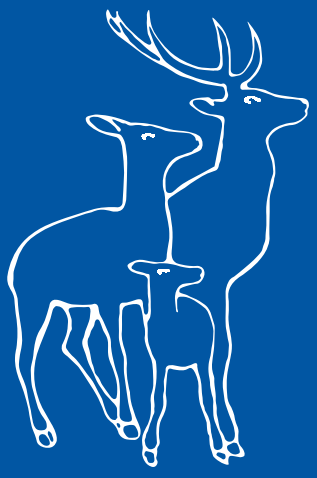


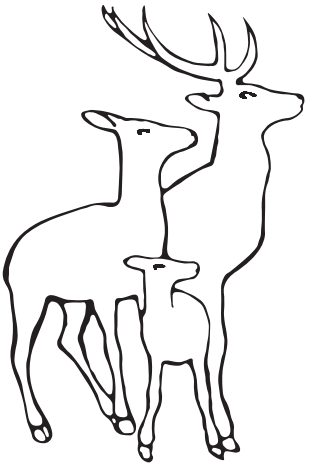
ISSN 0041-4301
Online ISSN 2791-6421
www.turkishjournalpediatrics.org

THE TURKISH JOURNAL OF PEDIATRICS

REVIEW ARTICLES

ORIGINAL ARTICLES

volume 65
number 3
May-June 2023



THE TURKISH JOURNAL OF PEDIATRICS

www.turkishjournalpediatrics.org

Volume 65 ▪ Number 3
May-June 2023

ISSN: 0041-4301
Online ISSN: 2791-6421

THE TURKISH JOURNAL OF PEDIATRICS

ISSN 0041-4301 Online ISSN 2791-6421
www.turkishjournalpediatrics.org

Cilt: 65 Sayı: 3, Mayıs-Haziran 2023

YAYIMLAYAN
Türkiye Milli Pediatri Derneği
Hacettepe Üniversitesi Çocuk Sağlığı Enstitüsü
Uluslararası Çocuk Merkezi

EDİTÖR ADRESİ
The Turkish Journal of Pediatrics
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Faks: +90 (312) 305 22 64
E-posta: editorial@turkishjournalpediatrics.org

YAYIN İDARE MERKEZİ
The Turkish Journal of Pediatrics Editör Ofisi
Hacettepe Üniversitesi
İhsan Doğramacı Çocuk Hastanesi
06100 Ankara
Tel : +90 (312) 305 26 76
Faks: +90 (312) 305 22 64

YAYININ TÜRÜ
Uluslararası hakemli dergi

YAYIN SIKLIĞI VE DİLİ
İki aylık ▪ İngilizce

BASIM YERİ
Meteksan Matbaacılık ve Teknik Sanayi A.Ş.
Beytepe No: 3, 06530 Bilkent, Ankara, Türkiye
Tel: +90 (312) 266 44 10 (Pbx)

BASIM TARİHİ: XX.XX.2023

YAYINCILIK HİZMETLERİ
Akdema Bilişim Yayıncılık ve Danışmanlık Tic. Ltd. Şti.
Kızılay Mah. Gazi Mustafa Kemal Bulvarı No: 23/8 06420
Çankaya/Ankara, Türkiye
Tel: +90 (533) 166 80 80 ▪ Web: www.akdema.com

ISSN 0041-4301 Online ISSN 2791-6421
www.turkishjournalpediatrics.org

Vol: 65 Number: 3, May-June 2023

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

EDITORIAL OFFICE
The Turkish Journal of Pediatrics
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Fax: +90 (312) 305 22 64
E-mail: editorial@turkishjournalpediatrics.org

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

PUBLICATION TYPE
International peer-reviewed journal

PUBLICATION FREQUENCY AND LANGUAGE
Bi-monthly ▪ English

PRINTED BY
Meteksan Matbaacılık ve Teknik Sanayi A.Ş.
Beytepe No: 3, 06530 Bilkent, Ankara, Türkiye
Tel: +90 (312) 266 44 10 (Pbx)

PRINT DATE: XX.XX.2023

PUBLISHING SERVICES
Akdema Informatics, Publishing, and Consultancy Trade LLC
Kızılay Mah. Gazi Mustafa Kemal Bulvarı No: 23/8 06420
Çankaya/Ankara, Türkiye
Tel: +90 (533) 166 80 80 ▪ Web: www.akdema.com

THE TURKISH JOURNAL OF PEDIATRICS

The Turkish Journal of Pediatrics is the 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 letters 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), Web of Science - Science Citation Index (SCI) Expanded, Turkiye Citation Index and ULAKBİM TR-Index.

CONTENTS

VOLUME: 65 NUMBER: 3 MAY-JUNE 2023

REVIEW ARTICLES

ORIGINAL ARTICLES

CONTENTS

VOLUME: 65 NUMBER: 3 MAY-JUNE 2023

The Turkish Journal of Pediatrics 2023; 65: 351-361
https://doi.org/10.24953/turkjped.2022.893

Review

Efficacy of antimicrobials or placebo compared to amoxicillin-clavulanate in children with acute otitis media: a systematic review

Katerina Tsergouli[1], Nikolaos Karampatakis[2], Theodoros Karampatakis[3]

[1]Department of Microbiology, Agios Pavlos General Hospital, Thessaloniki, Greece; [2]Department of Pediatrics, University General Hospital of Thessaloniki AHEPA, Thessaloniki, Greece; [3]Department of Microbiology, Papanikolaou General Hospital, Thessaloniki, Greece.

ABSTRACT

Background. Acute otitis media (AOM) is the inflammation of the middle ear. It constitutes one of the most frequent infections which affects children and usually occurs between 6 to 24 months of age. AOM can emerge due to viruses and/or bacteria. The aim of the current systematic review is to assess in children between 6 months and 12 years of age with AOM, the efficacy of any antimicrobial agent or placebo compared with amoxicillin-clavulanate, to measure the resolution of AOM or symptoms.

Methods. The medical databases PubMed (MEDLINE) and Web of Science were used. Data extraction and analysis were performed by two independent reviewers. Eligibility criteria were set, and only randomised control trials (RCTs) were included. Critical appraisal of the eligible studies was performed. Pooled analysis was conducted using the Review Manager v. 5.4.1 software (RevMan).

Results. Twelve RCTs were totally included. Three (25.0%) RCTs studied the impact of azithromycin, two (16.7%) investigated the impact of cefdinir, two (16.7%) investigated placebo, three (25.0%) studied quinolones, one (8.3%) investigated cefaclor and one (8.3%) studied penicillin V, compared to amoxicillin-clavulanate. In five (41.7%) RCTs, amoxicillin-clavulanate proved to be superior to azithromycin, cefdinir, placebo, cefaclor and penicillin V, while in seven (58.3%) RCTs its efficacy was comparable with other antimicrobials or placebo. The rates of AOM relapse after treatment with amoxicillin-clavulanate were comparable to those of other antimicrobials or placebo. However, amoxicillin-clavulanate was more effective in eradicating *Streptococcus pneumoniae* from the culture, when compared to cefdinir. The results of the meta-analysis were not evaluated due to substantial heterogeneity between studies.

Conclusions. Amoxicillin-clavulanate should be the treatment of choice for children between 6 months and 12 years of age with AOM.

Key words: child, otitis media, amoxicillin-clavulanic acid, antimicrobial agents, recurrence.

