes do not pay attention to this notion.[9,12]

In light of all this information, the aim of the present study was to investigate the relation between television, tablet/internet and smartphone exposures and temperament, while also investigating the moderator role of parental attitudes of children who are between the ages of 3-7. For this purpose, it was hypothesized that children who have a difficult temperament and are exposed to poor parenting attitudes would have excessive use of media, and the parental attitudes and child's temperament would be effective in their choices of screen media usage (i.e., television, tablet/internet and smartphone usage). As the second theory of the study, it was hypothesized that the child's temperament would directly affect the screen media usage and parenting attitudes would moderate the relation between the child's temperament and choices of screen media usage. As per these hypotheses; the role of parental attitudes in the relationship between the child's temperament traits and screen exposure was investigated in the present study.

Material and Methods

Sample

Two hundred and ten children and their families were chosen from day care units and among the healthy children in our hospital. Not being able to read and write to fill in the scales was the exclusion criterion of the study. This study was approved by Başkent University Institutional Review Board and Ethics Committee (Project no: KA15/262). All parents gave written informed consent for themselves and their children.

Measures

Sociodemographic information form and screen media assessment questionnaire: It is an information gathering tool prepared by the researchers for collecting sociodemographic and clinical data in accordance with the aim of the study. The form includes questions about the sociodemographic characteristics of the parents and developmental history of the child including birth history, primary caregiver in the first year, diseases, bad memories, sleep, and nutrition routines. In this questionnaire, when the child first used a television, smartphone, and tablet/ internet (as month/old), and the duration of daily television, smartphone and tablet/ internet (as an hour/day) use were also asked. In addition, the parents were asked about watching background television (whether the television is on while the child is playing in the room) and if there was a television in the child's own room. Also, they were asked what the child watches on TV (i.e., cartoons, educational programs, advertisings, all of them), what they did with a smartphone, tablet/internet (i.e., gaming, watching videos, talking on the phone, taking photos, etc.), and for what purpose these devices were given to their children (i.e., for spending time while the mother is doing housework, while feeding, for entertainment, for sleeping, other). Throughout this article, all variables concerning when the child used these digital devices (television, smartphone, tablet/ internet) and the time spent with these devices will be referred to as "screen media exposure".

Children's Behavior Questionnaire-Short Form (CBQ): This temperament scale was developed by Putnam and Rothbart.[13] CBQ was developed for assessing temperament, which is defined as individual changes in self-regulation and reactivity driven by processes such as genetics, environmental factors, maturation and experiences.

While reactivity is defined as the excitability of sensory, emotional and motor response systems, self-regulation includes systems for regulating them. CBQ was developed on this theoretical basis; it consists of 94 items and measures 15 dimensions of the temperament traits, namely: activity level, anger/frustration, approach, attentional focusing, discomfort, falling reactivity and soothability, fear, high intensity pleasure, impulsivity, inhibitory control, low intensity pleasure, perceptual sensitivity, sadness, shyness, smiling and laughter. On this scale, the parents were asked to rate their children on each item using a 7-point likert scale ranging from "extremely untrue" to "extremely true". There is also a "not applicable" option for situations that may be unrelated to the child. If the average score for a temperament trait is 7, it is concluded that the temperament trait is very intense for the child. If the average score is 1, it shows that the temperament trait is very weak. A score of 4 indicates that the temperament is neither intense nor weak.[14] The Turkish adaptation, validity and reliability study of the scale was conducted by Akın Sarı et al.[15] and the reliability coefficient was found as .78.

Parent Attitude Research Instrument (PARI): It is a 4-point Likert-type scale made of 60 items. The scale is used to evaluate the parental attitudes on child rearing. The scale was developed by Schaefer and Bell and Turkish adaptation was conducted by Le Compte et al.in 1978.[16,17] The internal consistency coefficient of the scale is between .58 and .88. PARI consists of five dimensions: excessive motherhood, democratic attitude and recognition of equality, hostile and rejective attitude, discord between parents, and an authoritarian attitude. An increase in scores, aside from the "democratic attitude and recognition of equality" dimension, shows negative parental attitudes.[17,18]

Statistical Analyses

The suitability of the variables to normal distribution was examined using the Kolmogorov – Smirnov and Shapiro Wilk tests. As the variables normally distributed mean value and standard deviation were used for descriptive variables. The relation between sociodemographic characteristics and screen media exposure, and the relation between

temperament traits and negative parental attitudes for television-computer duration were investigated by Pearson correlation analysis. The Independent sample t test was conducted for group comparison. One-way ANOVA was conducted for investigating group differences and Bonferroni correction was applied.

In this analysis, temperament traits were defined as independent variables because of their genetic origins and the screen media exposure features were defined as dependent variable. While investigating this relation, the parental attitudes were defined as the moderator variables since, as the environmental factors, they can influence present relation positively or negatively. As mentioned before the parental attitudes is an important factor for "goodness of fit". The moderator role of parental attitudes on the relationship between temperament traits and screen media exposure was investigated through regression analysis by using the Process macro of Hayes and Matthes.[19] Two-sided p-values less than .05 were considered statistically significant. All statistical analyses were conducted using SPSS 17.0 for Windows.

Results

A total of 210 children, 107 girls (51%) and 103 boys (49%), participated in this study. Sociodemographic characteristics, parental attitudes and temperament traits of the sample can be seen in Table I and Table II.

The analyses revealed that there were no differences between girls and boys in terms of the parameters such as age, number of siblings, birth order of children, age of speaking, birth history, illnesses, primary caregiver in the first year, sleeping and feeding problems, type of family, parents' age and occupation, and the parent/caregiver who is currently looking after the child. However, it was observed that the boys had more trauma history *(t = -2.299, p = .023)* and delay in walking *(t = -2.357, p = .015)* compared to girls. Moreover, considering gender differences, it was found that the boys

Table I. Demographic characteristic of the sample (N = 210).

	Median	*IQR*	*N*	*%*
Age				
Girls (Total)			107	100
Three			27	25.2
Four			27	25.2
Five			25	23.4
Six			28	26.2
Boys (Total)			103	100
Three			27	26.2
Four			25	24.3
Five			27	26.2
Six			24	23.3
Type of family				
Nuclear family			184	90.6
Traditional family			18	8.9
Divorced family			1	0.5
Number of siblings	2	1		
Birth order	1	1		
Parents' education level				
Mother				
Primary school			7	3.4
Secondary school			10	4.8
High school			62	29.8
University			110	52.9
Master degree			19	9.1
Father				
Illiterate			1	0.5
Primary school			6	2.9
Secondary school			8	3.9
High school			47	23
University			121	59.3
Master degree			21	10.3
Parents' employment status				
Mother				
Employed			129	62.6
Unemployed			81	37.4
Father				
Employed			206	99.5
Unemployed			4	0.5
Type of birth				
Vaginal birth			114	52.1
Cesarean			96	47.9

Table II. The descriptive characteristics of PARI and the temperament trait.

	M	SD	n
Parental attitudes			
Excessive motherhood	40.95	9.46	184
Democratic attitude and recognition of equality	24.05	2.96	184
Hostile and rejective attitude	29.54	7.14	181
Discord between parents	14.18	4.28	189
Authoritarian attitude	35.38	8.81	177
Temperament Traits			
Activity level	4.81	0.98	210
Anger/frustration	4.36	1.09	210
Approach	5.41	0.90	210
Attentional focusing	4.56	1.18	210
Discomfort	4.24	1.28	210
Falling reactivity and soothability	4.44	1.02	210
Fear	4.24	1.27	210
High intensity pleasure	4.67	1.11	210
Impulsivity	4.40	1.02	210
Inhibitory control	4.82	1.15	209
Low intensity pleasure	5.49	0.83	210
Perceptual sensitivity	5.90	1.07	210
Sadness	4.63	0.83	210
Shyness	3.74	1.38	210
Smiling and laughter	5.23	1.01	210

spend more time on the internet/ with a tablet *(t = -3.663, p = .000)* and more frequently have a television in their own room *(t = -2.461, p = .015)* when compared to girls. Considering the relationship between sociodemographic characteristics and screen exposure, the results revealed that the age they started watching television was significantly associated with having a traumatic experience *(t = 2.957, p = .010)*, sleep problems *(t = 3.199, p = .002)* and feeding problems *(t = 3.149, p = .002)*. Children with delayed speech were introduced to the tablet/internet later *(t = -2.678, p = .011)*. Daily internet/tablet duration was longer in children with delayed walking age *(r =.159, p =. 025)*. Apart from this, whether the child has a chronic disease, the type of family, the ages of mother and father, whether the mother and father are employed, the number of siblings, the siblings order and whether there is a delay in walking did not affect screen media exposure *(p > .05)*.

In terms of parent's education level, the results were inconsistent. First of all, as expected, when the mother's education level increased, the children's duration of daily television watching decreased (*r = -.137, p = .005*). However, the other significant findings revealed that, for the mothers who have a high education level, the children's age of starting watching television (*r = -.143, p = .044*) and using a smartphone (*r = -.144, p = .005*) decreased. Similarly, as the father's education level increased, the children's age they started using a smartphone decreased (*r = -.167, p = .024*) and the frequency of background TV increased (*r = .158, p = .029*). The age they started using a tablet/internet also varied according to the parental education level (for mother *r = -.198, p = .008*; for father *r = -.229, p = .002*). The higher the education level of fathers, the younger the age of being introduced to a tablet/internet; however, the education level of fathers did not affect the duration of television, smartphone or internet/tablet use.

Significant differences were also found in terms of the age they started watching television [$F(15,182) = 1.69$, $p = .044$,] and the time spent using a smartphone [$F(2,181) = 5.87$, $p = .003$]. Accordingly, after Bonferroni correction ($p < .05$), the age they started watching the television was the lowest for those who were raised by a babysitter *(m = 15.26, sd = 9.47)*, whereas their ages were higher when they were raised by mothers *(m = 20.59, sd = 10.51)* and grandmothers *(m = 23.51, sd = 11.52)*. Also, children who were cared for by a babysitter in the first year were introduced to smartphones earlier *(m = 26.8, sd = 14.1)* (Bonferroni correction $p < .01$), and children's ages were higher when they were raised by mothers *(m = 32.2, sd = 15.7)* and grandmothers *(m = 42.0, sd = 18.1)*. In addition, for those who were currently caring for the child, there were significant differences in the children's daily time spent on a smartphone [$F(3,192) = 3.06$, $p = .029$]. It is noteworthy that children who were currently cared for by a babysitter had the longest smartphone using time *(m = 1.88, sd = 1.87)* and the duration decreased for children who were cared for by grandmothers *(m = 1.39, sd = 1.87)*, by mothers *(m =.99, sd = 1.31)*, and by daycare center *(m =.75, sd = .87, non-significant)*, respectively. The reasons these children used screens and the duration of screen media exposure were also investigated, but there were no statistically significant results. These relationships are shown in Table III.

There were also certain significant correlations between parental attitudes/child's temperament and screen media exposure features, which are shown in Table IV.

In order to test the moderator role of parental attitudes on the relation between children's temperament traits and screen media exposure, a series of moderation analyses were performed. According to the results, 19 of 360 the models were significant and only these findings were reported. The significant results were evaluated based on the critical value obtained via the Johnson and Neyman18 technique, which identifies regions in the range of the moderator variable where the effect of the focal predictor on the outcome is statistically significant and not significant, and pick-a-point approach that involves selecting representative values (e.g., high, moderate, and low) of the moderator variable and then estimating the effect of the focal predictor at those values.[19] The results were presented according to temperament characteristics:

Anger/Frustration

When the scores of excessive mothering became lower than the critical value (CV = -.9877, B = -2.32, SE = 1.18, p = .05, 95% CI [4.6507, 0]), the negative relation between anger and age of starting to use a smartphone decreased (model: R^2 = .05, $F(3, 161) = 3.40$, p = .011, interaction: B = .28, SE = .11, p = .03). As children's level of anger increase, the duration of using a smartphone tended to increase; if the scores of excessive motherhing became higher than the critical value (CV = -3.7115, B = .19, SE = .09, p = .05, 95% CI [-4.8074, 0]), this relation tended to be stronger (model: R^2 = .10, $F(3, 175) = 6.78$, p = .0002, interaction: B = .02, SE = .01, p = . 01). As children's anger increase, the duration of using a smartphone tended to increase, and when the scores of authoritarian control became higher than the critical value (CV = -3.4589, B = .19, SE = .10, p = .05, 95% CI [0, .3856]), this relation tended to be stronger (model: R^2 = .17, $F(3, 171) = 11.76$, p = .000, interaction: B = .03, SE = .00, p = .000). Anger and age of starting using a smartphone had a negative association, and when the scores of authoritarian control became lower than the critical value (CV = -1.9245, B = -2.40, SE = 1.21, p = .05, 95% CI [0, .3900]), the strength of this relation became weaker (model: R^2 = .04, $F(3, 156) = 2.7$, p = .04, interaction: B = .29, SE = .13, p = .02).

Attentional focusing

When attentional focusing increased, the duration of internet usage decreased, and when the scores of hostility and rejection became higher than the critical value (CV = -2.2857, B = -.17, SE = .09, p = .05 , 95% CI [-.3551, 0]), the strength of this relation became weaker

Table III. The descriptive features of the ways children spend time on screen media and screen media exposure.

	What they watching on TV.			
	Cartoons (n = 192)	Educational programs (n = 6)	Advertisements (n = 3)	All of them (n = 1)
	M ± SD	*M ± SD*	*M ± SD*	*M ± SD*
Age they started watching TV	20.6m ± 10.8	23m ± 7.9	9m ± 4.2	24m ± 0
Age they started using smartphones	33.4m ± 16	50m ± 24.4	21.33m ± 17.24	24m ± 0
Age they started using a tablet/internet	36.9m ± 16.9	56m ± 19.5	39m ± 12.7	12m ± 0
Daily hours of watching TV	2.35h ± 2.86	1.3h ± 0.5	0.5h ± 0.5	5h ± 0
Daily hours of using a smartphone	0.9h ± 1.3	0.2h ± 0.4	0.6h ± 0.5	5h ± 0
Daily hours of using the internet	1h ± 1.24	0.4h ± 0.5	0.6h ± 0.54	5h ± 0

	What are they doing with the smartphones?				
	Playing games (n = 130)	Watching videos (n = 24)	Taking photos (n = 10)	Talking on the phone (n = 2)	All of them (n = 1)
	M ± SD	*M ± SD*	*M ± SD*	*M ± SD*	*M ± SD*
Age they started watching TV	20.03m ± 10.71	18.47m ± 9.39	23.4m ± 12.4	12m ± 4.9	24m ± 0
Age they started using smartphones	33.79m ± 15.70	32.16m ± 16.53	25.11m ± 12.57	19.2m ± 11.54	24m ± 0
Age they start using a tablet/ internet	35.85m ± 16.65	33.91m ± 18.88	33.66m ± 17.60	40.5m ± 16.52	12m ± 0
Daily hours of watching TV	2.21h ± 1.53	1.79h ± 1.22	1.9h ± 1.3	1.3h ± 1.1	5h ± 0
Daily hours of using a smartphone	1.26h ± 1.51	0.6 ± 0.6	0.25h ± 35	0.62h ± 0.94	5h ± 0
Daily hours of using the internet	1.1h ± 1.3	1h ± 0.8	0.3h ± 0.53	1.7h ± 1.4	5h ± 0

	What are they doing on the internet?				
	Playing games (n = 123)	Watching videos (n = 21)	No internet (n = 21)	Social media (n = 1)	All of them (n = 1)
	M ± SD	*M ± SD*	*M ± SD*	*M ± SD*	*M ± SD*
Age they started watching TV	21.04m ± 10.76	19.73m ± 11.57	24.10m ± 12	10m ± 0	24m ± 0
Age they started using smartphones	33.08m ± 16.35	32.24m ± 15.75	39.66m ± 19.1	42m ± 0	12m ± 0
Age they started using a tablet/internet	38.26m ± 17.48	34.15m ± 16.52	49.5m ± 17.18	42m ± 0	12m ± 0
Daily hours of watching TV	2.57h ± 3.42	1.78h ± 1.58	1.59h ± 1.04	1h ± 0	5h ± 0
Daily hours of using a smartphone	0.95h ± 1.2	0.9h ± 1.2	0.88h ± 1.73	2h ± 0	5h ± 0
Daily hours of using the internet	1.35h ± 1.2	1.1h ± 1.2	-	-	5h ± 0

m: months of age, h: hours of day

Table IV. The correlations between screen media exposure features and parental attitudes/temperament traits.

	Age they started watching TV	Age they started using a smartphone	Age they started using a tablet/ internet	Daily hours of watching TV	Daily hours of using a smartphone	Daily hours of using a tablet/ internet
	r	*r*	*r*	*r*	*r*	*r*
Parental attitudes						
Excessive motherhood	0.002	0.068	0.019	0.076	0.176*	0.130
Democratic attitude and recognition of equality	0.133	0.150	0.071	0.032	0.021	0.010
Hostile and rejective attitude	0.059	-0.067	-0.094	0.020	0.094	0.144
Discord between parents	-0.039	0.012	-0.124	0.017	0.172*	0.091
Authoritarian attitude	0.76	0.067	-0.048	0.127	0.253**	0.178*
Temperamental traits						
Activity level	-0.166*	-0.089	-0.125	0.028	0.016	0.095
Anger/frustration	-0.091	-0.123	-0.144	0.007	0.223**	0.106
Approach	-0.167*	-0.150*	-0.076	0.019	-0.072	-0.054
Attentional focusing	0.066	0.149	0.172*	0.009	-0.236**	-0.271**
Discomfort	-0.153*	-0.017	0.056	0.156*	-0.015	-0.029
Falling reactivity and soothability	0.008	-0.013	0.038	-0.024	-0.161*	-0.106
Fear	-0.025	0.118	0.114	-0.002	-0.021	-0.151*
High intensity pleasure	-0.094	-0.047	-0.102	0.000	0.204**	0.226**
Impulsivity	-0.157*	-0.71	-0.067	-0.032	0.043	0.092
Inhibitory control	0.000	0.131	0.214**	-0.103	-0.343**	-0.368**
Low intensity pleasure	-0.057	0.027	0.050	0.028	-0.146*	-0.174*
Perceptual sensitivity	-0.072	0.068	0.122	-0.026	-0.252**	-0.264**
Sadness	-0.013	0.082	0.171*	-0.121	-0.142*	-0.165*
Shyness	0.155*	0.187*	0.125	-0.085	-0.006	-0.154*
Smiling and laughter	-0.038	-0.055	-0.008	0.060	-0.081	-0.086

Note. *$p < .05$, **$p < .01$

(model: R^2 = .14, F(3, 173) = 9.47, p = .0000, interaction: B = -.04, SE = .01, p = .001). When attentional focusing increased, the duration of internet usage decreased, and if the scores of authoritarian control became higher than the critical value (CV = -6.3532, B = -.18, SE = .09, p = .05 , 95% CI [-.3720, 0]), the strength of this relation became weaker (model: R^2 = .13, F(3, 171) = 9.06, p = .0000, interaction: B = -.02, SE = .00, p = .015).

Discomfort

Discomfort and duration of watching TV had a positive association, and if the scores of authoritarian control became higher than the critical value (CV = -2.5923, B = .34, SE = .17, p = .05, 95% CI [0, .6912]), the strength of this relation tended to be stronger (model: R^2 = .07, F(3, 173) = 4.78, p = .0031, interaction: B = .05, SE = .02, p = .02).

Falling reactivity/soothability

As children's level of falling reactivity/ soothability increased, the age of starting to watch TV tended to decrase. If the scores of excessive mothering became lower than the critical value (CV= -4.5660, B = -1.84, SE = .93, p = .05, 95% CI [-3.6862, 0]), this relation tended to

become weaker; but if the scores became higher than the critical value (CV = 9.9264, *B* = 2.15, *SE* = 1.09, *p* = .05, 95% CI [0, 4.3028]), this relation tended to be stronger (model: R^2 = .05, *F*(3, 173) = 3.35, *p* = 0.02, interaction: *B* = .27, *SE* = .08, *p* = .001). Children's level of falling reactivity/soothability and the duration of using a smartphone had a positive but non-significant association (*p* = .059). When the scores of excessive mothering became higher than the critical value (CV = .2546, *B* = -.19, *SE* = .10, *p* = .05, 95% CI [-.3991, 0]), the strength of this relation became weaker and significant (model: R^2 = .09, *F*(3, 175) = 5.93, *p* = .0007, interaction: *B* = -.02, *SE* = .01, *p* = .011). As children's level of falling reactivity/soothability increased, the duration of using a smartphone tended to decrease. If the scores of authoritarian control became higher than the critical value (CV = -1.2450, *B* = -.20, SE = .10, *p*= .05, 95% CI [-.4193, 0]) the strength of this relation became weaker (model: R^2 = .12, *F*(3, 171) = 8.03, *p* = .0000, interaction: *B* = -.03, *SE* = .01, *p* = .015).

Inhibitory control

Although the negative relationship between the children's level of inhibitory control and age they started watching TV was non-significant (*p* = .69), if the scores of democratic attitudes became lower than the critical value (CV = -2.3883, *B* = -1.85, *SE* = .93, *p* = .05, 95% CI [-3.7034, 0]), this relationship tended to become weaker and significant (model: R^2 = .04, *F*(3, 173) = 2.93, *p* = .034, interaction: *B* = .56, *SE* = .24, *p* = .022). As children's level of inhibitory control increased, the duration of using internet tended to decrease. If the scores of hostility and rejection became higher than the critical value (CV = -8.0781, *B* = -.26, *SE* = .13, *p* = .05, 95% CI [-.5200, 0]), this relation tended to become weaker (model: R^2 = .18, *F*(3, 173) = 13.5, *p* = .0000, interaction: *B* = -.02, *SE* = .01, *p* = .049). As children's level of inhibitory control increased, the duration of internet usage tended to decrease. If the scores of authoritarian control became higher than the critical value (CV= -7.6616, *B* = -.22, *SE* = .11, *p* = .05, 95% CI [-.4547, 0]), this relation tended to become weaker (model: R^2 = .18, *F*(3, 171) = 12.90, *p* = .0000, interaction: *B* = -.02, *SE* = .01, *p* = .027).

Low intensity pleasure

When children's low intensity pleasure increased, the age they started watching TV decreased, but this relation was non-significant (*p* = .12). However, when the scores of democratic attitudes became lower than the critical value (CV = -.6130, *B* = -1.98, *SE* = 1.00, *p* = .05, 95% CI [-3.9797, 0]), the relationship became significant and the strength of this relation become weaker (model: R^2 = .05, *F*(3, 173) = 3.31, *p* = .021, interaction: *B* = .72, *SE* = .35, *p* = .044). When children's low intensity pleasure increased, the duration of tablet usage decreased, but this relation was non-significant (*p* = .10). However, if the scores of hostility and rejection became higher than the critical value (CV = .7861, *B* = -.22, *SE* = .11, *p* = .05, 95% CI [-.4462, 0]), their interaction became significant and the strength of this relation become weaker (model: R^2 = .08, *F*(3, 173) = 5.46, *p* = .0013, interaction: *B* = -.04, *SE* = .01, *p* = .005).

Sadness

As children's level of sadness increased, the age they started using a tablet tended to increase. If the scores of excessive mothering become higher than the critical value (CV = -.4271, *B* = 3.08, *SE* = 1.56, *p* = .05, 95% CI [0, 6.1644]) the strength of this relation tended to be stronger (model: R^2 = .06, *F*(3, 156) = 3.37, *p* = .019, interaction: *B* = .46, *SE* = .18, *p* = .014). As children's level of sadness increased, the duration of watching TV tended to decrease, but this association was non-significant (*p*=.061). However, when the scores of excessive mothering become higher than the critical value (CV = .3535, *B* = -.50, *SE* = .25, *p* = .05, 95% CI [-1.0096,0], the interaction became significant and the strength of relationship between sadness and duration of watching TV tended to become weaker (model: R^2 = .05, *F*(3, 178) = 3.19, *p* = .024, interaction: *B* = -.07, *SE* = .03, *p* = .016). As children's level of sadness increased, the duration of watching TV tended to decrease,

but this relation was non-significant (p = .63). If the scores of authoritarian control became lower than the critical value (CV = -12.6720, B = .89, SE = .45, p = .05, 95% CI [0, 1.7881]) this relation tended to become stronger; if the scores became higher than the critical value (CV = .2827, B = -.50, SE = .25, p = .05, 95% CI [-1.0189, 0]) this relationship tended to become weaker. In both cases, the interactions were significant (model: R^2 = .08, F(3, 173) = 5.43, p = .001, interaction: B = -.10, SE = .03, p = .0010).

Shyness

As children's level of shyness increased, the duration of watching TV tended to decrease. If the scores of authoritarian control become lower than the critical value (CV = -9.0304, B = .41, SE = .21, p = .05, 95% CI [0, .8319]), this relationship tended to become stronger; but if the scores became higher than the critical value (CV= -.4326, B = -.30, SE = .15, p = .05, 95% CI [-.6057, 0]) this relationship tended to become weaker (model: R^2 = .12, F(3, 173) = 8.23, p = .0000, interaction: B = -.08, SE = .01, p = .0000).

Discussion

This study investigated the relationship between screen media exposure and child's temperament, and the effects of parental attitudes on this relationship. The sample of the present study started watching television at about 20 months old; the average duration and the age of beginning to watching television were compatible with previous studies.[20-24] However, it was remarkable that the duration of smartphone usage was longer than in previous studies.[25] Studies conducted in the USA have shown that watching videos are the most commonly purpose of screen media usage.[25] In a recent study that investigated 3-5 year old's smartphone and tablet use in the USA revealed that the most common used applications are YouTube and YouTubeKids.[26] In the present study which was conducted in Turkey, it was found that playing video games are the most common purpose of screen media usage. These differences are thought to be associated with cultural differences and Turkey's being a developing country. Moreover, as in other developing countries, boys are perceived as more valuable than girls in the Turkish society.[27] The fact that there are more TVs in boys' rooms may be related to this situation. Similar to the present study, in a study conducted in the USA, it was found that male adolescents have more video game systems than girls and these systems are found to be more common in boys' rooms than girls.[28] In this study, contrary to Western studies, the higher the level of educationof the parents, the more media devices seem to exist in their houses.[25] In addition, the rate of working with care-givers was also higher for this group. This can be explained by the fact that families with higher education levels are likely to have a higher income and therefore purchasing power, making it easier for them to access screen media tools and private care services.

Children with a difficult temperament (i.e., activity level, discomfort, impulsivity) were permitted to watch television at earlier ages by their parents in this study. It was observed as a difficult temperament trait that, when the discomfort increased, the duration of watching television also increased. Studies showed that children with difficult temperament traits watch more television and this could be regarded as a coping mechanism.[10,29] Similarly, mothers of children who had difficulty in self-regulation at the age of 2 were also allowed more television time.[30] When these studies were evaluated together, the temperament trait seems to affect the screen exposure in early infancy as well as in the pre-school period. In a study conducted by Munzer et al.[8], it was found that children who had difficulty in self-regulation in the pre-school period were exposed to increased durations of screen and background TV. Similarly, in this study, self-regulation parameters such as attentional focusing and inhibitory control were observed to be lower for children having background television. The poor perceptual sensitivity in this study is related to background TV exposure. In other

words, temperament traits affect the preference of screen usage in positive and negative ways.

For the participants of this study, it is noteworthy that children with easy temperament traits did not have the internet at their homes. Parents of children with an easy temperament do not use the internet. Parent's relationship with electronic media devices is known to affect the use of these devices by children.[31] In studies, it was found that difficult temperament traits were related to internet addiction.[32,33] It is also known from previous studies that temperament traits are inherited.[34,35] Therefore, it seems that the easy temperament traits of the parents can limit their internet usage and in this way, their children can also be protected both environmentally and genetically.

No relationship was found between parental attitudes and television usage in this study. However, such a relationship was found between poor parenting attitudes and smartphone and tablet/internet usage durations. This shows us that media devices other than television are becoming more prominent for young children. As recognized from the literature, the smartphone is a device that creates an important screen exposure that is increasingly used in children between the ages of 0-8.[25] It was suggested that the parents of children between ages of 3 to 6 did not want their children to watch television, but they did not think the same way for smartphone usage. Studies revealed that the duration of television watching increases with a permissive parenting attitude.[36] Howe et al.[11] found that both permissive and authoritarian mothers allowed more television time. In a study conducted with 5-year-old children, it was found that authoritarian parents allowed less use of smartphone and game consoles for their children, but the duration of the television was not related to any parenting attitude.[37] It has also been shown that good parenting attitudes in mothers of elementary school children also decreased smartphone usage.[38] However, as revealed in the present study, authoritarian parents let their children use the internet/tablet and smartphone for more hours and used this screen exposure to get their own work done or to feed them. In other words, mothers use the screen as a "digital parent" and self-substitute. Cultural differences within the family can also explain the differences in screen media usage of these parenting attitudes. Therefore, it can be considered that the reasons for these differences should be determined by longitudinal studies.

In the literature, there are conflicting results regarding the relationship between parenting attitudes, temperament traits and screen media exposure. Some of them have found that parenting attitudes have an effect on screen media usage, while in some others, the temperament traits were found to be effective.[30,36,37,39] In a study conducted with adolescents, it was found that parenting attitudes affected temperament traits and made internet use problematic.[40]

In this study, it was seen that, when difficult temperament traits and bad parental attitudes were combined, screen media exposure increased. While smartphone exposure increased with the anger temperament trait, excessive mothering and authoritarian control parental attitudes could reinforce this relationship. While discomfort temperament traits prolonged television duration, authoritarian control parental attitudes increased this relationship. It was noted that the daily smartphone usage shortened as soothability increased and this relationship was affected by authoritarian control. Easy temperament traits such as attentional focusing and inhibitory control reduced the duration of internet usage, and poor parenting features such as hostility/rejection and authoritarian control weakened this relation. Additionally, for children with easy temperament traits, screen exposure was low, and as a moderator, bad parenting attitudes seem to reduce this relationship. In other words, when difficult temperament and bad parenting come together, negative results were observed; even if they have an easy temperament, children with bad parenting may be more exposed to screen media. It is stated in a study by Rubinet al.[41]

that even worse results could be observed when inhibited temperament traits and excessive mothering were combined. In this study, the reason for sad children to start using tablets/internet earlier may be due to the overprotection of mothers. Mothers endeavor to reduce the sadness of their children. Similarly, in the present study, it was found that the duration of daily television watching decreased as sadness increased and excessive mothering parental attitudes negatively affected this relationship. In other words, this parental attitude increased the duration of daily television watching. Apart from this, it was observed that parental attitude of authoritarian control had a binary effect on the relationship between unhappiness and shyness, and the duration of watching television. It was observed that daily television watching duration decreased as sadness and shyness increased, and these relations strengthened when the parental attitude of authoritarian control was low. However, it is noteworthy that when the authoritarian control increased, these relations were also negatively affected, namely the duration of watching television increased. As a result, the models of the present study showed that bad parenting attitudes increased screen media exposure while good parenting reduced it. In order to understand the effects of parental attitudes on the relationship between other temperament traits and screen media exposure, further studies should be conducted.

This is a comprehensive study that aimed to examine the association between temperament traits and screen media exposure, and the moderator role of parental attitudes on this relationship. In this study, the role of parental attitudes on the link between temperament traits and screen media exposure was investigated for the first time. These results can be a guide for future studies. In addition, the duration of television, smartphone and tablet/internet usages were examined separately and the differences between the usage purposes were also analysed. The study has some limitations, the first of which is the limited number of participants who contributed to the study. The second limitation is the fact that previous experiences were questioned while collecting data, which can be regarded as a cause for recall bias. In order to solve this problem, developing applications which monitor the use of screen media was suggested by Barr et al.[42]

In the light of the results of the present study, it was determined that the main reason for parents to expose their children to the screen at an early age may be due to their lack of information about the importance of the subject. Therefore, information programs about parenting, temperament management and the negative consequences of screen exposure may decrease the screen exposure time of pre-school children.

Acknowledgements

The authors would like to acknowledge Selvi Kayıpmaz for her statistical assistance, Artun Törenli for his valuable feedback while proof reading the article and all the participants who were involved in the study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BAS, HAT, ZTK; data collection: BAS, HAT, ZTK; analysis and interpretation of results: BAS, HAT, ZTK; draft manuscript preparation: BAS, HAT, ZTK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

This study was approved by Instituonal Review Board and Ethics Commitee (Project no: KA15/262) on 12.08.2015 and supported by Baskent University Research Fund.

Conflict of interest

The author has no conflict of interest to declare.

REFERENCES

1. Goldsmith HH, Buss AH, Plomin R, et al. Roundtable: what is temperament? Four approaches. Child Dev 1987; 58: 505-529.
2. Kristal J. The Temperament Perspective: Working with Children's Behavioral Styles. (1st ed) Baltimore: Paul H Brookes Publishing, 2005.
3. Shiner RL, Buss KA, McClowry SG, et al. What is temperament now? Assessing progress in temperament research on the Twenty-Fifth Anniversary of Goldsmith et al. Child Dev Perspect 2012; 6: 436-444.
4. Thomas A, Chess S. Temperament and Development. New York: Brunner/Mazel, 1977.
5. Hoghughi M. Parenting - An Introduction. In: MS, Long N (eds). Handbook of Parenting: Theory and Research for Practice. (1st ed) London: SAGE Publication, 2004: 1-17.
6. Akın Sarı B. Çocuk davranış listesi kısa formunun Türkçe geçerlilik-güvenilirlik çalışması ve 3-6 yaş çocuklarında mizacın etyolojisinin araştırılması. Yayınlanmamış Doktora Tezi, Gazi Üniversitesi Tıp Fakültesi, Ankara, 2009.
7. American Academy of Pediatrics. Council on Communications and Media. Media and Young Minds. Pediatrics 2016; 138: e20162591.
8. Munzer TG, Miller AL, Peterson KE, et al. Media exposure in low-income preschool-aged children is associated with multiple measures of self-regulatory behavior. J Dev Behav Pediatr 2018; 39: 303-309.
9. Yavuzer H, Şirin MR. 1. Türkiye Çocuk ve Medya Kongresi. Bildiriler Kitabı. Cilt 2, İstanbul: Çocuk Vakfı Yayınları, 2013.
10. Sherry JL. Toward an etiology of media use motivations: the role of temperament in media use. Commun Monogr 2001; 68: 274-288.
11. Howe AS, Heath AM, Lawrence J, et al. Parenting style and family type, but not child temperament, are associated with television viewing time in children at two years of age. PLoS One 2017; 12: e0188558.
12. Cole CF, Lee JH, Bucuvalas A, Sırali Y. Seven essential elements for creating effective children's media to promote peace building: lessons from International Coproductions of Sesame Street and Other Children's Media Programs. New Dir Child Adolesc Dev 2018; 2018: 55-69.
13. Putnam SP, Rothbart MK. Development of short and very short forms of the Children's Behavior Questionnaire. J Pers Assess 2006; 87: 102-112.
14. Rothbart MK, Ahadi SA, Hershey KL, Fisher P. Investigations of temperament at three to seven years: the Children's Behavior Questionnaire. Child Dev 2001; 72: 1394-1408.
15. Sarı BA, İşeri E, Yalçın Ö, Aslan AA, Şener Ş. Çocuk davranış listesi kısa formunun Türkçe güvenilirlik çalışması ve geçerliliğine ilişkin ön çalışma. Klinik Psikiyatri Dergisi 2012; 15: 135-143.
16. Schaefer ES, Bell RQ. Development of a parental attitude research instrument. Child Dev 1958; 29: 339-361.
17. LeCompte G, LeCompte A, Özer S. Üç sosyo-ekonomik düzeyde Ankaralı annelerin çocuk yetiştirme tutumları: bir ölçek uyarlaması. Türk Psikoloji Dergisi 1978; 1: 5-8.
18. Küçük AŞ. The validity of the Turkish form of the PARI subscales II, III, IV. Yayınlanmamış Yüksek Lisans Tezi, İstanbul: Boğaziçi Üniversitesi Sosyal Bilimler Enstitüsü, Eğitim Bilimleri Bölümü, 1987.
19. Hayes AF, Matthes J. Computational procedures for probing interactions in OLS and logistic regression: SPSS and SAS implementations. Behav Res Methods 2009; 41: 924-936.
20. Conners NA, Tripathi SP, Clubb R, Bradley RH. Maternal characteristics associated with television viewing habits of low-income preschool children. J Child Fam Stud 2007; 16: 415-425.
21. Zimmerman FJ, Christakis DA, Meltzoff AN. Television and DVD/video viewing in children younger than 2 years. Arch Pediatr Adolesc Med 2007; 161: 473-479.
22. Vandewater EA, Rideout VJ, Wartella EA, Huang X, Lee JH, Shim M. Digital childhood: electronic media and technology use among infants, toddlers, and preschoolers. Pediatrics 2007; 119: e1006-e1015.
23. Dennison BA, Erb TA, Jenkins PL. Television viewing and television in bedroom associated with overweight risk among low-income preschool children. Pediatrics 2002; 109: 1028-1035.
24. Jordan AB, Hersey JC, McDivitt JA, Heitzler CD. Reducing children's television-viewing time: a qualitative study of parents and their children. Pediatrics 2006; 118: e1303-e1310.
25. Commonsense Media. The Common Sense Census: Media Use by Kids Age Zero to Eight. Available at: https://www.commonsensemedia.org/research/the-common-sense-census-media-use-by-kids-age-zero-to-eight-2017.(Accessed onDecember 13, 2018).
26. Radesky JS, Weeks HM, Ball R, et al. Young children's use of smartphones and tablets. Pediatrics 2020; 146: e20193518.

27. Kagitcibasi C, Ataca B. Value of children and family change: a three-decade portrait from Turkey. Appl Psychol 2005; 54: 317-337.

28. Roberts DF. Media and youth: access, exposure, and privatization. J Adolesc Health 2000; 27(Suppl 2): 8-14.

29. Thompson AL, Adair LS, Bentley ME. Maternal characteristics and perception of temperament associated with infant TV exposure. Pediatrics 2013; 131: e390-e397.

30. Radesky JS, Silverstein M, Zuckerman B, Christakis DA. Infant self-regulation and early childhood media exposure. Pediatrics 2014; 133: e1172-e1178.

31. Asplund KM, Kair LR, Arain YH, Cervantes M, Oreskovic NM, Zuckerman KE. Early childhood screen time and parental attitudes toward child television viewing in a low-income Latino population attending the special supplemental nutrition program for women, infants, and children. Child Obes 2015; 11: 590-599.

32. Li X, Newman J, Li D, Zhang H. Temperament and adolescent problematic Internet use: the mediating role of deviant peer affiliation. Comput Hum Behav 2016; 60: 342-350.

33. Lee YS, Son JH, Park JH, Kim SM, Kee BS, Han DH. The comparison of temperament and character between patients with internet gaming disorder and those with alcohol dependence. J Ment Health 2017; 26: 242-247.

34. Qiu X, Martin GB, Blache D. Gene polymorphisms associated with temperament. J Neurogenet 2017; 31: 1-16.

35. Ebstein RP. The molecular genetic architecture of human personality: beyond self-report questionnaires. Mol Psychiatry 2006; 11: 427-445.

36. Jago R, Davison KK, Thompson JL, Page AS, Brockman R, Fox KR. Parental sedentary restriction, maternal parenting style, and television viewing among 10-to 11-year-olds. Pediatrics 2011; 128: e572-e578.

37. Veldhuis L, van Grieken A, Renders CM, Hirasing RA, Raat H. Parenting style, the home environment, and screen time of 5-year-old children; the 'be active, eat right' study. PLoS One 2014; 9: e88486.

38. Bae SM. The relationships between perceived parenting style, learning motivation, friendship satisfaction, and the addictive use of smartphones with elementary school students of South Korea: using multivariate latent growth modeling. Sch Psychol Int 2015; 36: 513-531.

39. Radesky JS, Schumacher J, Zuckerman B. Mobile and interactive media use by young children: the good, the bad, and the unknown. Pediatrics 2015; 135: 1-3.

40. Zhang G, Wu L, Zhou L, Lu W, Mao C. Television watching and risk of childhood obesity: a meta-analysis. Eur J Public Health 2015; 26: 13-18.

41. Rubin KH, Burgess KB, Hastings PD. Stability and social-behavioral consequences of toddlers' inhibited temperament and parenting behaviors. Child Dev 2002; 73: 483-495.

42. Barr R, Kirkorian H, Radesky J, et al. Beyond screen time: a synergistic approach to a more comprehensive assessment of family media exposure during early childhood. Front Psychol 2020; 11: 1283.

The Turkish Journal of Pediatrics 2021; 63: 832-845
https://doi.org/10.24953/turkjped.2021.05.011

Original Article

Evaluation of red meat allergy patients and review of the literature

Şeyhan Kutluğ, Gonca Hancıoğlu, Şefika İlknur Karadağ Kökçü, Recep Sancak, Fadıl Öztürk

Division of Pediatric Allergy and Immunology, Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey.

ABSTRACT

Background. It was reported that prevalence of red meat allergy in children was higher in our country than in western populations. However, the diagnosis of these patients is often delayed. The aim of the study was to present the clinical and laboratory characteristics of our red meat allergy patients.

Methods. The data were collected retrospectively from the files of children with red meat allergy. Also, 6 adults with red meat allergies were recorded in the families of the children. Patients with symptoms associated with red meat allergy and sensitive to beef or mutton in prick-to-prick tests were recorded.

Results. The median age of the 43 patients was 12 years (2-37), and 51% were male. Most of the patients were children (n=37, 86%). The median age was 10 years in children (2-17), and 54% were male. All of the children had dermatologic manifestations, 51% had respiratory symptoms, and 64% had anaphylaxis upon exposure to red meat. The anaphylaxis history was not associated with demographic, clinical and laboratory data. A total of 63% children had additional allergic diseases, and 75% of them were sensitive to both mutton and beef in prick-to-prick tests. The median total IgE level of the children was 327 (20-3550) IU/mL, median eosinophil count was 210/mm^3 (40-990) and mean vitamin D was 13.1 ± 1.2 mcg/L (n=27). Anaphylaxis occurred in 3 of 9 patients who received the open oral food challenge (OFC) test. After OFC, 3 patients continued to eat red meat without issues, and 1 patient was recommended to eat alternatives to red meat.

Conclusions. Clinical and laboratory findings were heterogeneous in children with red meat allergy. Anaphylaxis risk seems to be higher than other food allergies. OFC test is more helpful in both diagnosis and alternative red meat selection compared to laboratory findings.

Key words: anaphylaxis, children, food allergy, oral food challenge, red meat allergy.

Food allergy is an important disease because it is associated with high morbidity in children and adults.[1] It can cause nutritional deficiency and related consequences in children.[2,3] Also, the quality of life in all food-allergic patients and their families is negatively affected.[2] The frequency of IgE-mediated food allergy in children varies among countries and age groups, but is reported to be between 3.5% and 11%.[2] Food allergies have increased both in the general population and in children over the years,[2,4] and is becoming increasingly important because they constitute the most common cause of anaphylaxis in children.[5]

In Western societies, peanut, egg, sesame and milk have been found to be most responsible for food allergies in children.[2] Red meat allergy is rarely reported in children with food allergy.[6,7] However, this is not the case all over the world. Foods responsible for allergies vary according to eating habits, geographic regions and race.[2,7,8] There is no prevalence study on food allergy in children covering the entire country of Turkey. In some studies conducted in Turkey regarding the prevalence of food allergy in children, red

✉ Şeyhan Kutluğ
seyhankutlug@hotmail.com

Received 21st July 2020, revised 9th December 2020, 11st January 2021, accepted 20th February 2021.

meat allergy was rarely reported,[9-11] while in others it was frequently reported.[12,13] The conflicting results may be due to the fact that these studies were conducted in different regions of Turkey. Red meat allergy is reported more frequently in the Black Sea region of Turkey.[12,14-16] In a single-center study on the prevalence of red meat allergy in children in the black sea region, the primary beef allergy prevalence was reported to be 0.3%, based on the oral food challenge (OFC) test.[16] In a multi-center study in Turkey, it has been reported that beef is the second food responsible for anaphylaxis in children after milk.[15] For this reason, it is critical to identify patients with red meat allergy to prevent potentially life-threatening reactions at least in regions where red meat allergies are common. Patients with red meat allergy are diagnosed late, and even patients diagnose themselves in population where red meat allergy is rare.[17] It is also argued that red meat allergy is responsible for some spontaneous and idiopathic anaphylaxis.[18] For this reason, we want to present the features of our red meat allergy patients and raise awareness of red meat allergy by pediatricians. The aim of the study was to present the characteristics of our red meat allergy patients and review of the literature.

Material and Methods

Study Population

This retrospective study was conducted between January 2014 and December 2017 based on the records of patients admitted to Pediatric Allergy and Immunology Department. From these records, the individual that had been filled with any complaints after red meat consumption were selected. The records of the children whose complaints were associated with eating red meat and whose red meat sensitivity were shown with skin-prick tests were included in this study. Also, as we learned from the patients' clinical history, the files of patients over the age of 18 years in the same family with similar symptoms were included. Those who had taken other foods together with red meat, and who had described oral allergy syndromes were not included in the study. In the clinical history, it was called early-anaphylaxis if anaphylaxis developed at 0-2 hours after food intake, and delayed-anaphylaxis if anaphylaxis developed at 4-6 hours.[19] The study was approved by the Ondokuz Mayis University Ethics Committee of our institution (KAEK 2017/55). The demographic data of the patients, clinical history and characteristics, laboratory findings and any data that led to diagnosis were recorded from the files.

Laboratory Tests

Skin Prick Tests

Firstly, commercial antigens of beef and mutton were used in the skin-prick test to detect red meat sensitivity (Allergopharma, Reinbek, Germany). The fresh red meat was used in the prick-to-prick test to those who responded negatively to the commercial allergen solution or responded marginally.[2,19] Histamine (10 mg/mL) was used as positive control, and normal saline was used as negative control. If induration was 3 mm or higher in the prick test, the patient was considered sensitive. Raw beef and mutton were used as fresh meat (Fig. 1). In the presence of cooked red meat, prick-to-prick test was applied with it as well. Female and male beef/mutton were used in the prick-to-prick test for some patients who had given conflicting information about food allergy in the clinical history.

Serum-specific IgE

Beef specific-IgE (f27) and mutton specific-IgE (f88) were analyzed with Chemiluminescence Immunoassay (CLIA) method employing the IMMULITE® 2000 XPi (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) using 3gAllergy® kits. The detection range for specific IgE (sIgE) was ≥0.10-100 kU/L. The cutoff used for a positive test in these assays was 0.10 kU/L.

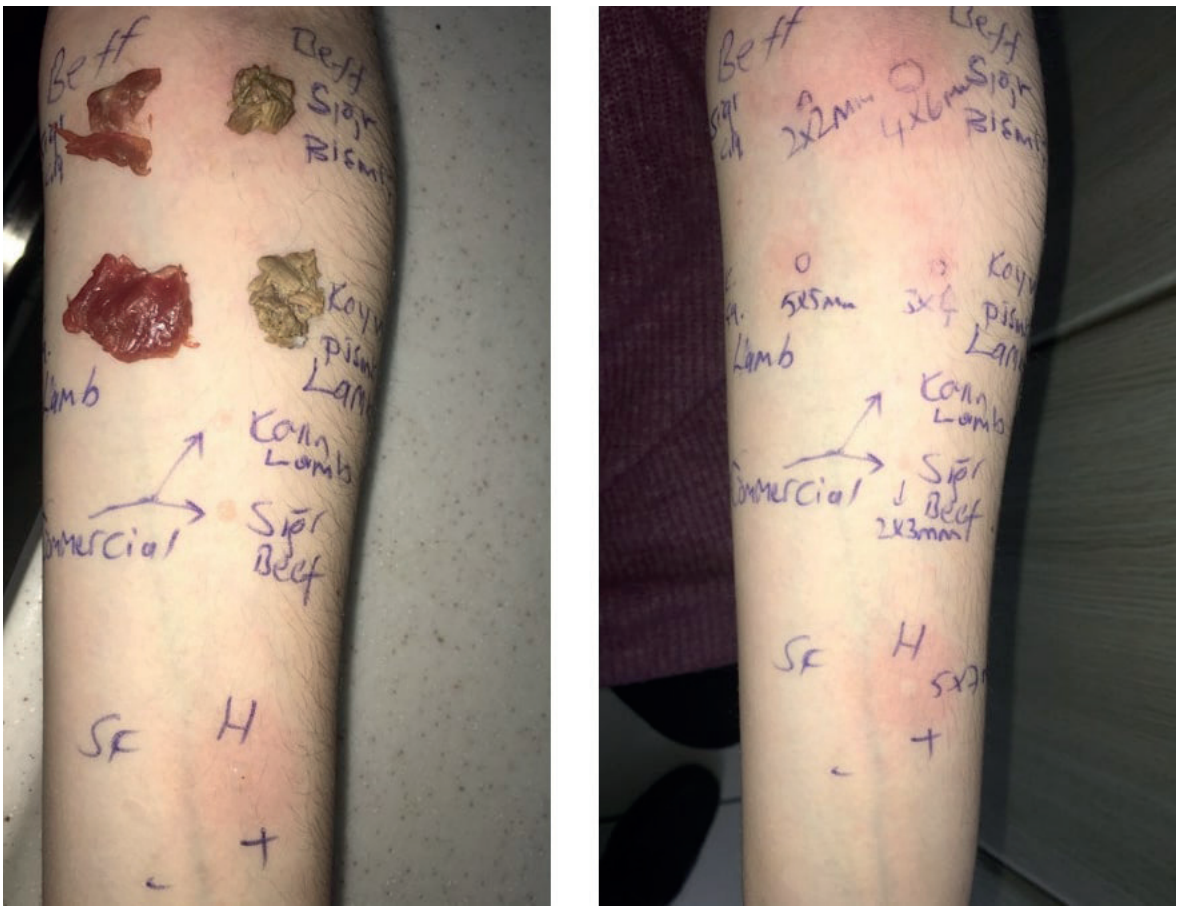


Fig. 1. The prick-to-prick test with raw and cooked red meat to detect red meat sensitivity in Patient 1.

Other laboratory records

In addition, the total IgE, eosinophil counts, hemoglobin, vitamin D (25 OH-D_3) level and blood groups of the patients were recorded.