Acute otitis media (AOM) is the inflammation of the middle ear. It constitutes one of the most frequent infections that affect children and usually occurs between 6to 24 months of age.[1] Around 80% of children are expected to suffer from an episode of AOM before entering school age[1,2], as the disease mainly appears in children aged between 6 and 24 months.[3] AOM can emerge due to viruses (coronaviruses, respiratory syncytial virus, and influenza viruses), as well as due to Gram-positive (*Streptococcus pneumoniae*) and Gram-negative (*Haemophilus influenzae*, and *Moraxella catarrhalis*) bacteria. Concurrent infection with both viral and bacterial factors have also been recorded.[4,5]

The main symptoms of AOM include mainly pain and/or fever, possibly combined with loss of appetite or vomiting. On the contrary, middle-

✉ Theodoros Karampatakis
tkarampatakis@yahoo.com

Received 10th October 2022, revised 5th February 2023, accepted 13th February 2023.

ear effusion, which is called the accumulation of fluid in the middle ear, is not usually associated with the presence of any symptoms.[6] AOM may be persistent for a long time, leading often to surgical procedures. Notably, the most frequent complication is a reduction in the mobility of the tympanic membrane, leading to hearing disorders, even hearing loss. This condition in children is so severe that it can have a negative impact on their psychological development, their speech development and their educational future.[6,7]

The appearance and spread of resistant bacteria, either through enzymatic mechanisms (production of β-lactamase) or through diminished susceptibility to penicillin, have crucially influenced the effective antimicrobial cure of AOM.[8] The goal of the current systematic review was to assess in children between 6 months and 12 years of age with AOM, the efficacy of any antimicrobial agent or placebo compared with amoxicillin-clavulanate and to measure the resolution of AOM or symptoms.

Methods

Search databases

To track all available data and information concerning the subject of this review, the medical databases PubMed (MEDLINE) and Web of Science were used. To secure full-literature tracking, a hand-search of references to related publications was also conducted.

Search plan

The search plan of the online medical databases mentioned above were comprised of Medical Subject Heading (MeSH) terms and 'free text' terms. Different spellings, synonyms, and terminology alterations over time were also considered. The final search was performed on December 3, 2021. The structured query for PubMed (MEDLINE) was:

A. Search terms for the status of AOM

#1 otitis media

#2 otitis media [MeSH Terms]

#3 middle ear inflam*

#4 middle ear inflammation [MeSH Terms]

#5 middle ear infect*

#6 #1 OR#2 OR#3 OR#4 OR#5

B. Search terms for the treatment with amoxicillin-clavulanate

#7 amoxicillin

#8 amoxicillin [MeSH Terms]

#9 amoxicillin clav*

#10 amoxi clavulanate [MeSH Terms]

#11 amoxicillin clavulanic acid [MeSH Terms]

#12 amox clav [MeSH Terms]

#13 acids, clavulanic [MeSH Terms]

#14 #7 OR#8 OR#9 OR#10 OR#11 OR#12 OR#13

C. Amoxicillin-clavulanate in AOM

#15 #6 AND #14

For Web of Science the search query was the following:

A. Search terms for the condition of AOM

Query #1:

acute media otitis (Topic) or acute media otitis (Title) or acute media otitis (Conference)

B. Search terms for the intervention amoxicillin-clavulanate

Query #2:

amoxicillin clavulanate (Topic) or amoxicillin clavunate (Title) or amoxicillin clavunate (Conference) or amoxicillin clavulanic acid (Topic) or amoxicillin clavulanic acid (Title) or amoxicillin clavulanic acid (Conference)

C. Amoxicillin-clavulanate in acute otitis media

(#1) AND #2

The filter with the term "English" was applied to both search engines to retrieve exclusively search results with publications in the English language.

Management of search results and study selection

Initially, all retrieved studies were introduced into the reference management software EndNote Version X7 (Clarivate, London, United Kingdom), where duplicates were deleted. Afterwards, for the selection of relevant studies all titles and abstracts were screened from the retrieved search results. In case it was not feasible to judge the potential suitability of the studies according to their title and/or abstract, full-text documents were assessed. Management of search results and study selection were performed independently by KT and NK. Any potential disagreements were arbitrated by TK.

Data extraction

The extraction of data was organised in Excel (Microsoft Corp., Redmond, WA, USA) format. The information retrieved from the remaining studies were: the first author of the publication, country, year of publication, journal of publication, the aim of the study, study population characteristics, study design, setting, sample size, intervention, comparator, measures used, analysis, primary and secondary outcomes of interest. Data extraction was conducted independently by KT and NK. Any probable disagreements were resolved by TK.

Data analysis

All data were summarised using basic descriptive statistics. The summary measure used was the distribution of frequency, expressed as a percentage of the total frequency (relative frequency, %). All descriptive statistics were conducted through the Statistical Package for Social Sciences (SPSS, 22nd version, IBM). The statistical significance was determined at $p<0.05$.

Eligibility criteria

Studies fulfilling the following criteria were eligible: Population: Studies on children between 6 months and 12 years of age, with AOM as defined in each study. A minimum sample size of 200 individuals was used to increase the power of the studies. There was no constraint regarding sex, race, or setting.

Intervention: All studies examining the impact of antimicrobials or placebos.

Comparator: Amoxicillin-clavulanate. There was no constraint on the days of treatment or the dosage used.

Outcomes: The main outcome was the disappearance of AOM otoscopic signs (bulging of the tympanic membrane, redness) or relief from its symptoms (pain or hearing disorders). The secondary outcomes included absence of middle ear fluid, microbiological eradication of the bacterial pathogen in culture, and relapse of AOM.

The types of studies included were exclusively randomised controlled trials (RCTs) which constitute the cornerstone of evidence-based medicine. The minimum study duration was set at 12 months. The publication date was set up to November 30, 2021. All publications written in languages other than English were excluded, as well as publications with no full-text available.

Assessment of methodological quality

The Critical Appraisal Skills Programme (CASP) for RCTs was used for the assessment of the methodological quality of the studies.[9] All RCTs fulfilling at least seven out of the 11 criteria set by the corresponding questions of the checklist were classified as being high-quality.

Statistical and pooled analysis

A separate pooled analysis was performed for each outcome. The 95% Confidence Intervals (CIs) and the pooled Odds Ratios (ORs) were calculated through the Cochran-Mantel-Haenszel test using the DerSimonian and Laird random effect model (since possible variability in the population of effects was assumed), as previously described.[10] Statistical heterogeneity of the study was assessed through poor overlap of 95% CIs at visual inspection, Higgin's and Thompson's I^2, tau-squared, and chi-square statistic. A value of I^2>50% was used to define substantial heterogeneity.[11] A p-value <0.1 was used to define statistical significance in heterogeneity. Pooled analysis was conducted through the Review Manager v. 5.4.1 software (RevMan). The level of statistical significance was set at p<0.05. For the outcomes of clinical success, microbiological eradication, and relapse, ORs>1 favoured antimicrobials or placebo, while ORs<1 favoured amoxicillin-clavulanate.

Results

Study basic retrieval and eligibility results

A total of 1086 unique records were initially retrieved. These studies were investigated for relatedness by title, abstract and availability of full-text. Ninety-one studies were reviewed for eligibility, identified as potentially relevant. Out of 91 studies, 79 were excluded: 21 as not being RCTs, 38 did not match the Population, Intervention, Comparator, Outcomes (PICO) question, and 20 were excluded due to a duration of less than 12 months. Finally, 12 studies were eligible for the CASP checklist. The PRISMA flow diagram of study retrieval and eligibility is shown in Figure 1.

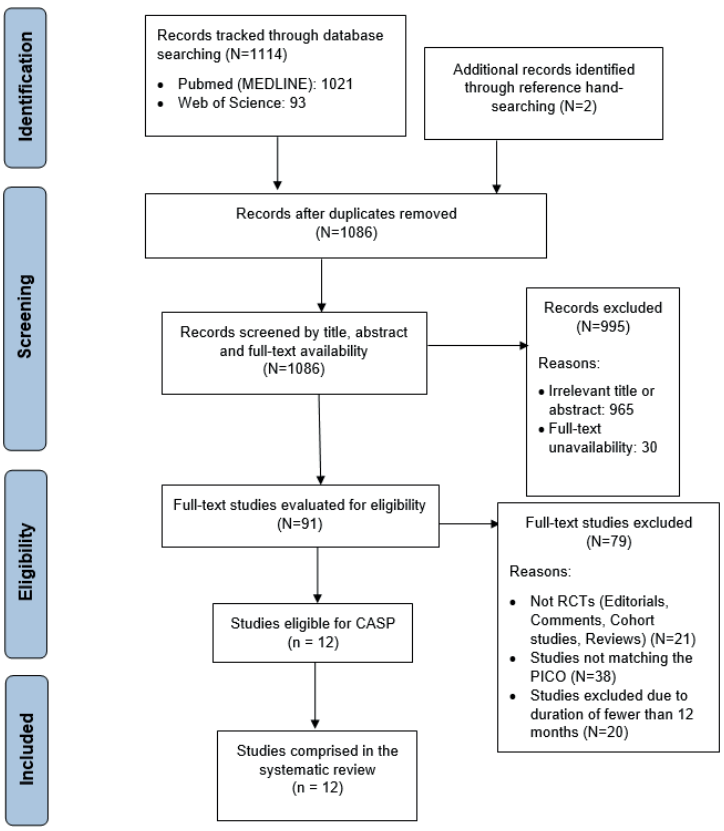


Fig. 1. PRISMA flow diagram of study retrieval and eligibility.

Assessment of methodological quality

Out of the 12 studies included, 10 were characterised as high-quality RCTs as they fulfilled more than seven out of 11 criteria.[12-21] Two of them displayed poor quality as they fulfilled only four and five criteria, respectively (Table I).[22,23]

Study characteristics

Out of 12 studies included, five (41.7%) were double-blind RCTs[12,14,16,21,23], while seven (58.3%) were investigator-blind RCTs[13,15,17-20,22], conducted mainly in the United States of America (USA) and South America (Table II). The study sample sizes ranged from 233 to 1586, with a median of 347.5 children (IQR = 99).[18,19] Out of 5577 children participating in all studies, 691 (12.4%) received azithromycin, 419 (7.5%) received cefdinir, 280 (5%) received placebo, 786 (14.1%) received levofloxacin, 453 (8.1%) received gatifloxacin, 119 (2.1%) received cefaclor and 171 (3.1%) received penicillin. A total of 2658 (47.7%) children received amoxicillin-clavulanate (Table II). Only two out of 12 (16.7%) included RCTs that applied to the administration of high-dose amoxicillin-clavulanate.[12,18] Nine out of 12 (75%) RCTs utilised amoxicillin-clavulanate in a 10-day treatment regimen.[12-18,20,22] Other RCTs utilised amoxicillin-clavulanate in 7-day[19] and 28-day courses.[21,23]

Main outcome

Three RCTs studied the impact of azithromycin compared to amoxicillin-clavulanate. Effective treatment with high- or single-dose azithromycin was comparable to treatment with amoxicillin-clavulanate[12,14], while amoxicillin-clavulanate displayed a higher rate of clinical success when compared with azithromycin (91.6% vs 73.7%, respectively, p<0.01), pertaining only to AOM cases caused *by Haemophilus influenzae*.[15] One study showed relevant clinical efficacy of cefdinir compared to amoxicillin-clavulanate[13], while another showed significantly higher rates of clinical success for amoxicillin-clavulanate compared to cefdinir (86.5% vs 71.0%, respectively, p<0.001).[22] One study displayed the non-inferiority of placebo compared to amoxicillin-clavulanate[16], while another revealed clear superiority of amoxicillin-clavulanate compared to placebo (61.0% vs 30.0%, respectively, p<0.001).[23] Regarding quinolones, which were studied through three different RCTs, gatifloxacin and levofloxacin

Table I. Randomized controlled trials eligible for Critical Appraisal Skills Programme (CASP).

	Arrieta A et al. [12]	Block SL et al. [13]	Block SL et al. [14]	Casey JR et al. [22]	Hoberman A et al. [15]	Hoberman A et al. [16]	Sáez-Llorens X et al. [17]	Noel GJ et al. [18]	Subba-Rao SD et al. [19]	Sher L et al. [20]	Thomsen J et al. [23]	Thomsen J et al. [21]
Focused	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Randomisation	✓	?	?	X	**?**	**?**	✓	✓	✓	**X**	**X**	**?**
Dropout	?	✓	?	X	✓	✓	**?**	**?**	**?**	✓	**?**	**X**
Blinding	✓	X	✓	?	**?**	✓	**?**	**?**	**?**	**?**	✓	✓
Baseline similar	✓	✓	✓	X	✓	✓	✓	**?**	✓	✓	**?**	✓
Equal treatment	✓	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	✓
Treatment effect	✓	✓	✓	✓	✓	✓	**?**	✓	✓	**X**	**?**	✓
Precise	✓	✓	✓	✓	✓	✓	✓	✓	✓	**?**	**X**	✓
Generalisable	?	X	?	?	**?**	✓	**?**	✓	✓	✓	**?**	✓
Outcome	✓	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	✓
Benefit vs harm	✓	?	?	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table II. Selected studies with their basic results – Primary outcome.

Author (Year)	Population age range	Study place	Sample size (Intervention/ Comparator)	Intervention	Comparator	Clinical success (%) Intervention/AMC	p-value (95% CI)
Arrieta A et al.[12]	6 months to 6 years with recurrent or persistent AOM	USA, South America	296 (151/145)	High-dose azithromycin	High-dose AMC	79.0/81.0	0.846 (-14.3 – 10.4)
Block SL et al.[13]	6 months to 6 years with AOM	USA	384 (256/128)	Cefdinir	AMC	83.3/86	>0.05 (N/A)
Block SL et al.[14]	6 months to 6 years with AOM	USA	346 (173/173)	Single-dose azithromycin	AMC	87.0/88.0	>0.05 (-9.2 – 6.5)
Casey JR et al.[22]	6 to 24 months with AOM	USA	325 (163/162)	Cefdinir	AMC	71.0/86.5	0.001 (N/A)
Hoberman A et al.[15]	6 to 30 months with AOM	USA, Europe, South America	730 (367/363)	Azithromycin	AMC	73.7/91.6*	<0.01 (6.37 – 29.42)
Hoberman A et al.[16]	6 to 23 months with AOM	USA	291 (147/144)	Placebo	AMC	74.0/80.0	0.14 (N/A)
Sáez-Llorens X et al.[17]	6 to 7 years with AOM	South America, Asia	413 (277/136)	Gatifloxacin	AMC	74.4/72.7	>0.05 (-8.3 – 10.7)
Noel GJ et al.[18]	6 to 5 years with AOM	USA, South America	1586 (786/800)	Levofloxacin	AMC	83.6/80.4	>0.05 (-7.18 - 0.81)
Subba Rao SD et al.[19]	1 to 12 years with AOM	South America, Asia	233 (119/114)	Cefaclor	AMC	78.6/91.4	0.008 (3.5 – 22.1)
Sher L et al.[20]	6 months to 7 years with AOM	USA, Central America	349 (176/173)	Gatifloxacin	AMC	84.7/78.6	>0.05 (-2.8 – 16.4)
Thomsen J et al.[23]	1 to 10 years with secretory AOM	Denmark	264 (133/131)	Placebo	AMC	30.0/61.0	<0.0001 (N/A)
Thomsen J et al.[21]	1 to 10 years with secretory AOM	Denmark	360 (171/189)	Penicillin V	AMC	19.0/44.0	<0.001 (N/A)

*Clinical success for the treatment of AOM caused by *Haemophilus influenzae*
AOM: acute otitis media, AMC: amoxicillin-clavulanate, N/A: not available, USA: United States of America

proved to have non-inferior cure rates when compared with amoxicillin-clavulanate.[17,18,20] Finally, amoxicillin-clavulanate displayed higher clinical success rates compared to cefaclor (91.4% vs 78.6%, p=0.008, respectively) and penicillin V (44.0% vs 19.0%, p<0.001, respectively).[19,21] The basic results of the 12 selected studies are summarised in Table II. Overall, the clinical success was 2111/2658 (79.4%) in the amoxicillin-clavulanate arm and 2154/2919 (73.8%) in the antimicrobials or placebo arm, with a statistically significant difference (OR=0.61, 95% CI 0.41 – 0.91, p=0.02, I^2= 87%).