Oral Food Challenges

The gold standard for food allergy diagnosis is double-blind placebo-controlled oral food challenge (DBPCOFC) test.[1] Our red meat allergy patients usually come from outside the city. For this reason, all our patients had preferred the open oral food challenge test (OFC). Beef and mutton are consumed as red meat in Turkey. Although our aim was to perform a challenging test on both meats, we left it to the consumption habit and request of the patient as to which red meat to use in the test. The OFC was prepared in line with current challenge protocols.[12,16] The titrated dose was given separately for beef and mutton as 1, 2, 7, 15, 25, 50 g with 20 min intervals. It was completed as not to exceed 100 g. The patients were monitored for at least 6 hours after the OFC.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics (22nd version, IBM Corp., NY, USA). Descriptive statistics were expressed as mean ± standard error or median, depending on the distribution of variables. Categorical variables were expressed as numbers and percentages. The normality test of numerical variables was verified with the Shapiro-Wilk test. Chi-Square and Fisher's Exact Tests were used to compare categorical variables. In comparison of quantitative data, independent sample *t*-test was used for those with normal distributions,

while nonparametric tests such as Mann Whitney-U and Kruskal-Wallis test were used in non-normal distributions of the data.

Results

Sixty-three people were admitted to the hospital with suspicion of red meat allergy between the dates mentioned above. In this study, records of 43 patients who were detected to be sensitive to red meat in the prick-to-prick test were presented. The demographic, clinical and laboratory data of the patients are presented in Table I. The median age of the 43 patients was 12 (2-37), and 51% were male. A total of 86% of the patients were between the ages of 2 and 17 years. A total of 86% of child patients were from the Ordu-Giresun in Turkey.

The data of the patients are summarized in Table II. All patients reported skin manifestations, and half of them reported respiratory symptoms upon exposure to red meat. In clinical history, anaphylaxis was described in 67% of patients. The most common accompanying allergic disease was respiratory allergic diseases such as asthma and allergic rhinitis. There was no allergic disease in 1/3 patients. Cow's milk allergy (CMA) was present in a two-year-old patient (patient number 29). CMA was diagnosed at 6 months of age based on convincing clinical history and skin-prick test results. The remaining 42 patients were consuming dairy products without any problems. Two of these 42 patients reported having CMA in infancy (patient number 21 and 22).

The mean age of the groups with and without anaphylaxis history were similar (p=0.136). There were no significant relationship between the presence of anaphylaxis history and sex, family history and the delay time in diagnosis (p=0.586, p=0.916, p=0.175, respectively). Although all the patients with parental consanguinity had anaphylaxis history, 69.6% of those who did not have parental consanguinity had anaphylactic history; however, this was not at a statistically significant level (p=0.146). No significant relationships were detected between the presence of anaphylaxis history and the presence of additional allergic disease, tick bite history, and mutton or beef sensitivity in the skin test (p=0.739, p=0.689, p=0.537, respectively). No significant relationships were detected between the presence of anaphylaxis history and sIgE, total IgE, eosinophil count, and vitamin D level (p=1.00, p=0.48, p=0.34, p=0.66, respectively).

The presence of a tick bite history did not affect history of early-anaphylaxis or delayed-anaphylaxis (p=1.00). No relationships were detected between early or delayed-anaphylaxis history and age, and sensitivity of mutton or beef in prick-to-prick test (p=1.00, p=0.580, respectively). Although the number of children with early-anaphylaxis history between 0-6 years of age was 1.5 times higher than those who had early-anaphylaxis history at the age of 6-18 years, the difference was not significant (p=0.06).

There was no significant relationship between the presence of additional allergic diseases and the sensitivity of mutton or beef, and it was not associated with vitamin D levels (p=0.397, p=0.184, respectively). There was no significant relationship between mutton sensitivity in the skin test and mutton sIgE (p=0.442). Similarly, no relationship was determined between beef sensitivity in skin test and beef sIgE (p=0.157).

The results of the patients that underwent the OFC are given in Table III. OFC was positive in 6 of the 9 patients. Three of 6 patients had developed anaphylaxis. Patient 36 had described moderate anaphylaxis after red meat consumption in his clinical history. He also had a history of vespula venom anaphylaxis. His sister had red meat allergy. He was applied the prick-to-prick test with beef. Since severe early-anaphylaxis developed after the skin test, the OFC test was not performed. The patient was advised to avoid all red meats. Adrenaline autoinjector was prescribed. Patient 1, who had only urticaria symptoms after eating red meat, did not develop a reaction after OFC with beef.

Table I. Characteristics of children and adults reporting reactions to red meat and sensitizied to red meat.

Characteristics	Total n= 43 (%)	Children n= 37 (86%)	Adult n= 6 (4%)
Age, y, median (min-max)	12 (2-37)	10 (2-17)	28 (22-37)
Sex, male (%)	22 (51.2%)	20 (54.1%)	2 (33.3%)
Clinical history, symptom/sign			
Urticaria or urticaria and angioedema	43 (100)	37 (100)	6 (100)
Respiratory (nasal congestion, cough, dyspnea)	24 (55)	19 (51)	5 (83)
Heart (tachycardia, bradycardia, collapse)	4 (9)	3 (8)	1 (16)
GIS (vomiting, diarrhea)	8 (18)	6 (16)	2 (33)
Anaphylaxis	29 (67)	24 (64)	5 (83)
Early (0-2 hours)	23 (53)	19 (51)	4 (66)
Delayed (2-6 hours)	6 (13)	5 (13)	1 (16)
Age of onset, median (min-max), year	5 (1-20)	4 (1-14)	7.5 (5-20)
Delay time in diagnosis, median (min-max), year	4 (0-28)	4 (0-15)	23 (4-28)
Timing of the symptoms in the clinical history			
0-2 hours	34 (79)	29 (78)	5 (83)
>2 hours	9 (21)	8 (22)	1 (16)
Family history	21 (48)	15 (40)	6 (100)
Parental consanguinity	8 (18)	7 (18)	1 (16)
City of residence			
Ordu-Giresun	38 (88)	32 (86)	6 (100)
Other cities	5 (11)	5 (13)	0 (0)
Comorbid allergic disease			
Asthma or allergic rhinitis	23 (53)	19 (51)	4 (66)
Atopic dermatitis or chronic spontan urticaria	9 (20)	8 (21)	1 (16)
Vespula or bee venom allergy	3 (7)	1 (2)	2 (33)
Cow's milk protein allergy	1 (2)	1 (2)	0 (0)
No	16 (37)	14 (37)	2 (33)
History of tick exposure	18 (41)	14 (37)	4 (66)
Positivity in skin-prick test			
Commercial antigen solution	3 (7)	3 (8)	0 (0)
Prick-to-prick	43 (100)	37 (100)	6 (100)
Beef only	5 (11)	4 (11)	1 (16)
Mutton only	6 (13)	5 (14)	1 (16)
Beef and mutton	32 (76)	28 (75)	4 (68)
Total IgE (IU/mL), median (min-max)	355 (20-3550)	327 (20-3550)	431 (33-1100)
Specific immunoglobulin E (kIU/L),			
Mutton, median (min-max), n=18	0 (0-7.25) (n=18)	0 (0-7.25) (n=17)	0 (n=1)
Beef, median (min-max), n=18	0.21 (0-31.2) (n=18)	0.23 (0-31.2) (n=17)	0.2 (n=1)
Eosinophil count (cell/mm^3), median (min-max)	210 (40-990)	210 (40-990)	170 (40-320)
Hemoglobin (gr/L), mean ± standart error	12.9 ± 0.20	12.8 ± 0.17	13.2 ± 0.99
25 OH-D_3 (mcg/L), mean ± standart error	13.3 ± 1.1 (n=32)	13.1 ± 1.2 (n=27)	14.5 ± 3.4 (n=5)

Table II. Detailed analysis of the patients.

Patient no	Age / Sex (y/M,F)	Symptom / sign	Age of onset	Timing (minute)	History of tick	Additional allergic disease	Prick-to-prick test (mm x mm)				sIgE beef kIU/L	sIgE mutton kIU/L	Blood group	25 OH-D_3 mcg/L
							Raw beef	Raw mutton	Cooked Beef/ mutton	Histamin				
1	9, M	UA	8	40	no	no	3x7	4x9	negative	5x6	0.18	7.25	Orh+	20.62
2	7, M	UA	3	60		no	4x4	3x3		5x4				
3	17, M	U	12	150	yes	no	4x4	4x6	4x4	4x4	0.00	0.00	Orh+	
4	7, M	UA, R, H	4	150	no	yes	4x4	3x3	negative	4x4				5.62
5	15, M	U, G	10	30	yes	no	2x2	5x6	3x7	3x3	0.15	0.00	Orh+	2.44
6	31, F	U,R	6	60	yes	yes	negative	3x3	3x4	5x5			Arh-	
7	8, M	U, G	4	5	no	no	5x5	4x4		5x5				
8	16, F	UA	4	15	no	yes	4x6	7x12	3x3	3x3	0.15	0.00		3.13
9	15, F	UA, R, G	7	5	yes	yes	7x7	5x6		4x4			Orh+	8.2
10	14, F	UA, R	3	60	yes	yes	4x7	3x3	negative	5x6	0.18	0.00	Orh+	17.32
11	16, M	U	9	60		yes	5x3	7x4	7x4	6x5				
12	6, M	UA, R	2	5	yes	yes	4x7	3x4		5x5			Orh+	4.1
13	11, M	UA, R	3	10	yes	yes	4x7	4x6	negative	4x6	20.00	0.00		21.43
14	4, M	U, R	2	15	no	yes	6x8		5x6	5x5	0.27	0.00	Orh+	15.1
15	7, F	UA	5	30	no	yes	3x3	3x3		5x6	0.23	0.00	Orh+	19.5
16	16, F	U, R	8	20		yes	3x3	4x4	8x8	6x6				18.31
17	10, M	UA, R	10	240	yes	no	2x2	3x5	3x4	4x6	31.20			8.85
18	24, F	U	20	30	yes	no	negative	4x6		4x4	0.20	0.00		8.75
19	16, F	U, G	14	30	no	no	7x9	7x9	3x3	4x4	0.74	0.29		15.86
20	12, M	U, R	5	60	yes	yes	2x2	3x3	3x3	5x5		0.00		18.62
21	6, F	UA, R, G	2	5	yes	yes	3x3	4x4		4x4				
22	9, F	U	5	30	yes	yes	4x4	3x6	3x3	4x4			Arh+	
23	10, F	UA, R	2	60		no	5x4	negative	3x2	6x5				
24	22, F	UA, R	17	240		yes	3x3	2x5	negative	4x4				4.5
25	17, M	U, R	9	20	yes	no	4x7	4x7	4x4	4x4				9.8

M: male, F: female, U: urticaria, UA: urticaria and angiodema, R: upper or lower respiratory symptom, G: gastrointestinal symptom, H: cardiovascular symptom, sIgE: specific immunoglobulin E

Table II. Continued.

Patient no	Age / Sex (y/M,F)	Symptom / sign	Age of onset	Timing (minute)	History of tick	Additional allergic disease	Prick-to-prick test (mm x mm)				sIgE beef kIU/L	sIgE mutton kIU/L	Blood group	25 OH-D_3 mcg/L
							Raw beef	Raw mutton	Cooked Beef/ mutton	Histamin				
26	13, F	U, G	5	5		no	3x6	3x5		3x5				
27	16, F	U, R	4	10	no	yes	6x5	4x4		5x5				20.6
28	14, F	UA, R	13	180	yes	no	3x4	4x5	8x9	5x8	0.00	0.00	Orh+	4.2
29	2, M	U	1	720	no	yes	15x17	11x15	9x10	5x7	1.52	4.62	Arh-	20.6
30	4, M	UA	2	240		yes	3x5	negative	3x3	6x6	0.23	0.00		14.3
31	4, F	UA	3	30	yes	yes	negative	4x4		4x6	0.00	0.00		9.78
32	8, M	U, R	4	30	no	yes	3x3	4x6	2x2	4x6	0.47	0.16		16.26
33	13, F	UA, R, H	10	15	yes	yes	4x4	3x3	4x5	4x4				5.5
34	16, M	UA	1	120		yes	3x3	3x3	3x4	4x4			Arh+	10.4
35	6, M	UA, R	4	30		yok	3x3	4x5		4x6				14.38
36	28, M	UA, R, G, H	5	30	yes	yes	4x4	5x5		6x6				23.6
37	14, F	U	9	60				4x4	4x4	4x4				
38	37, M	UA, R	9	30	yes	no	3x4	4x4	6x7	5x8				16.7
39	12, M	UA, R	5	60		no	3x3	2x2	2x2	4x5	0.00	0.00		8.9
40	28, F	U, R, G	5	10		yes	3x3	4x6		4x4				19.3
41	9, F	U, R, H	8	60	no	yes	4x11	6x11	4x6	5x5	0.23	0.00	Arh+	15.31
42	3, M	U	2	10		yes	5x5	3x3		5x5				
43	4, F	U	3	120	no	yes	negative	4x4	3x3	3x3				27.08

M: male, F: female, U: urticaria, UA: urticaria and angiodema, R: upper or lower respiratory symptom, G: gastrointestinal symptom, H: cardiovascular symptom, sIgE: specific immunoglobulin E

Table III. Characteristics of the patients undergoing oral food challenge (OFC) test (n=9) and developed anaphylaxis during the prick-to-prick test (n=1).

Patient no	Age/ Sex (y / M, F)	Clinical history	Positivity in sensitivity tests		Open OFC test		Follow-up after open OFC test
			Prick test	sIgE	OFC with beef	OFC with mutton	
1	9, M	Urticaria and angiodema	Beef and mutton	Beef and mutton	Negative	Delayed mild anaphylaxis 2nd test: Diffuse urticaria	Eating beef
3	17, M	Urticaria	Beef and mutton	Negative	Negative	Not done	Eating beef
4	7, M	Delayed mild anaphylaxis	Beef and mutton	ND	Delayed moderate anaphylaxis	Not done	Doesn't eat beef/ mutton
5	15, M	Delayed moderate anaphylaxis	Mutton	Beef	Delayed moderate anaphylaxis	Not done	Doesn't eat beef/ mutton
14	4, M	Early moderate anaphylaxis	Beef	Beef	Negative	Not done	Eating beef
28	14, F	Delayed moderate anaphylaxis	Beef and mutton	Negative	Urticaria	Not done	Doesn't eat beef/ mutton
29	2, M	Urticaria	Beef and mutton	Beef and mutton	Not done	Negative	Eating mutton
36 *	28, M	Early moderate anaphylaxis	Beef and mutton	Not done	Not done	Not done	Doesn't eat beef/ mutton
39	12, M	Early moderate anaphylaxis	Beef	Negative	Not done	Urticaria	Doesn't eat beef/ mutton
41	9, F	Early moderate anaphylaxis	Beef and mutton	Beef	Not done	Urticaria	Doesn't eat beef/ mutton

* His sister had red meat allergy. He also had a history of vespula venom anaphylaxis. Since the severe anaphylaxis developed during the prick-to-prick test, the OFC test was not performed.

Then OFC was conducted with mutton at a different time. Six hours later, the patient had widespread urticaria, nasal congestion, runny nose, and sneezing. Anaphylaxis training was provided to the patient, and adrenaline autoinjector was prescribed. The patient said that he wanted to eat mutton and applied the second OFC. Then, the second OFC was done at a different time. Widespread urticaria developed four hours after OFC. There were no other system involvements. However, he was told that the risk of anaphylaxis continued, and to avoid mutton.

All the patients who underwent the OFC test were questioned by phone twice within one year. It was found that the 4 patients who had negative results in the OFC test could eat the recommended red meat. Also, some patients who described mild anaphylaxis in their clinical history (i.e. patients 15, 35, 40) reported that they could eat red meat with antihistamines without any problems. Some patients (i.e. patient 3 and 19) reported that they had symptoms when they consumed large amounts of red meat. All patients who underwent the OFC test or reported anaphylaxis history were given anaphylaxis training, and adrenaline autoinjectors were prescribed.

Discussion

In this retrospective study, which included the highest number of red meat allergy patients in Turkey, the clinical characteristics of our patients were summarized. Most of our patients had an anaphylaxis history. The clinical and laboratory findings were very heterogeneous. These findings are not as helpful as the OFC test for diagnosis. The positivity and anaphylaxis rates in OFC tests were higher than other food allergies.[12,13,15] Also, the mutton and beef sensitivity in the prick-to-prick tests were evaluated separately in this study for the first time. In some red meat allergy patients, mutton or beef may be recommended as an alternative to red meat after OFC test.

Three types of diseases are reported for red meat allergy, which are primarily beef allergy, pork-cat syndrome and alpha-gal syndrome.[8] Although red meat allergy is rarely reported among other food allergies in the world, the case reports with alpha-gal syndrome have increased in recent years.[20-22] Recently identified alpha-gal syndrome is the appearance of anaphylaxis findings and symptoms 4-6 hours after red meat consumption with galactose-alpha-1.3-galactose (alpha-gal) sIgE, caused by the bite of some tick species,[19,23] which was included in the literature as the cause of delayed-anaphylaxis.[19] Alpha-gal sIgE was not measured in our case series. Our data were retrospectively collected. Alpha-gal syndrome is usually reported in adults,[8] and is reported very rarely in Turkey.[21,22] It should also be considered that alpha-gal sIgE might be elevated without red meat allergy, due to parasitic helminth infection, tick allergy, or a monoclonal antibody (i.e. cetuximab) allergy.[23] A total of 13% of our case series had reported delayed-anaphylaxis. We cannot be sure about the tick bite history especially in children. Awareness of tick bite has increased in our region due to Crimean Congo hemorrhagic fever disease. We questioned the tick bite history in detail from their parents. We did not find a relationship between tick bite history and presence of delayed-anaphylaxis history. No differences were reported even in individuals with alpha-gal syndrome in terms of alpha-gal sIgE among those who reported early and delayed-anaphylaxis.[20] Since there is no pork consumption in Turkey, pork-cat syndrome has not been reported so far.

Our patients with red meat allergy are primary beef allergies according to prick-to-prick tests. Likewise, another study in our region reported that children with red meat allergies had primarily beef allergies.[16] They conducted this study in the Eastern Black Sea region in Turkey, which included 4932 school children, the prevalence of primary beef allergy was reported to be 2.6% based on a questionnaire, and 0.3% based on the OFC test.[16] Most of our patients were from the Eastern Black Sea region

(Ordu-Giresun). In this prevalence study, reactions in the first 2 hours were recorded, whereas reactions between 2-6 hours were not recorded. Symptoms and signs may have developed after 2-6 hours in some of our patients undergoing OFC. For this reason, the expected red meat allergy prevalence in our region might be higher. In another prevalence study on challenge-proven food allergy in school children in the Eastern Black Sea Region, the most commonly detected food allergy was beef allergy, and then, milk, cocoa and egg allergies.[12] In another multi-centric prevalence study on food allergy in children in Turkey, it was reported that beef allergy was the second most frequent food allergy.[13] This information is different from studies conducted in western populations. Red meat allergy prevalence may vary according to geographical regions.[7,20] Red meat allergy awareness should be increased among pediatricians, at least in Turkey . In our patients, the age of onset of red meat allergy was approximately 4 years of age. This result was similar to the literature data.[14,16,24] The delay in diagnosis was 4 years. As reported previously, these patients were diagnosed late.[17]

Skin manifestations were reported in 66-93% of patients with red meat allergy.[16,20] In our case series, all patients had skin symptoms. Since our hospital is a tertiary hospital, not all patients might have been referred to us. We did not have any patients with only gastrointestinal tract (GIS) symptoms. Presentation with only GIS symptoms is reported rarely in the literature.[20] Food intolerance or poisoning may be considered in those who report complaints 4-6 hours after food intake. However, if the symptom repeats in the same person, or if only one of the few people who have taken the same food has symptoms, a food allergy should be considered. Food allergy is usually not considered in chronic spontaneous urticaria (CSU).[25] However, it was reported that we should consider red meat allergy disease in differential diagnosis in recurrent urticaria or in CSU.[16,20] Some of our patients had recurrent urticaria. Clinical variability may occur in patients with red meat allergy.[23,26] In these patients, clinical variability may not be due to potential cofactors (i.e. nonsteroid intake, alcohol, exercise) for food allergies.[23,26] In the same person, exposures to red meat at different times may cause acute urticaria or anaphylaxis, or may not cause any symptoms.[8,23,26] Clinical variability may depend on the amount of meat consumed, the allergen contained in the meat, and industrial processing.[8,26] But, the exact cause of this is not yet known. For this reason, there is no single diagnostic algorithm for red meat allergy.[8] Clinical history should be questioned carefully in these patients. Although it was not previously reported in the literature, we applied the prick-to-prick test with both female and male red meat to those who had contradictory clinical histories. Different sensitivity results in prick tests were detected in some patients. For this reason, it may be considered that one of the reasons for the variability in the history and laboratory findings might be due to the male/female status of the red meat.

The most important result of this case series is that both positivity and anaphylaxis rates in the OFC test were higher than in other food allergies.[12,13] This finding was also detected in beef allergic children in DBPCOFC test and in alpha-gal syndrome patients in open OFC test.[16,27] Also, it was reported that red meat allergies should be considered in idiopathic anaphylaxis patients.[17,18] We did not find a relationship between anaphylaxis history and demographic information, clinical history and skin tests of the patients. There is no study on predicting anaphylaxis in these patients. Some of our patients said that they could eat red meat by using antihistamines even if they had described mild anaphylaxis history. Similar notification is available in the literature.[28] However, precautions should be taken for anaphylaxis, due to clinical variability.

Skin test and sIgE are recommended as sensitivity tests in patients with suspected red meat allergy.[8] Some researchers report that sIgE is more sensitive than prick tests.[14,16] However, in our challenge-proven patients, mutton and beef

sIgE were negative in some. In our series, very few patients had sensitivity with commercial allergen in the skin test. In these patients, skin testing is recommended with fresh meat rather than commercial allergens.[19] Skin test and sIgE can be negative in some challenge-proven red meat allergy patients.[16] Furthermore, beef sensitivity tests may be negative in some patients who have had anaphylaxis with beef.[12] In our series, mutton sIgE was negative in 2 patients who had mutton allergy in the OFC test. It was reported that mutton sIgE is less sensitive than beef sIgE.[29] There is very heterogeneity in laboratory results in red meat allergy patients.[16,20,29] In the present study, no correlation was detected between the skin test and sIgE both for beef and mutton. Although red meat is not essential for a diet, it is more important for children and adolescents than adults.[7] Therefore, we wanted to recommend alternative red meat as mutton or beef for red meat allergic children. Furthermore, it is difficult for children to avoid allergens to which they are allergic.[3] We also performed the skin test and OFC test for beef and mutton separately. Some of our patients were able to eat alternative red meat without any problems after the OFC test. There is no study suggesting another red meat as an alternative in these patients.[8]

Most of our patients had other allergic diseases, the most common of which were respiratory allergies. It was reported that allergic diseases may be common in primary beef allergy patients.[8] In our case series, a patient with vespula venom allergy developed severe anaphylaxis during the prick-to-prick test. For this reason, if a patient with vespula venom allergy is performed a skin test or OFC test for red meat allergy research, extreme caution should be exercised for anaphylaxis. We could not measure the tryptase level in the patient to investigate the mast cell disorder. Recently, a combination of red meat allergy and venom allergy has been reported.[30] However, there is no clear explanation yet about the reason for this combination. It was reported previously that milk allergy was common in patients with beef allergy.[31] However, cow's milk allergy in our pediatric patients was not more common than in the general population. This finding is consistent with other reports in Turkey.[14,16] The rate of parental consanguinity in our patients was similar to the overall rate reported in Turkey. Half of our patients had a family history of allergies. There was a similar finding in another study in Turkey.[16] This finding suggests that polygenic and environmental factors may also be effective in red meat allergy, as in other allergic diseases.

Vitamin D levels of red meat allergy patients have never been reported. Vitamin D level and effect in allergic diseases continues to be investigated.[32,33] The mean vitamin D level of our pediatric patients was 13.1 mcg/L. Since this study was retrospective, we did not compare it with concurrent controls. However, the mean vitamin D level previously reported in healthy children of similar age and in the same geographic region was 16.9 mcg/L.[32] Low vitamin D levels compared to healthy children may be an issue that needs to be investigated prospectively. Our pediatric patients were not anemic despite avoiding eating red meat for a long time. They were not taking any iron replacement. This issue was not addressed in previous reports. We could not explain this unexpected finding. Unfortunately, we did not question the daily diet of the patients. It can be thought that iron sources other than red meat in the diet may be sufficient to prevent anemia when taken enough. Patients with red meat allergy due to alpha-gal syndrome are reported to have a B-negative blood group.[20,34] In our series, 14 patients tested for blood types had B-negative blood type. There are no reports of blood group of individuals with primary beef allergy in the literature. The antigens responsible for primary beef allergy (bovine serum albumin, immunoglobulin, myosin light chain kinase, parvalbumin, enolase, aldolase) should be compared biochemically with the blood group antigens.[8]

It was reported that the basophile activation test may be an alternative to OFC test in

patients with red meat allergy due to alpha-gal syndrome.[35] No study has been performed on this issue in patients with primary beef allergies. The most important limitation of our report was that the DBPCOFC test was not conducted. DBPCOFC is time-consuming and difficult to apply in a clinical setting. However, open OFC test can be used reliably in daily allergy practice in food allergies.[36] It was reported in the literature that the results of the open OFC test in red meat allergies were compatible with clinical and laboratory findings.[14,27] The second important limitation was that alpha-gal sIgE was not measured. Another limitation was that some allergic diseases reported to be associated in patients with red meat allergy (such as cow's milk allergy, cat dander, pork meat) were not investigated.

As a result, clinical history and sensitivity tests are important in patients with red meat allergy, as in all food allergies. However, they are not enough in red meat allergy. There are clinical and laboratory variability in these patients. This complicates the diagnostic algorithm. OFC testing is useful not only to confirm the diagnosis but also to suggest an alternative to red meat. More severe findings may occur in the OFC test compared to the clinical history. The risk of anaphylaxis in these patients is higher than in patients with other food allergies. Anaphylaxis does not mean that it will not develop in subsequent reactions, even if it is only a symptom of urticaria. Red meat allergy patients can respond differently to each repeated OFC test, therefore, should be followed even after negative OFC test. Anaphylaxis training and adrenaline autoinjector should be given to those who develop anaphylaxis in the OFC test and to red meat sensitive patients who report anaphylaxis in their clinical history.

Acknowlegement

We would like to thank the pediatric nurse Satı Aykut for help in the prick-to-prick test.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: study conception and design: ŞK, FÖ; data collection: ŞK, GH, ŞİKK; analysis and interpretation of results: ŞK, GH, FÖ, RS; draft manuscript preparation: ŞK, RS, FÖ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ondokuz Mayis University Ethics Committee of our institution (KAEK 2017/55).

Source of funding

There is no sources of financial assistance.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Abrams EM, Sicherer SH. Diagnosis and management of food allergy. CMAJ 2016; 188: 1087-1093.
2. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol 2018; 141: 41-58.
3. Mehta H, Groetch M, Wang J. Growth and nutritional concerns in children with food allergy. Curr Opin Allergy Clin Immunol 2013; 13: 275-279.
4. Keet CA, Savage JH, Seopaul S, Peng RD, Wood RA, Matsui EC. Temporal trends and racial/ethnic disparity in self-reported pediatric food allergy in the United States. Ann Allergy Asthma Immunol 2014; 112: 222-229.e3.
5. Lieberman P, Nicklas RA, Randolph C, et al. Anaphylaxis-a practice parameter update 2015. Ann Allergy Asthma Immunol 2015; 115: 341-384.
6. Steinke M, Fiocchi A, Kirchlechner V, et al; REDALL study consortium. Percevied food allergy in children in 10 European nations. A randomised telephone survey. Int Arch Allergy Immunol 2007; 143: 290-295.

7. Restani P, Ballabio C, Tripodi S, Fiocchi A. Meat allergy. Curr Opin Allergy Clin Immunol 2009; 9: 265-269.

8. Wilson JM, Platts-Mills TAE. Red meat allergy in children and adults. Curr Opin Allergy Clin Immunol 2019; 19: 229-235.

9. Doğruel D, Bingöl G, Altıntaş DU, Yılmaz M, Güneşer Kendirli S. Clinical features of food allergy during the 1st year of life: the ADAPAR birth cohort study. Int Arch Allergy Immunol 2016; 169: 171-180.

10. Barlık F, Güner ŞN, Barlık M, Söğüt A, Sancak R. Prevalence of food allergy in nursery and kindergarten children in Samsun. Turk Arch Pediatr 2013; 48: 288-293.

11. Kaya A, Erkoçoğlu M, Civelek E, Çakır B, Kocabaş CN. Prevalence of confirmed IgE-mediated food allergy among adolescents in Turkey. Pediatr Allergy Immunol 2013; 24: 456-462.

12. Orhan F, Karakas T, Cakir M, Aksoy A, Baki A, Gedik Y. Prevalence of immunoglobulin E-mediated food allergy in 6-9-year-old urban schoolchildren in the eastern Black Sea region of Turkey. Clin Exp Allergy 2009; 39: 1027-1035.

13. Mustafayev R, Civelek E, Orhan F, Yüksel H, Boz AB, Sekerel BE. Similar prevalence, different spectrum: IgE-mediated food allergy among Turkish adolescents. Allergol Immunopathol (Madr) 2013; 41: 387-396.

14. Orhan F, Sekerel BE. Beef allergy: a review of 12 cases. Allergy 2003; 58: 127-131.

15. Orhan F, Canitez Y, Bakirtas A, et al. Anaphylaxis in Turkish children: a multi-centre, retrospective, case study. Clin Exp Allergy 2011; 41: 1767-1776.

16. Topçu ZİK, Kaklıkkaya N, Baki A, Orhan F. Characteristics of beef allergy in schoolchildren in Turkey. Allergy Asthma Proc 2018; 39: 59-65.

17. Flaherty MG, Kaplan SJ, Jerath MR. Diagnosis of life-threatening alpha-gal food allergy appears to be patient driven. J Prim Care Community Health 2017; 8: 345-348.

18. Pattanaik D, Lieberman P, Lieberman J, Pongdee T, Keene AT. The changing face of anaphylaxis in adults and adolescents. Ann Allergy Asthma Immunol 2018; 121: 594-597.

19. Commins SP, Satinover SM, Hosen J, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. J Allergy Clin Immunol 2009; 123: 426-433.

20. Wilson JM, Schuyler AJ, Workman L, et al. Investigation into the α-Gal syndrome: characteristics of 261 children and adults reporting red meat allergy. J Allergy Clin Immunol Pract 2019; 7: 2348-2358.e4.

21. Unal D, Coskun R, Demir S, Gelincik A, Colakoglu B, Buyukozturk S. Successful beef desensitization in 2 adult patients with a delayed-type reaction to red meat. J Allergy Clin Immunol Pract 2017; 5: 502-503.

22. Keleş Ş, Gündüz M. Alpha gal specific IgE positivity due to tick bites and red meat allergy: the first case report in Turkey. Turk J Pediatr 2019; 61: 615-617.

23. Steinke JW, Platts-Mills TA, Commins SP. The alpha-gal story: lessons learned from connecting the dots. J Allergy Clin Immunol 2015; 135: 589-596.

24. Fiocchi A, Restani P, Riva E. Beef allergy in children. Nutrition 2000; 16: 454-457.

25. Zuberbier T, Bernstein JA. A comparison of the United States and International perspective on chronic urticaria guidelines. J Allergy Clin Immunol Pract 2018; 6: 1144-1151.

26. Wilson JM, Platts-Mills TAE. Meat allergy and allergens. Mol Immunol 2018; 100: 107-112.

27. Commins SP, James HR, Stevens W, et al. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. J Allergy Clin Immunol 2014; 134: 108-115.

28. Heber G, Abraham S, Bauer A. Urticaria and angioedema after consumption of red meat. J Dtsch Dermatol Ges 2013; 11: 673-674.

29. Brestoff JR, Zaydman MA, Scott MG, Gronowski AM. Diagnosis of red meat allergy with antigen-specific IgE tests in serum. J Allergy Clin Immunol 2017; 140: 608-610.e5.

30. Fischer J, Huynh HN, Hebsaker J, Forchhammer S, Yazdi AS. Prevalence and impact of type I sensitization to alpha-gal in patients consulting an allergy unit. Int Arch Allergy Immunol 2020; 181: 119-127.

31. Martelli A, De Chiara A, Corvo M, Restani P, Fiocchi A. Beef allergy in children with cow's milk allergy; cow's milk allergy in children with beef allergy. Ann Allergy Asthma Immunol 2002; 89(6 Suppl 1): 38-43.

32. Kutluğ S, Kılıç M, Bilgici B, Paksu Ş, Yıldıran A, Sancak R. An evaluation of vitamin D levels in children with seasonal allergic rhinitis during pollen season. Pediatr Allergy Immunol 2017; 28: 446-451.

33. Yepes-Nuñez JJ, Brożek JL, Fiocchi A, et al. Vitamin D supplementation in primary allergy prevention: systematic review of randomized and non-randomized studies. Allerg 2018; 73: 37-49.

34. Hamsten C, Tran TA, Starkhammar M, et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. J Allergy Clin Immunol 2013; 132: 1431-1434.

35. Mehlich J, Fischer J, Hilger C, et al. The basophil activation test differentiates between patients with alpha-gal syndrome and asymptomatic alpha-gal sensitization. J Allergy Clin Immunol 2019; 143: 182-189.

36. Lieberman JA, Cox AL, Vitale M, Sampson HA. Outcomes of office-based, open food challenges in the management of food allergy. J Allergy Clin Immunol 2011; 128: 1120-1122.

The Turkish Journal of Pediatrics 2021; 63: 846-854
https://doi.org/10.24953/turkjped.2021.05.012

Original Article

Motor functions, quality of life and maternal anxiety and depression in children with cerebral palsy of different intelligence levels

Elif Akçay[1], Yaşar Tanır[2], Serap Tıraş Teber[3], Birim Günay Kılıç[4]

[1]Department of Child and Adolescent Psychiatry, Ankara City Hospital, Ankara, Turkey [2]Department of Child and Adolescent Psychiatry, İstanbul University Faculty of Medicine, İstanbul; Departments of [3]Pediatric Neurology and [4]Child and Adolescent Psychiatry, Ankara University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Cerebral palsy (CP) is the most common motor disability in childhood. In addition to motor impairment, it is frequently accompanied by intellectual disability (ID). We aimed to investigate the associations between motor functions, quality of life (QoL) and maternal psychopathology in children with CP of different intelligence levels.

Methods. In total, 37 children and adolescents (16 females and 21 males) between 4 and 18 years of age diagnosed with CP were recruited from a Pediatric Neurology Outpatient Clinic. Gross Motor Function Classification System (GMFCS) and Bimanual Fine Motor Function (BFMF) were used for the children's motor functions assessment. Quality of life was determined by the caregivers with Pediatric Quality Of Life Inventory-Parent version (PedsQL-P). Maternal anxiety and depression levels were assessed using Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI).

Results. Moderate-severe ID (n=19)(13.5%, 37.8%) and normal IQ-mild ID (n=18) (32.4 %,16.2%) groups were evaluated in this study. GMFCS level 2 was more frequent in both groups. The majority of the severe-moderate ID group was at BFMF level 4, while the normal IQ-mild ID group was at BFMF level 2. PedsQL-P scores of children with CP, maternal BAI scores, and maternal BDI scores did not differ between the two groups ($p>0.05$). Psychosocial PedsQL scores had a moderate negative correlation with the maternal BAI scores ($r=-0.41$, $p<0.05$). There was also a moderate positive correlations between the ages of children and maternal BDI scores ($r=0.34$, $p<0.05$).

Conclusions. Our results demonstrated that maternal anxiety was correlated with psychosocial QoL in children with CP. Maternal depression scores increasing with the ages of the children with CP may also indicate the social support needs for mothers with children of chronic diseases. Further studies may reveal the associations with other biopsychosocial factors in children with CP of different intelligence levels by using longitudinal study designs with larger sample sizes.

Key words: anxiety, depression, cerebral palsy, child, quality of life.

Cerebral palsy (CP) is the most common motor disability in childhood.[1] Cerebral palsy (CP) is described as a nonprogressive neuromotor disorder that mainly affects the development of movement, muscle tone, and posture.[2] CP umbrella refers to very heterogeneous clinical presentations in children. In addition to motor impairments, there may be impairments in cognition, communication, hearing, vision, behavior, and epilepsy that may worsen the motor impairment, function, and quality of life (QoL).[3,4]

The most common comorbidities according to the Surveillance of Cerebral Palsy in Europe (SCPE) are speech/language impairments

✉ Elif Akçay
elifbayram07@gmail.com

Received 13th January 2021, revised 13th February 2021, accepted 24th February 2021.

(71%), severe intellectual disability (ID) (62%), epilepsy (39%), and visual impairment (22%).[3] CP is accompanied by different levels of ID.[5] Gabis and her colleagues' study[6] reported that 22.5% of children with CP had normal intelligence ,and 41.3% had moderate or severe ID. Moreover, it was suggested that more severe motor function impairments are associated with higher ID.[6] Bertoncelli et al.[7] reported that poor motor skills and epilepsy were associated with severe ID.

Severe motor function impairment in children with CP has been found to be more related to poor QoL in terms of physical health and autonomy. Additionally, children with low intelligence quotient (IQ) were found to have a higher risk of poor QoL in terms of social support.[8] Mobility performances relate to gross motor function levels; however, daily activities mostly depend on children and adolescents' intellectual ability with CP.[9] Caregiving a child who has limited self-mobility is highly psychologically and physically demanding.[10,11] Parents must spend more time caring for children with CP who have limited self-mobility. Greater limited self-mobility in children with CP is associated with greater stress and depressive symptoms among their mothers.[12] Because mothers are mostly primary caregivers, and important team workers of the rehabilitation and care services of children with cerebral palsy, they suffer from many socio-emotional problems and report higher anxiety levels.[13,14] Mothers of children with CP have been found to show depressive symptoms and poor QoL, and it was reported that 30% of mothers having CP children showed symptoms of depression.[15] Also, the prevalence of depression was reported at 20% to 30% in mothers of children with ID.[16,17] The mothers of children with ID reported that they were socially isolated due to caregiving their children and neglected their own social needs. Moreover, caregivers often neglect to receive mental health support for their own self-care needs.[18,19]

Consequently, since about half of children with CP have ID[20], more information is needed about the areas of life for children with CP who also have ID. Quality of life assessment is increasingly used as a mechanism to gain insight into a child's life, identify positive or challenging areas of life, and evaluate interventions. Psychopathology of caregivers may affect the QoL of children by causing inadequate care. Parents with psychiatric problems may subjectively evaluate their children's functionality more negatively. Moreover, it has been reported that caregivers of children with poor QoL experience more psychiatric problems than caregivers of healthy children.[13,21] In this study, we aimed to investigate the parent-proxy QoL of children with CP according to the severity of intelligence levels. In addition to QoL, we examined depression and anxiety in mothers of children with CP to identify challenges in CP children with severe ID.

Material and Methods

Participants and Procedures

In total, 37 children and adolescents (16 females and 21 males) between 4 and 18 years of age diagnosed with CP were recruited from a Pediatric Neurology Outpatient Clinic between April 2018 and September 2018. Also, 37 primary caregivers (all mothers) of children and adolescents with CP were invited to participate in the study. The participants were assessed by a child neurologist for the diagnosis of CP, their mother's literacy status, and abilities to fill out the questionnaires. Participants who reported any genetic disorder, severe visual and auditory impairments, neurological disorders except for epilepsy (e.g., acute cranial trauma, brain tumors) were not included in the study.

Ethical approval was obtained from the Ankara University Medical School Research Ethics Committee (ID-No: 04-215-18). Informed written consent was obtained from parents, and written assent was obtained from children and adolescents.

After the evaluation of the inclusion and exclusion criteria, sociodemographic and clinical

assessments were performed. Age, gender, family income levels, gestational age, maternal age, paternal age, birth weight, prematurity, types of birth, and presence of epilepsy, receiving special education were recorded. The cerebral palsy type was determined as spastic (hemiplegic, diplegic, tetraplegic), dyskinetic (athetosis, dystonia), ataxic or mixed types according to the "Surveillance of European Cerebral Palsy Group".[22] Gross Motor Function Classification System (GMFCS) and Bimanual Fine Motor Function (BFMF) were used for motor function assessment of the children by the child neurologist. GMFCS consists of five levels; from Level I (the most independent motor function) to Level V (the most restricted voluntary control of movement and ability to maintain postures, the most dependent motor function).[23] BFMF is designed to evaluate the upper extremity performance in children's daily living activities. Manipulation and gripping ability are evaluated at five levels. Scoring of GMFCS and BFMF are mostly in parallel with each other.[24]

The children's intelligence levels were evaluated by the Turkish version of the Wechsler Intelligence Scale for Children-Revised (WISC-R), Stanford-Binet Intelligence Test, and Ankara Developmental Screening Inventory (ADSI). The children with cerebral palsy were divided into two groups according to their intelligence levels; (i) Normal Intelligence Quotient (IQ)-Mild Intellectual Disability (ID) and (i) Moderate-Severe ID. Quality of life of the sample was determined by their caregivers with the Pediatric QOL Inventory, Parent version (PedsQL-P). Maternal anxiety and depression levels were assessed by using Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI).

Questionnaires

Pediatric QOL Inventory-Parent version (PedsQL-P): The PedsQL-P was used to assess health-related quality of life (HRQOL) during childhood. It has two subscales, psychosocial and physical health, and a total score, higher PedsQL-P scores indicate better HRQL. The reliability and validity of the PedsQL-P has been conducted in Turkish children and adolescents.[25,26]

Beck Anxiety Inventory (BAI): The BAI is a self-report scale used to assess the severity of anxiety symptoms. It is a Likert-type scale that is scored between "0" and "3" and comprises 21 items. Higher BAI scores indicate higher anxiety levels. The reliability and validity of the BAI has been conducted in Turkish populations.[27]

Beck Depression Inventory (BDI): The BDI is a self-report inventory used to assess the severity of depression symptoms. The scale is composed of 21 items that are scored between "0" and "3". Higher BDI scores indicate higher depression levels. The reliability and validity of the BDI has been conducted in Turkish populations.[28]

Intelligence Tests

Wechsler Intelligence Scale for Children-Revised (WISC-R) is used for intelligence assessment of children between the ages of 6 and 16 years.[29] The reliability and validity of the Turkish version has been conducted in Turkish children.[30] The intelligence level of the child is classified as normal, borderline, mild, moderate, and severe with WISC-R scores. Stanford-Binet Intelligence Test was used to assess children between the ages of 2 and 16 years and adapted to Turkish by Uğurel-Şemin.[31] The intelligence level is determined according to the standard scores in this test. Ankara Developmental Screening Inventory (ADSI) was used for determining the developmental levels of young children in Turkey.[32] It was administered to caregivers of children who were unable to express themselves, could not communicate, and could not be applied the WISC-R, Stanford-Binet test due to their intellectual levels. The ADSI includes 154 questions which assess 5 domains (language, cognitive, fine motor function, gross motor function, sociality, and self-care abilities) asked to the caregivers and are combined with the clinical observation

of the child for determining their intellectual levels. The total scores of these subscales give the general intellectual-developmental levels which are determined according to normative data matched for age.

Statistical analysis

We used the Shapiro-Wilk test to analyze data that was not normally distributed. According to the normal distribution, descriptive statistics were presented as mean ± standard deviation or median (min-max). Count and percentages were used to describe categorical variables; Continuous clinical variables were analyzed using Independent t-tests or the Mann-Whitney U test according to their distribution characteristics in group comparisons. Differences in categorical variables between the groups were examined using Pearson's chi-squared and Fisher exact analysis. Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23.0, (IBM SPSS Statistics; New York, USA), and all statistical tests were two-tailed with the significance level set at α = .05. Spearman's correlation coefficient was calculated to investigate the association among the clinical measures.

Results

Thirty-seven participants were included in the study; 19 of them were children with moderate-severe ID (moderate: 13.5%, severe: 37.8%), 18 of them were children with normal IQ (32.4 %) or mild ID (16.2%). There were no differences in age, maternal age, paternal age, and family income levels between the two groups (p>0.05). Perinatal factors (gestational age, birth weight, prematurity) were not different in the groups; however, there were differences in types of birth, gender, and presence of epilepsy (p= 0.012, p= 0.012, p= 0.012, respectively). Children with CP received special education for learning needs (Normal IQ-mild ID group: 77.8%; Moderate-severe ID group: 73.7%), and for their motor disabilities (Normal IQ-mild ID group: 88.9%; Moderate-severe ID group: 84.2%).

The CP types examinations indicated that the frequency of the spastic type was highest in both groups, and most of these children were spastic bilateral hemiplegia (Normal IQ-mild ID group: 55.6%; Moderate-severe ID group: 47.4%). GMFCS level 2 was more frequent in both groups according to gross motor functions examination of the children. There were no differences in severity of gross motor function (level 1-3=non-severe; level 4-5=severe) between the two groups (p=0.197). The majority of the severe-moderate ID group was at BFMF level 4, while the normal IQ-mild ID group was at BFMF level 2. There was a significant difference in severity of bimanual fine motor function (level 1-3= non-severe; level 4-5= severe) between the two groups ($X2$ (1, N = 37) = 17.79, p <0.001). Sociodemographic and clinical characteristics of the sample are shown in Table I.

Total PedsQL-P scores, Psychosocial PedsQL-P scores, Physical PedsQL-P scores of children with CP did not differ between normal IQ-mild ID group and moderate-severe ID group (t(35) = -.809, p = .424; t(35) = .098, p = 0.922; t(35) = -.088, p = .930, respectively). There were no differences in maternal BAI scores (U = 151.00, z = −0.610, p = .558) and maternal BDI scores (U = 156.50, z = −0.7443, p = .663) between two groups. . Results of maternal psychopathology and quality of life scores between the two groups are shown in Table II.

The correlations among the perinatal factors, gross and fine motor functions, intelligence levels, maternal anxiety scores, maternal depression scores, and PedsQL scores were calculated. Psychosocial PedsQL scores had a moderate negative correlation with the maternal BAI scores. PedsQL scores did not correlate with the other variables included in the correlational analyses. There were also moderate positive correlations between the children's ages and maternal BDI scores. There were also moderate positive correlations between epilepsy and female gender, GMFCS levels, and high negative correlations among the intelligence levels and BFMF level scores. All of the bivariate correlations between the variables are presented in Table III.

Table I. Sociodemographic and clinical characteristics of the sample.

	Normal IQ-mild ID (n=18)	Moderate-severe ID (n=19)	*p*
Age (mean ±SD) (year)	9.88 ± 3.68	11.65± 4.92	0.226
Gender			0.012*
Male	14 (77.8%)	7 (36.8%)	
Female	4 (22.2%)	12 (63.2%)	
Maternal age (mean± SD)	37.74±7.58	39.68±6.33	0.404
Paternal age (mean± SD)	41.72±7.61	44.15±7.41	0.331
Family income			0.638
Low	8 (44.4%)	7 (36.8%)	
Middle-high	10 (55.6%)	12 (63.2%)	
Gestational age (weeks)	34.66±5.50	35.84±4.89	0.496
Birth weight (grams)	2435.55±1115.90	2644.73 ±952.26	0.543
Prematurity	10 (55.6%)	8 (42.1%)	0.413
Types of birth			0.012*
Vaginal birth	6 (33.3%)	9 (47.4%)	
C/S	12 (66.7%)	10 (52.6%)	
Epilepsy	4 (22.2%)	12 (63.2%)	0.012*
Special education for cognitive ability			0.538
Yes	14 (77.8%)	14 (73.7%)	
No	4 (22.2%)	5 (26.3%)	
Special education for motor ability			0.527
Yes	16 (88.9%)	16 (84.2%)	
No	2 (11.1%)	3 (15.8%)	
CP type			N/A
Spastic bilateral hemiplegia	10 (55.6%)	9 (47.4%)	
Spastic unilateral hemiplegia	5 (27.8%)	4 (21.1%)	
Dystonic	-	1 (5.3%)	
Ataxic	2 (11.1%)	2 (10.5%)	
Mixed	1 (5.6%)	3 (15.8%)	
GMFCS			N/A
Level 1	6 (33.3%)	2 (10.5%)	
Level 2	7 (38.9%)	8 (42.1%)	
Level 3	1 (5.6%)	1 (5.3%)	
Level 4	1 (5.6%)	3 (15.8%)	
Level 5	3 (16.7%)	5 (26.3%)	
BFMF			N/A
Level 1	8 (44.4%)	1 (5.3%)	
Level 2	3 (16.7%)	2 (10.5%)	
Level 3	6 (33.3%)	2 (10.5%)	
Level 4	1 (5.6%)	9 (47.4%)	
Level 5	-	5 (26.3%)	

CP: cerebral palsy, C/S: cesarian section, GMFM: gross motor function measure, BFMF: bimanual fine motor function, IQ: intelligence quotient, ID: intellectual disability, SD: standard deviations. N/A: not applicable. * p<0. 05.

Table II. Quality of life scores and maternal psychopathology scores of the sample.

	Normal IQ-mild ID (n=18)	Moderate-severe ID (n=19)	p
Psychosocial PedsQL-P (mean± SD)	58.17±21.12	57.51±19.30	0.922
Physical PedsQL-P (mean± SD)	56.67±22.20	57.36±24.78	0.930
Total PedsQL-P (mean± SD)	55.48±16.09	59.97±17.56	0.424
BAI (mother) (median)(range)	11.50 (1-38)	13.50 (0-47)	0.558
BDI (mother) (median)(range)	11.36 (2-35)	11.72 (0-25)	0.663

PedsQL-P: the pediatric quality of life inventory parent version, BAI: beck anxiety inventory, BDI: beck depression inventory, IQ: intelligence quotient, ID: intellectual disability, SD: standard deviations.

Table III. Intercorrelations among study variables.