Secondary outcomes

Regarding the secondary outcomes of the study, Sher et al.[20] revealed similar microbiological eradication rates of the pathogens causing AOM between children treated with gatifloxacin and children treated with amoxicillin-clavulanate (81.2% vs 82.0%, respectively). Block et al.[13] disclosed higher rates of microbiological eradication in children treated with amoxicillin-clavulanate compared to cefdinir administered twice daily, in cases of AOM caused by *Streptococcus pneumoniae* (89.5% vs 55.2%, respectively, p=0.0019) (Table III). Overall, comparable microbiological eradication rates were observed between patients in the amoxicillin-clavulanate arm (256/301, 85.0%) and the antimicrobials or placebo arm (284/432, 65.7%) (OR=0.38, 95% CI 0.06 – 2.33, p=0.29, I^2= 95%). As far as the secondary outcome of relapse is concerned, three out of 12 (25.0%) RCTs provided data and showed no significant differences between azithromycin and amoxicillin-clavulanate by day 28-32[14], similar rates of relapse between placebo and

Table III. Studies reporting microbial eradication of the pathogen.

Author (Year)	Population age range	Study place	Sample size (Intervention/ Comparator)	Intervention	Comparator	Microbiological eradication rate (%) Intervention/ AMC	p-value (95% CI)
Block SL et al. [13]	6 months to 6 years with AOM	USA	384 (256/128)	Cefdinir	AMC	55.2/89.5*	0.0019 (N/A)
Sher L et al. [20]	6 months to 7 years with AOM	USA, Central America	349 (176/173)	Gatifloxacin	AMC	81.2/82.0	(N/A)

* AMC compared to cefdinir administered twice daily, for cases of AOM caused by *Streptococcus pneumoniae*
AOM: acute otitis media, AMC: amoxicillin-clavulanate, N/A: not available, USA: United States of America

Table IV. Studies reporting recurrence of AOM after treatment.

Author (Year)	Population age range	Study place	Sample size (Intervention/ Comparator)	Intervention	Comparator	Recurrence rate (%) Intervention/ AMC	p-value (95% CI)
Block SL et al.[14]	6 months to 6 years with AOM	USA	346 (173/173)	Single-dose azithromycin	AMC	N/A	N/A*
Hoberman A et al.[16]	6 to 23 months with AOM	USA	291 (147/144)	Placebo	AMC	16.0/19.0	0.056
Sáez-Llorens X et al.[17]	6 to 7 years with AOM	South America, Asia	413 (277/136)	Gatifloxacin	AMC	11.4/6.6	N/A*

* The authors claim that there were no significant differences between intervention and AMC without providing exact p-values
AOM: acute otitis media, AMC: amoxicillin-clavulanate, N/A: not available, USA: United States of America

amoxicillin-clavulanate before the day 21-25 visit (16.0% vs 19.0%, respectively, p=0.56)[16] and between gatifloxacin and amoxicillin-clavulanate (11.4% vs 6.6%) (Table IV).[17] Overall, the relapse rates were statistically comparable between patients in the amoxicillin-clavulanate arm (36/280, 12.9%) and the antimicrobials or placebo arm (54/424, 12.7%) (OR=1.15, 95% CI 0.53 – 2.50, p=0.72, I^2-60%).

Discussion

This study highlights the comparison of amoxicillin-clavulanate compared to other antimicrobials or a placebo in the treatment of AOM in children between 6 months and 12 years of age. This study revealed the superiority of amoxicillin-clavulanate compared to the other most commonly used antimicrobials for treating AOM, such as cefaclor and penicillin.[19,21] Out of three RCTs comparing azithromycin with amoxicillin-clavulanate, two of them revealed equal clinical success between the two antimicrobials.[12,14] The third one revealed the superiority of amoxicillin/clavulanate against azithromycin only when treating AOM cases caused by *H. influenzae*.[15] These findings are in accordance with a recently published meta-analysis focusing exclusively on amoxicillin/clavulanate and azithromycin highlighting the latter as compared to the former in treating AOM in children.[24] One study showed the non-inferiority of cefdinir compared to amoxicillin-clavulanate.[14] On the contrary, another study revealed the superiority of amoxicillin-clavulanate compared to cefdinir, despite being assigned as a poor quality RCT.[22] A previous study revealed that cefdinir is generally an effective antimicrobial showing clinical results faster than amoxicillin in children, focusing on the treatment of pharyngo-tonsillitis caused by group A beta-hemolytic *Streptococcus*, and including a small number of children.[25]

Three RCTs showed similar clinical success rates between amoxicillin-clavulanate and gatifloxacin, and amoxicillin-clavulanate and levofloxacin.[17,18,20] However, this finding is of minor clinical importance, as gatifloxacin has been withdrawn by the Food and Drug Administration (FDA) from sale due to reasons of safety and effectiveness in 2008.[26] In addition, the use of quinolones in children is controversial, and these antimicrobials should not be used in pediatric patients for routine infections.[27] One RCT showed clear superiority of amoxicillin-clavulanate vs placebo[23], while another displayed no statistical difference between them.[16] However, Hoberman et al.[16] underline that despite the comparable primary outcome between amoxicillin-clavulanate and placebo, children using the latter displayed higher rates of clinical failure. In any case, the use of a placebo in the control group in RCTs is a topic that raises severe ethical issues, and several considerations should be taken into account before deciding to use a placebo in them.[28]

Regarding the secondary outcomes of our review, despite the fact that no statistical differences were found regarding recurrence of AOM between amoxicillin-clavulanate and azithromycin, a placebo or gatifloxacin[14,16,17], amoxicillin-clavulanate proved to be more efficient in eradicating *S. pneumoniae* from the culture.[13] This is very important, as *S. pneumoniae* is the most common cause of AOM in children[29], and its rates of resistance to amoxicillin-clavulanate are relatively low.[30]

However, the robustness of our findings could not be strengthened through meta-analysis due to high heterogeneity between studies regarding both main and secondary outcomes. Thus, the results of quantitative synthesis could not be evaluated.

Our study presents several limitations. Initially, only studies in the English language were included. In addition, the initial search was performed using only two databases, instead of including others as well (e.g Cochrane Library

or EMBASE). Moreover, the NIH ClinicalTrials.gov (http://www.clinicaltrials.gov/) was not assessed to track terminated RCTs or those in progress. Furthermore, no actions were taken to resolve the observed heterogeneity when attempting to perform the meta-analysis. Finally, our systematic review did not take into consideration the importance of the duration of treatment, as underlined in previous RCTs.[31]

In conclusion, from the present systematic review, it can be concluded that amoxicillin-clavulanate should be the treatment of choice for children between 6 months and 12 years of age, with AOM. Although the effects of several antimicrobials or even placebos were proved to be comparable with that of amoxicillin-clavulanate according to several studies, none of them revealed any statistical superiority compared to amoxicillin-clavulanate.

Ethical approval

This study is a systematic review. As such, no ethical approval was required.