	2	3	4	5	6	7	8	9	10	11	12	13	14
1.Age	-0.19	0.03	-0.08	-0.01	-0.06	0.07	-0.07	-0.08	0.29	0.34*	-0.18	0.12	-0.04
2.Gender		-0.07	-0.23	0.13	0.28	0.37*	-0.45**	0.33*	0.03	-0.14	0.20	0.23	0.25
3.Gestational Age			0.78**	-0.87**	-0.21	-0.00	0.05	-0.03	-0.11	0.03	0.25	0.21	0.19
4.Birth Weight				-0.74**	-0.27	0.04	-0.01	-0.18	-0.01	-0.01	0.09	-0.01	0.01
5.Prematurity					0.17	0.05	0.003	-0.08	0.03	0.003	-0.19	-0.05	-0.07
6.GMFCS						0.64**	-0.31	0.38*	0.11	0.13	-0.03	-0.02	0.04
7.BFMF							-0.72**	0.28	0.18	0.12	-0.13	-0.01	-0.05
8.Intelligence level								-0.38*	-0.30	-11	0.14	0.01	0.01
9.Epilepsy									-0.01	-0.09	0.00	0.05	0.16
10.BAI (mother)										0.65**	-0.41*	-0.03	-0.20
11.BDI (mother)											-0.32	0.11	-0.13
12.Psychosocial PedsQL-P												0.49**	0.72**
13.Physical PedsQL-P													0.81**
14.Total PedsQL-P													

GMFM: gross motor function measure, BFMF: bimanual fine motor function, BAI: beck anxiety inventory, BDI: beck depression inventory, PedsQL-P: the pediatric quality of life inventory. Parent version.* p<0. 05; ** p<0. 01.

Discussion

We compared parent-proxy quality of life, maternal anxiety, and depression between the normal IQ-mild ID group and moderate-severe ID group in children with CP. We hypothesized that parent-proxy QoL, maternal psychopathology would be significantly different between the two groups, and our findings did not support this. However, there were differences in gender, types of birth, and presence of epilepsy between the two groups. There was a significant difference in BFMF severity between the two groups; however, GMFCS severity was not different between the two groups. Children with CP at different intelligence levels have contradictory results of QoL studies. Our findings were in line with studies showing that QoL scores were similar for children with and without cognitive impairment.[33,34] However, another large study found that CP children with cognitive impairment have lower QoL scores in the social support domain, compared to CP children with normal intelligence.[8]

Four large studies reported that children with CP have poor QoL, compared to the general population in all domains. The scores in the physical, but not the psychosocial domains, correlated with the level of motor impairment.[8,33,35,36] In our study, QoL scores

did not correlate with the level of gross and bimanual fine motor impairment. Furthermore, there was no correlation between maternal depression and parent proxy-reported QoL. In previous studies examining the relationship between parental depression and proxy reports of QoL, parental depression was negatively correlated with parent proxy-reported QoL, contrary to our results.[37,38] In our study, maternal anxiety scores correlated with the psychosocial domain of PedsQL-P, this result is consistent with previous studies which showed a negative correlation between BAI scores of mothers and the PedsQL P scores of their CP children.[39,40] Physical domains of QoL scores correlate well with the severity of motor impairment, but the psychosocial scores are low regardless of the impairment severity.[41] This finding underlines the importance of factors other than the severity of CP that may affect QoL in these children. According to our study results, maternal anxiety may be one of the factors affecting the psychosocial domain of QoL. In some studies, family variables such as parenting style and family functioning were found to be important factors affecting the psychosocial aspects of QOL of children with CP.[42,43] In future studies, parenting style, and family functioning can be examined along with maternal psychological status. In our study, the ages of children positively correlated with maternal BDI scores. It may be related to the increase in the mother's caregiving burden and the decrease in coping capacity as the child grows with chronic diseases.[44]

Presence of epilepsy correlated with a lower level of intelligence in our study. In Gabis and her colleagues' study[6], epilepsy was found in disabled individuals (33%), and both presence of epilepsy and GMFCS levels predicted 29.9% of variance in the IQ score. In our study, intelligence was associated strongly with BFMF but not GMFCS. CP with epilepsy is more often accompanied by ID than CP without epilepsy. Correspondingly, CP with ID is associated with a high risk of developing epilepsy.[45,46]

Our study has some limitations. It is a cross-sectional study, and had a small sample size, which precludes the determination of a causal relationship between clinical factors. Only the mother was evaluated as a caregiver. Familial characteristics such as family dynamics, parenting roles, and coping strategies were not evaluated. Emotional and behavioral assessment of children with CP were not applied, and structured psychiatric interviews for mothers were not implemented by researchers. Additionally, epilepsy rates are generally higher in the moderate-severe ID group as expected, and epilepsy could be an important factor that may affect the QoL of children with CP.

In conclusion, several biopsychosocial factors may be related to OoL in children with CP. Our results demonstrate that maternal anxiety was related to psychosocial QoL in children with CP. Maternal depression scores increased with the age of children with CP which may also indicate the social support needs of mothers with children of chronic diseases. We could not find an association with intelligence levels and QoL of children with CP, maternal anxiety, maternal depression in our study sample. Further studies may reveal the associations with other biopsychosocial factors in children with cerebral palsy of different intelligence levels by longitudinal study design with larger sample sizes.

Acknowledgement

We are extremely grateful to all children, their families for taking part.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EA, BGK; data collection: EA, YT, STT; analysis and interpretation of results: BGK, EA, YT, STT; draft manuscript preparation: EA, BGK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Ethical approval was obtained from the Ankara University Medical School Research Ethics Committee (ID-No: 04-215-18).

Source of funding

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

Conflict of interest

The author(s) declare no competing interests.

REFERENCES

1. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE). Dev Med Child Neurol 2000; 42: 816-824.
2. Patel DR, Neelakantan M, Pandher K, Merrick J. Cerebral palsy in children: a clinical overview. Transl Pediatr 2020; 9: 125-135.
3. Cans C, Guillem P, Arnaud C, et al. Prevalence and characteristics of children with cerebral palsy in Europe. Dev Med Child Neurol 2002; 44: 633-640.
4. Bax M, Goldstein M, Rosenbaum P, et al; Executive Committee for the Definition of Cerebral Palsy. Proposed definition and classification of cerebral palsy, April 2005. Dev Med Child Neurol 2005; 47: 571-576.
5. Beckung E, Hagberg G. Neuroimpairments, activity limitations, and participation restrictions in children with cerebral palsy. Dev Med Child Neurol 2002; 44: 309-316.
6. Gabis LV, Tsubary NM, Leon O, Ashkenasi A, Shefer S. Assessment of abilities and comorbidities in children with cerebral palsy. J Child Neurol 2015; 30: 1640-1645.
7. Bertoncelli CM, Altamura P, Vieira ER, Bertoncelli D, Thummler S, Solla F. Identifying factors associated with severe intellectual disabilities in teenagers with cerebral palsy using a predictive learning model. J Child Neurol 2019; 34: 221-229.
8. Arnaud C, White-Koning M, Michelsen SI, et al. Parent-reported quality of life of children with cerebral palsy in Europe. Pediatrics 2008; 121: 54-64.
9. Vos RC, Becher JG, Ketelaar M, et al; PERRIN+ Study Group. Developmental trajectories of daily activities in children and adolescents with cerebral palsy. Pediatrics 2013; 132: 915-923.
10. Carona C, Crespo C, Canavarro MC. Similarities amid the difference: caregiving burden and adaptation outcomes in dyads of parents and their children with and without cerebral palsy. Res Dev Disabil 2013; 34: 882-893.
11. Nam SJ, Park EY. Relationship between caregiving burden and depression in caregivers of individuals with intellectual disabilities in Korea. J Ment Health 2017; 26: 50-56.
12. Park EY, Kim JH. Activity limitation in children with cerebral palsy and parenting stress, depression, and self-esteem: a structural equation model. Pediatr Int 2020; 62: 459-466.
13. Raina P, O'Donnell M, Rosenbaum P, et al. The health and well-being of caregivers of children with cerebral palsy. Pediatrics 2005; 115: 626-636.
14. Yilmaz H, Erkin G, Nalbant L. Depression and anxiety levels in mothers of children with cerebral palsy: a controlled study. Eur J Phys Rehabil Med 2013; 49: 823-827.
15. Ones K, Yilmaz E, Cetinkaya B, Caglar N. Assessment of the quality of life of mothers of children with cerebral palsy (primary caregivers). Neurorehabil Neural Repair 2005; 19: 232-237.
16. Bailey DB Jr, Golden RN, Roberts J, Ford A. Maternal depression and developmental disability: research critique. Ment Retard Dev Disabil Res Rev 2007; 13: 321-329.
17. Singer GH. Meta-analysis of comparative studies of depression in mothers of children with and without developmental disabilities. Am J Ment Retard 2006; 111: 155-169.
18. Chandravanshi G, Sharma KK, Jilowa CS, Meena PS, Jain M, Prakash O. Prevalence of depression in mothers of intellectually disabled children: a cross-sectional study. Med J DY Patil Univ 2017; 10: 156-161.
19. Li-Tsang CWP, Yau MKS, Yuen HK. Success in parenting children with developmental disabilities: some characteristics, attitudes and adaptive coping skills. Br J Dev Disabil 2001; 47: 61-71.
20. Himmelmann K, Panteliadis CP. Clinical Characteristics. In: Panteliadis CP (ed). Cerebral Palsy (3rd ed) Cham: Springer, 2018: 75-87.
21. Brehaut JC, Kohen DE, Raina P, et al. The health of primary caregivers of children with cerebral palsy: how does it compare with that of other Canadian caregivers? Pediatrics 2004; 114: e182-e191.

22. Christine C, Dolk H, Platt MJ, Colver A, Prasauskiene A, Krägeloh-Mann I; SCPE Collaborative Group. Recommendations from the SCPE collaborative group for defining and classifying cerebral palsy. Dev Med Child Neurol Suppl 2007; 109: 35-38.

23. Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised Gross Motor Function Classification System. Dev Med Child Neurol 2008; 50: 744-750.

24. Elvrum AK, Andersen GL, Himmelmann K, et al. Bimanual fine motor function (BFMF) classification in children with cerebral palsy: aspects of construct and content validity. Phys Occup Ther Pediatr 2016; 36: 1-16.

25. Çakın Memik N, Ağaoğlu B, Coşkun A, Karakaya I. Çocuklar için yaşam kalitesi ölçeğinin 8-12 yaş çocuk formunun geçerlik ve güvenirliği. Çocuk ve Gençlik Ruh Sağlığı Dergisi 2008; 15: 87-98.

26. Cakin Memik N, Ağaoğlu B, Coşkun A, Uneri OS, Karakaya I. Çocuklar için yaşam kalitesi ölçeğinin 13-18 yaş ergen formunun geçerlik ve güvenilirliği. Türk Psikiyatri Dergisi 2007; 18: 353-363.

27. Ulusoy M, Sahin NH, Erkmen H. Turkish version of the Beck anxiety inventory: psychometric properties. J Cogn Psychother 1998; 12: 163-172.

28. Hisli N. Beck depresyon envanterinin üniversite öğrencileri için geçerliği, güvenirliği. Türk Psikoloji Dergisi 1989; 7: 3-13.

29. Wechsler D. WISC-R Manual for the Wechsler Intelligence Scale for Children Revised. New York: The Psychological Corporation, 1974.

30. Savasır I, Sahin N. Wechsler Çocuklar İçin Zeka Ölçeği Uygulama Kitapçığı. (Çeviren: Savaşır I, Şahin N). Ankara: Türk Psikologlar Derneği, 1995.

31. Uğural Şemin R. Stanford Binet Ölçeğinin İstanbul Çocuklarına Uygulanması. İstanbul: İstanbul Üniversitesi Fen Fakültesi Basım Atölyesi, 1987.

32. Savasır I, Sezgin N, Erol N. Ankara Gelişim Tarama Envanteri (AGTE). 2. ve 3. Basım. Ankara: Türk Psikologlar Derneği, 1998.

33. Wake M, Salmon L, Reddihough D. Health status of Australian children with mild to severe cerebral palsy: cross-sectional survey using the Child Health Questionnaire. Dev Med Child Neurol 2003; 45: 194-199.

34. McCarthy ML, Silberstein CE, Atkins EA, Harryman SE, Sponseller PD, Hadley-Miller NA. Comparing reliability and validity of pediatric instruments for measuring health and well-being of children with spastic cerebral palsy. Dev Med Child Neurol 2002; 44: 468-476.

35. Kennes J, Rosenbaum P, Hanna SE et al. Health status of school-aged children with cerebral palsy: information from a population-based sample. Dev Med Child Neurol 2002; 44: 240-247.

36. Vargus-Adams J. Longitudinal use of the Child Health Questionnaire in childhood cerebral palsy. Dev Med Child Neurol 2006; 48: 343-347.

37. Davis E, Mackinnon A, Waters E. Parent proxy-reported quality of life for children with cerebral palsy: is it related to parental psychosocial distress? Child Care Health Dev 2012; 38: 553-560.

38. Waters E, Doyle J, Wolfe R, Wright M, Wake M, Salmon L. Influence of parental gender and self-reported health and illness on parent-reported child health. Pediatrics 2000; 106: 1422-1428.

39. Soliman R, Altwairqi R, Alshamrani N, Al-Zahrani A, Al-Towairqi R, Al-Habashi A. Relationship between quality of life of children with cerebral palsy and their mothers' depression and anxiety. Saudi J Health Sci 2019; 8: 1-5.

40. Türkoğlu S, Bilgiç A, Türkoğlu G, Yilmaz S. Impact of symptoms of maternal anxiety and depression on quality of life of children with cerebral palsy. Noro Psikiyatr Ars 2016; 53: 49-54.

41. Aran A. Quality of life in children with cerebral palsy. In: Preedy VR, Watson RR (eds). Handbook of Disease Burdens and Quality of Life Measures. New York: Springer, 2010: 2453-2468.

42. Aran A, Shalev RS, Biran G, Gross-Tsur V. Parenting style impacts on quality of life in children with cerebral palsy. J Pediatr 2007; 151: 56-60.

43. Majnemer A, Shevell M, Rosenbaum P, Law M, Poulin C. Determinants of life quality in school-age children with cerebral palsy. J Pediatr 2007; 151: 470-475.

44. Ribeiro MF, Vandenberghe L, Prudente CO, Vila VD, Porto CC. Cerebral palsy: how the child's age and severity of impairment affect the mother's stress and coping strategies. Cien Saude Colet 2016; 21: 3203-3212.

45. Carlsson M, Hagberg G, Olsson I. Clinical and aetiological aspects of epilepsy in children with cerebral palsy. Dev Med Child Neurol 2003; 45: 371-376.

46. Švraka E. Children with cerebral palsy and epilepsy. In: Stevanović D, (ed). Epilepsy-Histological, Electroencephalographic and Psychological Aspects. Croatia: InTech Open Access Publicher, 2012: 251-276.

The Turkish Journal of Pediatrics 2021; 63: 855-866
https://doi.org/10.24953/turkjped.2021.05.013

Original Article

The relationship of motor development with sensory processing among infants born very preterm: a prospective case-control study

Özgün Kaya Kara[1], Sedef Şahin[2], Hasan Atacan Tonak[1], Mutluay Arslan[3], Barkın Köse[2], Koray Kara[4]

[1]Department of Physiotherapy and Rehabilitation, Akdeniz University Faculty of Health Sciences, Antalya; [2]Department of Occupational Therapy, Hacettepe University Faculty of Health Sciences, Ankara; [3]Department of Child Neurology, University of Health Sciences Gülhane Training and Research Hospital, Ankara; [4]Department of Child and Adolescent Psychiatry, University of Health Sciences Antalya Training and Research Hospital, Antalya, Turkey.

ABSTRACT

Background. Little is known about the relationship between sensory processing and motor development in very preterm infants. The purpose of this study was to explore the relationship of motor development with sensory processing among such infants with developmental delay and those who had typical development at the ages of 8 and 12 months.

Methods. This prospective case-control study included 61 preterm infants (31 males, 30 females, mean gestational age: 29.1 weeks). The infants had a gestational age of 32 weeks or less and a current corrected age of 8 months, and they had spent at least 15 days in the neonatal intensive care unit. Motor development was assessed with the Neuro-sensory Motor Developmental Assessment (NSMDA), and sensory processing was evaluated with the Test of Sensory Functions in Infants (TSFI).

Results. There were very strong positive correlations between the gross and fine motor scores of the NSMDA and the TSFI's subdomain scores and total scores ($r=0.85$-0.93, $p<0.001$). There were also very strong negative correlations between the functional level according to the NSMDA and the subdomain scores and total scores of the TSFI ($r=-0.89$-0.94, $p<0.001$).

Conclusions. The results show that sensory processing and motor development are related parts of the development of very preterm infants. In the early rehabilitation process, therapists should comprehensively take motor and sensory development into consideration.

Key words: preterm, infant, sensory processing, motor, development.

Neonatal Intensive Care Units (NICUs) are crucial for supporting vital functions and decreasing the rates of neonatal morbidity and mortality.[1] On the other hand, the special care procedures in the NICU have adverse effects on the short and long-term development of infants.[2,3] The NICU's environment consists of many stressors for preterm infants, who are subjected to excessive sensory stimuli such as loud sounds, bright lights, and painful medical procedures.[4] These excessive stimuli during a key period in the brain's development might affect the physiological responses of infants and cause negative changes in motor, neurological, and sensory development.[5-7] The reason for this is a lack of inhibitory control for selecting, controlling, and processing sensory stimuli in the developing premature brain.[8,9] Therefore, the relationship between the NICU and premature birth might set off a chain of adverse events that could lead to learning difficulties and sensory and motor dysfunction.[10-12]

✉ Özgün Kaya Kara
ozgun_kaya@yahoo.com

Received 6th January 2021, revised 1st March 2021, 8th March 2021, accepted 16th March 2021.

Sensory processing is the innate ability to interpret and process sensory inputs and to produce the most appropriate response to the environment.[8,9] Inadequate sensory processing may contribute to a sensory processing disorder.[3,8] This disorder involves difficulties in processing and transforming the sensory information used for the regulation of physiological, motor, and emotional or attentional responses in the organization of behavior.[13] This may result in excessive or insufficient responses to sensory stimuli in the child.[14]

Atypical sensory behaviors affect a child's participation in daily living activities,[14] which may cause a delay in developmental milestones.[15,16] Therefore, processing sensory inputs correctly is essential in normal neurodevelopment.[15,16] Particularly, impairments in the inputs of the vestibular, proprioceptive, and tactile sensory systems may cause problems in producing adaptive behavior, movement coordination, and the development of postural control and motor development.[5] Sensory processing disorder affects 39 to 52% of preterm infants, and infants born before 32 weeks of gestation are at greater risk.[2,3,11]

Most of the relevant research has focused on the cognitive development outcomes of preterm infants.[6,17-20] Only a few studies have investigated the relationship between the motor and sensory development of preterm infants in the first year of life, and the available results are conflicting.[3,8,21,22] Celik et al.[21] indicated that there is a significant association of gross motor function with sensory processing among infants born prematurely at corrected ages of 10-12 months, whereas Cabral et al.[8] could not find any relationship between motor function and sensory processing in premature infants at 4-6 months old.

In a recent study, de Paula Machado et al.[4] investigated the relationship between motor development, cognitive development, and sensory processing at the age of 12 months.[4] They indicated that early birth adversely affected sensory processing, and ocular-motor control in sensory processing was positively correlated with motor development.[4] However, they only provide insight into sensory processing and prematurity at the age of 12 months. The main possible reasons for these inconsistent results are the cross-sectional design of the studies and the heterogeneity of the sample groups. Myelinization begins in the 2nd trimester continues during the first year of life.[23] Therefore, observing motor and sensory development prospectively is clinically important for determining difficulties in sensory processing and motor delay in the first year of life, which is crucial for referral to necessary early interventions.[24]

There is quite limited research on sensory processing disorder during the first year of life in infants born preterm.[4,11,22] Furthermore, there is an essential need to focus on this subject in prospective studies. Thus, the aim of this study was to determine the association of sensory processing with motor development among infants born very preterm with developmental delay and very preterm infants who had typical development. We hypothesized that preterm infants with developmental delay would have poorer sensory processing and motor performance than preterm infants with typical development. We also hypothesized there is a relationship between improved fine and gross motor outcomes and better sensory processing.

Material and Methods

Approval for this prospective study was obtained from the Ethics Committee of the University of Health Sciences, Non-Interventional Clinical Researches Ethics Board Project No: 18/250). The families included were informed about the study, and the necessary permission was obtained with signed informed consent forms. The Declaration of Helsinki was applied in the study process.

Participants

We recruited 78 very preterm infants who were treated at a university hospital in the

department of pediatric neurology between October 2018 and March 2019. The inclusion criteria for infants in the preterm delayed group included (1) gestational age of 32 weeks or less and a current corrected age of 8 months; (2) having spent at least 15 days in the NICU; and (3) a diagnosis of developmental delay by a pediatric neurologist and child and adolescent psychiatrist according to clinical evaluation and the Denver Developmental Screening Test-II.[25] The inclusion criteria for the preterm comparison group were (1) gestational age of 32 weeks or less and a current corrected age of 8 months; (2) having spent at least 15 days in the NICU; and (3) normal motor development based on the Denver Developmental Screening Test-II.[25] Infants were excluded from the study if they had any congenital abnormalities, genetic syndromes, musculoskeletal disorders, or hearing or visual impairment.

Of the 78 participants, 36 were assigned to the preterm delayed group (preterm infants with developmental delay), and 42 were assigned to the preterm comparison group (preterm infants with normal development), after dropouts which have been shown in Fig. 1, 33 infants in the preterm delayed group and 28 infants in the preterm comparison group completed all the assessments.

Procedures

Approximately two hours after feeding, standardized assessments were performed on a large mattress on the floor or on a table with the infant in a sitting position on the mother's lap. The infants did not take any medication that would interfere with the assessment. The Neuro-sensory Motor Developmental Assessment (NSMDA) and the Test of Sensory Functions in Infants (TSFI) were used for the evaluations, which each took approximately 20 minutes. The NSMDA and TSFI tests were applied to infants at corrected ages of 8 and 12 months. The NSMDA was applied by the first author, who had 10 years of experience in the field of pediatric rehabilitation. The TSFI was applied by the second author, who had nine years of experience in pediatric rehabilitation.

Neuro-Sensory Motor Developmental Assessment (NSMDA)

The NSMDA consists of six sections that evaluate the movement function of children at 1 month to 6 years of age. It is a criterion-referenced test, and the categories evaluated are age-appropriate: (1) gross motor function, (2) fine motor function, (3) neurological status, (4) infant patterns of movement, (5) posture and balance, and (6) sensory-motor function. The scores of these six areas are summed to calculate a neurosensory motor developmental score. Development in each section is given points ranging from 1 (within normal limits) to 5 (no independent function). The scores of each section are summed to obtain functional grade scores for the motor performance classification of infants.

Total functional grade scores of 6–8 on the NSMDA were classified as normal motor function, scores of 9-11 indicated minimal motor problems, scores of 12-13 indicated mild motor problems, scores of 14-19 indicated moderate motor problems, scores of 20-25 indicated severe motor dysfunction, and scores of >25 indicated profound motor dysfunction. The biggest advantage of the NSDMA is its ability to differentiate between normal motor function and minimal, mild, moderate, or severe motor dysfunction.[26,27] The psychometric properties of the NSMDA have been identified for preterm[28,29] and extremely low-birth-weight infants.[30]

Test of Sensory Functions in Infants (TSFI)

The TSFI assesses the disturbances of sensorimotor integration that have a risk of occurrence in children aged 4 - 18 months. The TSFI consists of five subtests and 24 items that assess the functioning of the basic senses by observing the following features: sensitivity to deep pressure, the level of adaptive motor functions, visual-tactile coordination, control of eye movements, and the level of integration

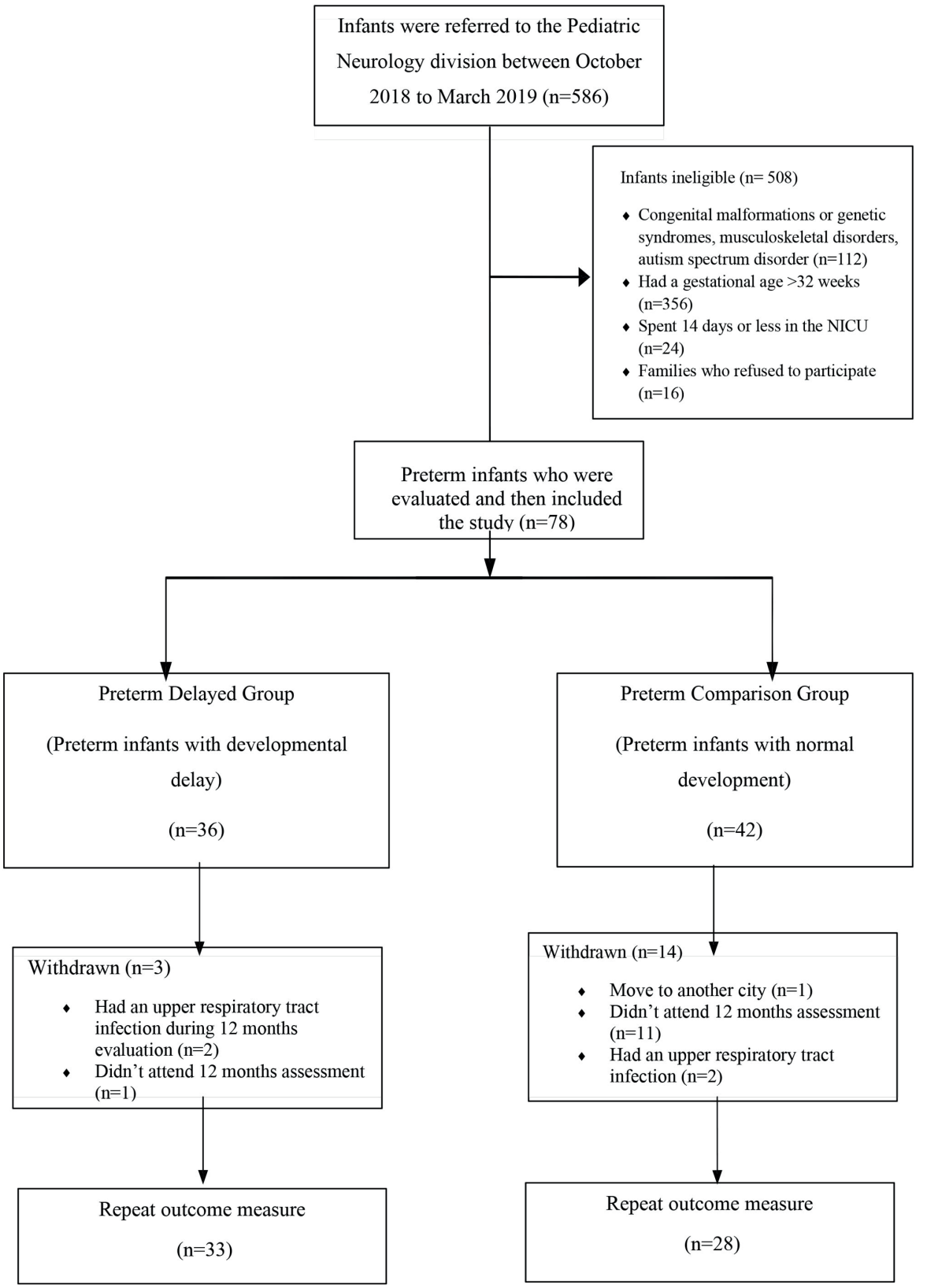


Fig. 1. Follow-up diagram.

of vestibular information. The range of the total score is 0 to 49, and higher scores show improved sensory processing.

The TSFI has cutoff values of four different age groups for both the subtests and the total score according to norm-referenced values of North American infants with typical development. Based on the cutoff values, sensory processing skills are evaluated as normal, risky, or abnormal.[31] Worldwide, the sensory processing of infants is usually assessed with the TSFI. It has commonly been used to determine the difficulties in sensory processing in preterm infants by Turkish researchers,[21] but a validation study for the Turkish population has not been performed yet.[32]

Statistical analysis

Statistical analyses were performed using the software SPSS version 21 for macOS (IBM SPSS Statistics; IBM Corporation, Armonk, NY, USA). The number of individuals needed in the study was determined to be 30 according to a power analysis using α=0.05 and β=0.20 (for 80% power).[21] The conformity of the variables to a normal distribution was determined by the Kolmogorov-Smirnov test along with histogram and box plots.

Descriptive statistics of the data were calculated with the mean and standard deviation. Mann-Whitney's U-test was used to describe the differences between groups at the ages of 8 and 12 months in the NSMDA sub-parameters and TSFI results. The relationship between the NSMDA and TSFI results of the preterm infants was analyzed with the Spearman correlation test. In the evaluation of the relationship, the correlation coefficients were classified as follows: 0 – 0.24: weak, 0.25 – 0.49: moderate, 0.50 – 0.74: strong, 0.75 – 1.00: very strong[33]. $p < 0.05$ was considered as statistically significant.

Results

The characteristics of the preterm infants are presented in Table I. There were no statistical differences between the groups. Baseline data showed that each group was well matched, including gestational age, birth weight, gender, maternal age, days in the neonatal care unit, etc.

Table II shows the median NSMDA and TSFI scores of the preterm infants and the differences at corrected ages of 8 and 12 months for each group. Compared to the average normal scores of the NSMDA subdomains, the preterm infants in the preterm delayed group showed decreases

Table I. Characteristics of the preterm infants.

Characteristics	Preterm delayed group (n=33)	Preterm comparison group (n=28)	p
	Mean (SD)	Mean (SD)	
Gestational age (weeks)	28.9 (2.3)	29.3(1.7)	0.46
Birth weight (g)	1154.66 (318.14)	1225.28 (258.2)	0.35
Corrected age (d) in first assessment	241.9 (2.8)	242.89 (4.8)	0.32
Maternal age (y)	31.09(5.07)	32.6 (4.4)	0.22
Days on Neonatal Intensive Care Unit	23.93 (7.18)	24.21 (6.08)	0.87
	n (%)	n (%)	
Gender (Males/Females)	31 (50.8)/30(49.2)	17 (60.7)/11(39.3)	0.2
Multiple births	9 (27.3)	14 (50)	0.11
Respiratory distress syndrome	16 (48.5)	9 (32.1)	0.29
Bronchopulmonary dysplasia	4 (12.1)	0	0.11
Intraventricular haemorrhage I/II	6(18.2)	8 (28.6)	0.37

Mann-Whitney's U-test for continuous variables and the Chi-square test for categorical variables.

Table II. Neurosensory Motor Developmental Assessment (NSMDA) and Test of Infant Sensory Profile Scores (TSFI) at 8-12 months corrected age

	Preterm delayed group (n=33)		Preterm comparison group (n=28)		Differences between groups at 8 months[a]		Differences between groups at 12 months[a]	
NSMDA	8 months Median (25%-75%)	12 months Median (25%-75%)	8 months Median (25%-75%)	12 months Median (25%-75%)	Z	p	Z	p
Gross motor (Mean average score for 8 months =30, for 12 months= 24)	15 (11-22)	17 (12-21)	32.5 (31-36.5)	31 (27.25-31)	-6.68	<0.001	-5.86	<0.001
Fine Motor (Mean average score for 8 months =15, for 12 months= 15)	10 (6.5-12.5)	11 (9-13)	19.5 (16-20)	16.5 (15-20)	-6.7	<0.001	-5.73	<0.001
Tactile (Mean average score for 8 months =12, for 12 months= 12)	4 (4-9)	8 (4-9.5)	12 (12-16)	12 (12-16)	-6.71	<0.001	-5.34	<0.001
Ocular (Mean average score for 8 months =12, for 12 months= 9)	6 (4-7)	8 (5.5-9)	12 (12-12)	12 (10-12)	-6.75	<0.001	-5.72	<0.001
Vestibular (Mean average score for 8 months =12, for 12 months= 12)	6 (4-8)	8 (4-9)	12 (12-12)	12 (12-12)	-7.0	<0.001	-5.32	<0.001
Functional Level (Normal=5-8)	15 (10-26)	17 (9-26)	6 (6-7)	7 (7-7.75)	-6.75	<0.001	-5.94	<0.001
TSFI								
Response to tactile deep pressure (Normal=9-10)	4 (2.5-8)	7 (4-8)	10 (9-10)	9.5 (9-10)	-6.63	<0.001	-5.79	<0.001
Adaptive motor functions (Normal=14-15)	5 (4-12)	12 (4-13)	14 (14-15)	14 (14-14.75)	-6.62	<0.001	-5.71	<0.001
Visual-tactile integration (Normal=9-10)	4 (2-7)	6 (3-7.5)	9 (9-10)	9 (9-9)	-6.63	<0.001	-5.78	<0.001
Oculomotor control (Normal=2)	0 (0-1)	1 (0-1)	2 (2-2)	2 (2-2)	-7.0	<0.001	-6.07	<0.001
Response to vestibular stimuli (Normal=10-12)	4 (3-9)	8 (4-9)	11 (10-11)	10 (10-10.75)	-6.53	<0.001	-5.78	<0.001
TSFI total score (Normal=44-49)	17 (11.5-37.5)	34 (14.5-40)	46 (45-47)	45 (44-46.75)	-6.52	<0.001	-5.83	<0.001

[a]Mann-Whitney's U-test, * p<0.05, NSMDA: neurosensory motor developmental assessment, TSFI: test of infant sensory profile scores

of 50% in gross motor scores (30 versus 15), 33.3% in fine motor scores (15 versus 10), 66.6% in tactile scores (12 versus 4), 50% in ocular scores (12 versus 6), and 50% in vestibular scores (12 versus 6) at 8 months. At 12 months, the preterm delayed group's subdomain scores

showed decreases of 29.1% in gross motor scores (24 versus 17), 26.6% in fine motor scores (15 versus 11), 33.3% in tactile scores (12 versus 8), 33.3% in ocular scores, and 33.3% in vestibular scores (12 versus 8) compared to the average normal scores.

In the preterm comparison group, the median scores of the NSMDA subdomains were in the normal range at 8 and 12 months. The median functional level indicated moderate motor problems for the preterm delayed group and normal motor function for the preterm comparison group at the ages of 8 and 12 months. The median TSFI total and subdomains scores were classified as risky-abnormal for the preterm delayed group and normal for the preterm comparison group at 8 and 12 months. There were also significant differences in the NSMDA subdomains and functional levels, TSFI subdomains, and the total TSFI score between the preterm delayed and preterm comparison groups at corrected ages of 8 and 12 months ($p<0.001$).

According to the NSMDA functional level score, 13 preterm infants were classified as having minimal-mild motor problems, and 20 had moderate to profound motor problems at the corrected age of 8 months. Based on the total scores of TSFI, 7 infants were in the risky group, and 27 were in the abnormal group at 8 months. At the corrected age of 12 months, the NSMDA indicated that 14 preterm infants were classified as having minimal-mild motor problems, and 19 had moderate to profound motor problems. Based on the total scores of TSFI, 8 infants were in the risky group, and 26 were in the abnormal group. Table III shows the classification of motor and sensory levels of the infants born very preterm.

There was a very strong positive correlation between the gross and fine motor scores of the NSMDA and the NSMDA sensory subdomains (tactile, vestibular, and ocular). Furthermore, there were very strong positive correlations between gross and fine motor scores of the NSMDA and the total and subdomain scores of the TSFI. There were very strong negative correlations between the functional level according to the NSMDA, the NSMDA subdomains, and the TSFI's total and subdomain scores ($p<0.001$; Table IV, Fig. 2).

Discussion

This prospective study investigated the correlation of motor development with sensory processing at the ages of 8 and 12 months among infants born very preterm. The results show that very preterm infants with developmental delay were in the risky-abnormal group of sensory processing and had moderate motor problems in motor development. In addition, there were very strong positive correlations between gross and fine motor function development and sensory processing at the ages of 8 and 12 months in infants born very preterm. Particularly, there were very strong negative correlations between sensory processing and motor performance.

Research indicates that very preterm infants have a higher risk of neurosensory motor disorders than their term-born peers.[34] Pin et

Table III. Classifications of motor and sensory level of preterm infants.

NSMDA Functional level N (%)	Normal	Minimal-mild motor problems	Moderate-profound
8 months	28 (45.9)	13 (21.3)	20 (32.8)
12 months	28 (45.9)	14 (23)	19 (31.1)
TSFI total score N (%)	Normal	Risky	Abnormal
8 months	27 (44.3)	7 (11.5)	27(44.3)
12 months	27 (44.3)	8 (13.1)	26 (42.6)

NSMDA: neurosensory motor developmental assessment, TSFI: test of infant sensory profile scores

Table IV. Relationship between NSMDA and TSFI scores.

NSMDA	Gross motor				Fine motor				Functional level			
	8 mo		12 mo		8 mo		12 mo		8 mo		12 mo	
	r	p	r	p	r	p	r	p	r	p	r	p
Tactile	0.93	<0.001	0.83	<0.001	0.90	<0.001	0.82	<0.001	-0.92	<0.001	-0.84	<0.001
Ocular	0.89	<0.001	0.82	<0.001	0.88	<0.001	0.87	<0.001	-0.90	<0.001	-0.93	<0.001
Vestibular	0.93	<0.001	0.92	<0.001	0.90	<0.001	0.82	<0.001	-0.93	<0.001	-0.84	<0.001
TSFI												
Response to tactile deep pressure	0.91	<0.001	0.85	<0.001	0.88	<0.001	0.85	<0.001	-0.89	<0.001	-0.89	<0.001
Adaptive motor functions	0.92	<0.001	0.89	<0.001	0.89	<0.001	0.85	<0.001	-0.90	<0.001	-0.91	<0.001
Visual-tactile integration	0.91	<0.001	0.92	<0.001	0.89	<0.001	0.90	<0.001	-0.90	<0.001	-0.94	<0.001
Oculomotor control	0.88	<0.001	0.92	<0.001	0.87	<0.001	0.88	<0.001	-0.89	<0.001	-0.92	<0.001
Response to vestibular stimuli	0.92	<0.001	0.89	<0.001	0.87	<0.001	0.87	<0.001	-0.89	<0.001	-0.92	<0.001
Total score	0.93	<0.001	0.88	<0.001	0.89	<0.001	0.87	<0.001	-0.90	<0.001	-0.90	<0.001

NSMDA: neurosensory motor developmental assessment, TSFI: test of infant sensory profile

al.[35] showed that at 8 months, preterm infants exhibit similar movements to their term-born peers in prone and supine positions. However, there were significant differences between them in motor performance in sitting and standing postures, which require more muscle activation and motor control against gravity.

Olsen et al.[36] investigated the neurodevelopmental results of 137 preterm infants at the age of 12 months who were born before 30 weeks of gestation. They demonstrated that the functional level of 76.6% of preterm infants was classified as mild to severe motor dysfunction according to the NSMDA. Similarly, the gross and fine motor scores of preterm infants with developmental delay in our study were below the average normal score. In addition, 33% of preterm infants in our study were classified as having minimal to profound motor dysfunction according to the functional level. Preterm infants have a higher risk for motor problems, so a multidisciplinary team approach in the NICU is crucial to detect and follow-up these preterm infants with motor delay and educate the family in an early period, especially in developing countries.

During the first year of life, the accuracy of neuromotor assessments is conflicting because motor development is not only fast and comprehensive but is also influenced by biological, environmental, and social factors. Burns et al.[26] indicated that the 8th month is the best evaluation month to predict normal or abnormal motor development in infants. Delays in gross and fine motor development could thus occur with increasing age. Therefore, in this prospective study, preterm infants were first assessed at 8 months to obtain information about motor performance and sensory processing about infants born very preterm.

Preterm infants are at high risk for sensory development from exposure to adverse sensory feedback, such as long-term intubation, heel-lance procedures, and intense sounds and lights in the NICU, as opposed to the safe environment of the uterus. Ryckman et al.[3] investigated sensory processing disorder in preterm infants born at 30 weeks or earlier when they had reached the age of 4-6 years. They demonstrated that 50% of the children had sensory processing disorder.

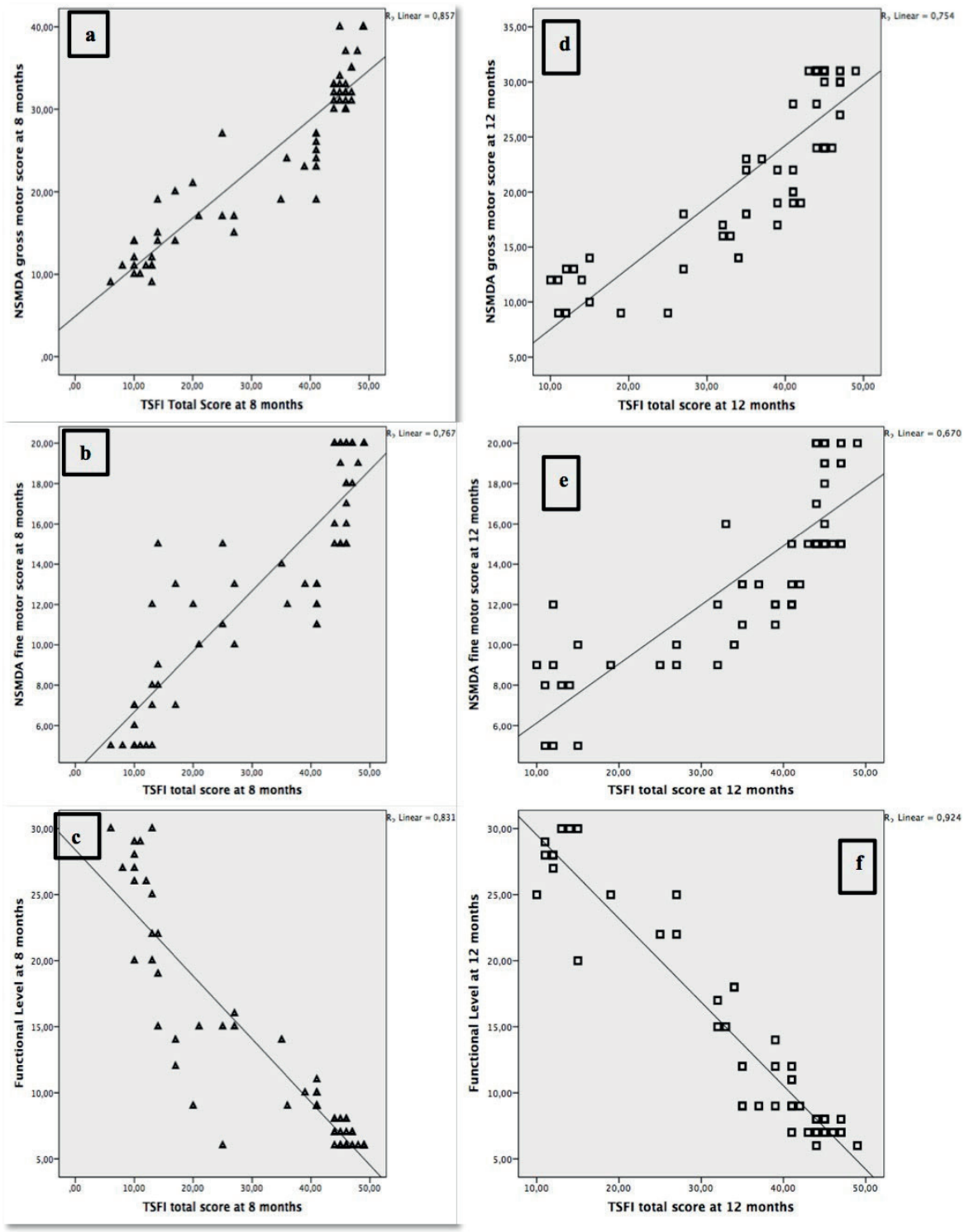


Fig. 2. (a, b, c) Relationship between gross motor, fine motor, and total TSFI scores at 8 months, **(d, e, f)** Relationship between gross motor, fine motor, and total TSFI scores at 12 months.

Similarly, Chorna et al.[22] found abnormal sensory reactivity at 12 months of age in 82% of infants born with weights of 1500 grams or less. Cabral et al.[8] showed significant differences in tactile deep pressure perception between term and preterm infants born at 37 weeks of gestation or earlier who stayed in the NICU for at least one day. Celik et al.[21] showed that there was a risk of sensory development issues in 60% of infants at the ages of 10-12 months who were born at 37 weeks or earlier and stayed in the NICU for at least 15 days.

The current study investigated the sensory processing of very preterm infants at the ages of 8 and 12 months. Similar to the literature, the TSFI indicated that infants born very preterm in this study had a higher risk in terms of oculomotor control, response to tactile deep pressure, visual-tactile integration, adaptive motor functions, and response to vestibular stimuli at the ages of 8 and 12 months. Furthermore, 44.3% of them at 8 months and 42.6% of them at 12 months had abnormal sensory processing. Findings from a recent study by de Paula Machado et al.[4] support our results in that there was a negative correlation between premature birth and sensory processing.

It is not surprising to observe the adverse effects of daily stressors in the NICU among preterm infants. NICU professionals should be aware of the increasing risk of sensory processing difficulties in preterm infants. In addition, neonatologists could improve strategies with physiotherapists and occupational therapists to prevent sensory processing problems during the first year of life, when cerebral plasticity is greater.

A few studies have investigated the relationship between motor development and sensory processing. Cabral et al.[8] indicated that 53% of preterm infants at the ages of 4-18 months had a risk of gross motor developmental delays. However, they did not show a statistically significant association between motor function development and sensory processing. A possible reason for this might be that they included preterm infants born at 37 weeks or earlier who stayed in the NICU for at least one day. Chorna et al.[22] showed that preterm infants who had abnormal reactivity at 12 months also had worse motor and language developmental scores than preterm infants who had normal reactivity at 12 months. Celik et al.[21] did a cross-sectional study that demonstrated a strong, significant, positive relationship between gross motor function and sensory processing in preterm infants at the age of 10-12 months.

In contrast to the literature, the present study prospectively investigated the relationship of motor performance with sensory processing in very preterm infants with developmental delay, along with very preterm infants with typical development at the ages of 8 and 12 months. As a result, we found that there were very strong positive correlations between the TSFI's total and subdomain scores and the NSMDA's fine and gross motor scores. Furthermore, there were very strong negative correlations between the TSFI's total and subdomain scores and functional levels. Recent findings add support to our results that improved sensory processing is related to better motor development at the age of 12 months among infants born preterm and full term.[4] These results reflect that sensory processing and motor function development are inseparable parts of infant development. In the early rehabilitation process, therapists should take development in its entirety into consideration.

No previous study has investigated the relationship between fine motor development and sensory processing. Our findings showed that there were very strong positive correlations between tactile, ocular, and vestibular processing and fine motor development. Chorna et al.[22] showed that 21% of preterm infants had a risk of vestibular issues, 49% had a risk of tactile issues, and 33% had a risk of ocular processing issues, which was supported by our results. Similarly, Celik et al.[21] found a moderate correlation between vestibular and ocular processing and motor development. In light of these findings, rehabilitation approaches that are aimed at

improving fine motor development should consist of supporting vestibular, ocular, and tactile sensory processing in preterm infants.

There were some limitations to this study. One of them is that no term control group was included. Furthermore, there was no long-term follow-up of the neurodevelopmental outcomes in this cohort. Future studies should determine the association of motor function development with sensory processing. Long-term follow-up of preterm infants and comparison to term infants should also be conducted.

Preterm infants had a high risk of motor developmental delays and sensory processing disorder. There were very strong relationships between motor function development and sensory processing. The effects of sensory-based early intervention programs for preterm infants should be researched.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ÖKK, SŞ, KK; data collection: ÖKK, SŞ, BK, MA; analysis and interpretation of results: HAT, KK; draft manuscript preparation: ÖKK, HAT. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Approval for this prospective study was obtained from the Ethics Committee of the University of Health Sciences, Non-Interventional Clinical Researches Ethics Board Project No: 18/250).

Source of funding

The authors have no financial relationships relevant to this article to disclose.

Conflict of interest

The authors declare that there are no conflicts of interest or funding.

REFERENCES

1. Anderson DE, Patel AD. Infants born preterm, stress, and neurodevelopment in the neonatal intensive care unit: might music have an impact? Dev Med Child Neurol 2018; 60: 256-266.
2. Crozier SC, Goodson JZ, Mackay ML, et al. Sensory processing patterns in children born very preterm. Am J Occup Ther 2016; 70: 7001220050p1-7.
3. Ryckman J, Hilton C, Rogers C, Pineda R. Sensory processing disorder in preterm infants during early childhood and relationships to early neurobehavior. Early Hum Dev 2017; 113: 18-22.
4. de Paula Machado ACC, de Castro Magalhaes L, de Oliveira SR, Bouzada MCF. Is sensory processing associated with prematurity, motor and cognitive development at 12 months of age? Early Hum Dev 2019; 139: 104852.
5. Mitchell AW, Moore EM, Roberts EJ, Hachtel KW, Brown MS. Sensory processing disorder in children ages birth-3 years born prematurely: a systematic review. Am J Occup Ther 2015; 69: 6901220030.
6. Pineda RG, Neil J, Dierker D, et al. Alterations in brain structure and neurodevelopmental outcome in preterm infants hospitalized in different neonatal intensive care unit environments. J Pediatr 2014; 164: 52-60.e2.
7. Victoria NC, Murphy AZ. The long-term impact of early life pain on adult responses to anxiety and stress: historical perspectives and empirical evidence. Exp Neurol 2016; 275(Pt 2): 261-273.
8. Cabral TI, Pereira da Silva LG, Tudella E, Simoes Martinez CM. Motor development and sensory processing: a comparative study between preterm and term infants. Res Dev Disabil 2015; 36C: 102-107.
9. Broring T, Oostrom KJ, Lafeber HN, Jansma EP, Oosterlaan J. Sensory modulation in preterm children: theoretical perspective and systematic review. PLoS One 2017; 12: e0170828.
10. Adams JN, Feldman HM, Huffman LC, Loe IM. Sensory processing in preterm preschoolers and its association with executive function. Early Hum Dev 2015; 91: 227-233.
11. Wickremasinghe AC, Rogers EE, Johnson BC, Shen A, Barkovich AJ, Marco EJ. Children born prematurely have atypical sensory profiles. J Perinatol 2013; 33: 631-635.
12. Cabral TI, da Silva LG, Martinez CM, Tudella E. Analysis of sensory processing in preterm infants. Early Hum Dev 2016; 103: 77-81.

13. Machado ACCP, Oliveira SR, Magalhaes LC, Miranda DM, Bouzada MCF. Sensory processing during childhood in preterm infants: a systematic review. Rev Paul Pediatr 2017; 35: 92-101.

14. Armstrong DC, Redman-Bentley D, Wardell M. Differences in function among children with sensory processing disorders, physical disabilities, and typical development. Pediatr Phys Ther 2013; 25: 315-321.

15. Hertzog D, Cermak S, Bar-Shalita T. Sensory modulation, physical activity and participation in daily occupations in young children. Can J Occup Ther 2019; 86: 106-113.

16. Bar-Shalita T, Granovsky Y, Parush S, Weissman-Fogel I. Sensory Modulation Disorder (SMD) and pain: a new perspective. Front Integr Neurosci 2019; 13: 27.

17. Nosarti C, Giouroukou E, Healy E, et al. Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome. Brain 2008; 131(Pt 1): 205-217.

18. Peterson BS, Vohr B, Staib LH, et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. JAMA 2000; 284: 1939-1947.

19. Spittle A, Orton J, Anderson PJ, Boyd R, Doyle LW. Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants. Cochrane Database Syst Rev 2015: CD005495.

20. Orton J, Spittle A, Doyle L, Anderson P, Boyd R. Do early intervention programmes improve cognitive and motor outcomes for preterm infants after discharge? A systematic review. Dev Med Child Neurol 2009; 51: 851-859.

21. Celik HI, Elbasan B, Gucuyener K, Kayihan H, Huri M. Investigation of the Relationship Between Sensory Processing and Motor Development in Preterm Infants. Am J Occup Ther 2018; 72: 7201195020p1--7201195020p7.

22. Chorna O, Solomon JE, Slaughter JC, Stark AR, Maitre NL. Abnormal sensory reactivity in preterm infants during the first year correlates with adverse neurodevelopmental outcomes at 2 years of age. Arch Dis Child Fetal Neonatal Ed 2014; 99: F475-F479.

23. Vaccarino FM, Ment LR. Injury and repair in developing brain. Arch Dis Child Fetal Neonatal Ed 2004; 89: F190-F192.

24. Kara OK, Sahin S, Yardimci BN, Mutlu A. The role of the family in early intervention of preterm infants with abnormal general movements. Neurosciences (Riyadh) 2019; 24: 101-109.

25. Yalaz K, Epir S. Denver Developmental Screening Test Manual, Standardization for Turkish Children. Ankara: Hacettepe University Press, 1983.

26. Burns YR, Ensbey RM, Norrie MA. The neuro-sensory motor developmental assessment Part II: predictive and concurrent validity. Aust J Physiother 1989; 35: 151-157.

27. Burns YR, Ensbey RM, Norrie MA. The neuro-sensory motor developmental assessment Part 1: development and administration of the test. Aust J Physiother 1989; 35: 141-149.

28. Spittle AJ, Lee KJ, Spencer-Smith M, Lorefice LE, Anderson PJ, Doyle LW. Accuracy of two motor assessments during the first year of life in preterm infants for predicting motor outcome at preschool age. PLoS One 2015; 10: e0125854.

29. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. Dev Med Child Neurol 2008; 50: 254-266.

30. Danks M, Maideen MF, Burns YR, et al. The long-term predictive validity of early motor development in "apparently normal" ELBW survivors. Early Hum Dev 2012; 88: 637-641.

31. DeGangi GA, Greenspan SI. Test of Sensory Functions in Infants (TSFI) Manual. Los Angeles, CA: Western Psychological Services, 1989.

32. Eeles AL, Spittle AJ, Anderson PJ, et al. Assessments of sensory processing in infants: a systematic review. Dev Med Child Neurol 2013; 55: 314-326.

33. Altman D. Practical Statistics for Medical Research. London: Chapman and Hall, 1992.

34. Raniero EP, Tudella E, Mattos RS. Pattern and rate of motor skill acquisition among preterm infants during the first four months corrected age. Rev Bras Fisioter 2010; 14: 396-403.

35. Pin TW, Darrer T, Eldridge B, Galea MP. Motor development from 4 to 8 months corrected age in infants born at or less than 29 weeks' gestation. Dev Med Child Neurol 2009; 51: 739-745.

36. Olsen JE, Allinson LG, Doyle LW, et al. Preterm and term-equivalent age general movements and 1-year neurodevelopmental outcomes for infants born before 30 weeks' gestation. Dev Med Child Neurol 2018; 60: 47-53.

The Turkish Journal of Pediatrics 2021; 63: 867-874
https://doi.org/10.24953/turkjped.2021.05.014

Original Article

The possible association between neonatal morbidities and amniotic fluid pH and electrolyte levels in infants of preeclamptic mothers

Seda Yılmaz Semerci[1], Burak Yücel[2], İbrahim Mert Erbaş[1], Osman Samet Günkaya[2], Merih Çetinkaya[1]

Divisions of [1]Neonatology and [2]Obstetrics and Gynecology, İstanbul Kanuni Sultan Suleyman Training and Research Hospital, İstanbul, Turkey.

ABSTRACT

Background. Preeclampsia is a pregnancy-specific syndrome associated with increased perinatal mortality characterized by hypertension and proteinuria. An increasing number of studies have been published on the effect of preeclampsia on neonatal morbidities. However, there is no study regarding the possible effect of preeclampsia on amniotic fluid pH and electrolytes. The aim of this study was to determine the possible role of amniotic fluid pH and electrolytes for the prediction of and/or association with preeclampsia and neonatal morbidities.

Methods. This was a prospective, case-control study. During cesarean section (C/S), 1 ml of amniotic fluid was aspirated before incision of membranes. Amniotic fluid pH and electrolytes were analyzed by blood gas machine and biochemistry laboratory concurrently. Maternal and neonatal demographic features and clinical outcomes, presence of respiratory morbidities were all recorded.

Results. Amniotic fluid pH, sodium and gestational age were found to be independent risk factors for preeclampsia. Subgroup analysis revealed that in early onset preeclampsia group mechanical ventilation duration, duration of 0_2 therapy, sepsis and intrauterine growth retardation (IUGR) were higher than infants in control group born before 32 gestational weeks. Also, in the early onset preeclampsia group pH and potassium were higher compared with the control group.

Conclusions. To the best of our knowledge, this is the first study that reported the value of amniotic fluid electrolyte analysis for the prediction of preeclampsia and neonatal morbidities in term and preterm infants. However, more studies including a larger number of infants are required to confirm the role of amniotic fluid analysis to predict preeclampsia and/or neonatal morbidities.

Key words: amniotic fluid, electrolytes, neonatal morbidities, pH, preeclampsia.

Amniotic fluid (AF) is a complex bio-environment with dynamic content. Amniotic fluid has many immunologic and biochemical properties as well as being a mechanical cushion for the fetus. Additionally, AF has a key role as an early diagnostic tool for fetal/neonatal disease. The pH of AF is proven to be affected by both maternal and fetal conditions such as preterm ruptures of membranes, gestational age and fetal distress.[1] As of the 20th gestational week fetal urine is the main component of AF, nearby amniotic membranous secretion and fetal lung liquid are determined as the other components. The volume of AF is primarily determined by intramembranous absorption of water and solutes from fetal venous structures.[2]

Preeclampsia (PE) is a pregnancy-specific syndrome associated with increased perinatal

✉ Seda Yılmaz Semerci
sedayilmazsemerci@gmail.com

Received 15th September 2020, revised 6th January 2021, 12th February 2021, accepted 18th February 2021.

This manuscript was presented at the World Congress of Perinatology at 11-14 September 2019 in İstanbul, Turkey.

mortality characterized by hypertension and proteinuria. Whereas early onset PE is diagnosed between 20-34 gestational weeks (GW), late onset PE is diagnosed after 34 GW.[3] Affecting 5-8% of the pregnancies worldwide, PE pathogenesis has two phases.[4] At the first phase, disorders of placentation and pseudoangiogenesis induce increased oxidative stress, causing endothelial dysfunction, which leads to maternal systemic inflammatory response syndrome and a clinical picture with typical symptoms and signs of PE.[5] Besides the maternal complications like cerebral edema or hemolysis, elevated liver enzymes, low platelet syndrome (HELLP syndrome), stroke, pulmonary edema; PE harbors severe fetal effects such as fetal distress, oliguria, abruption placenta, intrauterine growth restriction (IUGR). These factors make PE, a global reason for both, fetal and maternal mortality and morbidity.

AF content is related to antenatal steroids, preterm birth and GW, it has also been suggested to be affected from preterm premature ruptures of membranes, fetal distress and PE. Furthermore, AF electrolytes were shown to be predictors for neonatal morbidities.[6] Although PE was thought affect AF content and fetal/maternal conditions, there is no human study about this subject in the literature.

Therefore, the aim of this study was to determine the possible role of PE on AF pH and electrolytes and neonatal morbidities.

Material and Methods

This prospective, single center, case-control study was conducted in four months. The ethics committee of Kanuni Sultan Suleyman Training and Research Hospital approved the study (Ethics Committee approval number: KAEK//2015.7.7). Informed consent was obtained from parents. Preeclamptic mothers and their infants comprised the case group of the study. Pregnant women who did not develop PE, and their infants were taken as the control group. Congenital abnormalities, chromosomal disorders, blood contaminated AF, C/S without labour pain, other abnormalities of the pregnant mother, which might affect the results and those who declined to participate were all excluded. For the subgroup analysis; infants who were born under 34 GW from preeclamptic mothers and infants who were born under 34 GW from mothers who did not develop PE, were compared. To exclude the possible effect of GW on the parameters compared between groups; only the infants, who were born under 34 GW, were included in the group without early onset PE. Blood contaminated AF was determined if hematocrit was measured by the device. The procedure to avoid blood contamination of AF was to aspirate AF before the incision of membranes during the C/S. From all infants included, 1 ml of AF was aspirated during the C/S before incision of the membranes. pH value and electrolytes of AFs were analyzed by the blood gas machine (Siemens RAPIDLab®1200 Systems). Collected samples were analyzed both by the blood gas machine and laboratory of the study hospital with conventional biochemical methods concurrently. Maternal and neonatal demographic features and clinical outcomes, presence of morbidities such as sepsis, respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis (NEC) were all recorded. This study is registered in ClinicalTrials.gov with the number NCT02691559.

Statistical analysis

Clinical data were presented as means ± SD for parametric tests and categorical data displayed as median (interquartile range (IQR)) for nonparametric tests. The distribution of the variables were controlled by Kolmogorov-Smirnov test. Comparisons were performed with the t-test in case of normal distribution of the variable or the Mann-Whitney U test if the distribution is not normal. Mann-Whitney U test was used for the analysis of quantitative and Chi-Square test for qualitative data. A sample size calculation was performed based

on our observed results by using a one-sided McNemar's test. A sample size of 180 infants, at least 69 in each arm, is found to be sufficient to detect a clinically important difference between groups with 80% power and a 5% level of significance.

SPSS version 21.0 (SPSS, Chicago, IL) was used for statistical analysis. Statistical significance was accepted when the probability (P) value was < 0.05 and changes were referred to as significant at this P-value.

Results

A total of 382 infants were born via C/S in the study hospital during the study period. As 202 were excluded due to reasons given in Figure 1, AFs of 180 infants were included. Of these, 72 infants were born from preeclamptic mothers. A flow chart of participants has been given in Figure 1. The two distinct methods of the blood gas machine and conventional biochemistry results did not differ. Receiver operating characteristic analysis showed a statistically significantly lower birth weight and gestational age in the group with PE than the control group ($p<0.001$, $p<0.001$) (Table I). Besides hospitalization rate, RDS, NEC, sepsis, prematurity, IUGR were statistically significantly higher in the PE group when compared with the control group ($P <0.05$) (Table I). AF pH, Na, K were found to be statistically significantly higher in the PE group than the control group ($P <0.05$) (Table II). AF pH, Na and gestational age were found to be independent risk factors for PE in logistic regression analyses (Table III). Subgroup analysis revealed that in the early onset PE (EOP) group mechanical ventilation duration, duration of 0_2 therapy, sepsis and IUGR were higher than control group infants born before 34 GW (p 0.035, 0.012, 0.021, 0.011) (Table IV).

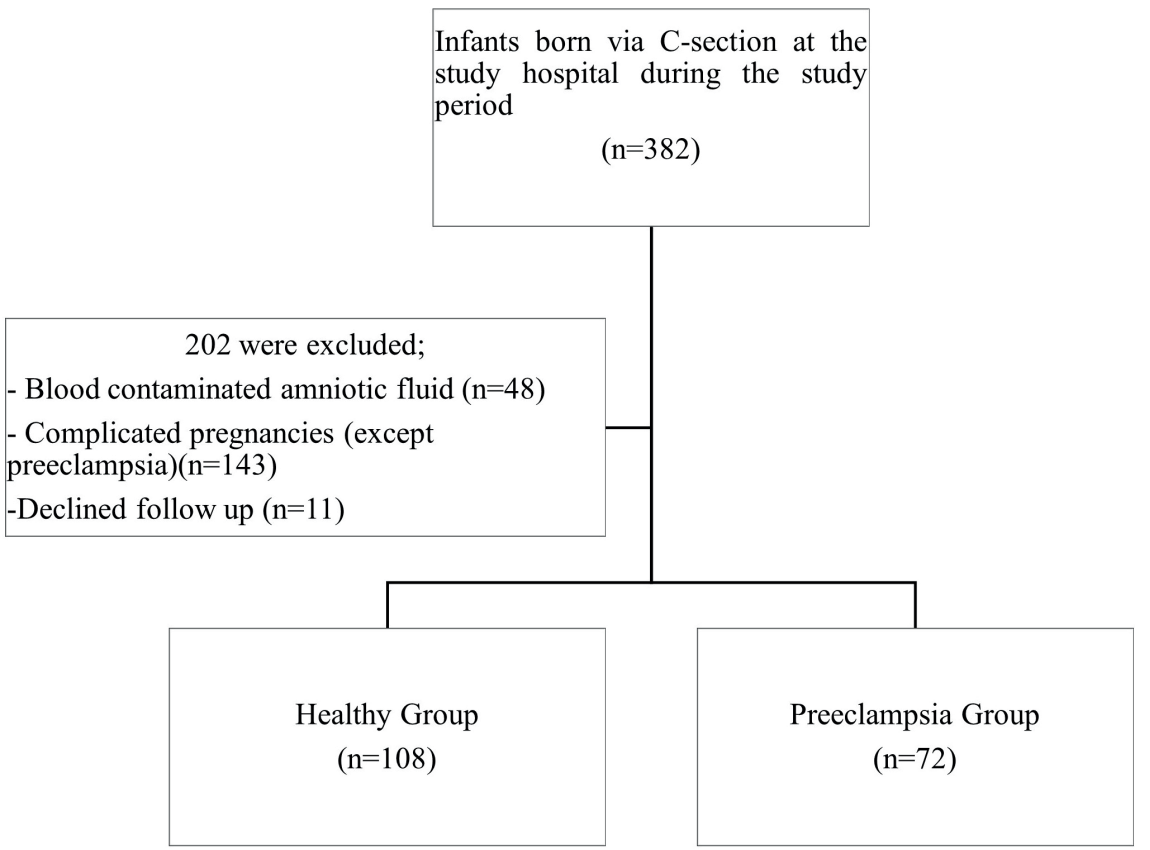


Fig. 1. Flow diagram of the study.

Table I. Comparison of the case and control groups for demographic features and neonatal morbidities.

		Preeclampsia (-) (n=108)		Preeclampsia (+) (n=72)		p
		Mean±SD/n-%	Median	Mean±SD/n-%	Median	
Maternal Age			28.0		27.5	0.288
Sex	Female	53	47.3%	31	43.1%	0.422
	Male	55	49.1%	41	56.9%	
Birth weight (g)			3173		2385	<0.001
Gestational age		37.2 ± 2.3		34.4 ± 3.8		<0.001
Hospitalization		26	23.2%	34	47.2%	0.001
Antenatal steroid		39	34.8%	27	37.5%	0.089
RDS		7	6.3%	19	26.4%	<0.001
TTN		18	16.1%	18	25.0%	0.136
NEC		3	2.7%	13	18.1%	<0.001
Sepsis		14	12.5%	27	37.5%	<0.001
Prematurity		33	29.5%	49	68.1%	<0.001
IVH		1	0.9%	4	5.6%	0.158
IUGR		4	3.6%	10	13.9%	0.043

Mann-Whitney u test / t test / Chi-square test
SD: standard deviation, RDS: respiratory distress syndrome, TTN: transient tachypnea of newborn,
NEC: necrotizing enterocolitis, IVH: intraventricular hemorrhage, IUGR: intrauterine growth restriction

Table II. Comparison of the pH and electrolyte values between case and control groups.

	Preeclampsia (-) (n=108)		Preeclampsia (+) (n=72)		p
	Mean±SD/n-%	Median	Mean±SD/n-%	Median	
pH	7.1 ± 0.1		7.2 ± 0.1		0.007
pC02 (mmHg)	42.6 ± 9.3		39.9 ± 9.2		0.054
HC03 (mmol/l)	13.9 ± 3.4		15.0 ± 5.5		0.955
Lactate (mmol/l)		7.1		7.4	0.588
Na (mEq/l)	118.1 ± 5.5		121.7 ± 5.9		<0.001
K (mEq/l)	4.1 ± 0.5		4.3 ± 0.7		0.019
Cl (mEq/l)	105.3 ± 5.5		103.8 ± 6.9		0.294
Ca (mmol/l)	1.1 ± 0.2		1.1 ± 0.2		0.076

Mann-Whitney u test / t test / Chi-Square test
SD: standard deviation, C02: carbon dioxide, HC03: bicarbonate,
Na: sodium, K: potassium, Cl: chlorine, Ca: calcium

The birth weight of the EOP group was lower than the control group (p 0.031) (Table III). In the EOP group, AF pH and K were statistically significantly higher than the control group (p 0.015, 0.036) (Table V).

Discussion

This study is unique as reveals that Na and pH values of AF might be predictors of PE in association with neonatal morbidities. In utero stress caused by PE was shown to be associated with epigenetic regulations on fetal DNA, amniotic epithelial cells and stromal cells via methylation changes; promising that amniotic membranes as a surrogate fetal tissue for the prediction of adverse intrauterine conditions.[7] However, there are limited studies inquiring about the effects of PE on AF content and neonatal morbidities.[8-10] Recently AF Na was proven to be related to respiratory morbidities in neonates.[6] Fetal urine production, lung liquid

Table III. Logistic regression analyses of data.

	Univariate Model					Multivariate Model				
	OR	%95 CI			p	OR	%95 CI			p
Maternal age	0.983	0.94	-	1.03	0.47					
Sex (Female/Male)	1.28	0.70	-	2.32	0.42					
Birth weight (g)	1.00	1.00	-	1.00	<0.001					
	0.72	0.70	-	0.82	<0.001	0.80	0.67	-	0.88	<0.001
Gestational week										
Hospitalization	2.96	1.56	-	5.60	0.001					
	5.17	1.77	-	15.06	0.003					
Antenatal steroid										
RDS	5.38	2.13	-	13.59	<0.001					
TTN	1.74	0.84	-	3.63	0.14					
NEC	8.01	2.19	-	29.22	0.002					
Sepsis	4.20	2.01	-	8.77	<0.001					
Prematurity	5.10	2.69	-	9.68	<0.001					
IVH	6.53	0.71	-	59.64	0.10					
IUGR	3.37	0.98	-	11.66	0.05					
pH	47.03	3.60	-	620	0.01	23.01	1.34	-	394.2	0.03
pC02 (mmHg)	0.97	0.94	-	1.00	0.06					
HC03 (mmol/l)	1.06	0.98	-	1.16	0.16					
p02 (mmHg)	1.00	1.00	-	1.01	0.30					
Lactate (mmol/l)	0.95	0.81	-	1.20	0.77					
Na (mEq/l)	1.11	1.04	-	1.17	<0.001	1.10	1.03	-	1.15	0.01
K (mEq/l)	1.92	1.13	-	3.31	0.01					
Cl (mEq/l)	0.97	0.90	-	1.02	0.13					
Ca (mmol/l)	0.36	0.08	-	1.97	0.27					

Logistic Regression
SD: standard deviation, OR: odds ratio, CI: confidence interval,
RDS: respiratory distress syndrome, TTN: transient tachypnea of newborn, NEC: necrotizing enterocolitis,
IVH: intraventricular hemorrhage, IUGR: intrauterine growth restriction, C02: carbon dioxide, HC03: bicarbonate,
Na: sodium, K: potassium, Cl: chlorine, Ca: calcium

secretion, swallowing and intramembranous absorption are the four main mechanisms of AF volume regulation.[2] The results of the studies investigating the effects of PE on neonatal morbidities are conflicting. However, it is known that PE is a syndrome, which causes increased inflammation and distress for both the fetus and mother. It was demonstrated that in the AF of preeclamptic mothers' vasoconstrictor mediators, such as endothelin is elevated in content, whereas the content of vasodilators, like nitric oxide is diminished.[11] As a result of this, disrupted placental perfusion in PE was proven to be related to fluctuations in oxygen levels, leading to oxidative stress in AF.[12] As a response to that increased oxidative stress caused by PE, oxygen radical absorbing capacity and Coenzyme Q10 levels of AF were pointed to be in relation to adverse neonatal outcomes including IUGR, preterm labor and preterm premature rupture of membranes due to intraamniotic infection.[13] Depending on those findings, PE is thought to own a two-sided reflection of its pathologies. One

Table IV. Comparison of the demographic features and neonatal morbidities between early onset preeclampsia and control group.

		Early onset preeclampsia (-) (n=15)			Early onset preeclampsia (+) (n=32)			p
		mean±SD/n-%			mean±SD/n-%			
Maternal age		28.8	±	6.8	28.8	±	5.5	0.99
Sex	Female	6		40%	15		20.80%	0.66
	Male	9		60%	17		23.60%	0.74
Birth weight (g)		2057	±	709.0	1044	±	728	0.031
Gestational week		32.4	±	2.0	31.9	±	2.6	0.54
Antenatal steroid		5.0		33%	12		37.50%	0.97
RDS		6.0		40%	18		56.30%	0.3
MV/day		1.5	±	3.9	4.14	±	7.1	0.035
02/day		4.9	±	14	16.51	±	26.57	0.012
BPD		1.0		7%	8		11.10%	0.1
NEC		3.0		20%	12		37.50%	0.2
Sepsis		6.0		40%	24		75%	0.021
IVH		1.0		6.60%	4		12.50%	0.55
IUGR		2.0		13.30%	10		31.30%	0.011

Mann-Whitney u test / t test / Chi-Square test
Standard deviation, RDS: respiratory distress syndrome, BPD: bronchopulmonary dysplasia,
NEC: necrotizing enterocolitis, MV: mechanical ventilation, 02: oxygen, IVH: intraventricular hemorrhage,
IUGR: intrauterine growth restriction

Table V. Comparison of the amniotic fluid pH and electrolyte values between early onset preeclampsia and control group.

	Early onset preeclampsia (-) (n=15)	Early onset preeclampsia (+) (n=32)	p
	mean±SD/n-%	mean±SD/n-%	
pH	7.1 ± 0.2	7.3 ± 0.2	0.015
pC02 (mmHg)	43.9 ± 4.9	40.5 ± 5.3	0.060
HC03 (mmol/l)	13.9 ± 3.4	15.0 ± 5.5	0.960
Lactate (mmol/l)	7.3 ± 2.3	7.2 ± 1.9	0.600
Na (mEq/l)	120.4 ± 6.2	123.2 ± 6.3	0.169
K (mEq/l)	4.2 ± 0.4	4.5 ± 0.6	0.036
Cl (mEq/l)	105.3 ± 5.5	103.8 ± 6.9	0.294
Ca (mmol/l)	1.1 ± 0.2	1.1 ± 0.2	0.076

Mann-Whitney u test / t test / Chi-Square test
SD: standard deviation, C02: carbon dioxide,
HC03: bicarbonate, Na: sodium, K: potassium, Cl: chlorine, Ca: calcium

is on the AF, which acts as a protective tissue for the fetus by re-organizing its antioxidant and secretory capacity, leading the changes in its content. The other reflection is on the fetus and later on the neonate, who is considered to be negatively affected by the oxidative stress and inflammation caused by PE. Therefore, higher hospitalization rate, RDS, NEC, sepsis, prematurity, IUGR, that were observed in the PE group of the study, are compatible with the

recent works concerning the topic/condition.[8-10] In addition, higher mechanical ventilation span, duration of 0_2 therapy, sepsis and IUGR that were detected in EOP group are supportive of this data.

Considering the first effect of PE on the fetus is decreased fetal urine output, for the findings of AF content, as a major content of AF, decreased disproportion of acidotic fetal urine in AF may explain elevated pH in PE group. Although antioxidant enzymes like catalase, glutathione reductase levels were reported to be decreased in AF of preeclamptic pregnancies, prooxidant enzymes such as xanthine oxidase were found to be elevated.[14] Therefore, this precipitous prooxidant capacity of AF can be a result of the inflammation caused by PE and it may be associated with higher AF pH levels in the PE group. Supporting this finding, Banadakoppa et al.[15] proved that especially alternative complement pathway complement activation products in AF at early pregnancy is linked to later development of EOP. Furthermore, higher Na^+ levels in the PE group may be related to increased Na^+ excretion from fetal kidneys that were affected in variable degrees by PE. Otherwise, maternal high serum potassium levels during the first half of pregnancy are demonstrated to be associated with a higher risk for the development of PE.[16] Even before the apparent clinical symptoms of PE, elevated AF K^+ levels, at the second trimester, is proven to be a predictor of PE.[17] Considering AF K^+ levels are correlated with maternal K^+ levels, in present study higher AF K^+ levels in the EOP group may be a result of PE. This also may be a result of the dysregulation of angiotensin converting enzyme (ACE) 2 pathway which is proven to have a role in microvascular endothelial dysfunction of PE.[18] The elevated early onset sepsis rate in the EOP group may also be associated with the endothelial impairment caused by PE.

With all these findings, it is possible to conclude that although the etiopathogenesis of PE and its effect on neonatal morbidities are not completely clarified, AF compositional changes before the development of clinical symptoms may be early biomarkers for both.

Nevertheless, the present study has a few limitations such as a restricted number of cases and lower diagnosis-sensitive numbers for participants with RDS and BPD, etc. Although we tried to choose the subgroups that were similar in terms of gestational age. We were not able to exclude all the factors which might have affected the results.

To the best of our knowledge, this is the first study that reported the value of AF electrolyte analysis for the prediction of PE and neonatal morbidities in term and preterm infants. However, more studies including a larger number of infants are required to confirm the role of AF analysis to predict PE and/or neonatal morbidities. Furthermore, this will create a need for more detailed studies for a better understanding of the molecular mechanisms underlying those changes in AF content and their relation to the maternal-fetal conditions.

Ethical approval

The ethics committee of Kanuni Sultan Suleyman Training and Research Hospital approved the study (Ethics Committee approval number: KAEK//2015.7.7).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SYS, MC; data collection: IME, OSG, SYS; analysis and interpretation of results: SYS, MC, BY; draft manuscript preparation: SYS, MC, BY, IME, OSG. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

None of the authors have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript.

Conflict of interest

The authors have no conflicts of interest to disclose.

REFERENCES

1. Underwood MA, Gilbert WM, Sherman MP. Amniotic fluid: not just fetal urine anymore. J Perinatol 2005; 25: 341-348.
2. Brace RA, Cheung CY. Regulation of amniotic fluid volume: evolving concepts. Adv Exp Med Biol 2014; 814: 49-68.
3. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol 2013; 209: 544.e1-544.e12.
4. Pennington KA, Schlitt JM, Jackson DL, Schulz LC, Schust DJ. Preeclampsia: multiple approaches for a multifactorial disease. Dis Model Mech 2012; 5: 9-18.
5. Shanmugalingam R, Hennessy A, Makris A. Aspirin in the prevention of preeclampsia: the conundrum of how, who and when. J Hum Hypertens 2019; 33: 1-9.
6. Yılmaz Semerci S, Yücel B, Erbas IM, Gunkaya OS, Talmac M, Çetinkaya M. The utility of amniotic fluid pH and electrolytes for prediction of neonatal respiratory disorders. J Matern Fetal Neonatal Med 2020; 33: 253-257.
7. Suzuki M, Maekawa R, Patterson NE, et al. Amnion as a surrogate tissue reporter of the effects of maternal preeclampsia on the fetus. Clin Epigenetics 2016; 8: 67.
8. Hansen AR, Barnés CM, Folkman J, McElrath TF. Maternal preeclampsia predicts the development of bronchopulmonary dysplasia. J Pediatr 2010; 156: 532-536.
9. Cetinkaya M, Ozkan H, Koksal N. Maternal preeclampsia is associated with increased risk of necrotizing enterocolitis in preterm infants. Early Hum Dev 2012; 88: 893-898.
10. Ozkan H, Cetinkaya M, Koksal N. Increased incidence of bronchopulmonary dysplasia in preterm infants exposed to preeclampsia. J Matern Fetal Neonatal Med 2012; 25: 2681-2685.
11. Pogorelova TN, Krukier II, Gunko VO, Nikashina AA, Alliluev IA, Larichkin AV. The imbalance of vasoactive components and arachidonic acid in the placenta and amniotic fluid in preeclampsia. Biomed Khim 2019; 65: 245-250.
12. Bilodeau JF. Review: maternal and placental antioxidant response to preeclampsia- impact on vasoactive eicosanoids. Placenta 2014; 35(Suppl): S32-S38.
13. Giannubilo SR, Tiano L, Ciavattini A, et al. Amniotic coenzyme Q10: is it related to pregnancy outcomes? Antioxid Redox Signal 2014; 21: 1582-1586.
14. Pogorelova TN, Gunko VO, Nikashina AA, Mikhelson AA, Alliluev IA, Mikhelson AM. Disbalance of redox processes in amniotic fluid at pre-eclampsia. Klin Lab Diagn 2018; 63: 483-489.
15. Banadakoppa M, Vidaeff AC, Yallampalli U, Ramin SM, Belfort MA, Yallampalli C. Complement split products in amniotic fluid in pregnancies subsequently developing early-onset preeclampsia. Dis Markers 2015; 2015: 263109.
16. Wolak T, Sergienko R, Wiznitzer A, Ben Shlush L, Paran E, Sheiner E. Low potassium level during the first half of pregnancy is associated with lower risk for the development of gestational diabetes mellitus and severe pre-eclampsia. J Matern Fetal Neonatal Med 2010; 23: 994-998.
17. Fotiou M, Michaelidou AM, Masoura S, et al. Second trimester amniotic fluid uric acid, potassium, and cysteine to methionine ratio levels as possible signs of early preeclampsia: a case report. Taiwan J Obstet Gynecol 2016; 55: 874-876.
18. Stanhewicz AE, Alexander LM. Local angiotensin-(1-7) administration improves microvascular endothelial function in women who have had preeclampsia. Am J Physiol Regul Integr Comp Physiol 2020; 318: R148-R155.

The Turkish Journal of Pediatrics 2021; 63: 875-883
https://doi.org/10.24953/turkjped.2021.05.015

Original Article

Evaluation of retinal nerve fiber layer and choroidal thickness with spectral domain optical coherence tomography in children with sickle cell anemia

Kamil Yılmaz[1], Hasan Öncül[2], Hülya Uzel[3], Kahraman Öncel[4], E. Deniz Yılmaz[5], Murat Söker[3]

Departments of [1]Pediatrics Infectious Diseases, [3]Pediatric Hematology and [5]Pathology, Dicle University Faculty of Medicine, Diyarbakır; Departments of [2]Ophthalmology and [4]Pediatric Hematology, University of Health Sciences Gazi Yaşargil Education Research Hospital, Diyarbakır, Turkey.

ABSTRACT

Background. The aim of this study is to examine the thickness of choroidal, macular and peripapillary retinal nerve fiber layer by spectral-domain optical coherence tomography (SD-OCT) in pediatric patients with sickle cell anemia (SCA) without retinopathy.

Methods. A total of 75 children (30 SCA patients (Group 1) and 45 healthy individuals (Group 2) were included in the study. Macular (central, superior, inferior, nasal, temporal), choroidal (subfoveal, at nasal distances from the central fovea of 1000 μm [N1], 2000 μm [N2], 3000 μm [N3], at temporal distances from the central fovea of 1000 μm [T1], 2000 μm [T2], 3000 μm [T3]) and RNFL (average, temporal, superotemporal, inferotemporal, nasal, inferonasal and superonasal) measurements were performed by SD-OCT. These parameters were compared with healthy children with similar demographic characteristics.

Results. The mean age was 14.11±3.86 (11-18) in sickle cell anemia patients and 13.15± 2.69 (10-18) in the healthy control group. Of the patients, 56.6% (n=17) of Group 1 and 44.4% (n=20) of Group 2 were male. Choroidal measurements made in the subfoveal, N1, N2, N3, T1, T2 and T3 quadrants showed that the choroid was thinner in 6 quadrants in SCA patients compared to the healthy group ($p = 0.003$, $p = 0.039$, $p = 0.035$, $p = 0.595$, $p = 0.006$, $p = 0.005$, $p = 0.047$, respectively). In RNFL measurements, there was significant thinning in the temporal, inferotemporal, and nasal quadrants of SCA patients compared to the healthy group. Changes in other quadrants were not significant.

Conclusions. SD-OCT is a useful imaging method in the diagnosis and screening in patients with SCA without retinopathy. Early diagnosis of retinopathy during subclinical disease will prevent visual loss in these patients.

Key words: choroidal thickness, retinal nerve fiber layer, sickle cell anemia, spectral-domain optical coherence tomography

Sickle cell anemia (SCA) is one of the most common hemoglobinopathies around the world. Hb S, abnormal hemoglobin, is formed as a result of glutamic acid replacing valine at position 6 of the globin chain. In this disease showing autosomal recessive inheritance, the term SCA is used for patients with homozygous Hb S, while the coinheritance of Hb S with other hemoglobin variants is called the sickling syndromes. The frequency of the SCA trait is 0.3-0.6% throughout Turkey, while this rate reaches 3-44% in some parts of the Cukurova region.[1]

Pathologies that occur in SCA target many organs and tissues. The pathogenesis of the disease includes anemia caused by chronic hemolysis, vascular damage, and organ and tissue ischemia due to a defect in blood flow.[2] The ocular manifestations of the disease may be observed

✉ Murat Söker
sokerm@hotmail.com

Received 18th August 2020, revised 28th November 2020, 23rd December 2020, accepted 29th January 2021.

in a wide spectrum ranging from the orbit to the retina. While anterior segment findings are frequently seen as comma-shaped vessels in the conjunctiva, cataract and iris atrophy may also develop in these patients.[3] Retinal vascular lesions observed in the posterior segment often occur secondary to neovascularization and ischemia in the peripheral retina. Patients are classified as proliferative or nonproliferative according to the presence of posterior segment neovascularization.[4] Visual loss usually occurs in the proliferative stage, in which vitreous hemorrhage, epiretinal membrane, and retinal detachment are observed.[5] However, in patients not progressing to the proliferative stage, decreased retinal sensitivity may be observed in addition to subclinical sectoral thinning in the peripapillary retinal nerve fiber layer (RNFL) and thinning in the fovea by spectral-domain optical coherence tomography (SD-OCT).[6,7] Besides, these patients may experience decreased vision due to pathologies such as abnormal perfusion-related macular infarction and ischemic optic neuropathy.[8]

Ocular structures such as RNFL and choroid with intense vascularization are expected to be affected in SCA, which is a disease presenting with hypoxia due to vascular occlusion and chronic anemia. Possible disorders in these structures may result in decreased visual function. The aim of this study was to evaluate RNFL, macular, and choroidal thickness measurements by SD-OCT in patients with SCA and compare these data with a demographically similar healthy group.

Material and Methods

This observational prospective clinical study was conducted between July 2017 and December 2019 after being approved by the ethics committee of Dicle University Faculty of Medicine (23.06.2017- report number:24). The study was conducted in accordance with the Helsinki Declaration, and a written informed consent form was obtained from all participants.

A total of 75 children (30 SCA patients (Group 1), 45 healthy individuals (Group 2) were included in the study, and both eyes of the participants were evaluated. The mean values of both eyes were recorded in the database. Group 1 consisted of children aged 11-18 years who were diagnosed with homozygous Hb SS (sickle cell anemia), whose parents were Hb S carriers, with a Hb S level > 40% and no Hb A according to the hemoglobin electrophoresis results. Group 2 consisted of healthy children aged 10-18 years without any chronic diseases. In Group 1, the ferritin level, mean number of vaso-occlusive painful crises per year, and blood transfusion requirement were determined by scanning patient files. Disease duration was determined as the period between diagnosis and study time. Exclusion criteria included the best-corrected visual acuity below 20/20, refractive error more than ± 1 D, presence of corneal pathology, presence of retinal/choroidal pathology other than SCA, intraocular pressure more than 21 mmHg, glaucomatous optic disc changes, axial length more than 24 mm, previous ocular trauma or surgery. Those with chronic additional systemic diseases, those unable to adapt to ophthalmologic examination, and those under 10 years of age were excluded from the study.

All participants underwent detailed ophthalmologic examination including refraction, cycloplegic refraction, best-corrected visual acuity, biomicroscopic examination, dilated fundus examination (via 90 D lens), intraocular pressure measurement (Reichert R7 non-contact tonometer, Reichert, USA), axial length measurement (AL-Scan Optical Biometer; Nidek, Gamagori, Japan) and central corneal thickness measurement (Pentacam® HR, OCULUS, Wetzlar, Germany) by the same clinician. SD-OCT (Heidelberg Engineering, Heidelberg, Germany) measurements were performed by the same clinician between 10.00-11.00 a.m. so that the measurements were not affected by diurnal variations.

Macular thickness was measured automatically in the central, superior, inferior, temporal,

and nasal quadrants using the ready-made package program of the instrument. RNFL measurements were performed automatically in 7 quadrants including the average, superonasal, superotemporal, nasal, inferonasal, inferotemporal, and temporal quadrants. Choroidal thickness was measured manually using the enhanced depth imaging OCT (EDI-OCT) mode of the instrument. The interface of the Bruch membrane was considered the anterior edge of the choroid, and the sclerochoroidal interface was considered the posterior border of the choroid. Choroidal thickness was measured in 7 different regions including the subfoveal region, nasal distances from the central fovea of 1000 μm [N1], 2000 μm [N2], 3000 μm [N3], temporal distances from the central fovea of 1000 μm [T1], 2000 μm [T2], 3000 μm [T3].

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS), Version 24.0 for Windows. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simonov test) whether or not they were normally distributed. Dats are presented as mean ± standard error. The chi-square test was used to compare these proportions in different groups. Comparisons between groups were performed using the Student's t-test. P values lower than 0.05 was considered statistically significant.

Results

A total of 75 children, 30 SCA patients (Group 1), and 45 healthy individuals (Group 2), were included in the study. The mean age was 14.11±3.86 (11-18) in sickle cell anemia patients and 13.15±2.69 (10-18) in the healthy control group. Of the patients, 56.6% (n=17) of Group 1 and 44.4% (n=20) of Group 2 were male. No difference was found between the groups in terms of age and gender distribution (p = 0.053, p=0.550, respectively). The demographic and ocular characteristics of the participants are shown in Table I.

The mean Hb S level was 59.99±8.44 g/dL, and the mean ferritin level was 470.3±79.4 μg/L in Group 1. Other clinical and laboratory characteristics of the patients with SCA are shown in Table II.

7 different choroidal thickness measurements made in the subfoveal, nasal and temporal quadrants (subfoveal, N1, N2, N3, T1, T2, T3) showed that the choroid was thinner in 6 quadrants in SCA patients compared to the healthy group (p = 0.003, p = 0.039, p = 0.035, p = 0.595, p = 0.006, p = 0.005, p = 0.047, respectively) (Table III). There was no statistical difference between the two groups in terms of macular thickness measurements (Table IV). RNFL analysis was performed in 7 quadrants (average, temporal, superotemporal, inferotemporal, nasal, inferonasal and superonasal). There was significant thinning in the temporal,

Table I. Baseline characteristics of sickle cell anemia patients and healthy controls.

Parameters	Sickle cell anemia (n=30)	Healthy controls (n=45)	p
Age (years)			
(Mean±SD /(Median age))	14.11±3.86(15.50)	13.15±2.69(13.00)	0.053
Gender (M/F)	17/13	20/25	0.550
Ocular blood pressure			
(Mean±SD)	12.94±0.82	12.88±1.34	0.876
Central corneal thickness(μm)			
(Mean±SD)	522.13±9.85	518.57±8.65	0.187
Axial length(mm)			
(Mean±SD)	24.96±5.61	23.65±2.90	0.239

M: male, F: female, SD: standard deviation

Table II. Clinical and laboratory characteristics of patients with sickle cell anemia.

Parameters	Mean±SD
HbS (g/dL)	59.99±8.44
Serum ferritin (μg/L)	470.33±79.4
Number of transfusions[a]	1.44±0.38
Number of crises[b]	2.72±1.52
Disease duration (year)[c]	11.27±4.88

[a]: the number of blood transfusions per year
[b]: the number of crises per year
[c]: time from being diagnosed with sickle cell anemia to study.

inferotemporal and nasal quadrants of SCA patients compared to the healthy group. Changes in other quadrants were not significant (p = 0.665, p = 0.043, p = 0.230, p = 0.018, p = 0.013, p = 0.706, p = 0.631, respectively) (Table V).

The number of annual crises was found to be positively correlated with the ferritin level and frequency of transfusion (p =0.017, p = 0.006, respectively). However, no correlation was found between the number of annual crises and choroidal thickness, macular thickness, and RNFL measurements.

Table III. Choroidal thickness values in sickle cell anemia patients and healthy controls.

Choroidal thickness (μm)	Sickle cell anemia (n:30) mean±SD	Healthy controls (n:45) mean±SD	p
Subfoveal	297.11±40.77	337.59±44.41	0.003
Nasal 1000	253.52±50.10	285.40±48.63	0.039
Nasal 2000	202.72±48.48	236.61±52.95	0.035
Nasal 3000	155.66±38.37	162.33±42.51	0.595
Temporal 1000	274.69±31.26	308.51±42.10	0.006
Temporal 2000	244.66±38.42	278.33±37.23	0.005
Temporal 3000	213.97±27.55	238.22±51.48	0.047

SD: standard deviation

Table IV. Macular thickness values in sickle cell anemia patients and healthy controls.

Macular thickness (μm)	Sickle cell anemia (n:30) mean±SD	Healthy controls (n:45) mean±SD	p
Central	258.55±14.84	259.59±19.39	0.849
Superior quadrant	345.05±10.44	344.38±9.26	0.823
Inferior quadrant	345.50±10.19	340.07±11.42	0.111
Temporal quadrant	330.30±11.27	327.66±10.52	0.428
Nasal quadrant	344.41±9.05	341.59±12.18	0.406

SD: standard deviation

Table V. RNFL thickness values in sickle cell anemia patients and healthy controls.

RNFL thickness (μm)	Sickle cell anemia (n:30) mean±SD	Healthy controls (n:45) mean±SD	p
Average	106.05±10.49	104.79±8.78	0.665
Temporal quadrant	75.05±8.30	69.72±8.44	0.043
Superotemporal quadrant	145.38±18.59	139.27±14.97	0.230
Inferotemporal quadrant	155.50±23.36	139.59±19.70	0.018
Nasal quadrant	73.22±16.57	84.92±13.72	0.013
Inferonasal quadrant	132.61±28.93	129.61±23.83	0.706
Superonasal quadrant	124.44±25.27	121.12±20.56	0.631

RNFL: retinal nerve fiber layer, SD: standard deviation

Discussion

The changes caused by SCA in the peripheral retina and macula have been known for a long time. Early histopathological changes have shown that patients with SCA develop thinning and atrophy in the inner retina, inner nuclear, and ganglion cell layers.[9-11] Retinal thinning is chronic and has a progressive course in patients with SCA.[12] Thinning in the temporal macula has been reported to occur in early childhood.[13] The researchers reported that macular thinning was associated with perifoveal flow defect and peripheral neovascularization.[14,15] Observing disorders such as microaneurysm in macula and an increase in the foveal avascular zone, especially in the temporal region, has been associated with terminal vessels in this region being more sensitive to occlusion.[16] The development of proliferative retinopathy is associated with the presence of temporal thinning in these patients, but it should be noted that even two-thirds of these patients may be asymptomatic.[11]

While homozygous hemoglobin S disease (Hb SS) is associated with more severe clinical symptoms in patients with SCA, Hb SC disease has been reported to be associated with more severe and earlier retinal disease.[2,17] Lim et al.[18] stated that patients with the Hb SC genotype were more prone to developing proliferative retinopathy, but macular thinning was observed more in Hb SS patients. More vaso-occlusive events were observed occuring in Hb SS patients; however, it was noted that proliferative retinopathy was paradoxically less developed in these patients. However, Cai et al.[12] did not detect any differences between Hb SS and Hb SC in terms of macular thinning. In our study, all of our patients were Hb SS, and Hb SC was not detected in our patient group.

Indirect ophthalmoscopy is usually the first line for the identification of signs of retinopathy but is dependent on operator experience and deep knowledge of the disease. Retinopathy can be detected in 10% of cases with standard fundus examination performed with a 90 D lens after dilatation. On the other hand, more sensitive methods such as wide-field fluorescein angiography (FA), SD-OCT, and optical coherence tomography angiography (OCTA) enable the early detection of sickle cell retinopathy. These imaging methods guide the formation of screening and treatment algorithms as well as a better understanding of the pathogenesis of the disease.

Minvielle et al.[19] showed microvascular abnormalities in the perifoveal and macular areas by FA in half of the patients without visual impairment and stated that this could be explained by capillary filling defects in the intermediate and deep plexuses. Another study reported that macular thinning areas on SD-OCT were associated with the degree of peripheral ischemia on wide-field FA.[13] However, many studies stated that there could be no findings on FA.[9-11] In the present study, none of the patients had retinal pathology that would require FA.

OCT Angiography is a new imaging technique that enables retinal vascular pathologies to be examined in more detail. The vascular loss was found to be the same in both genotypes as a result of evaluations by OCTA in patients with SCA.[20] However, in patients with proliferative retinopathy, vascular defects were observed to be higher in the deep plexus in the parafoveal, temporal, and nasal regions. Studies with OCTA demonstrated that vascular defects in retinal areas were associated with thinning in these areas. Han et al.[9] reported that retinal thickness measurements were correlated with foveal, parafoveal, superior and temporal vascular density, while visual acuity was correlated with foveal avascular zone, parafoveal vascular density in the superficial and deep plexuses. In the present study, we did not have the opportunity to perform OCTA to our patients. However, studies have revealed pathologies correlated with SD-OCT in the perfusion defect areas detected by OCTA.[21,22] Grego et al.[22] did not detect additional flow gap areas other than macular thinning identified by SD-OCT in OCTA, and stated that the SD-OCT findings

supported the OCTA findings in patients with SCA.

SD-OCT is a non-invasive, reproducible, and easily applicable imaging method. This method allows us to have an idea about possible macular, retinal, and RNFL changes that may occur as a result of ischemia and neovascularization in the retinal layers. Han et al.[9] showed that approximately 50% of eyes with SCA developed focal macular thinning without clinically significant maculopathy on SD-OCT. Besides subclinical foveal thinning and splaying, thinning in the outer retinal layer in the central, foveal temporal and parafoveal regions have been reported in patients without significant focal thinning.[21] Martin et al.[23] determined that 64% of SCA patients in early childhood had atrophy in the paramacular temporal region. The researchers linked this to chronic perfusion disorder in relation to the severity of the disease and stated that it could be used as a marker in other possible systemic complications. The choroid is the tissue with the highest rate of blood flow per volume in the body. The choroid, which has a rich vascular network, consists of melanocytes, nerves, extracellular fluid, and connective tissue. With the development of imaging methods, the choroid and retinal layers could be examined in more detail, and it became possible to gain information about retinal and choroidal changes in systemic and ocular diseases.[15] EDI-OCT is an imaging method that enables measuring choroidal thickness thanks to the choroid sections it provides. Although these measurements are not precise, it allows us to have information about blood flow to choroidal tissue.[24]

In the present study, choroidal thickness was evaluated in detail in 7 different quadrants. Choroidal thinning was observed in 6 quadrants in SCA patients compared to the healthy group. Choroidal thinning in these patients can be explained by slower flow and sickling of red blood cells in the choriocapillaris. In addition, anemia observed in these patients results in choroidal blood flow changes by leading to systemic vasoconstriction and cardiac output changes and may cause a decrease in choroidal thickness.

Grego et al.[22] reported that complications such as retinopathy and maculopathy are associated with hemolysis indices such as low hemoglobin and hematocrit rates, high reticulocyte percentage, and high total bilirubin levels. Vatansever et al.[25] stated that there was no difference in foveal flattening and temporal thinning between with and without a history of sequestration crisis in patients with SCA. In our study, no relationship was found between the number of crisis and macular thickness. However, we found that choroidal thickness and RNFL values were not related to the number of crisis. We think that this is because the main factor causing changes in the choroid and RNFL are parameters such as blood flow, anemia and hypoxia rather than the number of crisis.

RNFL is considered as a marker in the evaluation of retinal ganglion cell (RGC) functions. RGC plays an important role in the transmission of the visible image to the brain.[26] Vaso-occlusive changes leading to macular thinning in patients with SCA are likely to cause changes in peripapillary RNFL. It has been reported that peripapillary RNFL thinning may occur in ischemic retinopathy types such as diabetes and artery/vein occlusions.[27,28] An adult study reported that thinning was observed in the nasal and inferior quadrants in the case of Fe deficiency.[29] None of our patients had Fe deficiency, so this factor was not a confounding factor in this study. The degree of peripapillary RNFL thinning may related to the severity of macular thinning. Chow et al.[30] observed a significant thinning in the peripapillary RNFL of SCA patients with focal macular thinning compared to those without focal macular thinning. RNFL thinning was also observed in patients without focal macular thinning, but this difference was much less compared to those with focal macular thinning. On the other hand, Brasileiro et al.[10] did not

observe RNFL thinning in adult SCA patients without retinopathy compared to the control group. These changes in RNFL values of SCA patients pose a new problem. When performing RNFL analysis, these patients require different peripapillary RNFL thickness thresholds for glaucoma evaluations. Clinicians should be more careful when diagnosing glaucoma in these patients.