Author contribution

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

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Monasta L, Ronfani L, Marchetti F, et al. Burden of disease caused by otitis media: systematic review and global estimates. PLoS One 2012; 7: e36226. https://doi.org/10.1371/journal.pone.0036226
2. Marchisio P, Cantarutti L, Sturkenboom M, et al. Burden of acute otitis media in primary care pediatrics in Italy: a secondary data analysis from the Pedianet database. BMC Pediatr 2012; 12: 185. https://doi.org/10.1186/1471-2431-12-185
3. Klein JO. Otitis media. Clin Infect Dis 1994; 19: 823-833. https://doi.org/10.1093/clinids/19.5.823
4. Buttaravoli P, Leffler SM. Otitis Media, Acute. In: Buttaravoli P, Leffler SM, editors. Minor Emergencies (3rd ed). Elsevier; 2012: 137-141. https://doi.org/10.1016/b978-0-323-07909-9.00035-0
5. Coker TR, Chan LS, Newberry SJ, et al. Diagnosis, microbial epidemiology, and antibiotic treatment of acute otitis media in children: a systematic review. JAMA 2010; 304: 2161-2169. https://doi.org/10.1001/jama.2010.1651
6. Berman S. Otitis media in children. N Engl J Med 1995; 332: 1560-1565. https://doi.org/10.1056/NEJM199506083322307
7. Fougner V, Kørvel-Hanquist A, Koch A, et al. Early childhood otitis media and later school performance - a prospective cohort study of associations. Int J Pediatr Otorhinolaryngol 2017; 94: 87-94. https://doi.org/10.1016/j.ijporl.2017.01.016
8. Mather MW, Drinnan M, Perry JD, Powell S, Wilson JA, Powell J. A systematic review and meta-analysis of antimicrobial resistance in paediatric acute otitis media. Int J Pediatr Otorhinolaryngol 2019; 123: 102-109. https://doi.org/10.1016/j.ijporl.2019.04.041
9. Critical Appraisal Skills Programme. CASP Randomised Controlled Trial Standard Checklist. 2021. Available at: https://casp-uk.b-cdn.net/wp-content/uploads/2020/10/CASP_RCT_Checklist_PDF_Fillable_Form.pdf (Accessed on December 1, 2021).
10. Kontopantelis E, Springate DA, Reeves D. A re-analysis of the Cochrane Library data: the dangers of unobserved heterogeneity in meta-analyses. PLoS One 2013; 8: e69930. https://doi.org/10.1371/journal.pone.0069930
11. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560. https://doi.org/10.1136/bmj.327.7414.557

12. Arrieta A, Arguedas A, Fernandez P, et al. High-dose azithromycin versus high-dose amoxicillin-clavulanate for treatment of children with recurrent or persistent acute otitis media. Antimicrob Agents Chemother 2003; 47: 3179-3186. https://doi.org/10.1128/AAC.47.10.3179-3186.2003

13. Block SL, McCarty JM, Hedrick JA, et al. Comparative safety and efficacy of cefdinir vs amoxicillin/clavulanate for treatment of suppurative acute otitis media in children. Pediatr Infect Dis J 2000; 19: S159-65. https://doi.org/10.1097/00006454-200012001-00005

14. Block SL, Arrieta A, Seibel M, McLinn S, Eppes S, Murphy MJ. Single-dose (30 mg/kg) azithromycin compared with 10-day amoxicillin/clavulanate for the treatment of uncomplicated acute otitis media: a double-blind, placebo-controlled, randomized clinical trial. Curr Ther Res Clin Exp 2003; 64: 30-42. https://doi.org/10.1016/j.curtheres.2003.09.006

15. Hoberman A, Dagan R, Leibovitz E, et al. Large dosage amoxicillin/clavulanate, compared with azithromycin, for the treatment of bacterial acute otitis media in children. Pediatr Infect Dis J 2005; 24: 525-532. https://doi.org/10.1097/01.inf.0000164794.50281.1a

16. Hoberman A, Paradise JL, Rockette HE, et al. Treatment of acute otitis media in children under 2 years of age. N Engl J Med 2011; 364: 105-115. https://doi.org/10.1056/NEJMoa0912254

17. Sáez-Llorens X, Rodriguez A, Arguedas A, et al. Randomized, investigator-blinded, multicenter study of gatifloxacin versus amoxicillin/clavulanate treatment of recurrent and nonresponsive otitis media in children. Pediatr Infect Dis J 2005; 24: 293-300. https://doi.org/10.1097/01.inf.0000157088.37864.e6

18. Noel GJ, Blumer JL, Pichichero ME, et al. A randomized comparative study of levofloxacin versus amoxicillin/clavulanate for treatment of infants and young children with recurrent or persistent acute otitis media. Pediatr Infect Dis J 2008; 27: 483-489. https://doi.org/10.1097/INF.0b013e318168d2cb

19. Subba Rao SD, Macias MP, Dillman CA, Ramos BD, Kierszenbaum JS, Soliman AE. A randomized, observer-blind trial of amoxycillin/clavulanate versus cefaclor in the treatment of children with acute otitis media. Augmentin 415 Study Group. J Chemother 1998; 10: 460-468. https://doi.org/10.1179/joc.1998.10.6.460

20. Sher L, Arguedas A, Husseman M, et al. Randomized, investigator-blinded, multicenter, comparative study of gatifloxacin versus amoxicillin/clavulanate in recurrent otitis media and acute otitis media treatment failure in children. Pediatr Infect Dis J 2005; 24: 301-308. https://doi.org/10.1097/01.inf.0000157084.35865.ba

21. Thomsen J, Sederberg-Olsen J, Balle V, Hartzen S. Antibiotic treatment of children with secretory otitis media. Amoxicillin-clavulanate is superior to penicillin V in a double-blind randomized study. Arch Otolaryngol Head Neck Surg 1997; 123: 695-699. https://doi.org/10.1001/archotol.1997.01900070039006

22. Casey JR, Block SL, Hedrick J, Almudevar A, Pichichero ME. Comparison of amoxicillin/clavulanic acid high dose with cefdinir in the treatment of acute otitis media. Drugs 2012; 72: 1991-1997. https://doi.org/10.2165/11590320-000000000-00000

23. Thomsen J, Sederberg-Olsen J, Balle V, Vejlsgaard R, Stangerup SE, Bondesson G. Antibiotic treatment of children with secretory otitis media. A randomized, double-blind, placebo-controlled study. Arch Otolaryngol Head Neck Surg 1989; 115: 447-451. https://doi.org/10.1001/archotol.1989.01860280045015

24. Dawit G, Mequanent S, Makonnen E. Efficacy and safety of azithromycin and amoxicillin/clavulanate for otitis media in children: a systematic review and meta-analysis of randomized controlled trials. Ann Clin Microbiol Antimicrob 2021; 20: 28. https://doi.org/10.1186/s12941-021-00434-x

25. Brook I, Gober AE. Rate of eradication of group A beta-hemolytic streptococci in children with pharyngo-tonsillitis by amoxicillin and cefdinir. Int J Pediatr Otorhinolaryngol 2009; 73: 757-759. https://doi.org/10.1016/j.ijporl.2009.02.004

26. Food and Drug Administration. Determination That TEQUIN (Gatifloxacin) Was Withdrawn From Sale for Reasons of Safety or Effectiveness. 2008. Available at: https://www.federalregister.gov/documents/2008/09/09/E8-20938/determination-that-tequin-gatifloxacin-was-withdrawn-from-sale-for-reasons-of-safety-or (Accessed on December 10, 2021).