In the present study, SCA patients had significant RNFL thinning in the temporal, inferotemporal, and nasal quadrants compared to the healthy group. We think that these changes in peripapillary RNFL are associated with thinning and atrophy of macular inner retinal layers caused by perfusion defects. It should be kept in mind that RNFL losses in these patients may also be associated with hypomyelination, which occurs after nerve myelination and neurotransmitter synthesis defect.

This study had some limitations. Firstly, the number of patients is not enough, there is a need for a larger series of cases in this regard. The fact that cross-sectional examination was performed in these patients and that they were not ophthalmologically followed for a long time makes it difficult to have an idea about the progression of the disease. Long-term follow-up is needed to determine the relationship between the onset of disease symptoms and hematological parameters. A study of SCA children reported that proliferative disease developed in 43% of Hb SC patients and 14% of Hb SS patients in the 20 years.[2] Early diagnosis and treatment is the basis for preventing disease progression to the proliferative stage. The American Academy of Pediatrics recommends performing retinopathy screening in children for Hb SS and Hb SC by dilated fundoscopic examination, starting from 10 years of age.[31] Secondly, none of our patients underwent FA. However, it should be noted that FA is recommended only if there is a suspicious lesion in the fundus.[32] None of our patients had an indication for the FA application.

In the present study, thinning was observed in choroidal thickness and RNFL measurements, but there was no change in macular thickness in the evaluations of SCA patients by SD-OCT. The findings suggest that SD-OCT may be useful for the diagnosis and screening of retinopathy. Considering its widespread use and ease of image acquisition in pediatric populations, SD-OCT can be used more frequently in the screening examination of patients. Increasing awareness of the subclinical disease in this way will provide an opportunity for early identification of retinopathy and reducing possible visual loss.

Acknowledgement

We appreciate the University Medical School of Dicle for providing access to their patient data.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KY, HÖ; data collection: KY, HÖ, HU, KÖ; analysis and interpretation of results: KY, MS, EDY; draft manuscript preparation: KY, MS, HÖ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was conducted based on the rules of Declaration of Helsinki and approved by the Institutional Ethics Committee of Dicle University Faculty of Medicine. (23.06.2017-report number:24).

Source of funding

No funding was received from any source for this research.

Conflict of interest

The authors have no conflict of interests to declare. All the authors contributed to the study.

REFERENCES

1. Ünal S. Orak hücreli anemi tedavi ve izlem. Türk Hematoloji Derneği. HematoLog 2014; 4: 79-93.
2. Downes SM, Hambleton IR, Chuang EL, Lois N, Serjeant GR, Bird AC. Incidence and natural history of proliferative sickle cell retinopathy: observations from a cohort study. Ophthalmology 2005; 112: 1869-1875.
3. Fadugbagbe AO, Gurgel RQ, Mendonca CQ, Cipolotti R, dos Santos AM, Cuevas LE. Ocular manifestations of sickle cell disease. Ann Trop Paediatr 2010; 30: 19-26.
4. Elagouz M, Jyothi S, Gupta B, Sivaprasad S. Sickle cell disease and the eye: old and new concepts. Surv Ophthalmol 2010; 55: 359-377.
5. Moriarty BJ, Acheson RW, Condon PI, Serjeant GR. Patterns of visual loss in untreated sickle cell retinopathy. Eye (Lond) 1988; 2(Pt 3): 330-335.
6. Murthy RK, Grover S, Chalam KV. Temporal macular thinning on spectral-domain optical coherence tomography in proliferative sickle cell retinopathy. Arch Ophthalmol 2011; 129: 247-249.
7. Chow CC, Genead MA, Anastasakis A, Chau FY, Fishman GA, Lim JI. Structural and functional correlation in sickle cell retinopathy using spectral domain optical coherence tomography and scanning laser ophthalmoscope microperimetry. Am J Ophthalmol 2011; 152: 704-711.
8. Goldberg MF, Galinos S, Lee CB, Stevens T, Woolf MB. Editorial: macular ischemia and infarction in sickling. Invest Ophthalmol 1973; 12: 633-635.
9. Han IC, Tadarati M, Pacheco KD, Scott AW. Evaluation of macular vascular abnormalities identified by optical coherence tomography angiography in sickle cell disease. Am J Ophthalmol 2017; 177: 90-99.
10. Brasileiro F, Martins TT, Campos SB, et al. Macular and peripapillary spectral domain optical coherence tomography changes in sickle cell retinopathy. Retina 2015; 35: 257-263.
11. Hoang QV, Chau FY, Shahidi M, Lim JI. Central macular splaying and outer retinal thinning in asymptomatic sickle cell patients by spectral-domain optical coherence tomography. Am J Ophthalmol 2011; 151: 990-994.e1.
12. Cai CX, Han IC, Tian J, Linz MO, Scott AW. Progressive retinal thinning in sickle cell retinopathy. Ophthalmol Retina 2018; 2: 1241-1248.e2.
13. Ghasemi Falavarjani K, Scott AW, Wang K, et al. Correlation of multimodal imaging in sickle cell retinopathy. Retina 2016; 36(Suppl 1): S111-S117.
14. Do BK, Rodger DC. Sickle cell disease and the eye. Curr Opin Ophthalmol 2017; 28: 623-628.
15. Hood MP, Diaz RI, Sigler EJ, Calzada JI. Temporal macular atrophy as a predictor of neovascularization in sickle cell retinopathy. Ophthalmic Surg Lasers Imaging Retina 2016; 47: 27-34.
16. Stevens TS, Busse B, Lee CB, Woolf MB, Galinos SO, Goldberg MF. Sickling hemoglobinopathies; macular and perimacular vascular abnormalities. Arch Ophthalmol 1974; 92: 455-463.
17. Fox PD, Dunn DT, Morris JS, Serjeant GR. Risk factors for proliferative sickle retinopathy. Br J Ophthalmol 1990; 74: 172-176.
18. Lim WS, Magan T, Mahroo OA, Hysi PG, Helou J, Mohamed MD. Retinal thickness measurements in sickle cell patients with HbSS and HbSC genotype. Can J Ophthalmol 2018; 53: 420-424.
19. Minvielle W, Caillaux V, Cohen SY, et al. Macular microangiopathy in sickle cell disease using optical coherence tomography angiography. Am J Ophthalmol 2016; 164: 137-144.e1.
20. Mathew R, Bafiq R, Ramu J, et al. Spectral domain optical coherence tomography in patients with sickle cell disease. Br J Ophthalmol 2015; 99: 967-972.
21. Han IC, Tadarati M, Scott AW. Macular vascular abnormalities identified by optical coherence tomographic angiography in patients with sickle cell disease. JAMA Ophthalmol 2015; 133: 1337-1340.
22. Grego L, Pignatto S, Alfier F, et al. Optical coherence tomography (OCT) and OCT angiography allow early identification of sickle cell maculopathy in children and correlate it with systemic risk factors. Graefes Arch Clin Exp Ophthalmol 2020; 258: 2551-2561.
23. Martin GC, Albuisson E, Brousse V, de Montalembert M, Bremond-Gignac D, Robert MP. Paramacular temporal atrophy in sickle cell disease occurs early in childhood. Br J Ophthalmol 2019; 103: 906-910.
24. Spaide RF, Koizumi H, Pozzoni MC. Enhanced depth imaging spectral-domain optical coherence tomography. Am J Ophthalmol 2008; 146: 496-500.
25. Vatansever E, Vatansever M, Dinç E, Temel GÖ, Ünal S. Evaluation of ocular complications by using optical coherence tomography in children with sickle cell disease eye findings in children with sickle cell disease. J Pediatr Hematol Oncol 2020; 42: 92-99.
26. Mead B, Tomarev S. Evaluating retinal ganglion cell loss and dysfunction. Exp Eye Res 2016; 151: 96-106.

27. Lonneville YH, Ozdek SC, Onol M, Yetkin I, Gürelik G, Hasanreisoğlu B.The effect of blood glucose regulation on retinal nerve fiber layer thickness in diabetic patients. Ophthalmologica 2003; 217: 347-350.

28. Leung CK, Tham CC, Mohammed S, et al. In vivo measurements of macular and nerve fibre layer thickness in retinal arterial occlusion. Eye (Lond) 2007; 21: 1464-1468.

29. Akdogan E, Turkyilmaz K, Ayaz T, Tufekci D. Peripapillary retinal nerve fibre layer thickness in women with iron deficiency anaemia. J Int Med Res 2015; 43: 104-109.

30. Chow CC, Shah RJ, Lim JI, Chau FY, Hallak JA, Vajaranant TS.Peripapillary retinal nerve fiber layer thickness in sickle-cell hemoglobinopathies using spectral-domain optical coherence tomography. Am J Ophthalmol 2013; 155: 456-464.e2.

31. Section on Hematology/Oncology Committee on Genetics; American Academy of Pediatrics. Health supervision for children with sickle cell disease. Pediatrics 2002; 109: 526-535.

32. Habibi A, Arlet JB, Stankovic K, et al. French guidelines for the management of adult sickle cell disease: 2015 update. Rev Med Interne 2015; 36(5 Suppl 1): 5S3-5S84.

The Turkish Journal of Pediatrics 2021; 63: 884-892
https://doi.org/10.24953/turkjped.2021.05.016

Original Article

Can peripheral blood monocyte percentage and lymphocyte monocyte ratio at diagnosis predict survival in pediatric neuroblastoma patients?

Koray Yalçın[1], Gülen Tüysüz[1], Funda Tayfun Küpesiz[1], Selen Bozkurt[2], Alphan Küpesiz[1], Elif Güler[1]

Departments of [1]Pediatric Hematology/Oncology and BMT Unit and [2]Biostatistics and Medical Informatics, Akdeniz University Faculty of Medicine, Antalya, Turkey.

ABSTRACT

Background. Previous studies have shown that the immune system plays a critical role in cancer pathogenesis. The lymphocyte monocyte ratio (LMR) and monocyte percentage (MP) have been found to be prognostic factors in various types of adult cancers. But studies about pediatric tumors are scarce and to our knowledge, there are no studies evaluating the immune system effect in pediatric neuroblastoma patients. The aim of this study was to assess whether LMR and MP at diagnosis may have an effect on prognosis in neuroblastoma patients.

Methods. We retrospectively analyzed MP and LMR at diagnosis in 71 pediatric neuroblastoma patients treated between 2002 and 2016.

Results. The optimal cut-off values of LMR and MP were determined using the receiver operating characteristics curves (ROC) and area under the curve (AUC). We found that a low LMR (≤3.5) and a high MP (≥7.5%) were correlated with worse overall survival and shorter event-free survival in univariate analysis. Multivariate analysis revealed that elevated LMR was an independent factor for better OS and EFS.

Conclusions. In conclusion, LMR and MP might be valuable prognostic factors for predicting OS in neuroblastoma patients. Multicenter and prospective studies are warranted to confirm this hypothesis.

Key words: neuroblastoma, lymphocyte monocyte ratio, monocyte percentage, immune system.

The immune system has an important role on the outcome of cancer patients. It can prevent tumor outgrowth or conversely immune cells can help tumor outgrowth. Recent studies have shown that advanced cancer patients had a low lymphocyte count and this was associated with poor overall survival in various cancer types.[1-4] Monocytes also have a crucial role in tumor response. Inflammatory monocytes in peripheral blood are recruited by certain chemokines into the tumor microenvironment where they differentiate into tumor associated macrophages and promote angiogenesis, metastasis, immune suppression and chemo resistance.[5] The prognostic value of lymphocyte to monocyte ratio (LMR) has been investigated in hematologic malignancies and a low LMR was reported as an unfavorable prognostic factor in patients with diffuse large B cell lymphoma and Hodgkin lymphoma.[6,7] There are also numerous studies regarding the prognostic role of LMR in solid tumors such as soft tissue sarcomas, nasopharyngeal carcinoma, and ovarian cancer but all of these studies comprise only of adult patients. To our knowledge there is no study investigating the prognostic value of LMR and peripheral blood monocyte count in any childhood cancer.

Neuroblastoma is the most common extra cranial solid tumor of childhood and the most frequently diagnosed cancer in infancy.[8,9]

✉ Elif Güler
elifguler@akdeniz.edu.tr

Received 18th November 2020, revised 21st January 2021, accepted 22nd January 2021.

Neuroblastoma has a diverse pattern of clinical presentation; the disease course ranges from spontaneous regression to aggressive metastatic tumor.[10] It is regarded as one of the most common cancers that undergo spontaneous regression.[11-13] The induction of patients' immune response toward their own tumor cells is one of the mechanisms suggested to contribute to this phenomenon.

Age, stage, histology and genetic changes (ploidy, MYCN copy number, 11q deletion) are the well-known prognostic factors in neuroblastoma. Patients are stratified into risk groups and treated according to these prognostic factors. In this study, we aimed to analyze whether peripheral LMR and blood monocyte percentage (MP) might have an effect on survival and can be used for improving predictive ability of existing prognostic tools in neuroblastoma patients.

Material and Methods

Patients

Seventy-one neuroblastoma patients, who were diagnosed and treated between January 2002 and December 2016, were retrospectively evaluated. All patients were staged according to International Neuroblastoma Staging System (INSS).[14] Patients were treated according to national neuroblastoma protocols of Turkish Pediatric Oncology Group (TPOG); TPOG-Neuroblastoma 2003, and TPOG-Neuroblastoma 2009 protocols. The poor prognostic factors are older age at diagnosis [≥18 months for TPOG- 2009 and ≥12 months for TPOG 2003], advanced stage [stage 3-4], unfavorable histology, presence of genetic alterations (MYCN amplification, chromosome 1p, 11q and/or 17 q deletion). Being stage IV and older than 18 months for TPOG- 2009 and ≥12 months for TPOG 2003 made patients a high-risk group regardless of MYCN amplification and histology in both protocols. According to these parameters patients were stratified in to three risk groups: high, intermediate and low risk. Data including age, gender, clinical findings, stage, histopathology, genetic alterations, treatment modalities, survival of patients, peripheral blood MP and LMR were retrospectively analyzed from the files. The peripheral MP and LMR were determined from routine complete blood counts with five-part differential counts (absolute and percent of lymphocytes, monocytes, eosinophils, basophils and neutrophils) obtained at diagnosis using ADVIA 2120 Hematology System (Siemens, NY, USA). Peripheral MP was calculated by dividing the absolute monocyte count to total leukocyte count and multiplying by 100. Lymphocyte to monocyte ratio was calculated as dividing the absolute lymphocyte count to absolute monocyte count (ALC/AMC).

After obtaining the ethics approval from the local ethics committee (Akdeniz University KAEK -2020-735), the study was initiated with informed consent from the patients.

Statistical analysis

Statistical analyses were calculated by SPSS (version 20.0) software program. The choice of the best cutoff values of peripheral MP and the LMR for assessing survival was based on their utility as a marker for the clinically relevant binary outcome of death/survival using the receiver operating characteristics curves (ROC) and area under the curve (AUC). Chi-square test was used to determine relationships between categorical variables and Mann Whitney U test was used to compare the continuous variables related to two groups. Overall survival (OS) and event-free survival (EFS) were analyzed using the approach of Kaplan-Meier. Differences between survival curves were tested for statistical significance using the two-tailed log-rank test. The stepwise (backward selection) Cox proportional hazard model was used for the univariate and multivariate analyses to evaluate the variables under the prognostic factors' section to assess their impact on overall survival and event free survival. All p values are two-tailed and p values less than 0.05 were considered statistically significant.

Table I. Characteristics of patients.

Age at diagnosis (mean, months)	31.2 (1-204)
Gender	N (%)
Female	38 (53.5)
Male	33 (46.5)
Age at diagnosis	
< 12 months	17 (24.9)
≥12 months	54 (76.1)
Stage	
Stage I	5 (7)
Stage II	5 (7)
Stage III	13 (18.3)
Stage IV	40 (56.4)
Stage IVS	8 (11.3)
Histology	
Favorable	18 (25.4)
Unfavorable	42 (59.2)
NA	11 (15.5)
Chromosome 1p11q deletion	
Presence	5 (7)
Absence	5 (7)
Not determined	61 (86)
Risk group	
Low	11 (15)
Intermediate	15 (21)
High	45 (64)
MYCN amplification	
High	19 (41.3)
Normal	27 (58.7)
Not determined	25 (35.2)
Chemotherapy	
Yes	62 (87.3)
No	9 (12.7)
Surgery	
At diagnosis	19 (26.7)
Second look	29 (40.8)
No	23 (32.5)
Radiotherapy	
Yes	22 (31)
No	49 (69)
Autologous transplantation	
Yes	25 (35)
No	46 (65)
Relapsed	
Yes	22 (31)
No	49 (69)
Cellular status at diagnosis	
Mean leukocyte count /mm^3	8918 (2070-20100)
Mean absolute lymphocyte Count (ALC)/mm^3	3143 (300-12320)
Mean absolute monocyte Count (AMC)/mm^3	715 (100-2300)
Monocyte percentage (MP) %	8.2 (2-22)
Mean ALC/AMC (LMR)	5 (1-16)

Results

Clinical Characteristics

Totally 71 neuroblastoma patients were enrolled. Thirty-eight (53.5%) of the patients were female and the mean age at diagnosis was 31.2 months (range; 1–204 months). Patients were staged according to INSS; 5 patients had (7%) stage I, 5 (7%) patients had stage II, 13 (18.3%) patients had stage III, 40 (56.4%) patients had stage IV and 8 (11.3%) patients had stage IVS disease. Metastasis was detected in 48 patients (67.6%) at initial diagnosis. MYCN amplification were high in 19 (41.3%) of patients and non-amplified in 27 (58.7%) of patients. MYCN status were unknown in 25 (35.2%) patients. Eleven of them were ≥18 months (or ≥ 12 months) and had stage IV, 7 patients were ≥18 months (or ≥ 12 months) and had unfavorable histology, 7 patients had stage I –II disease. Forty-five patients (64%) were in high-risk group, 15 patients (21%) were in intermediate risk group and 11 (15%) patients were in low-risk group. Fifteen of patients with unknown MYCN status in high-risk group, while the others were in low-risk group. Clinical characteristics of patients are shown in (Table I).

At diagnosis, mean leukocyte count was 8918/mm^3 (range; 2070-20100), mean peripheral absolute lymphocyte count (ALC) was 3143/mm^3 (range; 300-12320), mean peripheral absolute monocyte count (AMC) was 715/mm^3 (range; 100-2300), mean MP was 8.2% (range; 2%-22%) and mean peripheral LMR was 5 (range; 1-16).

LMR and MP at diagnosis

Cut-off values for LMR and peripheral blood MP were determined according to ROC analysis. Peripheral MP of 7.5% or more had an AUC of 0.74 [95% confidence interval (CI), 0.63 to 0.86] with a sensitivity of 73% and specificity of 70% (Fig. 1). A LMR of 3.5 or less had an AUC of 0.75 (95% CI, 0.64 to 0.86) with a sensitivity of 80% and a specificity of 61% (Fig. 2). Area under the curve values from ROC analysis support the use of LMR of ≤3.5 and peripheral blood MP ≥7.5% as the cut-off values as markers of binary clinical outcome of survival. Patients were then assigned either to the high LMR (LMR > 3.5) group and low LMR (LMR ≤ 3.5) group. Forty patients were in high LMR group and 31 patients were in low LMR group. According to cut-off value of MP, 39 patients were in high MP (MP ≥7.5), 32 patients were in low peripheral MP (MP <7.5) group.

When the patients were classified according to peripheral MP levels, there were more patients in the high peripheral MP group with metastatic disease (p=0.005), stage 4 disease (p<0.001), unfavorable histology (p<0,001), MYCN amplification (p=0.003), high risk disease (p<0.001), and older age (≥12 months; p=0.015) compared to the low peripheral MP group. Also, in the low LMR group there were more patients with stage 4 disease (p=0.014) unfavorable histology (p=0,046) and high-risk disease (p=0.003) (Table II).

Outcome:

The median follow-up time after diagnosis was 22 months (range; 1-182 months). The 3 year OS and EFS rates were 51% ± 6.0 and 37% ± 6.1, respectively. The 3 year OS and EFS rates in low LMR group were significantly lower than that in the high LMR group (3-year OS: 38% ± 8.8 vs 62% ± 7.8 p=0.002 and 3-year EFS: 22% ± 7.7 vs 50% ± 8.5 p=0.003) (Fig. 3). The patients with high peripheral MP had significantly lower 3-year OS and EFS compared to the patients with low peripheral MP (3-year OS: 38.5% ± 7.8 vs 67.5% ± 8.5, p=0.005 and 3-year EFS: 24% ± 7.1 vs 54% ± 9.5 p=0.027) (Fig. 3).

The univariate analysis showed that stage IV disease, unfavorable histology, high risk disease, low LMR and high MP were associated with low EFS and OS (Table III). The multivariate analysis revealed that low LMR (Hazard ratio

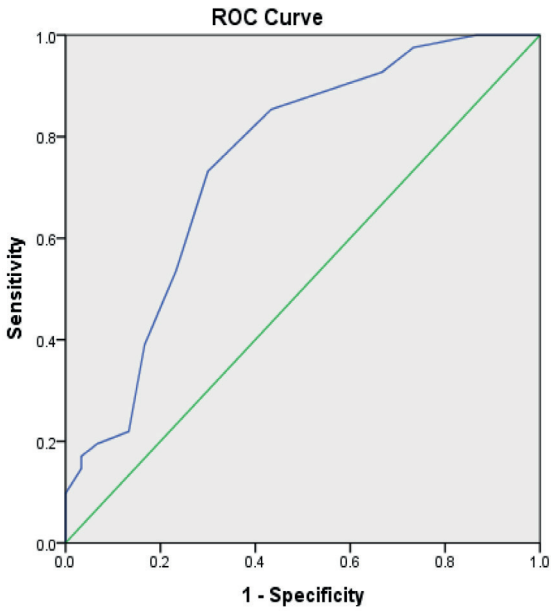


Fig. 1. Monocyte percentage of ≥7.5% had an AUC of 0.74 [95% confidence interval (CI), 0.63 to 0.86] with a sensitivity of 73% and specifity of 70%.

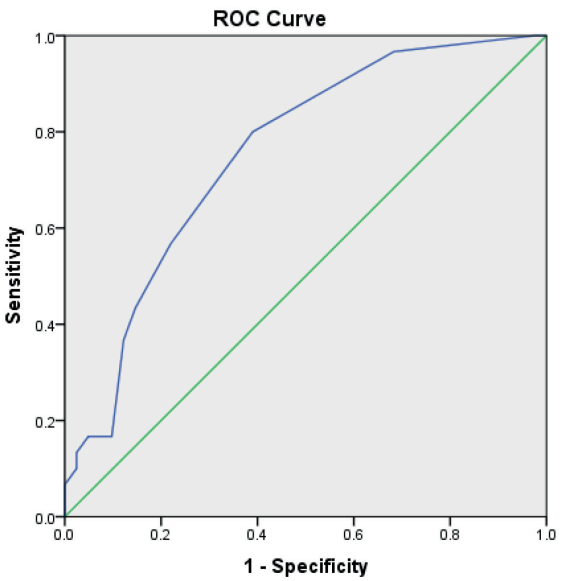


Fig. 2. Lymphocyte monocyte ratio of ≤ 3.5 had an AUC of 0.75 [95% confidence interval (CI), 0.64 to 0.86] with a sensitivity of 80% and specifity of 61%.

Table II. Characteristics of patients according to LMR and MP.

Variables	LMR		p-value	MP		p-value
	High (>3.5) (n=40)	Low (≤3.5) (n=31)		High (≥7.5%) (n=39)	Low (<7.5%) (n=32)	
Age at diagnosis						
<12 months	13 (32.5)	4 (12.9)	0.055	5 (12.8)	12 (37.5)	0.015
≥12 months	27 (67.5)	27 (87.1)		34 (87.2)	20 (62.5)	
Gender, N (%)						
Female	20 (50)	18 (58.1)	0.499	20 (51.3)	18 (56.2)	0.676
Male	20 (50)	13 (41.9)		19 (48.7)	14 (43.8)	
Stage, N (%)						
Stage 1,2,3,4S	22 (55.0)	8 (25.8)	0.014	8 (20.5)	22 (68.8)	<0.001
Stage 4	18 (45.0)	23 (74.2)		31 (79.5)	10 (31.3)	
Metastatic Disease						
Presence	25	23	0.29	32	16	0.005
Absence	15	8		7	16	
Risk Group, N (%)						
Low-Intermediate	20 (50.0)	5 (16.1)	0.003	5 (12.8)	20 (62.5)	<0.001
High	20 (50.0)	26 (83.9)		34 (87.2)	12 (37.5)	
Histology, N (%)						
Favorable	14 (40.0)	4 (16.0)	0.046	2 (6.7)	16 (53.3)	<0.001
Unfavorable	21 (60.0)	21 (84.0)		28 (93.3)	14 (46.7)	
MYCN amplification						
High	7 (29.2)	12 (54.5)	0.081	16 (59.3)	3 (15.8)	0.003
Normal	17 (70.8)	10 (45.5)		11 (40.7)	16 (84.2)	

(HR), 2.29; 95% CI, 1.11-4.75; p=0.025) and stage IV disease (HR, 2.97; 95% CI, 1.26-7.02; p=0.013) were the factors that were significantly associated with low 3-year OS. Low LMR (HR, 2.15; 95% CI, 1,07-4.30; p=0.03) and stage IV disease (HR, 3.13; 95% CI, 1.40-7.02; p=0.006) were also defined as independent factors for decreased 3-year EFS (Table III).

Discussion

Our study has shown that a low LMR might be a poor prognostic factor in pediatric neuroblastoma patients. In addition, a high MP was also associated with low EFS and OS even though the noteworthy association between high MP and clinical outcome could not be established in the multivariate analysis. Current literature has indicated that the combination of cellular components of the systemic inflammatory response, such as monocyte count, L/M ratio, and MP represent significant markers of clinical outcome in a wide variety of cancers.[15,16] Lymphocytes are regarded as the crucial factors in immune surveillance, and the presence of an immunologic antitumor reaction is based on lymphocytic infiltration into the tumor microenvironment.[17,18] The prognostic role of LMR was first described by Porrata et al.[6] in Hodgkin Lymphoma patients in 2011. Shortly after this study, in 2012, Li et al.[7] documented that LMR was also an independent prognostic factor of survival in diffuse large B-cell lymphoma patients. The association between low LMR and poor OS was shown later on in non-hematologic solid tumors. Li et al.[19] documented that pretreatment LMR level was a significant favorable factor for prediction of the clinical outcome in nasopharyngeal carcinoma patients. The following reports were similar

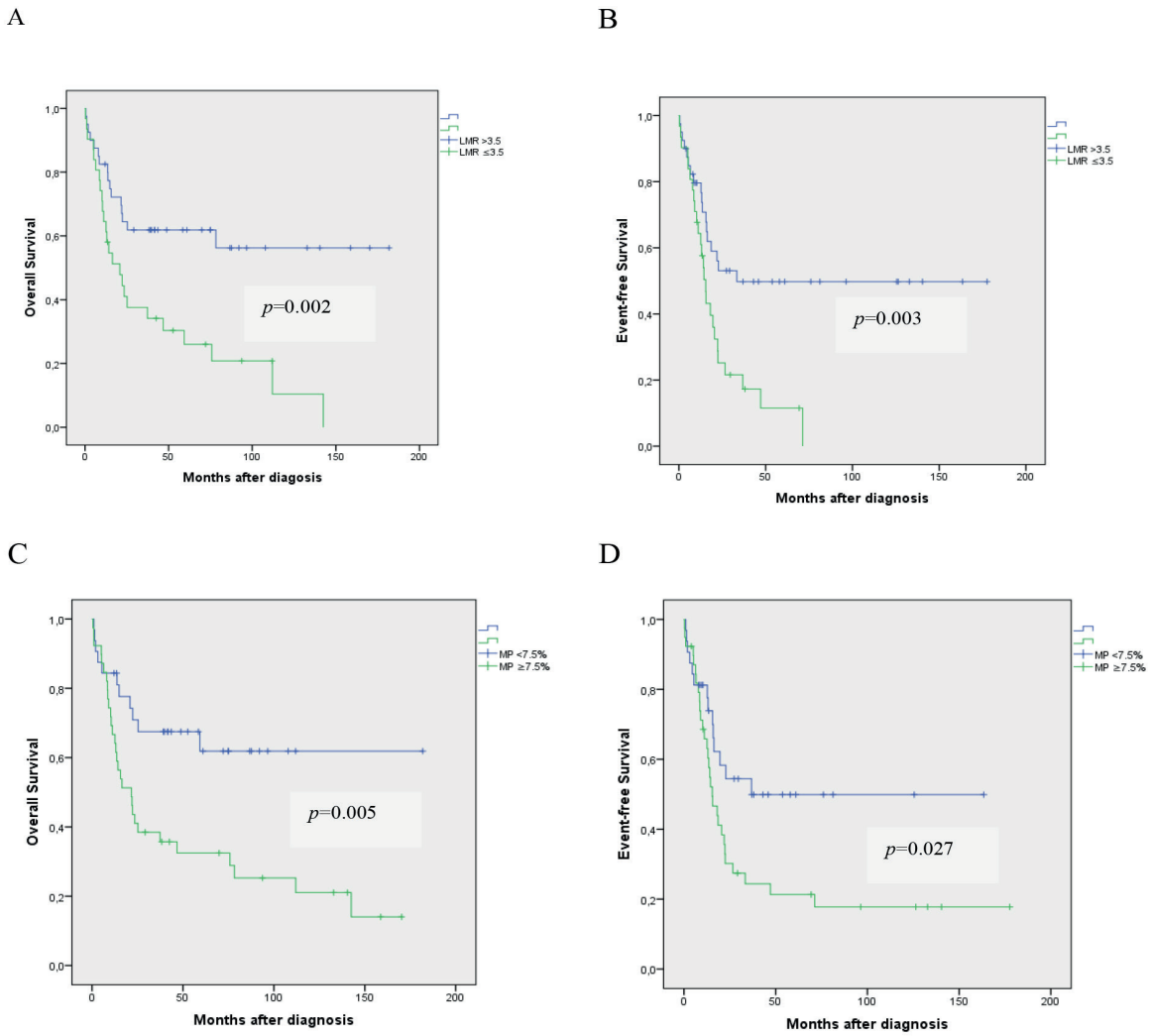


Fig. 3. Comparison of low and high LMR on OS (A), EFS (B) and low and high MP on OS (C) and EFS (D).
EFS: event-free survival, OS: overall survival, LMR: lymphocyte to monocyte ratio, MP: blood monocyte percentage

Table III. Univariate and multivariate analyses for OS and EFS.

		OS			EFS	
Covariate	HR	95% CI	p-value	HR	95% CI	p-value
Univariate analysis						
LMR ≤3.5	2.65	1.41-4.99	0.002	2.48	1.34-4.56	0.004
MP ≥7.5%	2.60	1.30-5.21	0.007	2.02	1.07-3.81	0.030
Stage 4 disease	3.13	1.49-6.57	0.003	3.13	1.54-6.35	0.002
Unfavorable histology	3.77	1.32-10.74	0.013	4.25	1.5-12.06	0.006
High risk	2.84	1.25-6.42	0.012	2.74	1.27-5.92	0.010
Multivariate analysis						
LMR ≤3.5	2.29	1.11-4.75	0.025	2.15	1.07-4.30	0.030
Stage 4 disease	2.97	1.26-7.02	0.013	3.13	1.40-7.02	0.006

LMR: lymphocyte to monocyte ratio
MR: monocyte percentage

to previously reported data; a lower LMR was associated with lymph nodes metastasis, tumor progression and poor 5-year cancer specific survival in esophageal squamous cell carcinoma patients.[20] The low LMR was related with more aggressive tumor behavior and worse long-term survival in resectable gastric adenocarcinoma patients.[21] Low LMR was significantly correlated with higher degree of tumor infiltration and poor prognosis also in other digestive system cancers such as pancreas and colorectal carcinomas.[22,23] Deng et al.[24] have retrospectively evaluated 317 newly diagnosed locally advanced rectal cancer patients and shown that the LMR was a valuable prognostic factor for predicting the OS in this group of patients. A meta-analysis showed that the patients with lower LMR had poorer OS in non-small cell lung cancer.[25] Low LMR was also associated with unfavorable survival in patients with ovarian cancer and could serve as a prognostic biomarker.[26]

The definite mechanism of the association between low LMR and poor survival of cancer patients are not totally explained. This relevance may de described through tumor infiltrating immune cells which contribute significantly in destruction or development of tumor growth. In tumor microenvironment the lymphocytes are considered as one of the most vital components of the host's cellular immunity. Therefore, a low lymphocyte count might be responsible for a fragile, inadequate reaction to tumor and thereby an exacerbated clinical outcome.[17]

Macrophage is another essential component of tumor infiltrating inflammatory cells. Tumor associated macrophages (TAM) are derived from peripheral blood monocytes and a positive correlation between TAM and peripheral blood monocyte count has been shown in previous studies.[27,28] Therefore, circulating level of monocytes may reflect formation or presence of TAMs. TAM may promote angiogenesis, metastasis, immune suppression and chemo resistance.[5,29-31] Macrophages do not only contribute to tumor growth but also impair effective anti-tumor lymphocyte response. Some clinical studies have indicated a significant correlation between elevated macrophage content and poor clinical outcome in soft tissue cancer patients.[32]

Metastatic disease at diagnosis is one of the most important prognostic factors for neuroblastoma.[10] It was shown that TAM enhance neo-angiogenesis, promote tumor cell migration and metastases.[27,30,33] The high peripheral monocyte count can reflect the presence or formation of TAM and has been reported as a poor prognostic factor in adult patients with various types of cancer.[33] Koh et al.[27] also reported that there was a positive correlation between TAM and MP in patients with Hodgkin lymphoma. We found that there were more patients with advanced stage and metastasis in the group who had high MP, unfortunately we could not evaluate tumor microenvironment. There is a unique study by Asgharzadeh et al.[34] which shows significantly greater numbers of infiltrating macrophages in tumor samples of neuroblastoma patients with metastatic (stage 4) disease. These findings support that number of peripheral monocyte count, which will convert to TAM, may have prognostic significance in neuroblastoma. However, there was a prognostic significance of high MP in neuroblastoma outcome according to univariate analysis, multivariate analysis did not reveal that high MP was an independent prognostic factor for EFS and OS in our study. Further studies including higher number of patients are needed to determine the prognostic value of peripheral monocytes count or MP in neuroblastoma patients.

The limitations of this study are the retrospective nature of the design, small number of patients, short follow up period and absence of tumor microenvironment evaluation. We evaluated LMR and MP on outcome of neuroblastoma patients. LMR was found as an independent prognostic factor for EFS and OS in this study. The peripheral blood count and cell count ratio can be determined readily and inexpensively by a standard automated complete blood count machine. LMR and MP may be new

prognostic factors for neuroblastoma and can be used for predicting survival which is a cost effective and easy-accessible biomarker. Our results should be verified by multi-centric and prospective studies which also evaluate the tumor microenvironment concomitantly.

Acknowledgement

The authors would like to thank all doctors and medical staff involved in medical care of neuroblastoma patients for their exceptional work.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KY, EG; data collection: KY, AK; analysis and interpretation of results: SB, GT, AK, EG; draft manuscript preparation: KY, GT, FTK, EG; All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

After obtaining the ethics approval from the local ethics committee (Akdeniz University KAEK -2020-735), the study was initiated with informed consent from the patients

Source of funding

We have nothing to disclose

Conflict of interest

All the authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES

1. Tredan O, Manuel M, Clapisson G, et al. Patients with metastatic breast cancer leading to CD4+ T cell lymphopaenia have poor outcome. Eur J Cancer 2013; 49: 1673-1682.
2. Liang L, Zhu J, Jia H, et al. Predictive value of pretreatment lymphocyte count in stage II colorectal cancer and in high-risk patients treated with adjuvant chemotherapy. Oncotarget 2016; 7: 1014-1028.
3. Milne K, Alexander C, Webb JR, et al. Absolute lymphocyte count is associated with survival in ovarian cancer independent of tumor-infiltrating lymphocytes. J Transl Med 2012; 10: 33.
4. Mehrazin R, Uzzo RG, Kutikov A, et al. Lymphopenia is an independent predictor of inferior outcome in papillary renal cell carcinoma. Urol Oncol 2015; 33: 388.e19-388.e.25.
5. Jinushi M, Komohara Y. Tumor-associated macrophages as an emerging target against tumors: creating a new path from bench to bedside. Biochim Biophys Acta 2015; 1855: 123-130.
6. Porrata LF, Ristow K, Colgan JP, et al. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma. Haematologica 2012; 97: 262-269.
7. Li ZM, Huang JJ, Xia Y, et al. Blood lymphocyte-to-monocyte ratio identifies high-risk patients in diffuse large B-cell lymphoma treated with R-CHOP. PLoS One 2012; 7: e41658.
8. Colon NC, Chung DH. Neuroblastoma. Adv Pediatr 2011; 58: 297–311.
9. Birch JM, Blair V. The epidemiology of infant cancers. Br J Cancer Suppl 1992; 18: S2-S4.
10. Irwin MS, Park JR. Neuroblastoma: paradigm for precision medicine. Pediatr Clin North Am 2015; 62: 225-256.
11. Yamamoto K, Hanada R, Kikuchi A, et al. Spontaneous regression of localized neuroblastoma detected by mass screening. J Clin Oncol 1998; 16: 1265-1269.
12. Cole WH, Everson TC. Spontaneous regression of cancer: preliminary report. Ann Surg 1956; 144: 366-383.
13. Papac RJ. Spontaneous regression of cancer: possible mechanisms. In Vivo 1998; 12: 571-578.

14. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 1993; 11: 1466-1477.

15. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008; 454: 436-444.

16. Chan JC, Chan DL, Diakos CI, et al. The lymphocyte-to-monocyte ratio is a superior predictor of overall survival in comparison to established biomarkers of resectable colorectal cancer. Ann Surg 2017; 265: 539-546.

17. Hoffmann TK, Dworacki G, Tsukihiro T, et al. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. Clin Cancer Res 2002; 8: 2553-2562.

18. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 2004; 21: 137-148.

19. Li J, Jiang R, Liu WS, et al. A large cohort study reveals the association of elevated peripheral blood lymphocyte-to-monocyte ratio with favorable prognosis in nasopharyngeal carcinoma PLoS One 2013; 8: e83069.

20. Huang Y, Feng JF. Low preoperative lymphocyte to monocyte ratio predicts poor cancer-specific survival in patients with esophageal squamous cell carcinoma. Onco Targets Ther 2015; 8: 137-145.

21. Hsu JT, Wang CC, Le PH, et al. Lymphocyte-to-monocyte ratios predict gastric cancer surgical outcomes. J Surg Res 2016; 202: 284-290.

22. Qi Q, Geng Y, Sun M, Wang P, Chen Z. Clinical implications of systemic inflammatory response markers as independent prognostic factors for advanced pancreatic cancer. Pancreatology 2015; 15: 145-150.

23. Neal CP, Cairns V, Jones MJ, et al. Prognostic performance of inflammation-based prognostic indices in patients with resectable colorectal liver metastases. Med Oncol 2015; 32: 144.

24. Deng YX, Lin JZ, Peng JH, et al. Lymphocyte-to-monocyte ratio before chemoradiotherapy represents a prognostic predictor for locally advanced rectal cancer. Onco Targets Ther 2017; 10: 5575-5583.

25. Wang Y, Huang D, Xu WY, Wang YW, Che GW. Prognostic value of pretreatment lymphocyte-to-monocyte ratio in non-small cell lung cancer: a meta-analysis. Oncol Res Treat 2019; 42: 523-531.

26. Lu C, Zhou L, Ouyang J, Yang H. Prognostic value of lymphocyte-to-monocyte ratio in ovarian cancer: a meta-analysis. Medicine (Baltimore) 2019; 98: e15876.

27. Koh YW, Shin SJ, Park C, Yoon DH, Suh C, Huh J. Absolute monocyte count predicts overall survival in mantle cell lymphomas: correlation with tumour-associated macrophages. Hematol Oncol 2014; 32: 178-186.

28. Mantovani A, Sica A, Locati M. New vistas on macrophage differentiation and activation. Eur J Immunol 2007; 37: 14-16.

29. Kitamura T, Qian BZ, Pollard JW. Immune cell promotion of metastasis. Nat Rev Immunol 2015; 15: 73-86.

30. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010; 141: 39-51.

31. Allavena P, Sica A, Garlanda C, Mantovani A. The yin-yang of tumor-associated macrophages in neoplastic progression and immune surveillance. Immunol Rev 2008; 222: 155-161.

32. Raj SKS, Kooshki M, Winters M, et al. Prognostic implications of tumor associated macrophages (TAMs) in soft tissue sarcoma. J Clin Oncol 2019; 37(Suppl 15): e22548.

33. Schmidt H, Bastholt L, Geertsen P, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer 2005; 93: 273-278.

34. Asgharzadeh S, Salo JA, Ji L, et al. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. J Clin Oncol 2012; 30: 3525-3532.

The Turkish Journal of Pediatrics 2021; 63: 893-902
https://doi.org/10.24953/turkjped.2021.05.017

Case Report

Cancer and constitutional Mismatch Repair Deficiency syndrome due to homozygous MSH 6 mutation in children with Café au Lait Spots and review of literature

Derya Özyörük[1], Emel Ünal Cabı[2], Nurdan Taçyıldız[2], Ferda Pınarlı[3], Ayşe Oğuz Erdoğan[4], Şahin Hanalioğlu[5], Arzu Yazal Erdem[1], Arzu Meltem Demir[6]

Departments of [1]Pediatric Hematology and Oncology, [4]Pathology and [6]Pediatric Gastroenterology, Health Sciences University, Ankara Childrens' Hematology and Oncology Hospital, Ankara; [2]Department of Pediatric Oncology, Ankara University Faculty of Medicine, [3]MYOGEN Genetic Diagnostic Center, Ankara; [5]Department of Neurosurgery, Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey.

ABSTRACT

Background. Constitutional mismatch repair deficiency (CMMRD) syndrome is a rare childhood cancer predisposition syndrome resulting from biallelic germline mutations of mismatch repair (MMR) genes. CMMRD syndrome is characterised by early onset malignancies in children.

Case. Here we present affected children of consanguinous parents diagnosed with CMMRD syndrome due to germline bi-allelic MSH 6 gene mutations with café au lait spots and multiple family cancers from Turkey and reported cases with CMMRD syndrome associated MSH 6 mutation in English literature. Hence, we reviewed English literature from 1990 to 2020 using Pub-Med database. Keywords used to search included constitutional mismatch repair deficiency syndrome, childhood cancer and MSH 6 gene mutation.

Conclusions. We emphasize that the inclusion of CMMRD syndrome in the differential diagnosis of a patient who presents with cafe´ au lait spots and/or hypopigmented skin lesions and cancer especially when consanguinity and/or a history of cancer coexist in children.

Key words: childhood cancer, constitutional mismatch repair deficiency syndrome, MSH 6 mutation.

Constitutional mismatch repair deficiency (CMMRD) syndrome is a rare childhood cancer predisposition syndrome resulting from biallelic germline mutations of the DNA mismatch repair (MMR) genes.[1] It is associated with a wide spectrum of malignancies including hematological, brain and intestinal tumors. It also frequently mimics clinical features of neurofibromatosis type 1 (NF1).[2-4] Although bi-allelic mutations in genes that regulate DNA mismatch repair, including MLH1, MSH2, MSH6 and PMS2 causes CMMRD syndrome, monoallelic germline mutations in one of these genes cause Lynch syndrome.[4-6] Therefore, individuals with bi-allelic mutations have a dysfunctional mismatch repair system from birth, CMMRD syndrome is characterised by early onset malignancies.[7-10] Hence, wreviewed English literature from 1990 to 2020 using Pub-Med database. Keywords used to search included constitutional mismatch repair deficiency syndrome, childhood cancer and MSH 6 gene mutation. Here we present affected children of consanguinous parents diagnosed with CMMRD syndrome due to germline bi-

✉ Derya Özyörük
dozyoruk@yahoo.com

Received 11th September 2020,
revised 26th November 2020, 11th January 2021,
1st February 2021, accepted 8th February 2021.

It was presented as a meeting abstract at the 51th Congress of the "Société Internationale d'Oncologie Pédiatrique" (SIOP) held in October 23-26, 2019, in Lyon, France. Also, it was presented as a meeting abstract in 61st American Society Hematology (ASH) Annual Meeting and Expositions held in December 7-10,2019 Orlando, FL, USA.

allelic MSH 6 gene mutations with café au lait spots and multiple family cancers from Turkey and reported cases with CMMRD syndrome associated MSH 6 mutation in English literature.

Case Report

A 8-year-old female was admitted to our clinic with diagnosis of a brain mass. In her past history; she was followed up with the diagnosis of NF-1 and familial mediterranean fever. Her parents were first-degree cousins with multiple cancer histories: one brother died from medulloblastoma and metachronous colon adenocarcinoma at 15 years of age and one sister died from brain tumor at 4 years of age. In addition, parents' uncle and aunt had colon adenocarcinoma and thyroid papillary cancer at 45 and 50 years of age, respectively. On physical examination, 8 to 10 cafe´ au lait spots and hypopigmented skin lesions with irregular borders on her body were found. Other findings of neurofibromatosis type 1 including neurofibromas, Lisch nodules, tibia pseudoarthrosis, sphenoid wing dysplasia, and optic glioma were not seen. Laboratory investigation was within normal limits other than low serum Ig G2 levels. Cranial magnetic resonance imaging (MRI) showed a partially enhancing mass (21x34x22mm) in the left cerebellar region, nonspesific subcortical white matter T2-FLAIR hyperintensities in frontal and parietal lobes. Also, the focal areas of hyperintense signal intensity in bazal ganglia, thalamus, mesencephalon and venous anomaly were noted. She underwent near-total resection of cerebellar mass and histopathology revealed classic desmoplastic medulloblastoma. The post-operative craniospinal MRI showed a left cerebellar hyperintensity (12x21mm) and enhancing lesion (4x8mm) without nodularity associated with recent surgery and other nonspesific intracranial findings were the same as the previous MRI. The index case was administered craniospinal irradiation (54 Gy) with a diagnosis of Medulloblastoma (Stage M2) according to Chang staging system followed by chemotherapy consisting of CCNU, cisplatin and vincristine. Because of positive family history and café au lait spots of the index case, we proceeded with genetic analysis that disclosed a novel homozygous single base insertion mutation in exon 5 of the MSH 6 gene (c.3261dupC p. Phe10881Leufs*5) (Fig. 1). NF type I and II genes were normal.

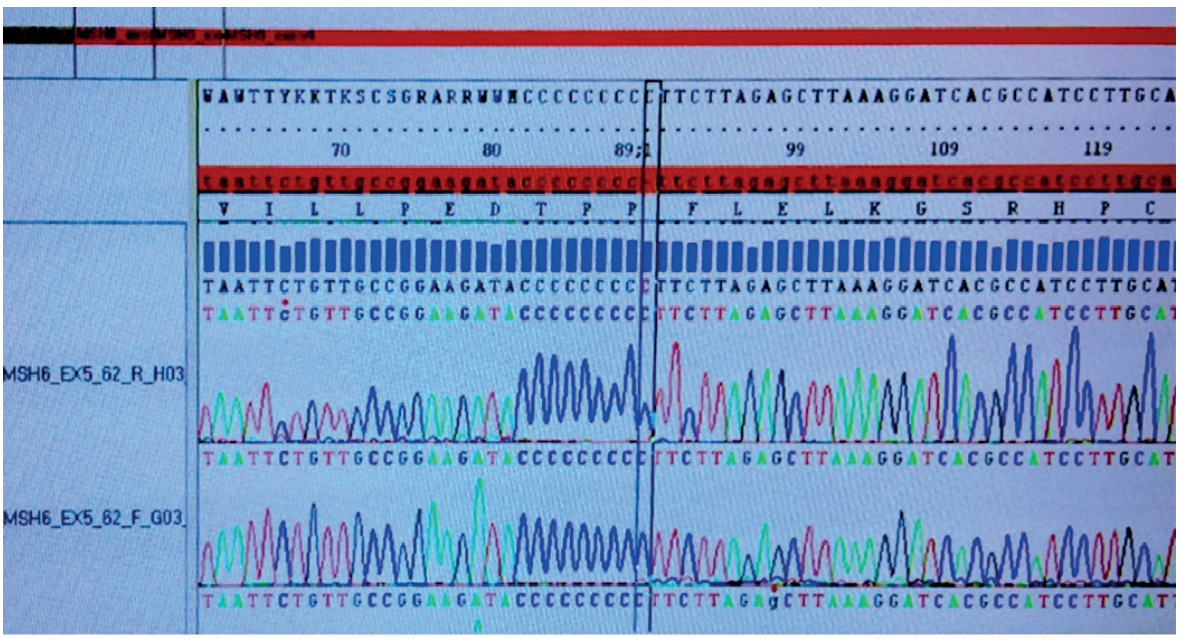


Fig. 1. MSH6 Gene Mutation of Index Case

The genetic screening of family members were performed for MSH 6 gene mutation. The family history is shown in the pedigree in Figure 2. After 5 cycles of conventional chemotherapy, the family declined the chemotharapy due to severe hematologic toxicities and recurrent infectious complications. The colonoscopy which was normal previously revealed a new occurance of colonic tubulovillous adenomas. The chemotherapy was stopped and anti-programmed death-1 (Anti-PD-1) drugs (nivolumab; 3 mg/kg/dose, every 2 weeks) were given for 24 doses. During the anti-PD-1 treatment, neuroimaging findings were stable and colonic polyps were gradually decreased in number and size. One year after the end of the treatment with anti-PD-1, MRI showed a new occurance of a right cerebellar heterogenously enhancing mass (33x41x28mm) with restricted diffusion causing midline shift. Also, colonoscopy revealed new colonic polyps. She underwent tumor resection but died post-operatively. Histopathology revealed medulloblastoma. Informed consent was received from the family.