27. Choi SH, Kim EY, Kim YJ. Systemic use of fluoroquinolone in children. Korean J Pediatr 2013; 56: 196-201. https://doi.org/10.3345/kjp.2013.56.5.196

28. Cheah PY, Steinkamp N, von Seidlein L, Price RN. The ethics of using placebo in randomised controlled trials: a case study of a Plasmodium vivax antirelapse trial. BMC Med Ethics 2018; 19: 19. https://doi.org/10.1186/s12910-018-0259-4

29. Venekamp RP, Schilder AGM. Antibiotic treatment in otitis media reduces middle ear effusion duration. J Pediatr 2014; 165: 641-642. https://doi.org/10.1016/j.jpeds.2014.06.035

30. Farajzadeh Sheikh A, Rahimi R, Meghdadi H, Alami A, Saki M. Multiplex polymerase chain reaction detection of Streptococcus pneumoniae and Haemophilus influenzae and their antibiotic resistance in patients with community-acquired pneumonia from southwest Iran. BMC Microbiol 2021; 21: 343. https://doi.org/10.1186/s12866-021-02408-7

31. Hoberman A, Paradise JL, Rockette HE, et al. Shortened antimicrobial treatment for acute otitis media in young children. N Engl J Med 2016; 375: 2446-2456. https://doi.org/10.1056/NEJMoa1606043

The Turkish Journal of Pediatrics 2023; 65: 362-376
https://doi.org/10.24953/turkjped.2022.1049

Review

Radiotherapy for pediatric non-rhabdomyosarcoma soft tissue sarcomas: a comprehensive review

Alper Kahvecioğlu, Melis Gültekin, Ferah Yıldız

Department of Radiation Oncology, Hacettepe University Faculty of Medicine, Ankara, Türkiye.

ABSTRACT

Background. For children with non-rhabdomyosarcoma soft tissue sarcomas, a risk-adapted treatment approach is generally used in order to minimize treatment-related morbidity and mortality in low-risk patients and maximize the benefit in high-risk patients. Our aim in this review is to discuss the prognostic factors, risk-adapted treatment options and the details of radiotherapy.

Methods. The publications reached by searching the keywords 'pediatric soft tissue sarcoma', 'non-rhabdomyosarcoma soft tissue sarcoma (NRSTS)', and 'radiotherapy' in Pubmed database were evaluated in detail.

Results. Today, based on prospective COG-ARST0332 and EpSSG studies, a risk-adapted multimodal treatment approach has become the standard in pediatric NRSTS. According to them, adjuvant chemotherapy/radiotherapy can be safely omitted in low-risk patients, while adjuvant chemotherapy/radiotherapy or both are recommended in intermediate and high-risk groups. Recent prospective studies for pediatric patients have reported excellent treatment outcomes with smaller radiotherapy fields and lower doses than adult series. The primary goal of surgery is maximal tumor resection with negative margins. In cases that are initially unresectable, neoadjuvant chemotherapy and radiotherapy should be considered.

Conclusions. A risk-adapted multimodal treatment approach is the standard in pediatric NRSTS . Surgery alone is sufficient in low-risk patients, and adjuvant therapies may safely be omitted. On the contrary, in intermediate- and high-risk patients, adjuvant treatments should be applied to reduce recurrence rates. In unresectable patients, the chance of surgery increases with the neoadjuvant treatment approach and thus treatment results may improve. In the future, outcome might be improved with further clarification of molecular features and targeted therapies in such patients.

Key words: Non-rhabdomyosarcoma soft tissue sarcoma, pediatric sarcoma, radiotherapy, sarcoma, treatment.

Pediatric soft tissue sarcomas originate from mesenchyme and accounts for 7% of all childhood cancers.[1] They are divided into two groups as rhabdomyosarcomas (RMS) and non-rhabdomyosarcoma soft tissue sarcomas (NRSTS) with an incidence of 40 and 60%, respectively.[2,3] NRSTS shows a bimodal age distribution in childhood, with the incidence being high in infants and adolescents.[4]

✉ Melis Gültekin
melisbahadir@yahoo.com.tr

Received 7th December 2022, revised 12th January 2023, accepted 13th February 2023.

There are several genetic syndromes and molecular alterations that have been associated with the development of NRSTS. The risk of developing malignant peripheral nerve sheath tumor (MPNST) in children with neurofibromatosis 1 (NF-1) is three times higher than in the general population.[5] The risk of NRSTS is also higher in Li-Fraumeni Syndrome, which is characterized by p53 gene mutation.[6] In addition, ionizing radiation and chemotherapy (CHT) may also play a role in their etiology. However, in most patients the etiology is unclear.

NRSTS are quite complex and heterogeneous group of tumors including nearly 50

histological subtypes. Pediatric NRSTS includes special histological subtypes such as infantile fibrosarcoma and the distribution of histological subtypes which are quite differed from adult cases. Synovial sarcomas, MPNST and fibrosarcomas are the most common histological subtypes in pediatric cases.[4] These tumors are usually observed in the trunk or extremities. The most common initial symptom is a painless mass. As they are usually tumors with local infiltrative growth pattern, symptoms associated with infiltration of adjacent neurovascular structures or organs may accompany. Regional lymph node (LN) metastases are generally rare but can be observed in some subtypes such as synovial, clear cell or epithelioid sarcomas.[7] Distant metastases are present in 15% of newly diagnosed cases, and the most common site is the lungs.[8]

A detailed anamnesis, including family history, is essential to detect underlying genetic disorders. A careful physical examination is required to determine the local characteristics of the tumor. Computed tomography (CT) or magnetic resonance imaging (MRI) is also recommended to evaluate the size and extension of the tumor, its relationship with adjacent structures, and treatment planning. Chest X-ray or thorax CT should be taken for evaluation of lung metastases. Fluorine-18-fluorodeoxyglucose positron emission tomography (PET-CT), widely used today, may also help in the staging of the disease.

In the presence of a soft tissue mass suspected of malignancy, a histological diagnosis must be obtained by core needle biopsy or open surgical biopsy. Classification of NRSTS causes challenges for pathologists and there is wide interobserver variability. Therefore, pathological examination should be performed by a pathologist with expertise in sarcomas, according to the World Health Organization (WHO) Classification.[9] Immuno histochemical study and molecular profiling are useful for accurate classification. The two most commonly used systems for the histological grading are those developed by the Pediatric Oncology Group (POG) and the Federation Nationale des Centers de Lutte Contre le Cancer (FNCLCC).[10] In the prospectively validated POG system, cases are graded as low, intermediate and high based on histological subtype, necrosis rate, and cellular pleomorphism.[11] According to this system, mortality rates are 15% in grade 1 and 2 tumors and 73% in grade 3 tumors.[12] In the FNCLCC system, grading is based on tumor differentiation, presence of necrosis and the number of mitoses.[13]

Although there is no standard consensus for pediatric NRSTS staging, the most commonly used staging system is the American Joint Committee on Cancer (AJCC). In this system, tumor size (T1 ≤5 cm, T2 >5 cm), tumor depth (a=superficial, b=deep), nodal involvement (N), distant metastasis (M) and histological grade (G) are taken into account.[14] Another staging system used after surgery is the 'Intergroup RMS Study Group (IRSG)' classification. It is determined based on the completeness of surgical resection: initial complete resection (R0) is classified as group 1, complete resection with microscopic residual disease (R1) and/or regional LN involvement (N1)refers to group 2, macroscopic residual disease (R2) or biopsy alone (not resected) is classified as group 3 and metastatic disease is classified as group 4. However, as a limitation, important factors such as tumor grade and width of surgical margins are not considered in this system.[15]

The most significant poor prognostic factors in pediatric NRSTS are tumor grade (high), tumor size of >5 cm, positive or close surgical margins, and presence of metastatic disease.[16,17] In a meta-analysis including unresectable patients, age, delayed complete surgical resection, histological subtype, response to neoadjuvant CHT, tumor site and presence of radiotherapy (RT) were also defined as prognostic factors. Trunk, intra-abdominal or intrathoracic localization, MPNST histology, age >10 years were poor prognostic factors for survival. NF-1-associated MPNSTs had the worst CHT response and survival rate in all NRSTS.[18]

Researchers at St. Jude Children's Research Hospital identified three risk groups with significantly different overall survival (OS) rates according to the prognostic features. Group 1 includes completely resected and non-metastatic patients, Group 2 includes unresectable and non-metastatic patients, and Group 3 includes metastatic patients, with 5-year OS rates of 89%, 56%, and 15%, respectively.[8,16,17] In the Children's Oncology Group (COG) risk classification used today, the cases are divided into low-, intermediate-, and high-risk groups according to resection width, POG tumor grade, tumor size, and presence of distant metastases. Five-year OS rates are 90%, 50%, and 15% for the low-, intermediate-, and high-risk groups, respectively.[16,17,19]

Treatment

A multidisciplinary approach is mandatory in the treatment of this special disease. In the past, due to the rarity of prospective studies on NRSTS in the pediatric age group, cases were often treated similar to adult patients. Following the definition of prognostic factors in the two large single-center series in pediatric NRSTS, prospective studies including a multimodal risk-adapted treatment approach were designed by the COG and the European pediatric Soft Tissue Sarcoma Study Group (EpSSG).[16,17,20] These comprehensive studies have led to the definition of a standard of care for pediatric NRSTS and both will be discussed in detail below.[19,21] Again, the INternational Soft Tissue SaRcoma ConsorTium (INSTRuCT), formed by COG, EpSSG and Cooperative Weichteilsarkom Studiengruppe, aimed to provide treatment standardization and improve treatment outcomes in pediatric NRSTS, and has recently published its recommendations.[22] In general, patients are classified according to key prognostic factors such as presence of distant or LN metastases, histologic grade, primary tumor size (≤5 cm vs. >5 cm) and extent of surgical resection, and a risk-adapted treatment approach is applied (Table I).

Surgery

While determining the local treatment method, tumor grade, tumor size, tumor localization, surgical margins and patient age should be considered. The main treatment component of the multimodal treatment strategy is complete surgical resection with negative margins prior to or after CHT and/or RT. One of the most important goals should be to avoid any microscopic or macroscopic disease left behind. Although negative surgical margins after resection are essential, surgical procedures with high morbidity or mutilation should be avoided since similar local control rates can be achieved with modern RT techniques. A cornerstone randomized trial including both adult and pediatric patients with soft tissue sarcoma showed that limb-sparing surgery and adjuvant RT had similar survival rates when compared to amputation.[23]

Table I. INSTRuCT risk-adapted treatment recommendations for NRSTS.[22]

IRS Group	Grade	Tumor Size	Surgical Status	Treatment
I-II	Low	-	R0-R1	Surgery alone
I	High	≤5 cm	R0	Surgery alone
II	High	>5 cm*	R1	Adjuvant RT
I-II	High	>5 cm**	R0-R1	Adjuvant CHT (4-6 cycles of I&D) ± RT
III	High	>5 cm	Unresectable or delayed resection planned	Neoadjuvant CHT (6 cycles of I&D) and RT
Metastatic	-	-	-	Neoadjuvant CHT (6 cycles of I&D) and RT

CHT: chemotherapy, D: doxorubicin, I: ifosfamide, INSTRuCT: INternational Soft Tissue SaRcoma ConsorTium, IRS: Intergroup Rhabdomyosarcoma Study, NRSTS: non-rhadbomyosarcoma soft tissue sarcomas, R0: negative resection margins, R1: microscopic tumor infiltration, RT: radiotherapy.
*Eventually size ≤5 cm.
**Eventually synovial sarcoma IRS group II with ≤5 cm tumor and/or axial location.

Since lymphatic metastasis is rare, routine LN dissection is not recommended. However, clinically suspicious LNs should be sampled, especially in tumors of specific histological types at risk of regional LN metastasis. Although this is an evolving area, the utility of sentinel LN biopsy has been reported in pediatric NRSTS at high risk for nodal involvement and can be considered in some cases.[24] The optimal management of pathologically confirmed metastatic LNs is unknown due to the rarity of these cases, but overall, LN dissection with adjuvant RT is generally the preferred approach.

Systemic Treatment

NRSTSs are generally accepted chemoresistant tumors except synovial sarcoma. Although they are defined as chemoresistant, CHT plays a vital role in selected patients. It has been shown that the regimen with the highest response rates among the different chemotherapeutic agents was a combination of ifosfamide and doxorubicin.[20] CHT is generally used with an aim to increase the resectability rates of unresectable tumors and is always used with RT since the highest resectability rates are achieved with combined approaches rather than CHT alone.[17] Also, CHT can provide systemic disease control in metastatic patients. Again, it can be applied as adjuvant therapy to provide systemic control in tumors with high metastatic potential in the postoperative period. In a retrospective analysis of 36 patients it was shown that patients with high-grade or tumors larger than 5 cm had better 5-year metastasis-free survival and OS rates with adjuvant CHT than those who had not.[25] However, a large trial in pediatric patients failed to demonstrate any survival benefit with adjuvant CHT compared to observation alone.[26]

With a better definition of molecular features and the integration of genetic data in NRSTS, molecular targeted therapies such as specific tyrosine kinase inhibitors, such as imatinib, sunitinib, pazopanib etc., can be used as new agents in pediatric NRSTS. The prospective ARST1321 study showed that pathological near complete response rates increased with the addition of pazopanib to neoadjuvant chemoradiotherapy (CRT).[27] However, comprehensive prospective studies are needed to clarify whether survival rates are improved with targeted therapies.

Radiotherapy

RT plays an essential role in the treatment of NRSTS. It can often be applied to patients with a high risk of local recurrence, either preoperatively or postoperatively. Indications for adjuvant RT in current clinical practice are determined by surgical margin status, tumor grade, tumor size, invasion of adjacent structures, histological subtype, age, and underlying genetic syndromes (e.g., Li Fraumeni Syndrome). Surgery alone is a sufficient therapy for patients with localized, low-grade tumors with negative surgical margins. If the surgical margin is close or positive, re-excision should be the first choice and RT in these patients is generally reserved for recurrence. With this approach, excellent survival rates can be achieved with re-excision and adjuvant RT, even if there is recurrence.[16] Exceptionally, if limb-sparing surgery cannot be performed in case of recurrence or the morbidity of the surgery to be performed is unacceptable, adjuvant RT may be considered without delay. In the presence of high-grade tumors >5 cm or marginally-resected high grade tumors, adjuvant RT is recommended to increase local control.[28]

The treatment approach used in pediatric NRSTS in recent years is based on the risk grouping, in which more intensive treatments are applied to increase survival in high-risk cases and de-escalated treatment approach in low-risk patients in order to avoid treatment related morbidity. In summary, treatment plans vary from surgery alone to more aggressive neoadjuvant or adjuvant CHT and RT regimens. This approach was tested in the recently published prospective 'ARST0332' trial designed by COG.[19] In this study patients

were categorized into low-, intermediate-, and high-risk groups according to the POG tumor grade, tumor size, distant metastasis status, initial extent of surgery and surgical margins, and four different treatment arms (A-D) were defined (Table II). Five hundred twenty-nine patients under 30 years of age with more than 30 histological subtypes were included in this study. The absence of microscopic tumor cells in the surgical margins was accepted as R0 resection, while the adequate surgical margin was determined as ≥5 mm. According to this protocol, surgery alone was performed in low-risk patients, except those with high-grade tumors and R1 resection. Adjuvant RT with a total dose of 55.8 Gy was applied to patients with high-grade tumors and positive microscopic margins. Adjuvant CHT containing ifosfamide and doxorubicin plus adjuvant RT (55.8 Gy) starting after the second cycle of CHT was applied to resected patients in the intermediate and high-risk groups. Initially unresectable patients underwent surgery after neoadjuvant CRT. After surgery, adjuvant CHT and RT boost were applied according to the surgical margin status. The total dose of neoadjuvant RT was 45 Gy. After surgery, 10.8 Gy boost was applied to patients who underwent R1 resection, and 19.8 Gy boost to patients who underwent R2 resection or were unresectable. No adjuvant therapy was applied for low-grade tumors that were initially metastatic if all lesions were grossly resected. However, metastasis-directed RT at a dose of 50 Gy in 25 fractions was applied to all residual metastatic lesions at the end of the therapy. Whole lung or whole abdomen or pelvis RT was not recommended. At the end of a median follow-up period of 6.5 years, risk groups were shown to have a significant predictive effect on survival rates. The 5-year OS and event-free survival (EFS) rates were 96.2% and 88.9%, 79.2% and 65%, and 35.5% and 21.2% for low-, intermediate-, and high-risk patients, respectively. According to the ARST0332 study results, the oncological outcomes were excellent with surgery alone in low-risk patients, and late toxicities of adjuvant treatments could be avoided safely in these patients. In addition, it was underlined that a lower adjuvant RT dose (55.8 Gy at adjuvant setting and 45 Gy at neoadjuvant setting) rather than conventional doses provided satisfyingly high local control rates.