Discussion

CMMRD is considered in children with glioma, leukemia, colorectal cancer and other hereditary nonpolyposis colon cancers and any of the findings: 1) cafe´ au lait spots and/or hypopigmented skin lesions 2) history of parental consanguinity, or 3) positive family

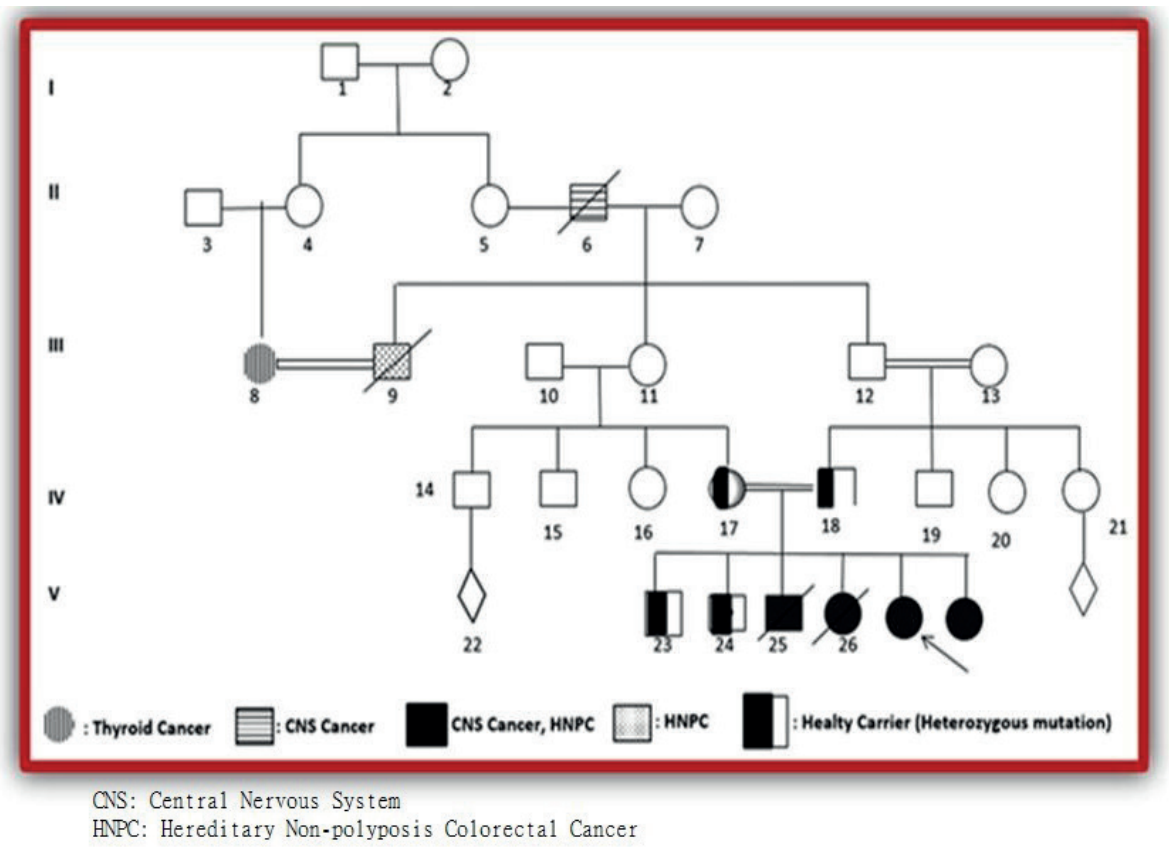


Fig. 2. Five generation family tree representing cases with MSH6 mutation and/or cancers: Parents and two siblings (11 and 6years old males) are heterozygous; Sibling six-month-old is homozygous; 15-year-old deceased sibling with medulloblastoma and metastatic colorectal carcinoma was homozygous for MSH6 mutation; Other 4-year-old deceased sibling with brain tumor has no genetic test. CNS: Central Nervous System; HNPC: Hereditary Non-polyposis Colorectal Cancer

history of hereditary nonpolyposis colon cancers. In addition, a three-point scoring system for the suspected diagnosis of CMMRD in a pediatric/young adult cancer patient has been recommended by European Consortium Tumours highly specific for CMMRD syndrome.[9,11,12]

The global prevalence of CMMRD is currently unknown, but rate is expected to increase in regions with a high prevalence of consanguineous marriages in especially low and middle income countries. Recently, a modified surveillance protocol for these countries with limited resources has been reported. This surveillance protocol consisted of complete blood count and fecal occult blood every 6 months, upper endoscopy/ colonoscopy and abdominal ultrasound annually, and brain MRI or CT every 6 months in children. In addition, whole body MRI was advised for surveillance as recommended in developed countries where it is available and/or reimbursed.[13]

According to a review of 146 cases of CMMRD syndrome conducted by Wimmer et al[9], the most common mutation was PMS2 mutations (60% of cases). Twenty-two percent and 20% of cases are caused by MLH1 or MSH2 and MSH6 mutations, respectively.[9]

We reviewed English literature from 1990 to 2020 using Pub-Med database. Keywords used to search included constitutional mismatch repair deficiency syndrome, childhood cancer and MSH 6 mutation. We could find approximately 38 patients diagnosed with CMMRD syndrome due to MSH 6 mutations among 20 cases and/or case series from 63 articles. The characteristics of the 38 pediatric patients with CMMRD syndrome including our patients are shown in Table I.[1,12-31] The median age of onset of the first tumour was 8 years (range, 1 to 17 years). The 38 individuals with CMMRD had a total of 66 tumours (Table I). Nineteen patients had one malignancy, 13 patients had two malignancy and 7 patients had more than two malignancy. Of the 66 tumors 28 (42%) were brain tumours, 15 (23%) haematological malignancies, 19 (29%) GI poliposis and/or colon cancer, and 4 (6%) other tumours. Among the CNS tumours, the most prevalent type were glioblastoma multiforme and other high grade glial tumors (n:20; 71%). On the other hand, we found a limited number of medulloblastoma cases (n:5;25%) in patients with CMMRD with MSH6 gene mutations including with our patient.

The cafe´ au lait spots and/or hypopigmented skin lesions mimicking NF 1 features were determined in 29 patients diagnosed with CMMRD syndrome including with our patients in Table I. The cafe´ au lait spots and/ or hypopigmented skin lesions history of seven patients were not available. The cafe´ au lait spots in patients with CMMRD usually differ in colour and shape from typical NF1-associated cafe au lait spots. Regardless of the genetic basis underlying clinical findings in patients with CMMRD, the majority presented cafe au lait and/or other signs indicative NF 1, although a minority fullfilled the NIH criterion for NF 1 diagnosis.[2] This phenotypic overlap caused to misdiagnosis of CMMRD patients as having NF1 and impeded proper management and genetic counselling of these patients and their families similar to our patients in the past. As reported case and case series of CMMRD syndrome increase, misdiagnosis of these cases will be less in the future.

CMMRD syndrome is an autosomal recessive cancer predisposition syndrome resulting from bi-allelic mutations in MMR genes. Although consanquinity is highly suggestive for CMMRD syndrome, it can be seen in non-consanquineous families usually as compound heterozygous mutations.[11] In the present review, there were consanquinity in 22 patients (58) including our patient. The consanguinity history of seven patients were not available.

Immunotherapies are directed against inhibiting receptors, such as PD-1 protein and one of its ligands programmed death-ligand 1 (PD-L1). Nivolumab, is anti-PD-1 mAb, binds PD-1 and stimulates memory response to tumor antigen-specific T cell proliferation.[3]

Table I. The clinical features and tumors of patients with CMMRD.

Reference	First tumor (age)	Second tumor (age)	Third tumor (age)	Fourth tumor (age)	Features	Family history
Menko et al [14] 2004	Oligodendroglioma (10y)	CRC (12y)			CAL	Consang. Family cancer was positive
Hedge et al [15] 2005	Lymphoma (5y)	CRC (8y)			CAL	No Consang. Family cancer was positive
	GBM (8y)				CAL	No Consang. Family cancer was positive
Ostergaad et al [16] 2005	Pilocytic astrocytoma (9y)	Anaplastic astrocytoma(10y)	T-cell lymphoma (10y)		CAL, frecklesIg A deficiency	No Consang. Family cancer was positive
	Spinal glioblastoma (3y)				CAL, frecklesIg A deficiency	No Consang. Family cancer was positive
Scott et al [17] 2007	Medulloblastoma (7y)	AML (10y)	CRC (13y)		CAL,2 hairy nevus, hypopigm. macules IgA and G2 deficiencies	Consang. Family cancer was positive
Poley et al [18] 2007	NHL (4y)	Anaplastic oligodendroglioma (6y)			CAL	Consang. Family cancer was positive
Auclair et al [19] 2007	GI polyposis/CRC (9y)				CAL, Lisch nodules	Consang. Family cancer was positive
Etzler et al [20] 2008	Medulloblastoma (6 y)	MDS/AML (9 y)			CAL	Consang. Family cancer was positive
	GBM (9 y)				CAL, hypopigm. macules	Consang. Family cancer was positive
Rahner et al [21] 2008	CRC (17 y)				SLE, vitiligo	No Consang. No Family cancer
Peter et al [22] 2009	T-cel lymphoma (8y)				CAL, lisch nodules	No Consang. Family cancer was positive
Ripperger et al [23] 2010	T-cell lymphoma (6y)	CRC (13 y)			CAL	Consang. Family cancer was positive

AML: acute myeloblastic leukemia, CAL: café-au-lait, Consang: consanguinity, CRC: colorectal cancer, Hypopigm: hypopigmented, TLL: T-cell lymphoblastic lymphoma, GBM: glioblastoma multiforme, SLE: systemic lupus erythematosus, GI: gastrointestinal, mo: mounts, WT: Wilms tumor, NA: not available

Table I. Continued.

Reference	First tumor (age)	Second tumor (age)	Third tumor (age)	Fourth tumor (age)	Features	Family history
Ilencikova et al [24] 2012	Gliomatosis cerebri (11y)	T-cell lymphoma (11y)			CAL	Consang. Family cancer was positive
Hoel et al [25] 2014	T-cell lymphoma (20 mo)	CRC (13 y)			No CAL	Consang. No Family cancer
Bougard et al [26] 2014	GI polyposis (14y)	CRC (17y)	CRC (19y)	Urinary tract carcinoma (24y)	CAL	Consang. Family cancer was positive
Bakry et al [12] 2014	T cell lymphoma (10 y)	GI polyposis (12.5y)	GBM (8y)		CAL	Consang. No Family cancer
	Anaplastic olygodendroglioma (10y)				CAL	Consang. Family cancer was positive
	Anaplastic astrocytoma (11y)				CAL	Consang. Family cancer was positive
	GI polyposis (11y)				CAL	No Consang. No Family cancer
	T-cell lymphoma (6 y)	GI polyposis (11y)			CAL, freckles	No Consang. No Family cancer
	GBM (12,5y)				CAL	No Consang. No Family cancer
El-Hasid et al [27] 2015	AML (26 mo)				CAL, hypopigm. macules	Consang. No Family cancer
Lovaine et al [1] 2015	GBM (6y)				CAL	Consang. Family cancer was positive
	CRC/polyp (11y)				CAL	Consang. No Family cancer
	T-cell lymphoma (6y)	T-cell lymphoma (11y)	GBM (14y)	CRC /polyps (14y)	CAL, hypopigm. macules	Consang. No Family cancer
Al Harbi et al [28] 2018	GBM (5y)				CAL	Consang.Family history was positive

AML: acute myeloblastic leukemia, CAL: café-au-lait, Consang: consanguinity, CRC: colorectal cancer, Hypopigm: hypopigmented, TLL: T-cell lymphoblastic lymphoma, GBM: glioblastoma multiforme, SLE: systemic lupus erythematosus, GI: gastrointestinal, mo: mounts, WT: Wilms tumor, NA: not available

Table I. Continued.

Reference	First tumor (age)	Second tumor (age)	Third tumor (age)	Fourth tumor (age)	Features	Family history
Citak et al [29] 2019	WT (2y)	GBM (4y)	T-ALL (6y)		CAL, inguinal freckling	Consang. No Family cancer
Athanasiadis et al [30] 2020	GBM (13y)	CRC (13y)			CAL	Consang. Family cancer was positive
Our patients	Medulloblastoma (8y)	GI polyposis (9)			CAL, hypopigm. macules Ig G2 deficiency	Consang. Family cancer was positive
	Medulloblastoma (12y)	CRC (13 y)			CAL, hypopigm. macules freckles	Consang. Family cancer was positive
Guerrini-Rousseau L et al [31]	High grade glioma (11y)				NA	NA
	High grade glioma (13y)				NA	NA
	Medulloblastoma (7y)	CRC (22)	High grade glioma (25y)		NA	NA
	High grade glioma (17y)				NA	NA
	Medulloblastoma (1y)				NA	NA
	High grade glioma (3y)				NA	NA
	High grade glioma (13y)	High grade glioma (13y)			NA	NA

AML: acute myeloblastic leukemia, CAL: café-au-lait, Consang: consanguinity, CRC: colorectal cancer, Hypopigm: hypopigmented, TLL: T-cell lymphoblastic lymphoma, GBM: glioblastoma multiforme, SLE: systemic lupus erythematosus, GI: gastrointestinal, mo: mounts, WT: Wilms tumor, NA: not available

The optimal duration of immunotherapy is unknown, especially in the pediatric setting. Because the clinical findings remained stable in our index patient, nivolumab was stopped after 24 doses. Immunotherapy using immune checkpoint inhibitors have shown great promise in both adult and pediatric malignancies. First remarkable and durable responses reported were from two siblings with CMMRD-associated recurrent multifocal glioblastoma whom were treated with nivolumab (immune checkpoint inhibitor).[6] After that, another study reported a 5-year-old female with CMMRD and relapsed glioblastoma multiforme whom was treated with nivolumab had durable response.[28] Recently, the European C4CMMRD consortium has reported that the outcome of patients with constitutional mismatch repair deficiency (CMMRD) and brain tumor from the C4CMMRD database. According to their report, 8 patients with high grade glial tumor were administered immunotherapy with anti-PD1 antibodies at relapse. They observed disease progression in 7 of these patients within the first two months of immunotherapy and 6 of them died at 5.2 months (ranges between 1.8–9.5months) after the first injection. Therefore, they concluded that the prognosis of patients with a CMMRD-related brain tumor (especially glioblastoma) is not as good as originally thought.[31]

Although significant progress has been made about cancer immunotherapy, there is no sufficient experience with prophylactic immunotherapy to prevent cancer formation especially in cancer predisposing syndrome in childhood. Because the surveillance does not quarantee detection of precancerous lesions or cancer at a curable stage, it causes a great psychological burden in families who have children with homozygous MMR gen mutations. Recently, cancer immunoprevention has been emphasized especially for healthy cases with homozygous mutation of MMR genes during surveillance to decrease the tumorogenesis.[32,33]

In conclusion, CMMRD syndrome is a rare and challenging disease. Because of the dismal prognosis of after cancer occurance, further studies are required to prevent cancer with immunotherapy in patients with CMMRD syndrome. In addition, we emphasize the inclusion of CMMRD syndrome in the differential diagnosis of a cancerous patient who present with cafe´ au lait spots and/or hypopigmented skin lesions especially when consanguinity and/or a history of family cancer coexist in children.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DÖ; data collection: DÖ; analysis and interpretation of results: DÖ, EUC; draft manuscript preparation: DÖ, EUC, NT, FP, EUC, NT, FP, AOE, SH, AYE, AMD. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

REFERENCES

1. Lavoine N, Colas C, Muleris M, et al. Constitutional mismatch repair deficiency syndrome: clinical description in a French cohort. J Med Genet 2015; 52: 770-778.
2. Wimmer K, Rosenbaum T, Messiaen L. Connections between constitutional mismatch repair deficiency syndrome and neurofibromatosis type 1. Clin Genet 2017; 91: 507-519.
3. Westdorp H, Kolders S, Hoogerbrugge N, de Vries IJM, Jongmans MCJ, Schreibelt G. Immunotherapy holds the key to cancer treatment and prevention in constitutional mismatch repair deficiency (CMMRD) syndrome. Cancer Lett 2017; 403: 159-164.
4. Wimmer K, Etzler J. Constitutional mismatch repair-deficiency syndrome: have we so far seen only the tip of an iceberg? Hum Genet 2008; 124: 105-122.
5. Alexander TB, McGee RB, Kaye EC, et al. Metachronous T-lymphoblastic lymphoma and Burkitt lymphoma in a child with constitutional mismatch repair deficiency syndrome. Pediatr Blood Cancer 2016; 63: 1454-1456.

6. Bouffet E, Larouche V, Campbell BB, et al. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. J Clin Oncol 2016; 34: 2206-2211.

7. Ramchander NC, Ryan NA, Crosbie EJ, Evans DG. Homozygous germ-line mutation of the PMS2 mismatch repair gene: a unique case report of constitutional mismatch repair deficiency (CMMRD). BMC Med Genet 2017; 18: 40.

8. Ramachandra C, Challa VR, Shetty R. Constitutional mismatch repair deficiency syndrome: do we know it? Indian J Hum Genet 2014; 20: 192-194.

9. Wimmer K, Kratz CP, Vasen HF, et al; EU-Consortium Care for CMMRD (C4CMMRD) Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). J Med Genet 2014; 51: 355-365.

10. Durno CA, Sherman PM, Aronson M, et al; International BMMRD Consortium. Phenotypic and genotypic characterisation of biallelic mismatch repair deficiency (BMMR-D) syndrome. Eur J Cancer 2015; 51: 977-983.

11. Vasen HF, Ghorbanoghli Z, Bourdeaut F, et al; EU-Consortium Care for CMMR-D (C4CMMR-D). Guidelines for surveillance of individuals with constitutional mismatch repair-deficiency proposed by the European Consortium "Care for CMMR-D" (C4CMMR-D). J Med Genet 2014; 51: 283-293.

12. Bakry D, Aronson M, Durno C, et al. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. Eur J Cancer 2014; 50: 987-996.

13. Kebudi R, Amayiri N, Abedalthagafi M, et al; International RRD Consortium on Low-Resource Settings Panel. Challenges and specific strategies for constitutional mismatch repair deficiency syndrome in low-resource settings. Pediatr Blood Cancer 2020; 67: e28309.

14. Menko FH, Kaspers GL, Meijer GA, Claes K, van Hagen JM, Gille JJ. A homozygous MSH6 mutation in a child with cafe-au-lait spots, oligodendroglioma and rectal cancer. Fam Cancer 2004; 3: 123-127.

15. Hegde MR, Chong B, Blazo ME, et al. A homozygous mutation in MSH6 causes Turcot syndrome. Clin Cancer Res 2005; 11: 4689-4693.

16. Østergaard JR, Sunde L, Okkels H. Neurofibromatosis von Recklinghausen type I phenotype and early onset of cancers in siblings compound heterozygous for mutations in MSH6. Am J Med Genet A 2005: 139A: 96-105.

17. Scott RH, Mansour S, Pritchard-Jones K, Kumar D, MacSweeney F, Rahman N. Medulloblastoma, acute myelocytic leukemia and colonic carcinomas in a child with biallelic MSH6 mutations. Nat Clin Pract Oncol 2007; 4: 130-134.

18. Poley JW, Wagner A, Hoogmans MM, et al; Rotterdam Initiative on Gastrointestinal Hereditary Tumors. Biallelic germline mutations of mismatch-repair genes: a possible cause for multiple pediatric malignancies. Cancer 2007; 109: 2349-2356.

19. Auclair J, Leroux D, Desseigne F, et al. Novel biallelic mutations in MSH6 and PMS2 genes: gene conversion as a likely cause of PMS2 gene inactivation. Hum Mutat 2007; 28: 1084-1090.

20. Etzler J, Peyrl A, Zatkova A, et al. RNA-based mutation analysis identifies an unusual MSH6 splicing defect and circumvents PMS2 pseudogene interference. Hum Mutat 2008; 29: 299-305.

21. Rahner N, Höefler G, Högenauer C, et al. Compound heterozygosity for two MSH6 mutations in a patient with early onset colorectal cancer, vitiligo and systemic lupus erythematosus. Am J Med Genet A 2008; 146A: 1314-1319.

22. Peters A, Born H, Ettinger R, Levonian P, Jedele KB. Compound heterozygosity for MSH6 mutations in a pediatric lymphoma patient. J Pediatr Hematol Oncol 2009; 31: 113-115.

23. Ripperger T, Beger C, Rahner N, et al. Constitutional mismatch repair deficiency and childhood leukemia/lymphoma--report on a novel biallelic MSH6 mutation. Haematologica 2010; 95: 841-844.

24. Ilencikova D. Constitutional mismatch repair-deficiency syndrome (CMMR-D)- a case report of a family with biallelic MSH6 mutation. Klin Onkol 2012; 25(Suppl): S34-S38.

25. Hoell JI, Gombert M, Ginzel S, et al. Constitutional mismatch repair-deficiency and whole-exome sequencing as the means of the rapid detection of the causative MSH6 defect. Klin Padiatr 2014; 226: 357-361.

26. Bougeard G, Olivier-Faivre L, Baert-Desurmont S, et al. Diversity of the clinical presentation of the MMR gene biallelic mutations. Fam Cancer 2014; 13: 131-135.

27. Elhasid, R, Dvir R, Keidar R, et al; BMMRD Consortium. Management of acute myeloblastic leukemia in a child with biallelic mismatch repair deficiency. J Pediatr Hematol Oncol 2015; 37: e490-e493.

28. AlHarbi M, Ali Mobark N, AlMubarak L, et al. Durable response to nivolumab in a pediatric patient with refractory glioblastoma and constitutional biallelic mismatch repair deficiency. Oncologist 2018; 23: 1401-1406.

29. Citak EC, Sagcan F, Gundugan BD, et al. Metachronous Wilms tumor, glioblastoma, and T-cell leukemia in an child with constitutional mismatch repair deficiency syndrome due to novel mutation in MSH6 (c.2590G>T). J Pediatr Hematol Oncol 2021; 43: e198-e202.

30. Athanasiadis DI, Athanasiadou KI, Voulgaridou A, et al. Coexistence of constitutional mismatch repair deficiency syndrome and Lynch syndrome in a family of seven: MSH6 mutation and childhood colorectal cancer – a case series. Acta Gastroenterol Belg 2020; 83: 479-481.

31. Guerrini-Rousseau L, Varlet P, Colas C, et al. Constitutional mismatch repair deficiency-associated brain tumors: report from the European C4CMMRD consortium. Neurooncol Adv 2019; 1: vdz033.

32. Lenders EKSM, Westdrop H, Brüggeman RJ, et al. Cancer prevention by aspirin in children with Constitutional Mismatch Repair Deficiency (CMMRD). Eur J Hum Genet 2018; 26: 1417-1423.

33. Unal E, Ozyoruk D, Tacyildiz N, et al. ANTI-PD-1 treatment in a family with constitutional mismatch repair deficiency syndrome with multiple cancers from Turkey. Is cancer immunoprevention with checkpoint inhibitors HAS a role in Cases with homozygous mutationis? Blood 2019; 134(Suppl 1): 5632.

The Turkish Journal of Pediatrics 2021; 63: 903-908
https://doi.org/10.24953/turkjped.2021.05.018

Case Report

A fatal interstitial lung disease in an anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibody negative patient with juvenile dermatomyositis

Osman Yeşilbaş[1], Mehmet Yıldız[2], Can Yılmaz Yozgat[3], Irmak Tahaoğlu[4], Hakan Yazan[5], Erkan Çakır[5], Amra Adrovic[2], Sezgin Şahin[2], Kenan Barut[2], Özgür Kasapçopur[2]

Departments of [1]Pediatric Critical Care Medicine, [4]Pediatrics and [5]Pediatric Pulmonology, [3]Medical Student, Bezmialem Vakıf University Faculty of Medicine, İstanbul; [2]Department of Pediatric Rheumatology, İstanbul University Cerrahpaşa Medical School, İstanbul, Turkey.

ABSTRACT

Background. Juvenile dermatomyositis associated interstitial lung disease, rarely seen in pediatric age groups, has adverse effects on survival. Anti-melanoma differentiation associated gene 5, one of the identified autoantibodies in juvenile dermatomyositis, preferentially affects the lung tissue and may cause rapidly progressive interstitial lung disease. It is a major cause of mortality in juvenile dermatomyositis. In this case report, we present a pediatric patient diagnosed with juvenile dermatomyositis without anti-melanoma differentiation associated gene 5 antibody positivity.

Case. A six-year-old male patient admitted to the Pediatric Intensive Care Unit with symptoms of respiratory failure, 1.5 months after the diagnosis of juvenile dermatomyositis. Thorax computed tomography examination revealed pneumomediastinum, a trace of left-sided pneumothorax, atelectasis on the left posterior lung region, ground-glass opacity, minimal subpleural patchy consolidation, and subcutaneous emphysema especially on the sides of the chest wall. Broad-spectrum antibiotics were started. His nasal swab sample was positive in terms of influenza B; therefore, oseltamivir was added to the treatment. Autoimmune myositis antibodies panel was examined but all of them including anti-melanoma differentiation associated gene 5 antibody resulted as negative. There was no notable reduction in lung infiltrations with the patient's current treatment regimen. On the 12th day of Pediatric Intensive Care Unit admission, thorax computed tomography scan revealed progressed radiological lung findings compatible with rapidly progressive interstitial lung disease secondary to juvenile dermatomyositis. Despite intensive medical and extracorporeal treatments such as pulse steroid, intravenous immunoglobulin, methotrexate, cyclophosphamide, rituximab, therapeutic plasma exchange and, extracorporeal membrane oxygenation, the patient died on the 35th day.

Conclusions. Juvenile dermatomyositis patients should be carefully monitored for the development of interstitial lung disease. Rapidly progressive interstitial lung disease with a high mortality may develop shortly after diagnosis, even if the anti-melanoma differentiation associated gene 5 antibody is negative.

Key words: anti-melanoma differentiation associated gene 5, child, juvenile dermatomyositis, interstitial lung disease, rapidly progressive interstitial lung disease.

Juvenile dermatomyositis (JDM), one of the juvenile-onset myositis, is a very rare systemic autoimmune muscle disease and vasculopathies of unknown etiology accompanied by characteristic skin manifestations such as Gottron's papules or periorbital heliotrope rash. It also has systemic manifestations, such as Raynaud's syndrome, arthritis, cardiac dysfunction, dysphagia, and various forms of pulmonary disease.[1-3] Interstitial lung disease (ILD), the most common form of lung

✉ Osman Yeşilbaş
drosmanyesilbas@gmail.com

Received 21st July 2020, revised 4th October 2020, accepted 23rd December 2020.

involvement, has adverse effect on survival. Anti-melanoma differentiation associated gene 5 (anti-MDA5), one of the identified autoantibodies in DM with an incidence of 7-38%, preferentially affects the lung tissue and may cause rapidly progressive ILD (RP-ILD). It is a major cause of mortality in both adult and juvenile DM patients, particularly in East-Asian cohorts.[2,3]

In this case report, we present a very interesting pediatric patient diagnosed with juvenile DM (JDM) without anti-MDA5 antibody positivity. The disease has progressed to RP-ILD emerged with spontaneous pneumothorax and pneumomediastinum in a short time after diagnosis and our patient died despite intensive immunosuppressive and extracorporeal treatments such as therapeutic plasma exchange (TPE) and extracorporeal membrane oxygenation (ECMO).

Case Report

A six-year-old male patient was admitted to the Pediatric Intensive Care Unit (PICU) with symptoms of respiratory failure. The patient was conscious and had a Glasgow Coma Score of 15. His vital signs were: blood pressure 106/65 mmHg, pulse rate 140/min., respiratory rate 36/min., body temperature 37°C. He had subcostal/intercostal retractions and nasal flaring. Lung auscultation revealed bilateral crackles. Also, he had subcutaneous emphysema on his neck and chest, and heart auscultation revealed deep heart sounds. His oxygen saturation was between 80% and 85% in room air; thus, noninvasive ventilation (NIV) with high flow nasal cannula (HFNC) was started. It was learned that he had been diagnosed with JDM approximately one and half months ago with signs and symptoms of typical Gottron's papules (Fig. 1A) and periorbital heliotrope rash, fatigue, bilaterally distal and proximal interphalangeal arthritis (Fig. 1A), and oral aphthae (Fig. 1B). Hydroxychloroquine, prednisolone, and methotrexate had been started for JDM therapy.

On the PICU admission, blood gas, serum biochemistry, and electrolytes were within the normal range. C-reactive protein and procalcitonin levels were negative. On chest X-ray, there were bilateral infiltrates, subcutaneous emphysema on the sidewalls of the chest and neck without any signs of pneumothorax (Fig. 2). Thorax computed tomography (CT) examination revealed pneumomediastinum, a trace of left-sided pneumothorax, atelectasis on the left posterior lung region, ground-glass opacity, minimal subpleural patchy consolidation, and subcutaneous emphysema especially on the sides of the chest wall (Fig. 3).

Since the patient was still on immunosuppressive therapy, we decided to continue with

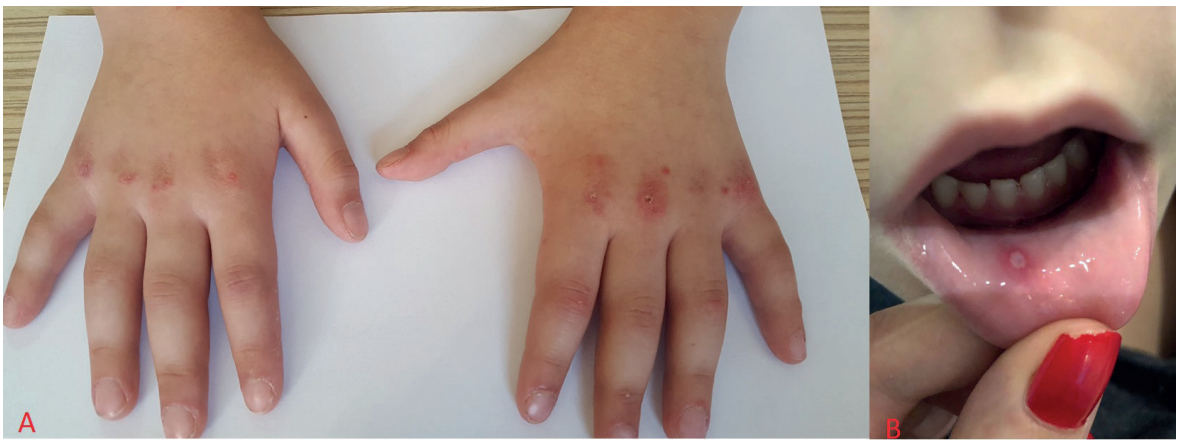


Fig. 1. A. Typical Gottron's papules and *bilaterally* distal and proximal interphalangeal arthritis. **B.** The patient's oral aphthae.

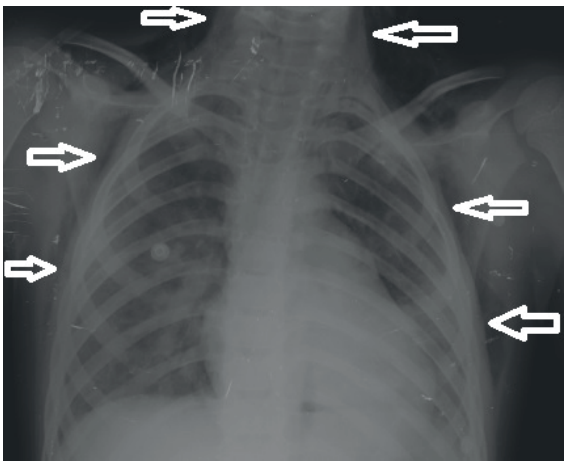


Fig. 2. Bilateral infiltrates, subcutaneous emphysema on the sidewalls of the chest and neck (white arrows).

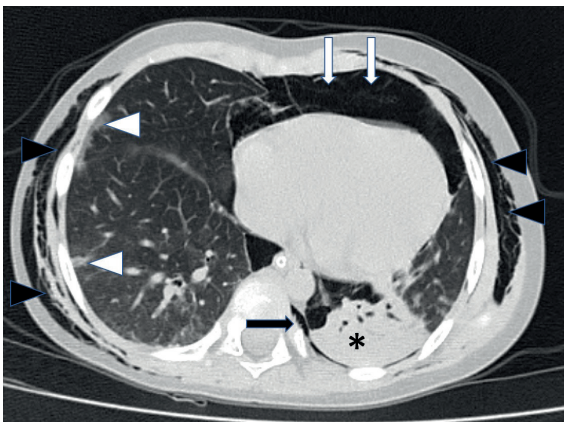


Fig. 3. First thorax computed tomography examination; pneumomediastinum (white arrows), a trace of left-sided pneumothorax (black arrow), atelectasis on the left posterior lung region (asterisk), ground-glass opacity, minimal subpleural patchy consolidation (white arrowheads), and subcutaneous emphysema especially on the lateral sides of the chest wall (black arrowheads).

intravenous (IV) piperacillin-tazobactam, teicoplanin, fluconazole, trimethoprim-sulfamethoxazole and enterally azithromycin treatments which were started in the external center prior to the PICU admission. The virus panel examination, which was done by polymerase chain reaction (PCR) of the patient's nasal swab sample, was positive in terms of influenza B; therefore, oseltamivir (2x60 mg) was added to the treatment and teicoplanin was terminated. A tube was placed in the mediastinum and left chest under operating room conditions. Before the procedure, he was intubated and after the procedure he was started on invasive mechanical ventilator support in the PICU. A transthoracic echocardiographic examination revealed normal heart functions. Hydroxychloroquine and prednisolone were discontinued and methylprednisolone (2 mg/kg/day) was initiated. Autoimmune myositis antibodies panel (*anti-MDA5, anti PM-Scl, anti Jo-1, anti Ku, anti PL-7, anti PL-12, anti-NXP2, anti-EJ, anti-SRP, anti MI2 alfa, anti MI2 beta, anti-SAE1, anti-TIF1g, anti-PM/scl75, anti-OJ antibodies*) was examined but all resulted as negative. Five days later, the patient was extubated and NIV was started with HFNC. Fluconazole and trimethoprim-sulfamethoxazole treatments were terminated. Three days later, body temperature and acute phase reactants increased, new lung infiltrations occurred, and he was reintubated. Teicoplanin and trimethoprim-sulfamethoxazole treatments were restarted, and piperacillin-tazobactam treatment was replaced by meropenem. We decided to terminate methylprednisolone treatment by gradually decreasing it due to the patient's lung infection. The tests which were done especially for tuberculosis and immunodeficiencies were negative. There was no notable reduction in lung infiltrations with the patient's current treatment regimen. Galactomannan antigen, cytomegalovirus PCR, and all the cultures resulted as negative. On the 12th day of the PICU admission, thorax CT scan revealed progressed radiological lung findings compatible with RP-ILD secondary to JDM (Fig. 4); thus, five-day pulse steroid (30 mg/kg/day), IV immunoglobulin (IVIG, 2 g/kg), and methotrexate (15 mg/m^2/week) were started. Laboratory tests (anti-nuclear antibody, anti-dsDNA, ENA panel, C_3, C_4, p-ANCA, c-ANCA, lupus anticoagulant, anticardiolipin, antiphospholipid, and anti-glomerular basement membrane antibodies) for other concomitant vasculitis and rheumatic diseases were negative. Fluconazole was added to the patient's treatment for possible fungal pneumonia. On the 18th day, the patient had no

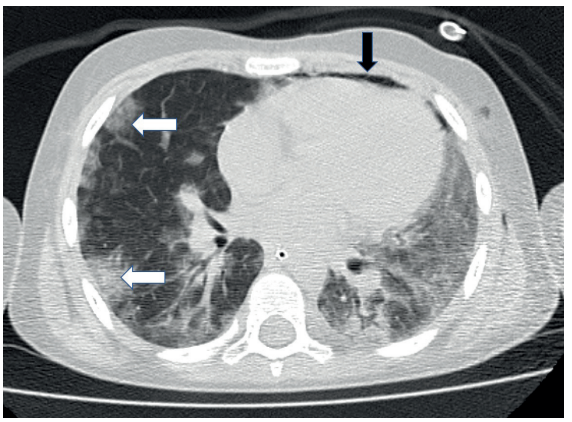


Fig. 4. Second thorax computed tomography examination; minimal pneumomediastinum (black arrow), ground-glass opacity, progressed subpleural patchy consolidation (white arrows), and widespread nonspecific interstitial pneumonia.

improvement in lung findings; therefore, high dose IV cyclophosphamide treatment (1000 mg/m2/month) and daily TPE were started for RP-ILD secondary to JDM. Despite all these intensive treatments, hypoxia worsened, severe pediatric acute respiratory distress syndrome (PARDS) was diagnosed, and venoarterial ECMO was promptly initiated on the 20th day of PICU admission. On the 23rd day of PICU admission, rituximab (375 mg/m^2) was started as a rescue therapy for RP-ILD and TPE terminated but the patient, who did not respond to any of these advanced medical and extracorporeal treatments, died on the 35th day due to multiple organ failure.

Informed consent was received from the family.

Discussion

Lung involvement secondary to JDM is very rare in the pediatric age group. In our previous study, a total of 50 patients with JDM were reviewed retrospectively and none of our patients had lung involvement.[4] In all age groups, the most common lung involvement in DM and polymyositis (PM) is ILD, and its prevalence rate is reported to be 23-65%. Nonspecific interstitial pneumonia, organizing pneumonia/bronchiolitis, obliterans organizing pneumonia, usual interstitial pneumonia, diffuse alveolar damage, and pulmonary capillaritis are other forms of ILD.[1] RP-ILD is uncommon, but it is still one of the significant causes of death in JDM.[3,4] Kobayashi et al.[5] retrospectively examined 8 patients with RP-ILD secondary to JDM, in which 5 of these patients died. Initial thorax CT findings were found to be the most prevalent subpleural curvilinear shadow, and the others are: ground-glass opacity, pleural effusion, traction bronchiectasis, and consolidation around bronchovascular bundles. It was reported that four of these patients developed air leaks such as pneumomediastinum and pneumothorax during the disease. The retrospective study concluded that the anti-MDA5 antibody level was significantly higher in the RP-ILD patient group. Biopsy or autopsy results of all deceased patients were compatible with the findings of diffuse alveolar damage.[5] Even though we did not perform any biopsy or autopsy to our patient, the patient's initial thorax CT showed ground-glass opacity, atelectasis, subpleural patchy consolidation, pneumomediastinum, and pneumothorax. Intriguingly, the anti-MDA5 antibody was found to be negative in our patient who developed RP-ILD in approximately one and half months after the first symptoms and diagnosis of the disease, and unfortunately, he lost his life shortly after. Sato et al.[3] examined 29 JDM and JPM patients in their retrospective study and reported that 3 of them developed RP-ILD. While the anti-MDA5 antibodies were positive in 2 of these patients, this antibody was not studied in the other patient.[3] Bakhshaee et al.[6] reported a case report of a 21-year-old female patient with RP-ILD secondary to DM presenting with subcutaneous emphysema, pneumomediastinum, and pneumothorax similar to the condition of our patient. In their study, Ye et al.[7] have reported that pneumothorax or pneumomediastinum has an incidence rate of 8,6% among ILD secondary to DM/PM. We think that the detection of influenza B in our patient may trigger the development of RP-ILD and may have an additional contribution to the severity of the patient's clinical situation.

Steroids are the first-line treatment in the acute presentation of ILD secondary to juvenile-onset myositis. A 3-day pulse dose steroid and then 1 mg/kg daily dose of prednisolone is the most preferred treatment in patients with ILD who require hospitalization and whose conditions were suspected to progress into RP-ILD. The other preferred immunosuppressive agents according to the clinical course of the disease are mycophenolate mofetil, azathioprine, methotrexate, calcineurin inhibitors such as cyclosporine and tacrolimus, cyclophosphamide, hydroxychloroquine, IVIG, and rituximab.[1,8,9] The two most prevalently used agents in the treatment of RP-ILD are pulse dose steroid and high dose IV cyclophosphamide. According to the literature, it has been reported that rituximab, tofacitinib, TPE are effective in RP-ILD patients who do not respond to standard immunosuppressants.[9-13] Daily TPE treatment as a rescue therapy was started to our patient who did not respond to pulse dose methylprednisolone, high dose IV cyclophosphamide, and IVIG treatments. Despite 5 sessions of TPE, his lung findings worsened significantly, he was taken to ECMO support and the rituximab treatment was started. The patient who did not respond to all these intensive treatments died on the 35th day of PICU admission. The main reasons why pulsed dose steroid and high dose IV cyclophosphamide treatments were not started immediately after hospitalization to PICU were: the anti-MDA5 antibody result was negative and opportunistic bacterial infections and/or influenza B may have also played a role in the sudden deterioration of the lung findings.

In conclusion, JDM patients should be carefully monitored for the development of ILD. RP-ILD with a high mortality may develop shortly after diagnosis, even if the anti-MDA5 antibody is negative.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: OY, MY, CYY, IT; data collection: OY, AA, KB, HY, SŞ; analysis and interpretation of results: OY, EÇ, ÖK; draft manuscript preparation: OY, MY, CYY. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Schnabel A, Hellmich B, Gross WL. Interstitial lung disease in polymyositis and dermatomyositis. Curr Rheumatol Rep 2005; 7: 99-105.
2. Li D, Tansley SL. Juvenile dermatomyositis-clinical phenotypes. Curr Rheumatol Rep 2019; 21: 74.
3. Sato S, Uejima Y, Nanbu M, et al. Clinical analysis and outcome of interstitial lung disease complicated with juvenile dermatomyositis and juvenile polymyositis. Mod Rheumatol 2017; 27: 652-656.
4. Barut K, Aydin PO, Adrovic A, Sahin S, Kasapcopur O. Juvenile dermatomyositis: a tertiary center experience. Clin Rheumatol 2017; 36: 361-366.
5. Kobayashi N, Takezaki S, Kobayashi I, et al. Clinical and laboratory features of fatal rapidly progressive interstitial lung disease associated with juvenile dermatomyositis. Rheumatology (Oxford) 2015; 54: 784-791.
6. Bakhshaee M, Jokar MH, Mirfeizi Z, Atabati E, Tarighat S. Subcutaneous emphysema, pneumomediastinum and pneumothorax in a patient with dermatomyositis. Iran J Otorhinolaryngol 2017; 29: 113-116.
7. Ye S, Chen XX, Lu XY, et al. Adult clinically amyopathic dermatomyositis with rapid progressive interstitial lung disease: a retrospective cohort study. Clin Rheumatol 2007; 26: 1647-1654.
8. Shappley C, Paik JJ , Saketkoo LA. Myositis-related interstitial lung diseases: diagnostic features, treatment, and complications. Curr Treatm Opt Rheumatol 2019; 5: 56-83.

9. Schnabel A, Reuter M, Biederer J, Richter C, Gross WL. Interstitial lung disease in polymyositis and dermatomyositis: clinical course and response to treatment. Semin Arthritis Rheum 2003; 32: 273-284.

10. Ogawa Y, Kishida D, Shimojima Y, Hayashi K, Sekijima Y. Effective administration of rituximab in anti-MDA5 antibody-positive dermatomyositis with rapidly progressive interstitial lung disease and refractory cutaneous involvement: a case report and literature review. Case Rep Rheumatol 2017; 2017: 5386797.

11. Endo Y, Koga T, Suzuki T, et al. Successful treatment of plasma exchange for rapidly progressive interstitial lung disease with anti-MDA5 antibody-positive dermatomyositis: a case report. Medicine (Baltimore) 2018; 97: e0436.

12. Abe Y, Kusaoi M, Tada K, Yamaji K, Tamura N. Successful treatment of anti-MDA5 antibody-positive refractory interstitial lung disease with plasma exchange therapy. Rheumatology (Oxford) 2020; 59: 767-771.

13. Wendel S, Venhoff N, Frye BC, et al. Successful treatment of extensive calcifications and acute pulmonary involvement in dermatomyositis with the Janus-Kinase inhibitor tofacitinib - A report of two cases. J Autoimmun 2019; 100: 131-136.

The Turkish Journal of Pediatrics 2021; 63: 909-912
https://doi.org/10.24953/turkjped.2021.05.019

Case Report

An unusual neurologic presentation of pediatric neuroinvasive West Nile virus infection: ophthalmoplegia

Senem Ayça[1], Gülşen Akkoç[2], Halit Özdemir[3], Hatice Nilgün Selçuk Duru[4]

Departments of [1]Paediatric Neurology, [2]Paediatric Infectious Diseases, [3]Family Medicine and [4]Child Health and Diseases, Haseki Training and Research Hospital, İstanbul, Turkey.

ABSTRACT

Background. West Nile virus (WNV) is an uncommon arbovirus infection and is usually asymptomatic in pediatric patients and due to its rarity is not very well known by clinicians.

Case. We present a 5-year-old girl admitted to the Pediatric Emergency Service with fever, vomiting, neck stiffness, walking difficulty and sudden deviation of eyes who was diagnosed with a neuroinvasive WNV infection.

Conclusions. Ophthalmoplegia is an unusual presentation of neuroinvasive WNV and there are no published pediatric cases with ophthalmoplegia in the literature.

Key words: West Nile Virus, ophthalmoplegia, ataxia, children.

West Nile virus (WNV) is an uncommon arbovirus infection and is usually asymptomatic in pediatric patients. Infections due to WNV have increased in frequency and include previously virus-free regions, especially since 2018.[1] Some European countries including Balkan countries and Turkey had higher numbers of reported WNV cases than the previous years.[2]

Neuroinvasive WNV infection presentations include aseptic meningitis, meningoencephalitis and acute flaccid paralysis syndrome. Brainstem encephalitis, cerebellitis, movement disorders, cranial neuropathies, polyneuropathy/radiculopathy, chorioretinitis and optic neuritis are also recognized WNV neurological presentations.[3] Knowledge of WNS infections and atypical neurological presentations is important for pediatricians and neurologists because of the increased number of cases. Herein, we present a case of pediatric neuroinvasive WNV with ophthalmoplegia, which is an unusual presentation. To the best of our knowledge, this is the first published pediatric case with ophthalmoplegia in the literature.

✉ Senem Ayça
senemkaleci85@gmail.com

Received 19th May 2020, revised 30th December 2020, accepted 9th January 2021.

Case Report

A previously healthy 5-year-old girl with fever, vomiting, fatigue, and myalgia presented to a pediatric clinic in September 2019. She was prescribed amoxicillin and antipyretics. A day after admission, additional symptoms such as frontal headache, deviation of eyes, meaningless speech, and walking imbalance progressed and she was admitted to our tertiary hospital in Istanbul, Turkey. Her medical history was uneventful. She was born as a child of non-consanguineous parents through spontaneous vaginal birth. Her parents recalled her being bitten by mosquitoes a few weeks ago in Esenler/İstanbul.

On admission, she was lethargic with a 39.2°C body temperature, and a truncal maculopapular rash was observed. She had isochoric pupils with normal light reflexes, but she had a sudden medial deviation of bilateral eyes and she had

limited abduction paresis of the bilateral eyes. She had deep tendon reflexes with normal response and her muscle strength was normal. Her plantar reflex was flexor bilaterally. She had neck stiffness, and signs of meningeal irritation were positive. She had walking difficulty and left-sided ataxia. The rest of her general physical examination was unremarkable. The results of a blood investigation were within normal ranges (C-reactive protein 1.1 mg/L; erythrocyte sedimentation rate (ESR) 15 mm/h). Lumbar puncture was performed, cerebrospinal fluid (CSF) cytochemical analysis findings were as follows; white blood cells 1200/mm^3 (polymorphonuclear cells and lymphocytes were both seen), red blood cells 116/mm^3, protein 70 mg/dL, glucose 63 mg/dL (at the same time the blood glucose was 75 mg/dL). CSF analyses performed using polymerase chain reaction (PCR) assay for herpes simplex virus type 1-2, human herpesvirus type 6-7, varicella-zoster virus (VZV), parechovirus, and enterovirus were negative. Serology of VZV, Epstein-Barr virus, cytomegalovirus, toxoplasmosis, parvovirus b19, and mycoplasma pneumonia were tested. Ceftriaxone and acyclovir treatment and dexamethasone was started for presumed infectious meningitis. She had nausea, vomiting, and diarrhea, so she was rehydrated.

Ophthalmologic consultation and fundoscopic examination was normal except for bilateral 6^{th} cranial nerve palsy. Brain and spine magnetic resonance imaging (MRI) scans showed normal results. An electroencephalogram revealed no abnormalities. Three days after admission, she had normal oculomotor movements and had no fever, vomiting, and diarrhea. She had mild left-sided ataxia, which resolved within two weeks.

The serology of the above mentioned microorganisms were negative except for serum mycoplasma pneumonia. Immunoglobulin (Ig)-M was borderline positive and clarithromycin treatment was added. National arbovirus and viral zoonotic diseases laboratory test results for WNV reverse transcription (RT)-PCR was negative, immune fluorescent agglutination (IFA) IgM and IgG were positive. Serological testing of serum and cerebrospinal fluid (CSF) remains the gold standard for the WNV diagnosis.[4] The result of positive IFA IgM for WNV, clinical meningitis findings with a history of being bitten by mosquitoes verified the diagnose of WNV. In the region of Esenler, where she had a history of being bitten by mosquitos adult WNV cases had previously been seen.

WNV IgM antibodies are detectable in most patients within 3 to 8 days of symptom onset and IgG antibodies are also detectable shortly after IgM antibodies.[5] The WNV serology of the patient was performed 4 days after symptom onset and both IgM and IgG antibodies were detected. Plaque reduction neutralization assay (PRNA) could be an additional diagnostic modality for this patient because flaviviruses might elicit cross-reactive test results. As a limitation PRNA could not be performed because of her parents' incompatibility. Antibiotherapy was stopped on the 10th day and she was discharged. Seven days after discharge her follow-up neurologic and systemic re-examinations were normal.

Written informed consent was obtained from the parents of the patient.

Discussion

WNV neuroinvasive infection has been defined as an illness with evidence of an acute infectious process with clinical evidence of meningitis, encephalitis or acute flaccid paralysis.[4] For a definitive diagnosis of WNV infection, specific antibodies should be detected in serum or CSF samples. Detection of WNV IgM in CSF is diagnostic of neuroinvasive disease. RT-PCR is not recommended for the diagnosis of WNV infection because peak viremia occurs 3-4 days before symptom onset and WNV RNA is no longer detectable.[5] RT-PCR is only recommended for immunocompromised patients.[5] Pleocytosis with a predominance of polymorphonuclear cells, and the presence of abnormal appearing reactive lymphocytes and elevated protein with normal glucose are the

characteristic CSF findings of neuroinvasive WNV.[6] In our patient, there were signs of meningeal irritation and CSF analysis revealed pleocytosis with polymorphonuclear cells and lymphocytes. RT-PCR was negative because peak viremia had ended. Detection of WNV IgM was diagnostic for this patient.

Serologic studies showed that WNV cases are 80% asymptomatic.[7] Only 10% of symptomatic WNV cases develop neuroinvasive disease. WNV infection with neuroinvasive involvement causes encephalitis (50%), meningitis (37%), and acute flaccid paralysis (6%).[8] Also, movement disorders such as tremor, ataxia, and myoclonus may be present during WNV illness.[9]

In the literature, there are only two pediatric case reports describing ataxia in neuroinvasive WNV infection.[10,11] A 43-year-old adult was reported with WNV who presented with ataxia and ocular dysmetria, which was reminiscent of Bickerstaff's encephalitis, and was treated with high-dose corticosteroids and intravenous immunoglobulin.[12] There are no pediatric WNV case reports presenting with ataxia and ophthalmoplegia. In our case, we did not consider the diagnosis of Miller Fisher syndrome or Bickerstaff's encephalitis because of the accompanying fever, rash and CSF pleocytosis. CSF analysis and serology results excluded other viral etiologies for the differential diagnosis.

Age and sex play a role in disease severity. Older males are at greater risk for developing neuroinvasive involvement, people of younger age are more likely to present with milder forms or asymptomatic infection.[13] Prognostic data on WNV neuroinvasive disease are scarce, especially for children because of the limited pediatric WNV cases. In adult studies, almost all mortality is confined to patients with neuroinvasive disease. The death rate is approximately 9% in neuroinvasive WNV cases and in patients with WNV encephalitis it is approximately 12–15%.[14] It has been shown that sequelae from neuroinvasive WNV are highly variable and younger age at infection is the most important predictor of recovery.[15] In our case, the patient made a complete recovery, and we think that children with neuroinvasive WNV infections have a good prognosis.

To the best of our knowledge, this is the first published report of neuroinvasive WNV infection presenting with ophthalmoplegia. It is therefore essential for pediatricians to be familiar with the wide spectrum of neurologic manifestations of WNV disease.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SA, GA; data collection: HÖ; analysis and interpretation of results: HNSD; draft manuscript preparation: SA, GA. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

None.

REFERENCES

1. Duggal NK, Langwig KE, Ebel GD, Brault AC. On the fly: interactions between birds, mosquitoes, and environment that have molded West Nile virus genomic structure over two decades. J Med Entomol 2019; 56: 1467-1474.
2. ECDC. Epidemiological update: West Nile virus transmission season in Europe, 2018. European Centre for Disease Prevention and Control, Available at: https://www.ecdc.europa.eu/en/news-events/epidemiological-update-west-nile-virus-transmission-season-europe-2018 (Accessed on October 24, 2019).
3. Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, Campbell GL. Virology, pathology, and clinical manifestations of West Nile virus disease. Emerg Infect Dis 2005; 11: 1174-1179.
4. Sejvar JJ, Haddad MB, Tierney BC, et al. Neurologic manifestations and outcome of West Nile virus infection. JAMA 2003; 290: 511-515.
5. American Academy of Pediatrics. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, (eds). Red Book: 2018-2021 Report of the Committee on Infectious Diseases. (31st ed) Itasca, IL: American Academy of Pediatrics, 2018.

6. Tyler KL, Pape J, Goody RJ, Corkill M, Kleinschmidt-DeMasters BK. CSF findings in 250 patients with serologically confirmed West Nile Virus meningitis and encephalitis. Neurology 2006; 66: 361-365.

7. Lindsey NP, Staples JE, Lehman JA, Fischer M; Centers for Disease Control and Prevention (CDC). Surveillance for human West Nile virus disease -United States, 1999-2008. MMWR Surveill Summ 2010; 59: 1-17.

8. Sejvar JJ, Marfin AA. Manifestations of West Nile neuroinvasive disease. Rev Med Virol 2006; 16: 209-224.

9. Lenka A, Kamat A, Mittal SO. Spectrum of movement disorders in patients with neuroinvasive West Nile virüs infection. Mov Disord Clin Pract 2019; 6: 426-433.

10. DeBiasi RL, Parsons JA, Grabert BE. West Nile virus meningoencephalitis in an immunocompetent adolescent. Pediatr Neurol 2005; 33: 217-219.

11. Moon TD, Nadimpalli A, Martin EB, Ortiz MA, Van Dyke RB. Balance and gait abnormalities of a child with West Nile virus infection. Pediatr Infect Dis J 2005; 24: 568-570.

12. Popescu A, Jain S, Massier A, Gopalakrishna KV, Bambakidis P. West Nile infection presenting with ocular dysmetria, ataxia, and hyperreflexia reminiscent of Bickerstaff's encephalitis. Am J Med 2008; 121: e3-e4.

13. Petersen LR, Brault AC, Nasci RS. West Nile virus: review of the literature. JAMA 2013; 310: 308-315.

14. CDC. Centers for Disease Control and Prevention. Available at: http://www.cdc.gov (Accessed on November, 2020).

15. Ali M, Safriel Y, Sohi J, Llave A, Weathers S. West Nile virus infection: MR imaging findings in the nervous system. AJNR Am J Neuroradiol 2005; 26: 289-297.

The Turkish Journal of Pediatrics 2021; 63: 913-916
https://doi.org/10.24953/turkjped.2021.05.020

Case Report

Cardiac arrest due to a fatal dose of propranolol successfully treated with intravenous lipid infusion

Edin Botan[1], Tanıl Kendirli[1], Emrah Gün[1], Serdar Balsak[1], Mehmet Ramoğlu[2], Ercan Tutar[2]

Divsions of [1]Pediatric Critical Care Unit and [2]Pediatric Cardiology, Department of Pediatrics, Ankara University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Background. Beta blockers (BB) are used for very different indications in both adults and children. There can be mild adverse effects with normal doses. When taken in toxic doses, this can have fatal results in children. There are some standard therapies during BB poisoning such as insulin and glucagon but there is not enough knowledge concerning intravenous lipid infusion therapy (ILI).

Case. Herein we present a case of propranolol poisoning in a previously healthy 2-year-old girl. In this patient, cardiac arrest developed twice, and cardiopulmonary resuscitation was performed for 5 and 20 minutes, respectively. We initiated inotropes, insulin, calcium and glucagon with a lack of response to all medical treatment. We used ILI and the patient improved after this treatment. She recovered without any disability.

Conclusions. ILI treatment should be considered with life-threatening BB poisoning which is unresponsive to standard therapies.

Key words: beta blockers, poisoning, propranolol, children, intravenous lipid infusion therapy.

Beta blockers (BB) are commonly used drugs in adults but their use in children is rare.[1] They are frequently used for the treatment of hypertension, ischemic heart disease and arrhythmia in adults.[1] The indications of BB usage in children include supraventricular tachycardia, atrial fibrillation/flutter, prevention of cyanotic spells in tetralogy of Fallot, thyrotoxicosis, migraine headache prophylaxis and hypertension.[1] Propranolol, metoprolol, esmolol and carvedilol are the most frequently used BB in children. Propranolol is a non-cardioselective BB with membrane stabilizing activity.[2]

✉ Edin Botan
edinbotan@hotmail.com

Received 29th May 2020, 2nd September 2020, accepted 3rd December 2020.

This study was presented at the 16th National Pediatric Emergency Medicine and Intensive Care Congress, 2-5 October 2019, Antalya, Turkey.

Intravenous lipid infusion (ILI) treatment is primarily used in poisoning with lipophilic local anesthetic and chemical agents, but there are few reports of its usage in calcium channel blocker (CCB) and BB poisoning.[3] The use of ILI in propranolol poisoning has rarely been reported in adults and scarcely in children.[4] Herein we present an infant who was successfully treated by ILI who developed severe cardiac arrest (CA) due to a fatal dose of propranolol ingestion.

Case Report

A previously healthy 2-year-old girl was brought to our Pediatric Emergency Care Service due to vomiting and drowsiness that had started 6 hours ago. On admission, her Glasgow Coma Scale (GCS) was 6, cardiac arrest developed and we started cardiopulmonary resuscitation (CPR), na intraosseous line was established and one dose of epinephrine was given after endotracheal intubation. Return of spontaneous

circulation (ROSC) was achieved in 5 minutes. After ROSC the Patient's cebtral pulses were weak and peripheral pulses were absent. The patient was given a high-speed infusion of 0.9% saline. Blood glucose level was 96mg/dL. She was transferred to the pediatric intensive care unit (PICU). The patient was monitored, connected to a mechanical ventilator and a central venous catheter was inserted. % 0.9 Serum Intravenous fluid rescue therapy was given twice at 20mL/kg with rapid saline infusion, epinephrine infusion was started at a dose of 0,1mcg/kg/min. The patient's venous blood gases revealed a pH: 7.10, pCO2: 45, pO2: 20.9 HCO3: 13.5, lactate: 5.2mmol/L, during PICU admission. Biochemical parameters and other blood values were found within normal ranges. Cardiac arrest developed again after 15 minutes of PICU admission. We started CPR again and the patient received adrenaline 7 times and one dose of bicarbonate. The bradycardia continued and 1 mg of atropine was administered intravenously three times. We prepared for extracorporeal cardiopulmonary resuscitation (ECPR) if spontaneous circulation was not restored. ROSC occurred in 20 minutes and her rhythm was pulsed ventricular tachycardia, therefore we performed electrical cardioversion 3 times as 1-2-2 joule/kg respectively, and amiodarone infusion was started. As the patient's QTc> 0.55s on electrocardiography, amiodarone was discontinued. At that time, she required a high dose of inotropic support (0.3mcg/kg/min of epinephrine, 0.1mcg/kg/min of norepinephrine, 10mcg/kg/min of dopamine).

When the patient's history was deepened, we learned that 5 tablets of her grandmother's drug (40mg, Dideral®, propranolol), which is equivalent to 17/mg/kg dose, was not in its drug box and we strongly suspected that patient had propranolol poisoning. She received activated charcoal at 1gr/kg, meanwhile, the patient was hypotensive (53/35mmHg) in spite of the high dose of inotropes and vasopressors, we initiated insulin 1U/kg intravenous bolus and switched to 0.5 U/kg/hr infusion. 10% calcium gluconate infusion at a dose of 1 mL/kg, and subcutaneous glucagon at a dose of 0.5mg (because there is no intravenous form) were administered. Despite all attempts findings of decompensated shock continued and we decided to start ILI at the 60th minute of PICU admission. ILI was given at a dose of 1.5mL/kg IV bolus (for 3 minutes) and 0.25mL/kg/hour infusion was started for 30 minutes. Hemodynamic parameters of the patient improved in hours and we were able to decrease the epinephrine and norepinephrine doses rapidly after ILI treatment. After ILI treatment, consequent blood gases and biochemical values are given in (Table I). At the 24th hour of the PICU admission, the patient's vital signs, blood gases, blood glucose, biochemical parameters, and electrocardiogram findings returned to normal, and she was extubated.

At the end of the 24th hour, all therapies were discontinued. The Patient was transferred to the pediatric ward on the 2nd day and she was discharged on the 3rd day of admission with full recovery.

Written informed consent was obtained from the parents of the patient

Table I. The patient's blood gases and certain biochemical values after ILI treatment.

	pH	PCO2	HCO3	Lactate	BUN	Creatinine	AST	ALT	ILI treatment
0.hour	7.10	45	13.5	5.2	13	0.55	174	83	
2.hour	7.39	45.2	27.2	7.0	10	0.38	135	78	1.5CC/KG*
4.hour	7.50	28.4	23.8	3.9					
8.hour	7.45	34.7	23.3	2.2	7	0.27	80	61	
24.hour	7.38	36.1	21.2	2.8	7	0.20	54	54	

BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ILI: intravenous lipid infusion

Discussion

Propranolol overdoses have potentially life-threatening effects such as bradycardia, hypotension and CA similar to CCBs.[5,6] At the same time BBs can cause toxic effects regardless of dose. Initial therapy in BBs poisoning is similar to other toxic drugs; determine and support the airway, breathing and circulation (ABC) troubles. In symptomatic patients IV glucagon (initially in reversal of BB symptoms) and calcium (especially in calcium channel blocker poisoning), fluid therapy, atropine, and inotropes are basics therapies.[4,6,7] In our case, hyperinsulinemic euglycemia treatment and ILI were applied when there was no response to symptomatic treatment in the case of cardiovascular collapse following a high dose of BB intake.

ILI therapy is also highly recommended as a salvage treatment for severe poisoning with lipophilic anesthetic and other drugs.[8] Sebe et al.[5] reported a series of 15 adult patients who were treated with ILI secondary to BB, CCB, and paracetamol intoxication, 14 of them recovered, whereas one patient died. Le Fevre et al.[9] reported a case of a 25-year-old patient with a dramatic response to ILI following pulseless electrical activity arrest due to a mixed amitriptyline and propranolol overdose. Amanda et al.[10] reported a complete clinical recovery with the administration of ILE in combination with high-dose insulin (HDI) in a 7-month-old symptomatic pediatric patient with acute propranolol toxicity. But their case was that of multidrug poisoning and it is unclear which drug caused the cardiotoxicity. Differently, our case only took propranolol leading to CA and our patient fully recovered with ILI treatment.

Rarely, BB and CCB poisoning does not respond to standard treatment, these intoxications may be fatal and extracorporeal support like ECMO and plasma exchange (PEX) may be needed.[6,9,11] Kolcz et al.[11] reported a case of 15-year-old girl with cardiogenic shock after alcohol, propranolol and verapamil overdose. Their patients successfully recovered with ECPR and PEX.

Our patient did not respond to fluids or high dose inotropic support and although we prepared for ECMO this was not necessary as she regained spontaneous circulation after 20 minutes of CPR. We didn't consider any extracorporeal therapies because our patient's clinical situation recovered rapidly after ILI treatment.

In conclusion, BB poisoning can be life-threatening by causing cardiovascular collapse in children. When patients are unresponsive to the classical treatment options in these poisonings ILI treatment should be considered.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EB, EG; data collection: EB, SB; analysis and interpretation of results: EB,TK,MR; draft manuscript preparation: TK,ET. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors have no potential conflict of interest with the present article.

REFERENCES

1. Farzam K, Jan A. Beta Blockers. Last Update: July 9, 2021. Available at: https://www.ncbi.nlm.nih.gov/books/NBK532906/ (Accessed on September 20, 2021)
2. Brubacher JR. Chapter 61 β-Adrenergic Antagonists. In: Nelson LS, Lewin NA, Howland MA, Hoffman RS, Goldfrank LR, Flomenbaum NE (eds). Goldfrank's Toxicologic Emergencies (9th ed) New York: McGraw-Hill, 2011.
3. Ozcan MS, Weinberg G. Intravenous lipid emulsion for the treatment of drug toxicity. J Intensive Care Med 2014; 29: 59-70.

4. Graudins A, Lee HM, Druda D. Calcium channel antagonist and beta-blocker overdose: antidotes and adjunct therapies. Br J Clin Pharmacol 2016; 81: 453-461.

5. Barrueto FJ. Beta blocker poisoning. UpToDate. 2018. Available at: https://www.uptodate.com/contents/beta-blocker-poisoning. (Accessed on November 04, 2020).

6. Sebe A, Dişel NR, Açıkalın Akpınar A, Karakoç E. Role of intravenous lipid emulsions in the management of calcium channel blocker and β-blocker overdose: 3 years experience of a university hospital. Postgrad Med 2015; 127: 119-124.

7. Seegobin K, Maharaj S, Deosaran A, Reddy P. Severe beta blocker and calcium channel blocker overdose: role of high dose insulin. Am J Emerg Med 2018; 36: 736.e5-736.e6.

8. Weinberg G. Lipid rescue resuscitation from local anaesthetic cardiac toxicity. Toxicol Rev 2006; 25: 139-145.

9. Le Fevre P, Gosling M, Acharya K, Georgiou A. Dramatic resuscitation with Intralipid in an epinephrine unresponsive cardiac arrest following overdose of amitriptyline and propranolol. BMJ Case Rep 2017; 2017: bcr2016218281.

10. Thompson AM, Franco Palacios CR, Henriksen MN. Intravenous lipid emulsion and high-dose insulin as adjunctive therapy for propranolol toxicity in a pediatric patient. Am J Health Syst Pharm 2016; 73: 880-885.

11. Kolcz J, Pietrzyk J, Januszewska K, Procelewska M, Mroczek T, Malec E. Extracorporeal life support in severe propranolol and verapamil intoxication. J Intensive Care Med 2007; 22: 381-385.

The Turkish Journal of Pediatrics 2021; 63: 917-921
https://doi.org/10.24953/turkjped.2021.05.021

Case Report

Diphallia: a case report of a rare anomaly evaluated by magnetic resonance imaging

Márcio Luís Duarte[1,2], Lucas Ribeiro dos Santos[2,3], André de Queiroz Pereira da Silva[1,4], Marcelo de Queiroz Pereira da Silva[1]

[1]WEBIMAGEM Telerradiologia, São Paulo, São Paulo, Brazil; [2]Master´s degree in Evidence-Based Health UNIFESP, São Paulo, São Paulo, Brazil; [3]Faculdade de Ciências Médicas de Santos, Santos, São Paulo, Brazil; [4]Hospital São Rafael, Imperatriz, Maranhão, Brazil.

ABSTRACT

Background. Diphallus, also known as penile duplication, is a rare malformation, seen once in every 5 to 6 million births. Newborns showing this condition present higher mortality rates due to malformations and infections. The underlying etiology of this malformation is uncertain, but it is thought to be associated with trauma, drug use, or infections that may affect fetal the mesoderm between the 23rd and 25th day of pregnancy. Our objective is to describe this rare malformation - diphallus - through magnetic resonance imaging, as well as additional findings.

Case. A Three-month-old male patient with a 33-week ultrasound demonstrating genital malformation presented to our clinic. At birth, the physical examination revealed diphallia and imperforated anus. Surgical procedures were carried out right after birth to correct the anus malformation. The child did not present any alteration in skin color, and no signs of pain were shown in the abdomen, pelvis, and penises palpations. Urination was observed only through the right penis. Magnetic resonance imaging (MRI) showed two penile structures, each one presenting developed with corpus cavernosum. The penis located on the right showed a complete urethral path in the corpus spongiosum to the vesical floor while the penis located on the left was bigger and did not present a urethral path.

Conclusions. Penile duplication is a rare condition that is often, associated with other malformations, especially anorectal. To fully understand the extension of congenital anomalies and to determine the optimal surgical approach, MRI yields detailed imaging of the entire pelvic region, providing a thorough anatomical frame of reference, and should be routinely incorporated into presurgical evaluation.

Key words: infant, newborn, urogenital abnormalities, anorectal malformations, magnetic resonance imaging.

Duplication of the penis, also referred to as diphallia, is a rare congenital anomaly, with an estimated incidence of 1:5 million live births;[1] over one hundred cases have been reported since the first documentation by Wecker in 1609.[2]

Cases of diphallia differ greatly from one another, varying from double glans with a common shaft, to complete duplication of the phallus (which is even more infrequent).[3,4] Other anomalies may coexist, such as the ectopic scrotum, hypospadias, and double bladder.[5] Gastrointestinal malformations, presenting imperforate anus, colon duplication, and vertebral deformities can often occur with diphallia.[5] Newborns with this condition have an increased risk of death due to malformations and the associated infections.[6]

Diphallia can be classified as true diphallia or bifid phallus, both of which can be further classified as complete or partial duplication. In complete penile duplication, each penis presents two corpus cavernosum and one corpus spongiosum; in partial diphallia, one of them presents rudimentary structures.[7,8]

✉ Márcio Luís Duarte
marcioluisduarte@gmail.com

Received 29th July 2020, revised 11th November 2020, 3rd December 2020, accepted 9th December 2020.

Bifid phallus, which is responsible for only one-third of the reported cases of diphallia, presents only one corpus cavernosum in each penis, and according to the location of separation, it can be classified as complete when it occurs at the base of the shaft, or partial when it is located as the glans.[7,8] The bifid phallus is responsible for only one-third of the reported cases of diphallia.[7,8] Anatomical differentiation may be difficult, and subsidiary diagnostic techniques are most often necessary to plan the therapeutic approach.

Herein, we report a case of a partial true diphallia, correlating with magnetic resonance imaging (MRI) findings. Informed consent was obtained from the patient´s parents for the publication of this case report.

Case Report

A 3-month-old male patient presented to the pediatrics department, with a previous 33-weeks gestational ultrasound demonstrating genital malformation, with possible diphallia, a precise diagnosis could not be reached due to fetus position. At birth, the patient presented with diphallia with two testicles, one in each hemi-scrotum (Fig. 1) and imperforate anus. The mother denied the use of any medications,

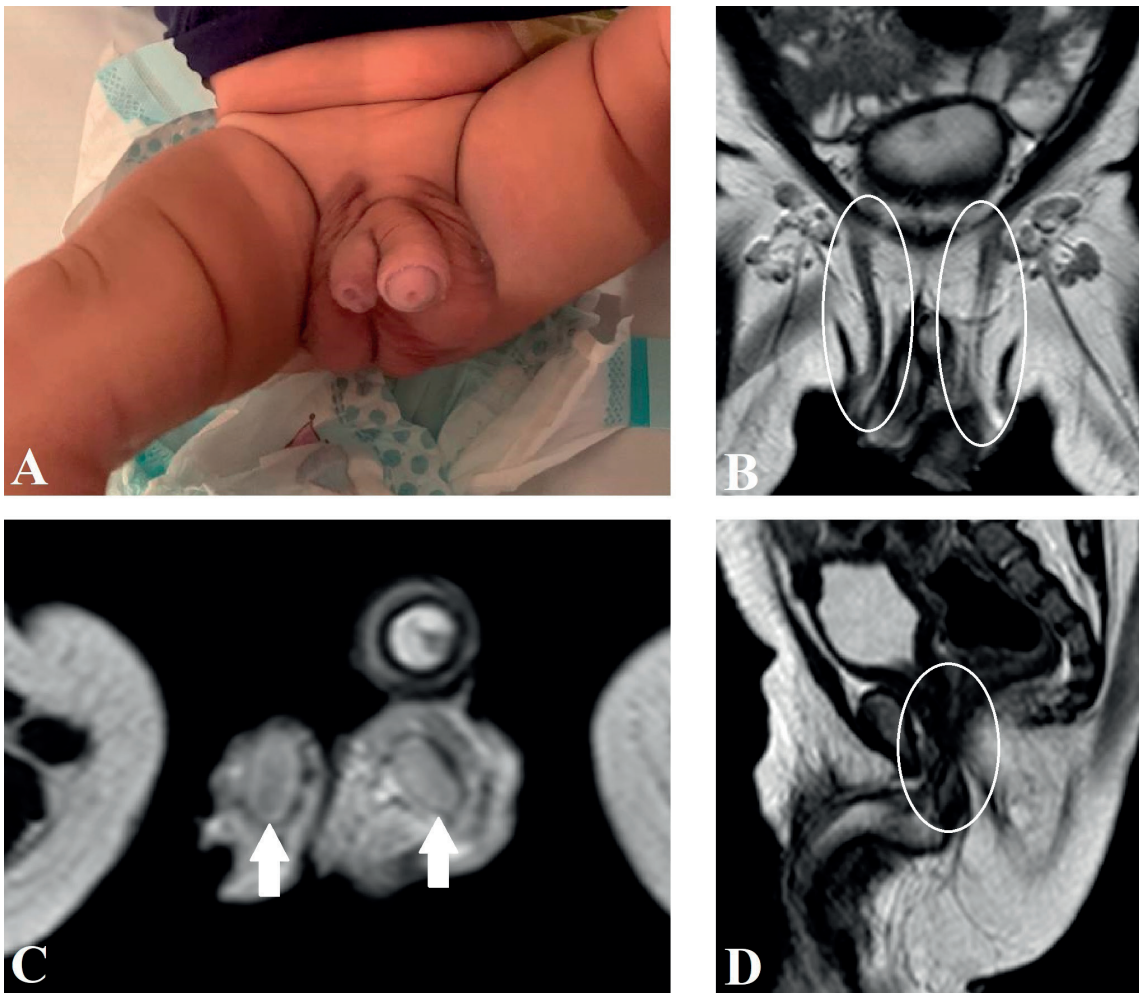


Fig. 1. A: A visual demonstrating two penises - diphallia. B: The MRI in the coronal section in the T2 sequence showing normal spermatic cords (white circles). C:MRI in the axial section in the T2 sequence showing normal testicles (white arrows). D: MRI in the sagittal section in the T2 sequence, the rectum appears to join in the urethra - a defect of the urogenital sinus (white circle).

alcohol nor drugs during pregnancy. At birth, the patient underwent surgery to correct the imperforate anus. At the consultation, he presented with 2 penises, but only through the right penis was urine outflow observed. The remainder of the physical examination was unremarkable, as anal imperforation had already been corrected.

The patient underwent MRI (Fig. 2), which demonstrated two penile structures. The penis located on the right contained a complete urethral path, through the corpus spongiosum, connecting to the bladder; the penis on the left was larger but did not have a complete urethral path, presenting with an incomplete rudimentary membranous and prostatic urethra. MRI also showed anorectal atresia with a fistulous path, 2mm thick, directing to the prostatic/membranous urethra on the right. On MRI two preserved, well located, testicles were observed. After the MRI, the patient has been prepared for penile surgery.

Discussion

Diphallia is a rare condition, which may be complicated by other congenital anomalies. The cloacal-like (urethrorectal fistula) associated malformation presented by our patient has been previously reported in the literature, being repaired by simple excision.[8]

The underlying etiology of this malformation is uncertain, but it might be associated with trauma, drug use, or infections that may affect the fetal mesoderm between the 23rd and 25th day of pregnancy.[7,9,10] It was found that the karyotype in cases of diphallia is normal, except for the case described by Karna and Kapur, associated with a translocation between 46, XY, t(l:14)(p36.3;q24.3.[11]

Compared to bifid phallus, diphallia usually presents with more complex associated malformations, such as other genitourinary malformations (bladder and urethral duplication, vesical exstrophy, renal abnormalities, and bifid scrotum), as well as anorectal malformations (bowel duplications, fistulas, and anal atresia), and vertebral anomalies.[8,12]

True diphallia rarely occurs in isolation and usually presents with other anomalies, such as hypospadias, epispadias, and ectopic urethra.[4] The hypoplastic or blind-ended urethra, as seen in our patient. that presents with only one complete and well-developed urethra can also occur.

Penile duplication, as well as associated anomalies, may urge multiple procedures, at different points of time, to achieve full functional and aesthetic conditions. It is fundamental to depict urethral pathways, and which (if one or both) present urine outflow; in most cases, the conventional urodynamic study can determine such characteristics, as well as other renal tract malformations.[4] However, MRI can further depict other minor associated anomalies, as well as help to decide the surgical approach, as it permits evaluation of penile structures; in fact, comprehensive studies comparing MRI to other imaging techniques have already demonstrated its superiority, yielding greater accuracy.[14,15]

Also, to determine which penis has the full functional capacity, the presence of two corpus cavernosum and one corpus spongiosum is decisive, as well as spermatic cords, as MRI demonstrated in our patient; such structures will determine which penis will be excised. In more complex cases, where there is only one corpus cavernosum in each penis, the joining of two corporal bodies with penile reconstruction has been reported.[4,6,10,16,17]

Every patient with diphallia needs to be carefully examined for the assessment of associated malformations.[10] The great variability of the anatomy and the presentation in cases of diphallus result in different strategies for the management of malformation.[4,10] Surgical correction is individualized to achieve adequate urinary continence and erection with adequate aesthetics.[4,6,9]

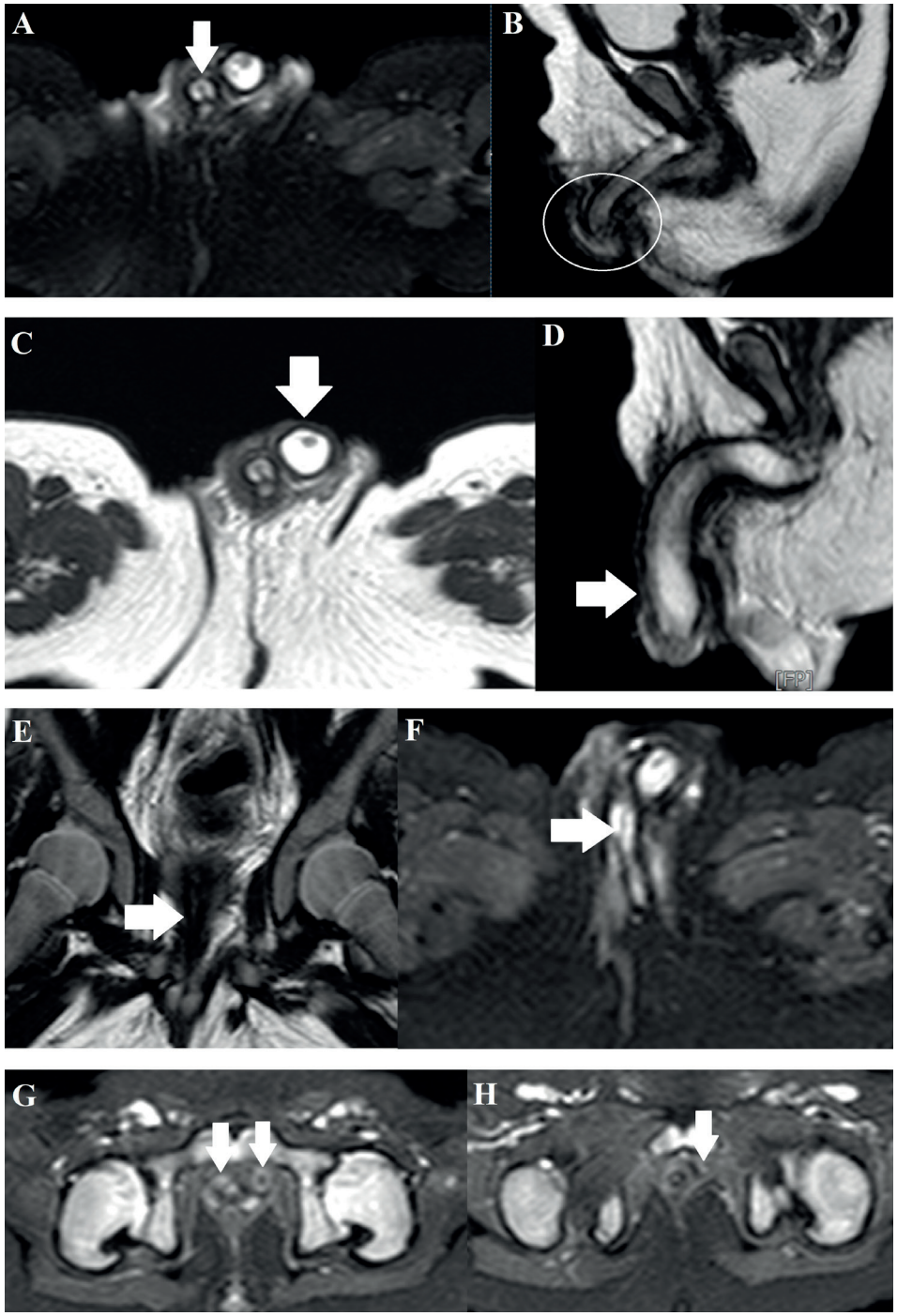


Fig. 2. A: MRI in the axial section in the T2 FAT SAT sequence. B: MRI in the sagittal section in the T2 sequence showing the appearance of the urethra of the right penis (white arrow and circle). C: MRI in the axial view and D: in the sagittal view in the T2 sequence showing a single mass of the corpus cavernosum in the left penis (white arrows). E: MRI in the coronal section in the T2 sequence. F: MRI in the axial section in the T2 FAT SAT sequence showing the right urethra opening into the corpus spongiosum (white arrows). G: MRI in the axial section in T2 FAT SAT sequence showing the two urethrae (white arrows). H: MRI in the axial section in the T2 FAT SAT sequence showing the left urethra ending in a blind bottom (white arrow).

Penile duplication is a rare condition that is often associated with other malformations, especially anorectal. To fully understand the extension of congenital anomalies and to determine the optimal surgical approach, MRI yields detailed imaging of the entire pelvic region, providing a thorough anatomical frame of reference, and should be routinely incorporated into presurgical evaluation.

Acknowledgment

To Ms. Roberta Neves Gago Rodrigues for the translation adjustments and spelling correction.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MLD, LRDS, MDQPDS; data collection: MLD, LRDS; analysis and interpretation of results: MLD, LRDS, ADQPDS; draft manuscript preparation: MLD, LRDS, ADQPDS, MDQPDS.All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Torres-Medina E, Sanchez-Puente JC, Aragon-Tovar A. Diphallia, report of one case and review of literature. Rev Mex Urol 2009; 69: 32-35.
2. Neugebauer FL. 37 Fälle von Verdoppelung der äusseren Geschlechtsteile. Monatschr Geburtsh Gynäk 1898; 7: 645-648.
3. Sharma KK, Jain R, Jain SK, Purohit A. Concealed diphallus: a case report and review of the literature. J Indian Assoc Pediatr Surg 2000; 5: 18-21.
4. Karagözlü Akgül A, Uçar M, Çelik F, Kırıştıoğlu İ, Kılıç N. Complete penile duplication with structurally normal penises: a case report. Balkan Med J 2018; 35: 340-343.
5. Carvalho AP, Ramires R, Soares J, Carvalho LF, Filinto M. Surgical treatment of complete penile duplication. Actas Urol Esp 2008; 32: 941-944.
6. Bakheet MA, Refaei M. Penile duplication and two anal openings; report of a very rare case. Iran J Pediatr 2012; 22: 133-136.
7. Frolov A, Tan Y, Rana MW, Martin JR 3rd. A rare case of human diphallia associated with hypospadias. Case Rep Urol 2018; 2018: 8293036.
8. Gyftopoulos K, Wolffenbuttel KP, Nijman RJ. Clinical and embryologic aspects of penile duplication and associated anomalies. Urology 2002; 60: 675-679.
9. Mukunda R, Bendre PS, Redkar RG, Hambarde S. Diphallus with anorectal malformation-case report. J Pediatr Surg 2010; 45: 632-634.
10. Mirshemirani A, Roshanzamir F, Shayeghi S, Mohajerzadeh L, Hasas-Yeganeh S. Diphallus with imperforate anus and complete duplication of recto-sigmoid colon and lower urinary tract. Iran J Pediatr 2010; 20: 229-232.
11. Karna P, Kapur S. Diphallus and associated anomalies with balanced autosomal chromosomal translocation. Clin Genet 1994; 46: 209-211.
12. Karaca I, Turk E, Ucan AB, Yayla D, Itirli G, Ercal D. Surgical management of complete penile duplication accompanied by multiple anomalies. Can Urol Assoc J 2014; 8: E741-E743.
13. Muramatsu M, Shishido S, Nihei H, et al. Urinary reconstruction in vertebral, anorectal, cardiac, trachea-esophageal, renal abnormalities and limb defects association with chronic renal failure and penile duplication. Int J Urol 2015; 22: 125-127.
14. Zhan Y, Wang J, Guo WL. Comparative effectiveness of imaging modalities for preoperative assessment of anorectal malformation in the pediatric population. J Pediatr Surg 2019; 54: 2550-2553.
15. Thomeer MG, Devos A, Lequin M, et al. High resolution MRI for preoperative work-up of neonates with an anorectal malformation: a direct comparison with distal pressure colostography/fistulography. Eur Radiol 2015; 25: 3472-3479.
16. Corrêa Leite MT, Fachin CG, de Albuquerque Maranhão RF, Francisco Shida ME, Martins JL. Penile duplication without removal of corporal tissue: step by step of an excellent cosmetic result. J Pediatr Urol 2014; 10: 567-570.
17. Tirtayasa PM, Prasetyo RB, Rodjani A. Diphallia with associated anomalies: a case report and literature review. Case Rep Urol 2013; 2013: 192960.

The Turkish Journal of Pediatrics 2021; 63: 922-926
https://doi.org/10.24953/turkjped.2021.05.022

Case Report

Cockayne syndrome type: a very rare association with hemorrhagic stroke

Başak Atalay[1], Mine Sorkun[1], Elif Yüksel Karatoprak[2]

Departments of [1]Radiology and [2]Pediatric Neurology, İstanbul Medeniyet University Faculty of Medicine, Göztepe Training and Research Hospital, İstanbul, Turkey.

ABSTRACT

Background. Cockayne Syndrome (CS) is a rare autosomal recessive disorder that is mainly characterized by neurodevelopmental delay, cutaneous photosensitivity, and cachectic dwarfism. Genetic diagnosis is supported by the typical physical appearance and imaging findings of these patients.

Case. In our case, a 16-year-old female previously diagnosed as CS presented with right-sided hemiparesis. Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) images showed diffuse cerebral and cerebellar atrophies, volume loss of brain stem, calcification of the bilateral basal ganglia, hemorrhage on the posterior limb of the left internal capsule, thalamus, and posterior periventricular area.

Conclusions. Cockayne syndrome is rarely associated with stroke; we report the clinical and neuroradiologic findings of CS presenting with a hemorrhagic stroke.

Key words: Cockayne syndrome, hemorrhagic stroke, magnetic resonance imaging, computed tomography.

Cockayne Syndrome (CS) is a rare autosomal recessive disorder with a prevalence of 2.5 per million.[1] It was first described in 1936 by Edward Cockayne.[2] Cockayne Syndrome is a multisystem disorder classified as a nucleotide excision repair disease characterized by neurodevelopmental delay, cutaneous photosensitivity, pigmentary retinopathy, neurosensory hearing loss, dental caries, cachectic dwarfism with senile-like appearance, dementia, endocrinopathies, progressive spasticity, ataxia, peripheral neuropathy, osteopenia, kyphosis, and joint contractures.[3,4]

Neurological manifestations are the main cause for morbidity of the disease. Herein we report a rare case of CS presenting with hemorrhagic stroke.

✉ Başak Atalay
basak_hosgoren@yahoo.com

Received 24th November 2020, revised 10th January 2021, accepted 14th February 2021.

Case Report

A 16 year old female was admitted to our hospital with right-sided hemiparesis for two weeks. She was diagnosed with CS at the age of ten years clinically and genetically (CS type A, Homozygous C.593-594dup frameshift pathogenic variation in ERCC8 gene). Her weight was 11 kg, and her blood pressure was 90/60 mmHg. Examination revealed characteristic manifestations of CS such as cachectic dwarfism, progeroid face, bilateral cataracts, intellectual disability, microcephaly, joint contractures in the lower extremities, and dental caries (Fig. 1). Her history included full term, birth with 2500 gr weight, and normal developmental milestones until the age of one. Then, developmental delay and growth failure was detected. Her current examination revealed right sided hemiparesis with Medical Research Council Scale for muscle strength of 3/5 in the right upper and lower extremities and spasticity and contractures in the extremities. The patient had sensorineural hearing loss. Tests for lipid profile, renal and hepatic functions, serum

Fig. 1. Characteristic face, cachectic phenotype, joint contractures of the patient with Cockayne syndrome are shown.

homocysteine level, antithrombin III, proteins C and S, factor V Leiden, anticardiolipin antibodies, antiphospholipid antibodies, and antinuclear antibodies were normal.

Magnetic resonance imaging (MRI) showed diffuse cerebral and cerebellar atrophies with ventricular dilatation and widened sulci, volume loss of brain stem, and bilateral basal ganglia calcification. On T1 weighted images, there was high signal intensity on the posterior limb of the left internal capsule, thalamus, and posterior periventricular area suggesting hemorrhage. Restricted diffusion was seen on the posterior limb of the internal capsule and thalamus due to late subacute hemorrhage (Fig. 2).

Subsequent computed tomography (CT) revealed calcification in the bilateral basal ganglia and periventricular white matter of frontal and parietal lobes; CT also demonstrated hemorrhage on the posterior limb of the left internal capsule and thalamus (Fig. 3). No significant pathology was detected in CT and MR angiography studies regarding intracranial vascular structures. The patient previously diagnosed as CS, presented with hemorrhagic stroke during the course of the disease.

Permission was obtained from the parents and informed consent was obtained from the family.

Discussion

Cockayne Syndrome is a rare multisystem disorder characterized by a variety of clinical features, including severe neurological manifestations. Patchy and segmental demyelination, neuronal loss without major brain malformations, calcifications, vascular changes with accelerated atherosclerosis and arteriolosclerosis, severe cerebellar atrophy, and ventricular enlargement are the main neuropathologic findings that can clinically manifest as microcephaly, intellectual disability, tremors, ataxia, seizures, strokes and subdural hemorrhages.[1]

The diagnosis is based on characteristic phenotype, clinical features, radiological findings, and specific genetic tests for DNA analysis, which measure the recovery of RNA transcription after exposure to ultraviolet (UV) radiation.[1,5] Neuroimaging findings, including calcifications mostly in the basal ganglia and cerebral cortex, cerebral and cerebellar atrophy with subsequent ventricular dilation, abnormal white matter signal intensity on MRI are the cardinal features suggesting the diagnosis of CS. The high signal intensity of the cerebral white matter on T2 weighted images demonstrates the tigroid patterns of demyelination, which represents sparing of the perivascular white matter.[1,3] Our patient had neuroimaging findings compatible with CS.

ERCC6 (CSB) and ERCC8 (CSA) genes were identified as the two major genes responsible for CS.[6] The disease is classified as classical form Type I, early-onset Type II, and late-onset Type III. Type II patients show symptoms in the congenital period, while Type III patients may be affected in late childhood or adulthood. Indeed CS patients demonstrate similar clinical and neuroradiological findings, but the onset and progression of the disease vary among subgroups. With a progressive manner of

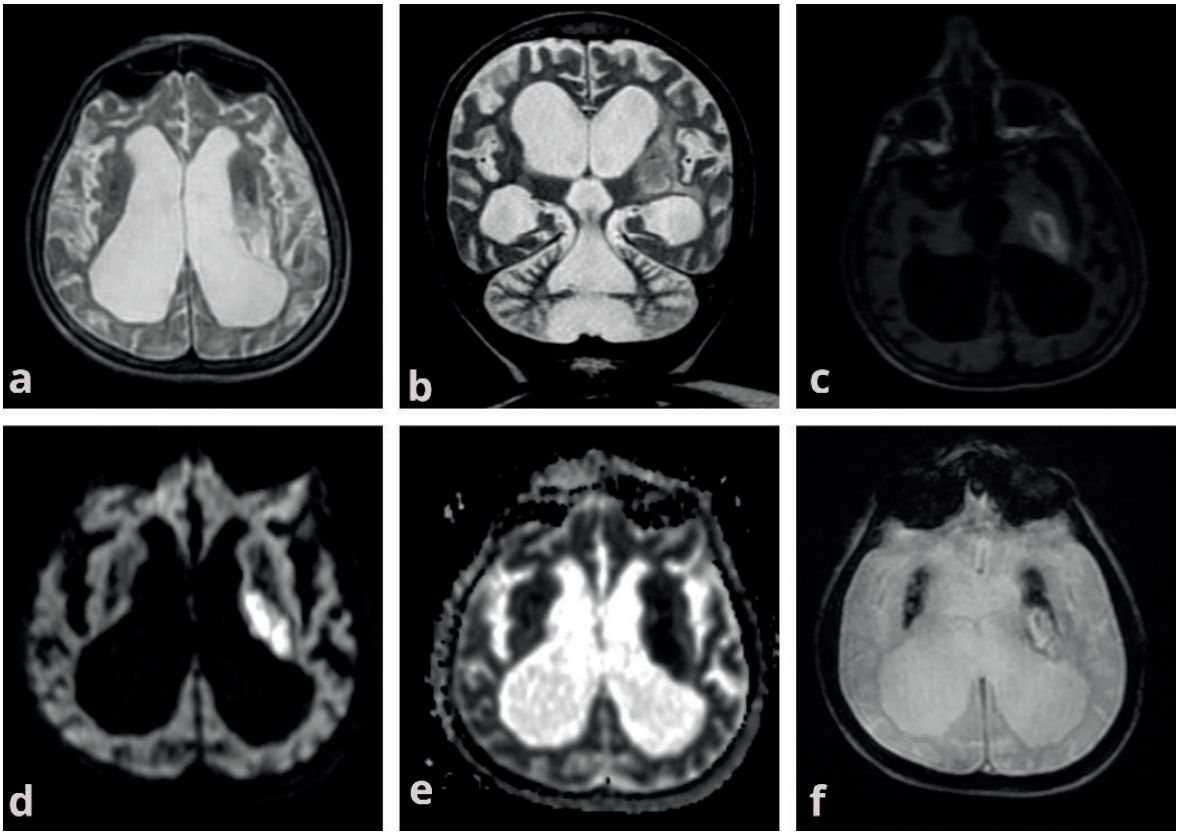


Fig. 2. Magnetic Resonance Imaging demonstrated diffuse cerebral and cerebellar atrophies with ventricular dilatation, and widened sulci, volume loss of brain stem on T2 weighted images (a,b). On T1 weighted images, there was high signal intensity on the posterior limb of the left internal capsule and thalamus which suggested subacute hemorrhage (c). Diffusion-weighted images revealed restricted diffusion on the posterior limb of the left internal capsule, thalamus, and posterior periventricular area with a decrease in the ADC value (d,e). Gradient echo imaging showed small areas of hypointensity in basal ganglia suggesting calcification (f).

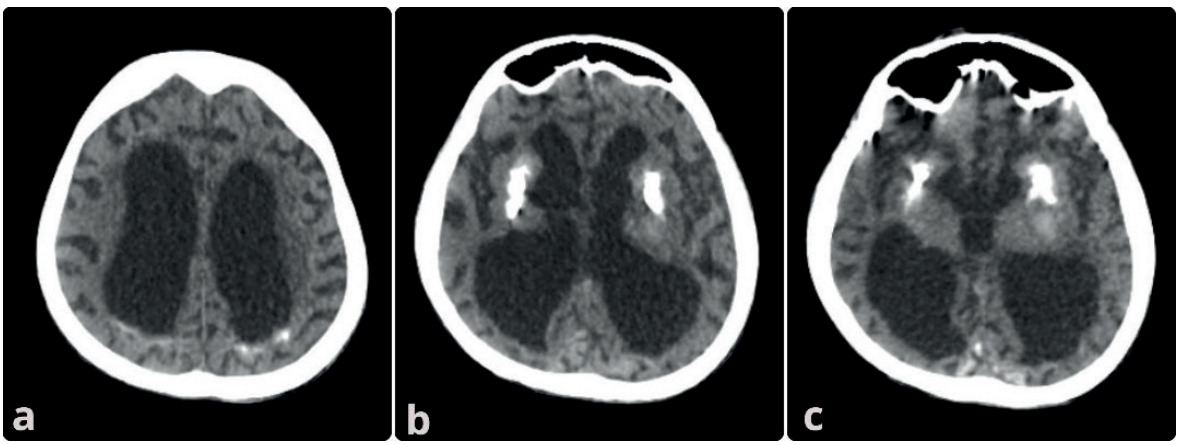


Fig. 3. Computed tomography (CT) revealed calcification in the bilateral basal ganglia and periventricular white matter of frontal and parietal lobes (a,b,c). CT also demonstrated hemorrhage on the posterior limb of the left internal capsule and thalamus (b,c).

the disease, symptoms worsen with time. Development is very limited, especially in type II CS patients with a congenital form. Unlike Type II, in Type I classical form of the disease, development is normal in the first months of life, while motor delay begins at the end of the first year. Patients with CS Type III may have mild intellectual disability and learning difficulties. There is no threshold among the subtypes of the disease. Approximately 65% of patients show CSB gene mutation. Besides according to CSA and CSB linked mutations, there are no specific symptoms or severity of the disease. However, more severe forms (Type II) appear to be associated with CSB gene mutation, and milder forms (Type I) are associated with CSA gene mutation.[7] Based on the genetic analysis (ERCC8 gene, CSA mutation) and clinical onset of the disease, our case was accepted as Type I, the classical form of CS. Solving the underlying molecular mechanisms with larger data will elucidate the CS genotype-phenotypecorrelation.

The normal human aging process and its consequences are seen in the clinical features of CS. Premature onset of hypertension, microvascular pathology, which is characterized by string vessels, accelerated atherosclerosis, and arteriolosclerosis, are observed in the course of the disease and can lead to stroke.[1,8,9] In our case, there were no radiological and laboratory findings suggesting cerebral atherosclerotic or thrombotic risk factors. Still, we havenot ruled out microvascular pathology since histopathological evaluation could not be performed. Literature on arterial disease in patients with CS is sparse, including ischemic or hemorrhagic strokes and transient ischemic attacks.[4,9-12] Mizuguchi and Itoh[12] declared a case of CS with hemorrhagic infarct in the parietal and insular cortex and demonstrated cerebrovascular changes histologically. The radiological findings of hemorrhagic stroke in CS had not been reported before. According to the literature, our case is the first to report the association between CS and hemorrhagic stroke radiologically.

In a recently published paper, Inceer et al.[13] reported a Cockayne syndrome case as a rare cause of hemiplegia presented with subdural hematoma. Similar to this case report, bilateral subdural hematoma has been reported in another CS case with hemiplegia in the literature.[14] Another CS case with hemiplegia, had normal brain imaging, while cerebral angiography showed stenotic plaques.[11] Although chronic subdural hematoma is mostly seen in elderly patients; case reports show that it can be seen with CS, which has premature aging signs.[11,13] Various vascular changes may occur in patients with CS; pathological studies indicate that these patients have an increase in small arteries and arterioles in the subarachnoid space filled with non-atherosclerotic fibrotic tissue. The subarachnoid space and vascular structures increase with brain atrophy and subdural hemorrhages are related to enlarged subarachnoid spaces.[8]

In conclusion, it should be noted that besides the typical neurological features of CS, cerebral arterial disease may develop during the course of the disease. Although it is a rare association, hemorrhagic stroke may be accompanied by CS based on early atherosclerosis.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BA, MS; data collection: BA, MS; analysis and interpretation of results: BA, MS, EYK; draft manuscript preparation: BA, MS, EYK. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest regarding the content of this article.

REFERENCES

1. Karikkineth AC, Scheibye-Knudsen M, Fivenson E, Croteau DL, Bohr VA. Cockayne syndrome: clinical features, model systems and pathways. Ageing Res Rev 2017; 33: 3-17.
2. Cockayne EA. Dwarfism with retinal atrophy and deafness. Arch Dis Child 1936; 11: 1-8.
3. Koob M, Laugel V, Durand M, et al. Neuroimaging in Cockayne syndrome. AJNR Am J Neuroradiol 2010; 31: 1623-1630.
4. Rapin I, Weidenheim K, Lindenbaum Y, et al. Cockayne syndrome in adults: review with clinical and pathologic study of a new case. J Child Neurol 2006; 21: 991-1006.
5. Simon B, Oommen SP, Shah K, Mani SE, Gibikote S. Cockayne syndrome: characteristic neuroimaging features. Acta Neurol Belg 2015; 115: 427-428.
6. Henning, KA, Li L, Iyer N, et al. The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. Cell 1995; 82: 555-564.
7. Laugel V. Cockayne syndrome: the expanding clinical and mutational spectrum. Mech Ageing Dev 2013; 134: 161-170.
8. Hayashi M, Miwa-Saito N, Tanuma N, Kubota M. Brain vascular changes in Cockayne syndrome. Neuropathology 2012; 32: 113-117.
9. Weidenheim KM, Dickson DW, Rapin I. Neuropathology of Cockayne syndrome: evidence for impaired development, premature aging, and neurodegeneration. Mech Ageing Dev 2009; 130: 619-636.
10. Crome L, Kanjilal GC. Cockayne's syndrome: case report. J Neurol Neurosurg Psychiatry 1971; 34: 171-178.
11. Shirasaki N, Hayashi M, Handa Y, et al. Cockayne's syndrome presenting cerebral ischemic attack: case report. No To Shinkei 1986; 38: 871-875.
12. Mizuguchi M, Itoh M. A 35-year-old female with growth and developmental retardation, progressive ataxia, dementia and visual loss. Neuropathology 2005; 25: 103-106.
13. Inceer BS, Bulut O, Savaş Y. Cockayne syndrome as a rare cause of hemiplegia: review of the literature accompanied by a case report. Turk J Neurol 2019; 25: 36-38.
14. Shimoizumi H, Matsui M, Ito S, Miyao M, Kobayashi S. Cockayne syndrome complicated by acute subdural hemorrhage. Brain & Development 1995; 17: 363-377.

The Turkish Journal of Pediatrics 2021; 63: 927-931
https://doi.org/10.24953/turkjped.2021.05.023

Case Report

Ileal atresia and severe cerebral injury after fetoscopic laser photocoagulation treatment for twin-to-twin transfusion syndrome

Ayşe Anık[1], Abdullah Barış Akcan[1], İrem Çulcuoğlu[2], Ali Onur Erdem[3], Sezen Karaca Özkısacık[3], Münevver Kaynak Türkmen[1]

Departments of [1]Neonatology, [2]Pediatrics and [3]Pediatric Surgery, Aydın Adnan Menderes University Faculty of Medicine, Aydın, Turkey.

ABSTRACT

Background. Twin-to-twin transfusion syndrome (TTTS) is a significant cause of perinatal morbidity and mortality. Fetoscopic laser photocoagulation (FLP) is the optimal treatment option for twin-to-twin transfusion syndrome; but can cause central nervous system, extremity and intestinal system injury.

Case. We report the case report of ileal atresia and severe cerebral infarction co-occurrence after fetoscopic laser photocoagulation treatment. It is uncertain as to whether ileal atresia occurred due to ischemia associated with TTTS, the treatment with FLP, or a combination of both.

Conclusions. Cases with prenatal ultrasonographic abnormalities after FLP should have a close assessment to detect bowel complications. Despite many developments in its management, TTTS remains an important risk factor for cerebral injury.

Key words: twin-to-twin transfusion syndrome, fetoscopic laser photocoagulation, ileal atresia, cerebral injury.

Twin-to-twin transfusion syndrome (TTTS) is observed in 10-15% of monochorionic pregnancies and is a significant cause of perinatal morbidity and mortality. The main cause of TTTS is vascular anastomosis in the placenta. If TTTS is not treated, the perinatal mortality rate reaches 80-90%.[1] Fetoscopic laser photocoagulation (FLP) is the optimal treatment option for TTTS. There are very few cases of central nervous system, extremity, and intestinal system injury that develop after FLP treatment.[2] This case report presents a neonate with ileal atresia and severe cerebral ischemia that developed after FLP treatment for TTTS.

Case Report

A 32-year old mother presented with TTTS, Quintero stage I, at 18 weeks of gestation. No congenital anomalies were found in either twin by ultrasonography (USG); and FLP was performed at 18 weeks of gestation without any complication. Fetal chromosome analysis showed a 46, XY karyotype. Fetuses were monitored once or twice a week by USG. At the 32 weeks of pregnancy, magnetic resonance imaging (MRI) showed widespread cystic leukoencephalomalacia in the left cerebral hemisphere parenchyma and ascites (free fluid in the abdominal cavity) in the recipient fetus, while the donor fetus was normal (Fig. 1). Follow-up monitoring by USG showed no flow in the middle cerebral artery, which was compatible with MRI findings. At 33 weeks of gestation, a cesarean section was performed for persistent uterine contractions. The donor twin, with a birth weight of 1850 g, with a hemoglobin level of 13.1 g/dl, was hemodynamically stable and developed mild acute respiratory distress. The follow up of the donor was uneventful, and physical examination of the baby and abdomen and brain MRI were normal. The recipient

✉ Ayşe Anık
drayseank@yahoo.com

Received 23rd May 2020, accepted 26th November 2020.

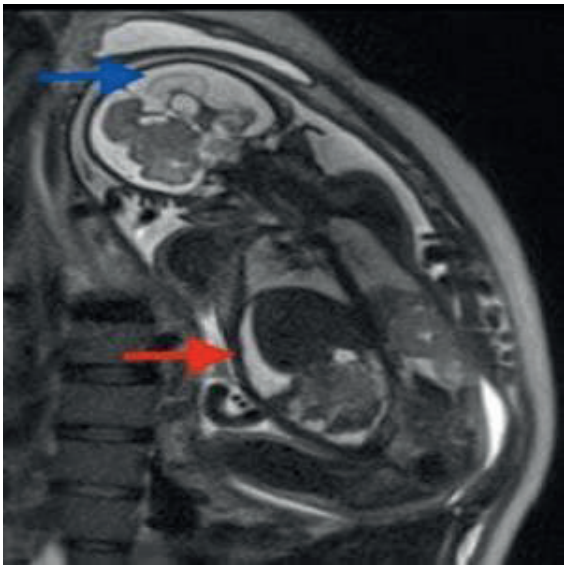


Fig. 1. Prenatal magnetic resonance imaging: Widespread cystic leukoencephalomalacia in left cerebral hemisphere (blue arrow) and ascites (red arrow) in recipient fetus.

twin, with a birth weight of 2250 g, with a hemoglobin level of 16.1 g/dl, had abdominal distention and exhibited signs and symptoms of intestinal obstruction after 24 hours of birth. Laparotomy was performed on day 2 of life, an atretic segment of ileum that was 30 cm apart from the ileocecal valve was resected and primary end-to-end anastomosis was performed (Fig. 2). Pathological examination of the specimen revealed abnormal villous configuration, luminal obliteration, narrowing, fibrosis and calcification, consistent with ileal atresia. Cranial MRI showed brain volume loss and periventricular cystic encephalomalacia areas in the left cerebral hemisphere (Fig. 3). Newborn screening for inherited metabolic disorders was normal. The baby was exclusively fed breast milk and discharged at 37 weeks of corrected age. An informed consent was obtained from the parents before the study.

Discussion

This case report presents the association of ileal atresia and severe neurologic injury observed in the recipient twin in a monochorionic pregnancy with FLP treatment for TTTS.

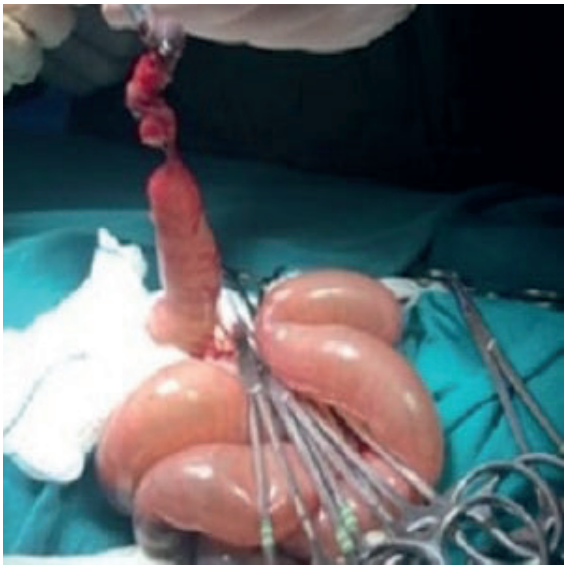


Fig. 2. Surgical image: Ileal atresia.

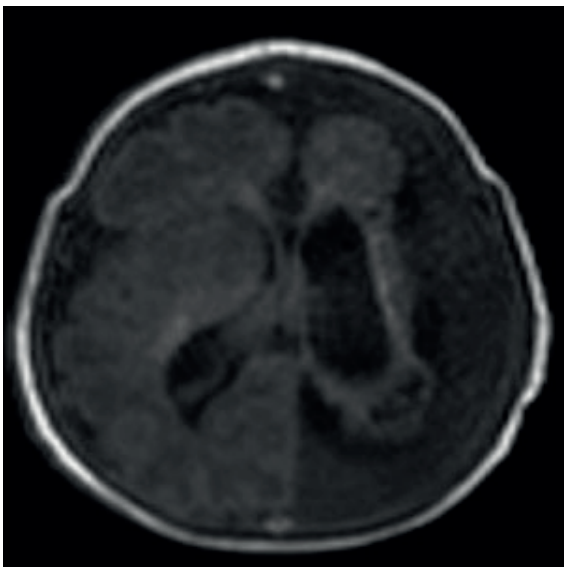


Fig. 3. Postnatal magnetic resonance image: Widespread cystic leukoencephalomalacia, widespread parenchyma loss and compensatory expansion of left lateral ventricle in left cerebral hemisphere.

Small bowel atresia is a rare congenital malformation. Duodenal atresia develops as a result of disrupted rechanneling of the intestine, while jejunoileal atresia is thought to result from a mesenteric vascular accident.[3] The EUROCAT study reported the prevalence of jejunoileal atresia as 0.7 per 10,000 live births

among babies from singleton pregnancies with normal karyotype, while the same study reported that 21 of 423 jejunoileal atresia cases were twin cases with unstated chorionic status.[3] Similarly, Cragan et al.[4] reported the increased incidence of jejunoileal atresia in monozygotic twin pregnancies.

Several cases of congenital intestinal atresia or ileal perforation after FLP have been reported (Table I). In monochorionic twins, it is thought that mesenteric ischemia linked to hypoperfusion and hyperviscosity secondarily to vascular anastomosis or thromboembolisms after FLP treatment may play a role in small intestine atresia. A literature search revealed 16 cases who developed jejunoileal atresia/perforation after FLP treatment, with our patient being the 17th case. Jejunoileal atresia was more common in the recipients than those of donors (Table I). Bowel complications can be seen in both fetuses by different etiologies. While, polycythemia and hyperviscosity due to transfusion may cause vascular accidents in the recipient twin; anemia in the donor twin may cause persistent hypoperfusion of the intestine, and the resulting hypoxemic damage may lead to bowel necrosis and perforation. Moreover, thrombosis of placental vessels could cause intestinal ischemia in both the recipient and the donor twin. Schnater et al.[5] speculated that thrombo-emboli may be induced by the laser coagulation of the placental vessels. Of the cases given in Table I, isolated fetal ascites was detected in 4 cases, as in our patient. This case report is important as it should be kept in mind that isolated fetal ascites noted on ultrasound after FLP treatment for TTTS is not necessarily secondary to fetal anemia but may be secondary to in utero bowel perforation. Due to the small number of cases available currently, more detailed studies are needed to reveal the exact underlying pathologies of bowel complications after FLP treatment.

To the best of our knowledge, this is the first case with co-occurrence of ileal atresia and severe cerebral infarction after FLP treatment.

Table I. Type of bowel problems associated with twin-to-twin transfusion syndrome after fetoscopic laser photocoagulation.

Case No.	Lesion	Affected twin	Associated anomalies	Authors
1	Ileal atresia	R	No	Arul et al. [13]
2	Ileal atresia	R	No	
3	Ileal atresia	R	No	Schnater et al. [5]
4	Ileal atresia	R	No	Morikawa et al. [14]
*5,6	D: Sigmoid perforation, R: Ileocecal perforation	D, R	No	Detlefsen et al. [15]
7	Ileal atresia and perforation	R	Pulmonary stenosis	Saura et al. [16]
8	Jejunal atresia	D	No	
**9,10	D: Transverse colon perforation, R: Jejunal perforation	D, R	No	Marcellin et al. [17]
11	Jejunal atresia, sigmoid perforation	D	No	Sanchez-Galan et al. [18]
12	Ileal atresia	R	No	
13	Ileal atresia	D	No	
14	Caecal perforation	R	No	
15	Ileal atresia and perforation	D	No	Piek et al. [19]
16	Jejunal atresia and perforation	R	No	Tan et al. [20]
17	Ileal atresia and perforation	R	Brain injury	Current case

D, donor; R, recipient. *Case 5 is donor and Case 6 is recipient in the same pregnancy, **Case 9 is donor and Case 10 is recipient in the same pregnancy

The cause of cerebral injury in TTTS cases is still not clearly defined. The risk factors for neurological morbidity have been reported as high Quintero stage, low birth weight, low gestational age at birth, and advanced gestational age at laser surgery[6]. Injury patterns are classified as hemorrhagic (intraventricular hemorrhage, periventricular hemorrhagic infarcts) or ischemic white matter injury (periventricular leukomalacia) according to USG.[7] Both forms of injury may develop in the antenatal period linked to disrupted cerebral blood flow.[8] In TTTS, compromised cerebral perfusion and ischemic injury of the central nervous system may occur due to vascular anastomoses, independent of FLP treatment.[9] There are some studies reporting lower rates of neurologic complications and higher survival rates after FLP treatment. Rossi et al.[10] reported less mortality and fewer neurologic sequelae in fetuses that were treated with laser photocoagulation. Miralles-Gutiérrez et al.[11] reported the global mean rate of neurological injury in twins treated with FLP as 14.07% and emphasized that the rate was higher than the results found in dichorionic twins but lower than the results found in twins treated with serial amnio-reductions or conservative management. Lopriore et al.[7] reported the incidence of antenatally acquired severe cerebral lesions in the TTTS group as 10% (8/84) and 2% (2/108) in the non-TTTS group (OR 5.58, 95% CI 1.05 to 39.21, p= .02). They emphasized the need for fetal imaging before and after the intervention to determine the effect of FLP on cerebral injury.[7] Conversely, a study conducted by Weisz et al.[12] showed ischemic changes in white matter on fetal diffusion-weighted magnetic resonance imaging taken 24-96 hours after FLP treatment. They proposed these changes may be associated with FLP treatment. As a result, it is unknown whether cerebral injury develops before, during or after FLP treatment.

In conclusion, this case report emphasizes that intestinal complications should be kept in mind in TTTS after FLP therapy, especially in case of isolated fetal ascites. Despite many developments in its management, TTTS remains an important risk factor for cerebral injury.

Author contribution

The authors confirm contribution to the paper as follows: case report conception and design: AA, ABA, MKT; data collection: AA, İÇ; draft case report preparation: AA, AOE, SKÖ, ABA, MKT. All authors reviewed the results and approved the final version of the case report.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Berghella V, Kaufmann M. Natural history of twin-twin transfusion syndrome. J Reprod Med 2001; 46: 480-484.
2. Slaghekke F, Lopriore E, Lewi L, et al. Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014; 383: 2144-2151.
3. Best KE, Tennant PW, Addor MC, et al. Epidemiology of small intestinal atresia in Europe: a register based study. Arch Dis Child Fetal Neonatal Ed 2012; 97: F353-F358.
4. Cragan JD, Martin ML, Waters GD, Khoury MJ. Increased risk of small intestinal atresia among twins in the United States. Arch Pediatr Adolesc Med 1994; 148: 733-739.
5. Schnater JM, van Zalen-Sprock RM, Schaap AH, Festen S, Aronson DC. Ileal atresia and thrombo-embolic liver calcifications diagnosed after treatment with intrauterine laser coagulation therapy for twin-to-twin transfusion syndrome: report of 2 cases. J Pediatr Surg 2005; 40: 875-876.
6. Lopriore E, Ortibus E, Acosta-Rojas R, et al. Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstet Gynecol 2009; 113(2 Pt 1): 361-366.
7. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. Am J Obstet Gynecol 2006; 194: 1215-1220.

8. Volpe JJ. Intracranial hemorrhage: germinal matrix-intraventricular hemorrhage of the premature infant. In: Volpe JJ (ed). Neurology of the Newborn (5th ed) Chapter 11. Philadelphia: Saunders, 2008: 517-590.

9. Banek CS, Hecher K, Hackeloer BJ, Bartmann P. Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. Am J Obstet Gynecol 2003; 188: 876-880.

10. Rossi AC, D'Addario V. Laser therapy and serial amnioreduction as treatment for twin-twin transfusion syndrome: a metaanalysis and review of literature. Am J Obstet Gynecol 2008; 198: 147-152.

11. Miralles-Gutiérrez A, Narbona-Arias I, González-Mesa E. Neurological complications after therapy for fetal-fetal transfusion syndrome: a systematic review of the outcomes at 24 months. J Perinat Med 2018; 46: 991-997.

12. Weisz B, Hoffmann C, Ben-Baruch S, et al. Early detection by diffusion-weighted sequence magnetic resonance imaging of severe brain lesions after fetoscopic laser coagulation for twin-twin transfusion syndrome. Ultrasound Obstet Gynecol 2014; 44: 44-49.

13. Arul GS, Carroll S, Kyle PM, Soothill PW, Spicer RD. Intestinal complications associated with twin-twin transfusion syndrome after antenatal laser treatment: report of two cases. J Pediatr Surg 2001; 36: 301-302.

14. Morikawa M, Sago H, Yamada T, et al. Ileal atresia after fetoscopic laser photocoagulation for twin-to-twin transfusion syndrome--a case report. Prenat Diagn 2008; 28: 1072-1074.

15. Detlefsen B, Boemers TM, Schimke C. Necrotizing enterocolitis in premature twins with twin-to-twin transfusion syndrome. Eur J Pediatr Surg 2008; 18: 50-52.

16. Saura L, Muñoz ME, Castañón M, et al. Intestinal complications after antenatal fetoscopic laser ablation in twin-to-twin transfusion syndrome. J Pediatr Surg 2010; 45: E5-E8.

17. Marcellin L, Quintana A, Essaoui M, et al. Meconium peritonitis in both fetuses with early twin-to-twin transfusion syndrome. Twin Res Hum Genet 2012; 15: 527-531.

18. Sánchez-Galán A, Encinas JL, Antolín E, et al. Intestinal complications in twin-to-twin transfusion syndrome (TTTS) treated by laser coagulation (LC). Cir Pediatr 2017; 30: 33-38.

19. Piek MW, Oepkes D, Lopriore E, Hulsker CC. Ileal blowout due to ileal atresia in a donor with twin-to-twin transfusion after fetoscopic laser surgery. Ann Pediatr Surg 2018; 14: 42-43.

20. Tan LN, Cheung KW, Philip I, Ong S, Kilby MD. Isolated ascites in a monochorionic twin after fetoscopic laser ablation is not necessarily secondary to recurrence or anaemia: bowel complications in twin-to-twin transfusion syndrome after fetoscopic laser ablation. Fetal Diagn Ther 2019; 45: 285-294.

The Turkish Journal of Pediatrics 2021; 63: 932-936
https://doi.org/10.24953/turkjped.2021.05.024

Case Report

Umbilical artery to common femoral artery (CFA) transposition, a novel technique for limb salvage in a newborn: a case report

Hamidreza Hosseinpour[1], Ali Bahador[2], Ali Tadayon[2]

[1]Department of Surgery, Shiraz Laparoscopic Research Center, [2]Department of Pediatric Surgery, Shiraz University of Medical Sciences, Shiraz, Iran.

ABSTRACT

Background. Cardiac catheterization is a course of action with a low rate of complication; however, the potential risk factors should be considered before the procedure. The risk of arterial complications increases in sick premature infants, especially in the first days of life.

Case. A four-day-old neonate with cyanotic heart disease (tricuspid atresia) was referred to our tertiary center for patent ductus arteriosus (PDA) stenting by cardiac catheterization. During catheterization, the stent escaped and was trapped in the left external iliac artery. Following the stent retrieval, the left external artery was disrupted entirely and caused pulse-less left lower extremity. The patient was immediately transferred to the operating room to repair the artery. Retracted ends of the artery had caused a 3-4 cm space between them. In this rare and emergency situation, the left umbilical artery was used to maintain the common femoral artery's (CFA) blood supply. Therefore, an end to end anastomosis of the distal part of the left umbilical artery with the proximal part of the left CFA was done successfully and uneventfully.

Conclusions. The complications of cardiac catheterization that lead to surgical repair are almost challenging, and adequate preoperative planning should be performed. In selective cases, the umbilical artery can be used to maintain the blood flow to the common femoral artery.

Keywords: cardiac catheterization, complication, surgical repair, newborn, rupture.

Congenital heart disease (CHD) is a progressive global problem in infants. Catheter-based intervention has improved the survival and quality of life.[1] It affects nearly 800 per 100,000 births and has caused death in about 6% of US infants during the past decade.[2]

For patients with established CHD, cardiac catheterization procedures can offer less invasive interventional therapy, for patients with complex lesions.[3] Although the overall rate of complications in catheterization procedures is very low, they can cause various unintentional cardiac and vascular adverse events including cardiac arrest, prolonged ischemia, pseudo-aneurysm, and even vessel injury and perforation.[4]

One of the most infrequent but emergent complications of cardiac catheterization is stent loss, which can often be successfully handled percutaneously.[5] However, our patient went through a complete disruption of the left external iliac artery during stent retrieval.

Following recognized disruption of the left external iliac artery, our patient underwent emergency exploration of the left femoral area and retro-peritoneum and successful repair of the artery by performing end to end anastomosis of the distal left umbilical artery to the proximal common femoral artery (CFA).

✉ Ali Tadayon
alitad4@gmail.com

Received 20th October 2020, revised 9th January 2021, accepted 11th February 2021.

Case Report

A 4-day-old female who weighed 2.3 kg and was the product of a normal vaginal delivery was referred to our pediatric cardiologist in a tertiary referral center due to cyanosis. On arrival, the patient was cyanotic, with oxygen saturation (O_2) of 60% at room air that increased to 65-68% with oxygen therapy. The heart rate was 160 bpm with a respiratory rate of 66 per minute and a blood pressure of 70/45 mmHg. She was mildly tachypneic with a single S2 and a 3/6 pansystolic murmur in the left subclavicular area, and there was no evidence of hepatomegaly. The cardiac axis was 60º, and the chest radiography showed decreased pulmonary vascularity.

She was admitted to our unit for echocardiography and further evaluation. Bedside transthoracic echocardiography revealed tricuspid atresia, pulmonary atresia, hypoplastic right ventricle (RV), medium-sized atrial septal defect (ASD), medium-size patent ductus arteriosus (PDA), very small (pinhole) size ventricular septal defect (VSD), good left ventricular (LV) systolic function, thickened mitral valve, and severe pulmonary hypertension.

After primary stabilization (hydration, correcting acidosis, preparing packed red blood cell (RBC), and starting prostaglandin E_1 with a dose of 0.05 μg/kg/min), the patient was transferred to catheterization laboratory for cardiac catheterization for PDA stenting and atrial septostomy. Prostaglandin was discontinued three hours prior to the procedure. Under general anesthesia, multiple attempts were made for the left axillary artery access, but they all failed due to tortuous PDA, and our cardiologist was unable to get into the PDA through the left axillary artery. Meanwhile, intravascular heparin was started for him with a dose of 50 U/kg. The right axillary artery was then cannulated for PDA stenting under fluoroscopy guidance, using a Rontis Coronary stent system (Rontis AG, Europa Ultra, Switzerland, 4*20 mm). The stent was dislodged to the left external iliac artery (probably due to the short length and relatively large (3.5 mm) diameter of PDA. For retrieving the lost stent, the left femoral artery was accessed, and, after multiple attempts, the stent was removed via an introducer and a 4 mm snare. After the procedure, aortogram failed to show any flow passing through the left iliac artery (Fig. 1). Doppler ultrasound (DUS) of the left lower extremity confirmed the loss of color flow from the femoral artery up to the posterior tibialis. Pediatric surgery consultation was done, and the patient was immediately transferred to the operating room for iliac artery repair.

In supine position, the patient was prepped and draped in the usual sterile surgical fashion. Under general anesthesia, a vertical incision was made from the left lower quadrant to the inguinal canal and proximal part of the thigh. Exploration of the left external iliac, left CFA and vein was done. The left external iliac artery was utterly disrupted. At first sight, the distal end was present, but the proximal end was not found. Thus, we extended the incision

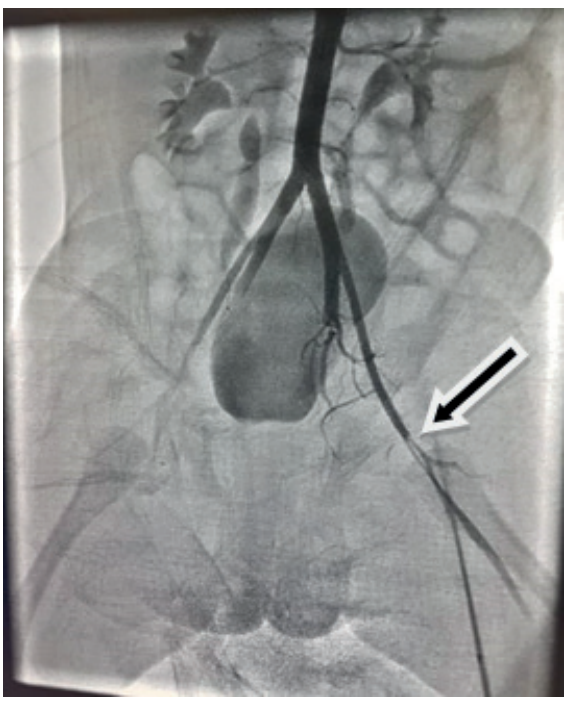


Fig. 1. Abnormal angiography of the left femoral artery

vertically and then the proximal end, which was retracted for about 4-5 cm, was seen in the retroperitoneum. At first, we tried to anastomose the two ends of the artery with each other, but the vessels were under tension that led to narrowing of the lumen and increased risk of future thrombosis. In this emergent situation, we had to find a way to maintain the CFA's blood supply to prevent left lower extremity ischemia. There were some potentially impossible options like reverse saphenous vein graft anastomosis that all could cause more severe complications due to vein diameter at this age.

During our exploration, we incidentally found the end of the left umbilical artery, which was pulsatile, so the idea of using the umbilical artery as the supplier of CFA came to our mind. The left umbilical artery was incised from its end near the umbilicus. There was no blood flow, so Fogarty catheterization (NO 3.5) was performed for embolectomy, which was effective with a good jet flow. Then, we put a bulldog vascular clamp at the end of the umbilical artery and tried to perform Fogarty catheterization of the proximal end of CFA until the back-flow perfusion appeared. At this time, the umbilical artery was rotated downward and brought down near the proximal end of CFA, and following spatulating the smaller vessel, an end to end anastomosis was done by simple interrupted sutures with prolene 8-0 under loupe magnification. The distal end of the external iliac artery was ligated with silk 3-0. The femoral artery had a good flow with a satisfying pulse. Figure 2 shows the left external iliac artery after anastomosis. After homeostasis, the fascia was closed with PDS 3-0, and the skin was closed with nylon 3-0. Anti-coagulation therapy was started for her immediately after the procedure with intravascular administration of heparin every 12 hours.

The postoperative course was uneventful, and the patient was transferred to neonatal intensive care unit (NICU) for medical therapy and stabilization before another attempt for a percutaneous procedure or a modified Blalock-Taussig shunt. Following the next 48 hours, the common femoral, popliteal and posterior pedal pulses were bilaterally and symmetrically present, and no sign of ischemia or bleeding was present. Unfortunately, the patient eventually died due to her cardiac conditions and clearly unrelated to her last procedure.

Consent was obtained from the parents regarding the publication of this case report.

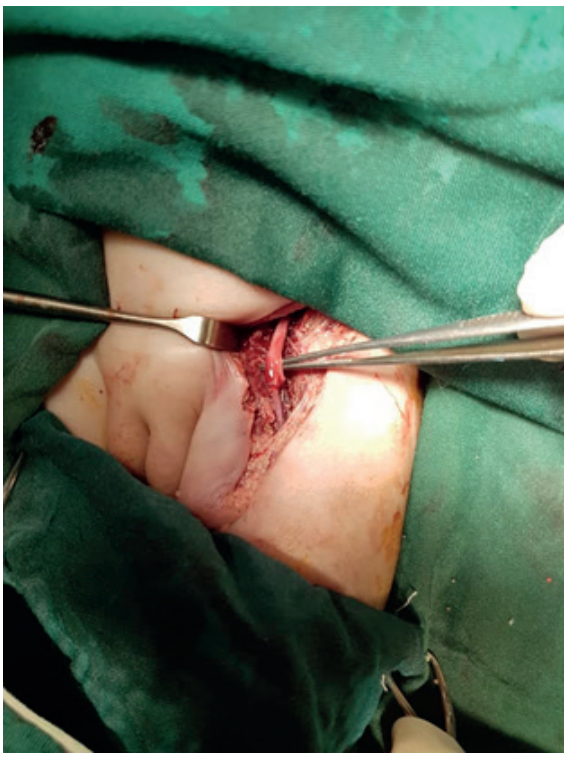


Fig. 2. Repaired left external iliac artery after the procedure

Discussion

Angiography is a well-known diagnostic and interventional procedure that is used worldwide with well-established low rate of complications. According to a study conducted by Hessel et al.[6], complications originating from the access-site of catheterization are the most common ones, and generally among transfemoral and transaxillary approaches, the transfemoral technique is the method of choice because it is both easier and safer. In another study, it is claimed that the overall rate of major vascular complications is about 4.85%.[7]

In a large single-center experience, conducted by Brilakis et al.[8], it was revealed that stent loss, as a stressful complication, still occurs with an incidence of 0.32% in general. Their results revealed that there was an 86% rate of successful retrieval, and 26% of them were done by snare loop. Among all, 11,773 patients who participated in this study and 38 patients who went through stent loss as a complication, in only three patients, the stent was subsequently immobilized in the femoral arteries, which was retrieved by small-balloon and two-wire technique. Although facing this stressful experience is somehow difficult, our pediatric cardiology team tried to retrieve the stent by snare loop immediately, but it caused subsequent complications afterward.

The best approach to a susceptible artery injury is a complete, detailed vascular examination to recognize any changes which result in a pale and pulseless extremity.[9] This is what our pediatric cardiology team had done in the Cath lab. As soon as they noticed the absence of palpable pulse in the left lower extremity, and while the aortogram failed to show any flow in the left iliac artery, DUS was done and the report confirmed the diagnosis.

A study by McMillan et al.[10] noted that surgical repair post- arterial injury in neonates is unlikely (less than 0.4%), and if surgical intervention is required, it is probably complex and necessitates a vascular graft. The main problem with our case was the distance between the two ends of the disrupted artery, which was about 3-4 cm. That means there was no choice except using vascular autograft, which was impossible due to the emergency situation and age of the neonate; that is when the idea of using umbilical artery occurred.

There were some graft options in this situation. First, we could ligate the artery to stop the bleeding and hope for collateral arteries to supply the left lower limb[11], which was impossible, as the limb was going cold and cyanotic. The next option was harvesting the jugular vein as an autograft[12], a very sophisticated procedure, as the patient was a neonate with a relatively short neck. One of the controversial options was the utilization of the contralateral saphenous or femoral vein, again as an autograft[13], which was also not feasible due to the procedure's time-consuming nature. So, using the umbilical artery was a promising way of maintaining the limb's blood supply, at least transiently.

At the umbilicus, the umbilical vessels enter the abdomen. The two umbilical arteries are then extended as the hypogastric arteries and moved to the back of the anterior abdominal wall and the sides of the bladder to their origin from the internal iliac arteries. The anatomy of the arteries is shown in Figure 3. With age, the parts of the hypogastric arteries continuing between the sides of the bladder and the umbilicus are eliminated, and they develop as fibrous cords, the lateral umbilical ligaments. The umbilical vein and ductus venosus are eliminated; the vein turns into the ligamentum teres, while the ductus develops into the ligamentum venosum of the liver.[14-16]

Although there are some other options (such as using femoral vein or intra-arterial shunt) for management of iliac artery disruption, this procedure worked as a bridge operation, which

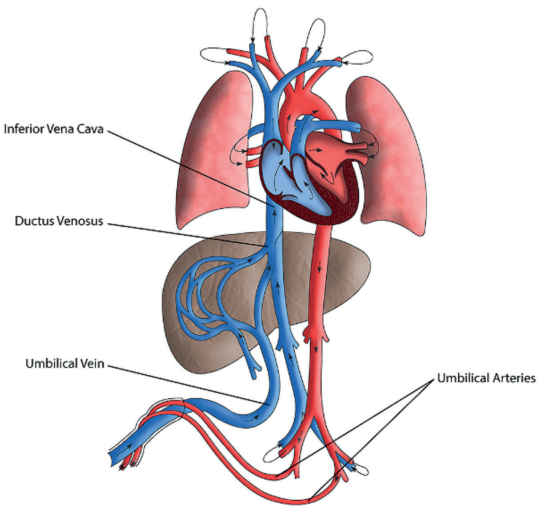


Fig. 3. Anatomy of the umbilical arteries and veins

provided enough time to save the limb and keep the extremity warm until the patient got stable.

Cardiac catheterization is a course of action with a low rate of complications; however, potential risk factors should be considered before the procedure. On the other hand, complications that lead to surgical repair are almost always challenging and adequate preoperative planning should not be performed. Hence, in selective cases, the umbilical artery can be used to maintain the blood flow to the common femoral artery.

Acknowledgements

The authors would like to thank Shiraz University of Medical Sciences, Shiraz, Iran and also Center for Development of Clinical Research of Nemazee Hospital and Dr. Nasrin Shokrpour for editorial assistance.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AB, AT; data collection: HH; draft manuscript preparation: HH. All authors reviewed the results and approved the final version of the manuscript.

Conflict interest

The authors declare that they have no competing interests.

REFERENCES

1. Zimmerman MS, Smith AGC, Sable CA, et al; GBD 2017 Congenital Heart Disease Collaborators. Global, regional, and national burden of congenital heart disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Child Adolesc Health 2020; 4: 185-200.
2. Abouk R, Grosse SD, Ailes EC, Oster ME. Association of US state implementation of newborn screening policies for critical congenital heart disease with early infant cardiac deaths. JAMA 2017; 318: 2111-2118.
3. Gao Y, Wang P, Su Y, et al. Cardiac catheterization procedures in children with congenital heart disease: increased chromosomal aberrations in peripheral lymphocytes. Mutat Res 2020; 852: 503163.
4. Tokel K, Gümüş A, Ayabakan C, Varan B, Erdoğan İ. Complications of cardiac catheterization in children with congenital heart disease. Turk J Pediatr 2018; 60: 675-683.
5. Iturbe JM, Abdel-Karim AR, Papayannis A, et al. Frequency, treatment, and consequences of device loss and entrapment in contemporary percutaneous coronary interventions. J Invasive Cardiol 2012; 24: 215-221.
6. Hessel SJ, Adams DF, Abrams HL. Complications of angiography. Radiology 1981; 138: 273-281.
7. Kugelmass AD, Cohen DJ, Brown PP, Simon AW, Becker ER, Culler SD. Hospital resources consumed in treating complications associated with percutaneous coronary interventions. Am J Cardiol 2006; 97: 322-327.
8. Brilakis ES, Best PJ, Elesber AA, et al. Incidence, retrieval methods, and outcomes of stent loss during percutaneous coronary intervention: a large single-center experience. Catheter Cardiovasc Interv 2005; 66: 333-340.
9. McCann RL, Schwartz LB, Pieper KS. Vascular complications of cardiac catheterization. J Vasc Surg 1991; 14: 375-381.
10. McMillan I, Murie JA. Vascular injury following cardiac catheterization. Br J Plast Surg 1984; 71: 832-835.
11. Mays ET, Wheeler CS. Demonstration of collateral arterial flow after interruption of hepatic arteries in man. New Engl J Med 1974; 290: 993-996.
12. Holsworth IG, Kyles AE, Bailiff NL, Hopper K, Long C, Ilkiw JE. Use of a jugular vein autograft for reconstruction of the cranial vena cava in a dog with invasive thymoma and cranial vena cava syndrome. J Am Vet Med Assoc 2004; 225: 1205-1210.
13. Perry MO, Thal ER, Shires GT. Management of arterial injuries. AnnSurg 1971; 173: 403-408.
14. Emmanouilides GC, Rein BI. Abdominal aortography via the umbilical artery in a newborn infant. Radiology 1964; 82: 447-4450.
15. Gray H, Lewis WH. Anatomy of the Human Body. (20th ed) Philadelphia: Lea & Febiger, 1918.
16. Paster SB, Middleton P. Roentgenographic evaluation of umbilical artery and vein catheters. JAMA 1975; 231: 742-746.

The Turkish Journal of Pediatrics 2021; 63: 937-939
https://doi.org/10.24953/turkjped.2021.05.025

Entero-encephalopathy due to *FBXL4*-related mtDNA depletion syndrome

Josef Finsterer

Klinik Landstrasse, Messerli Institute, Vienna, Austria.

With interest we read the article by Köse et al.[1] about an 11-months-old Turkish male with entero-ecephalopathy due to the compound heterozygous variants c.772G>T (p.Gly258) and c.1061G>C (p.Trp354Ser) in *FBXL4*. The patient manifested clinically with dysmorphism, developmental delay, axial hypotonia, cerebral atrophy, intractable myoclonic epilpesy, optic atrophy, dysphagia, gastro-intestinal dysmotility, intestinal bleeding, and severe lactic acidosis. We have the following comments and concerns.

It is well-known that *FBXL4* variants may cause secondary mtDNA depletion.[2] Thus, we should know the mtDNA copy number in different tissues to assess if the phenotype correlated with tissue variations of the mtDNA copy number.

FBXL4 variants cause multiple respiratory chain complex dysfunction.[3] Thus, we should know if biochemical investigations were carried out and which of the respiratory chain complexes showed reduced activity.

FBXL4 variants have been reported to manifest with recurrent infections.[3] Since the patient had recurrent pneumonia we should know if immunological parameters (leukocyte counts, interleukines) were within normal limits or abnormal. Involvement of the immune-system in mitochondrial disorders (MIDs) has been previously reported.[4] In this respect we should know if gastro-intestinal bleeding was due to infectious gastro-enteritis and if the patient experienced other recurrent infectious diseases.

The index patient had inherited one of the variants each from the mother and father respectively, and the brother had inherited only the variant from the mother. We should know if any of these mutation carriers was prospectively investigated and if any of them manifested clinically or subclinically with typical MID features.

Epilepsy was classified as intractable but only phenobarbital (PB), levetirazetam (LEV), vigabatrin (VGB), and topiramate (TPM) were tried. Mitochondrial myoclonic epilepsy may favourably respond to benzodiazepines.[5] We should know if benzodiazepines were ever tried and why epilepsy was initially treated with a combination of three anti-seizure drugs (ASDs).

From some of the ASDs it is well-known that they are potentially mitochondrion-toxic and may exhibit severe side effects. Particularly, PB may exert adverse effect on respiratory chain functions. It is also conceivable that gastrointestinal compromise was increased due to the application of VGB as VGB can be associated with severe gastro-intestinal side effects.

Since *FBXl4* variants may manifest with stroke-like episodes (SLEs)[6] of which epilepsy is a dominant feature, we should know the results of multimodal MRI after onset of epilepsy. In particular diffusion weighted imaging, apparent diffusion coefficient, perfusion weighted imaging, and oxygen-extraction MRI should be shown, to assess if epilepsy was associated with a stroke-like lesion.

✉ Josef Finsterer
fifigs1@yahoo.de

Received 17th August 2020, accepted 6th September 2021.

The authors mention that abdominal sonography was normal but show an abdominal X-ray with massive intestinal distension. The authors should explain this discrepancy and if it was attributable to different time points at which the investigations were carried out.

According to figure 1, facial dysmorphism also included downslanting palpebrae, hypertelorism, and broad nose root.

In summary, this interesting case report has a number of shortcomings and reveals some discrepancies which should be solved. We should know the degree of mtDNA depletion, the amount of respiratory chain complex dysfunction, if the cellular or humoral immune system was affected, and if the heterozygote mutation carriers manifested clinically or not.

Key words: mitochondrial, mtDNA, depletion, multisystem, epilepsy.

REFERENCES

1. Köse E, Köse M, Edizer S, et al. Different clinical presentation in a patient with two novel pathogenic variants of the FBXL4 gene. Turk J Pediatr 2020; 62: 652-656.
2. Wang S, Lin L, Wang Y, et al. Novel homozygous mutation in the FBXL4 gene is associated with mitochondria DNA depletion syndrome-13. J Neurol Sci 2020; 416: 116948.
3. El-Hattab AW, Dai H, Almannai M, et al. Molecular and clinical spectra of FBXL4 deficiency. Hum Mutat 2017; 38: 1649-1659.
4. Finsterer J, Zarrouk-Mahjoub S. Affection of immune cells by a C10orf2 mutation manifesting as mitochondrial myopathy and transient sensory transverse syndrome. Acta Neurol Belg 2017; 117: 969-970.
5. Finsterer J, Scorza FA, Fiorini AC, Scorza CA. Mitochondrial myoclonic epilepsy requires specific treatment. Seizure 2020; 78: 168-169.
6. Ebrahimi-Fakhari D, Seitz A, Kölker S, Hoffmann GF. Recurrent stroke-like episodes in FBXL4-associated early-onset mitochondrial encephalomyopathy. Pediatr Neurol 2015; 53: 549-550.

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

Engin Köse

Division of Pediatric Metabolism and Nutrition, Department of Pediatrics, Ankara University Faculty of Medicine, Ankara, Turkey. enginkose85@hotmail.com

Dear Editor,

We read with interest the ''letter to editor'' entitled ''Entero-encephalopathy due to FBXL4-related mtDNA depletion syndrome'' which evaluated the article written by Köse et al.[1]

The author asked about the biochemical investigations for the respiratory chain complexes activity. Although we agree with the reviewer that this should have been evaluated I am afraid we were unable to assess the respiratory chain complexes activity.

The author mentioned that FBXL4 variants have been reported to manifest with recurrent infections due to involvement of the immune-system in mitochondrial disorder and gastrointestinal bleeding may be due to gastrointestinal infection.[2] However, the stool findings of patient (stool culture, microscopic assessment) were unremarkable. Furthermore, laboratory parameters (white blood cell account, C-reactive protein) was not compatible with infection. With these findings, in this patient gastrointestinal infection was ruled out.

Another issue brought up in this letter queried if any of the mutation carriers were prospectively investigated and if any of them manifested clinically or subclinically with typical mitochondrial disorders features. All parents and sibling were evaluated for the findings of mtDNA depletion findings. However, no clinical findings were revealed.

The author stated that Mitochondrial myoclonic epilepsy may favorably respond to benzodiazepines.[3] We initiated benzodiazepine (midazolam) infusion for refractor epilepsy. However, a poor response was seen. Secondly, other antiepileptic drugs were initiated one by one. A ketogenic diet was tried for intractable epilepsy but it failed. At last, epilepsy was controlled with 4 antiepileptic drugs. Gastrointestinal symptoms and findings did not persist in the clinical follow-up except for intestinal dysmotility. In other words, no side effects of vigabatrin treatment were seen.

The results of the multimodal MRI after the onset of epilepsy was questioned. For the index patient, diffusion MRI was performed at 2 and 7 month-old-age. In both MRIs, no findings of stroke-like lesions were detected. We agree that this information should have initially been added and thank the author for this question.

We mentioned that abdominal sonography was normal but showed an abdominal X-ray with massive intestinal distension in the report. Abdominal sonography was performed to assess dysmorphological findings, the presence of intra-abdominal bleeding and to evaluate for acute abdomen. We detected a massive intestinal distension with X-ray (at the same time with abdomen sonography) and physical examination and in clinical follow-up intestinal dysmotility and distension persisted. However, gastrointestinal bleeding discontinued.

We agree with the author concerning the additional facial dysmorphism findings such as down slanting palpebrae, hypertelorism, and broad nose root.

We thank the author for their interest in our work and hope that we were able to sufficiently address all the questions.

Key words: mitochondrial, mtDNA, depletion, multisystem, epilepsy, FBLX4.

REFERENCES

1. Köse E, Köse M, Edizer S, et al. Different clinical presentation in a patient with two novel pathogenic variants of the FBXL4 gene. Turk J Pediatr 2020; 62: 652-656.
2. El-Hattab AW, Dai H, Almannai M, et al. Molecular and clinical spectra of FBXL4 deficiency. Hum Mutat 2017; 38: 1649-1659.
3. Finsterer J, Scorza FA, Fiorini AC, Scorza CA. Mitochondrial myoclonic epilepsy requires specific treatment. Seizure 2020; 78: 168-169.

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 margins of 2.5 cm or 1 inch all round, and pages must be numbered on the right upper corner. Line numbers should be added to each page on the left margin.

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 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, ethical approval, funding, conflict of interest, authors contribution.

Types of Articles:

Original Article: Original articles should report scientific findings within the pediatric field of interest. They must include a structured abstract of less than 300 words, with the following headings: background, methods, results and conclusions to appear after the title page. The manuscript should be structured with the following headings: introduction, materials and methods, results, and conclusions. The introduction should put the study in context with the current literature and reflect the purpose of the study. The materials and methods 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. The manuscript should be no longer than 3,500 words.

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 a structured abstract with the following headings: background, case and conclusions that summarizes the case(s), a brief introduction, 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: Structured abstract with the following headings: background and objectives, material and methods results and conclusions, 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). 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 structured abstract 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. It is critically important that all significant findings should be presented in the abstract. The abstracts should be structured with the following headings for an original article: Background, Methods, Results and Conclusions and Background, Case Report, Conclusion for a case report.

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).

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. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, a footnote must give full credit to the original source. Tables should be added to the end of the main manuscript following the references.

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 added to the end of the manuscript after the tables.

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.

Publication Ethics and Informed Consent:

The Turkish Journal of Pediatrics takes issues of publication ethics very seriously and subscribes to its principles on how to deal with acts of misconduct thereby committing to investigate allegations of misconduct in order to ensure the integrity of research. Scientific articles sent to journals should take into account the recommendations of the International Committee of Medical Journal Editors (ICMJE) and the International Standards for Editors and Authors of COPE (Committee on Publication Ethics).

The Materials and Methods section and the Title Page 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.

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 and Funding: 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 added to the title page. If there is no conflict of interest, this section must include a "The authors declare no conflict of interest" statement. All sources of funding should also be mentioned in the title page.

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.

Acknowledgement: An "acknowledgement(s)" section may be added to thank those who helped the study or preparation of the article, if any. The acknowledgements should be added to the title page. This section contains statements of gratitude for personal, technical or material help, etc.

Authors contribution:

We require that all authors take responsibility for the content of the work submitted for review. An author contribution section should be added to the title page

The contributions of all authors must be described in the following manner: The authors confirm contribution to the paper as follows: study conception and design: X. Author, Y. Author; data collection: Y. Author; analysis and interpretation of results: X. Author, Y. Author. Z. Author; draft manuscript preparation: Y. Author. Z. Author. All authors reviewed the results and approved the final version of the manuscript.

Please note that changes to the list of contributors are not permitted after the article has been accepted.

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 prevalence of…….

Names of journals are to be abbreviated in accordance with the style of Index Medicus.

References to journal articles:

Example: Oral R, Coohey C, Zarei K, et al. Nationwide efforts for trauma-informed care implementation and workforce development in healthcare and related fields: a systematic review. Turk J Pediatr 2020; 62: 906-920.

References to books:

Example: Strasburger VC, Brown RT, Braverman PK, Rogers PD, Holland-Hall C, Coupey SM. Adolescent Medicine: A Handbook for Primary Care. Philadelphia: Lippincott Williams & Wilkins, 2006: 13-21.

References to chapters in books:

Example: Macumber IR, Flynn JT. Systemic hypertension. In: Kliegman RM, St Geme III JW, Blum NJ, Tasker RC, Shah SS, Wilson KM (eds). Nelson Textbook of Pediatrics (21st ed) Vol 2. Philadelphia: Elsevier, 2020: 2490-2499.

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.

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.

Copyright: All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means without the prior written permission of the copyright owners. Permission is not required to copy summaries of papers or of articles on the condition that a full reference to the source is given. Multiple copying of the contents without permission is always illegal.

No manuscript will be considered for review until all authors have completed an uploaded the copyright form.

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.

Checklist

- 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
  - Ethical approval
  - Funding
  - Conflict of interest
  - Acknowledgement
  - Authors contribution
- Article
  - Original articles: Title, Structured abstract, Key words, Introduction, Material and Methods, Results, Discussion, References, Tables, Figure legends
  - Case Reports: Title, Structured abstract, Key words, Introduction, Case Report, Discussion, References, Tables, Figure legends
- Manuscript should be uploaded in a blinded manner; any affiliation must be covered.
- Declaration of informed consent and ethical approval should be given in the material methods section and on the title page
- Tables should be added to the bottom of the main manuscript document
- Figures should be uploaded separately
- Figure legends should be added to the bottom of the main manuscript following the tables.

Printed by METEKSAN in Ankara, Turkey