Table II. COG's ARST0332 Study: A risk-adapted treatment approach in NRSTS.[19]

Risk Group	Prognostic Factors				Treatment (Treatment Arm)
	Grade	Tumor Size	Metastasis*	Resection Status of Primary Tumor	
Low	Low	≤5 cm/>5 cm	(-)	Grossly resected (R0/R1)	Observation (A)
	High	≤5 cm	(-)	Microscopic margins (-)	Observation (A)
	High	≤5 cm	(-)	Microscopic margins (+)	Adjuvant RT (B)
Intermediate	High	>5 cm	(-)	Grossly resected (R0/R1)	Adjuvant CHT and RT (C)
	High	>5 cm	(-)	Unresected/R2**	Neoadjuvant CRT, surgery, adjuvant CHT ± RT (D)
High	Low	≤5 cm/>5 cm	(+)	Grossly resected (R0/R1)	Observation (A) or Adjuvant CHT and RT (C)***
	High	≤5 cm/>5 cm	(+)	Grossly resected (R0/R1)	Adjuvant CHT and RT (C)
	High	≤5 cm/>5 cm	(+)	Unresected/R2**	Neoadjuvant CRT, surgery, adjuvant CHT ± RT (D)

CHT: chemotherapy, COG: Children's Oncology Group, CRT: chemoradiotherapy, NRSTS: non-rhabdomyosarcoma soft tissue sarcomas, RT: radiotherapy.
*Lymph node and/or distant metastasis.
**Unresectable or high-grade tumor >5 cm where delayed resection planned.
***If all disease resected (A) or not (C).

Venkatramani et al.[29] also separately reported the characteristics and treatment outcomes of patients diagnosed with synovial sarcoma, the most common NRSTS, in the ARST0332 study. When 138 available patients were examined, risk-adapted treatment was found to be effective and safe. All parameters used in risk classification had a significant predictive effect on the outcomes. Adjuvant CHT and RT could be avoided in almost one third of patients. The 5-year OS rate was reported as 100% in patients who underwent surgery alone for low-risk disease. Sixty-nine (50%) of the synovial sarcoma patients were initially considered unresectable and treated with neoadjuvant CRT. The dose of RT in these patients was 45 Gy which is lower than the doses used in the postoperative adjuvant RT approach and gross total resection was performed in 87% of them. Since less than 20% had a microscopic residual disease, a boost of 10.8 Gy was applied postoperatively in the study. Although synovial sarcoma is considered as a chemosensitive tumor, it is interesting that high (>90%) necrosis rate was detected in only 28% of patients after neoadjuvant CRT. In the following ARST1321 Phase 2 study, it was shown that the addition of pazopanib to neoadjuvant CRT increased rates of pathological near complete response in children and adults with advanced NRSTS.[27] The long-term results of these trials will reveal the effect of pathological response rates on survival outcome.

Similar to the COG, the NRSTS 2005 study of EpSSG also examined the risk-adapted treatment in pediatric patients with NRSTS (Table III).[21] The EpSSG study included two

Table III. EpSSG's NRSTS 2005 Study: A risk-adapted treatment approach in NRSTS.[21]

Surgery Alone		
Synovial Sarcoma	IRSG I, ≤5 cm	
	IRSG I, ≤5 cm, any grade	Upfront surgery, no adjuvant treatment.
Adult type NRSTS	IRSG I, >5 cm, grade 1	
	IRSG II, any size, grade 1	
Adjuvant RT*		
	IRSG I, >5 cm, grade 2	
Adult type NRSTS	IRSG II, ≤5 cm, grade 2–3	Adjuvant RT (54.0 Gy)
	IRSG II, >5 cm, grade 2	
Adjuvant CHT (with or without RT)		
	IRSG I, >5 cm	4 cycles I&D
Synovial Sarcoma	IRSG II, ≤5 cm	3 cycles I&D + RT (50.4 Gy)
	IRSG II, >5 cm	
	Axial site or resected N1	3 cycles I&D + RT (54 Gy) with 2 cycles I + 1 cycle I&D
Adult type NRSTS	IRSG I–II, >5 cm, grade 3 or resected N1	
Neoadjuvant CHT (with or without RT)		
Synovial Sarcoma	IRS Group III (unresected) or unresected N1	3 cycles I&D + Surgery + RT** (50.4-59.4 Gy) with 2 cycles I + 1 cycle I&D ± 1 cycle I&D
Adult type NRSTS		

CHT: chemotherapy, D: doxorubicin, EpSSG: European pediatric Soft Tissue Sarcoma Study Group, I: ifosfamide, IRSG: Intergroup Rhabdomyosarcoma Study Group, N: nodal stage, NRSTS: non-rhabdomyosarcoma soft tissue sarcomas, RT: radiotherapy

* Following upfront surgery.

**50.4 Gy after R0, 54.0 Gy after R1, and 59.4 Gy after R2 resection.

different treatment protocols under the headings of synovial sarcomas and adult type NRSTS to create subgroups as homogeneous as possible. Unlike the COG study, metastatic patients were not included in this study. Patients with synovial sarcoma were stratified according to surgical stage, tumor size, nodal involvement, and tumor localization. The risk classification in the EpSSG study also included the IRSG classification system based on surgical resection status, which was previously mentioned, and grading was based on the FNCLCC grading system. In this study patients were divided into four treatment groups: surgery alone, adjuvant RT, adjuvant CHT (± RT), or neoadjuvant CHT (± RT). The main CHT regimen was ifosfamide plus doxorubicin. With a median follow up of 80 months, 5-year OS and EFS rates were 98.1% and 91.4 % in the surgery alone group, 88.2% and 75.5% in the adjuvant RT group, 75.8% and 65.6% in the adjuvant CHT group and 70.4% and 56.4% in the neoadjuvant CHT group, respectively. As a conclusion, the authors stated that risk-adapted treatment was safe and feasible.

Timing of Radiotherapy

RT can be used as preoperative, intraoperative, postoperative or as definitive therapy but it is usually recommended in the postoperative setting for NRSTS.[23,30] Preoperative RT, on the other hand, is increasingly popular because of its various advantages. A randomized trial in adult patients failed to show any local control or survival benefit with preoperative RT, but there are no studies confirming this for the pediatric population.[31] Advantages of preoperative RT include performing less morbid surgeries in large (>5 cm) tumors and tumors that are difficult to operate at the beginning, reducing the risk of tumor seeding during surgery, increasing the biological effect of radiation in tumors with intact vascularization and better oxygenation, better determination of target volumes during RT planning, smaller irradiated volumes with exclusion of surgically manipulated tissues, incision scars, and drain sites with lower RT doses and improved long-term functional outcomes.[31,32] However, there are possible disadvantages like increased wound complications, rare but possible progression during RT, and the inability to perform definitive surgery in cases with progressive tumors.[31] There are conflicting reports on whether acute wound complications are more common with preoperative RT. It has been reported in the literature that preoperative RT causes wound complications in approximately 11-29% of adult series.[33,34] In the ARST0332 study, which included the pediatric population, 11% of the patients who underwent delayed surgery following neoadjuvant CRT had wound complications requiring surgical intervention.[19] The slightly lower incidence of wound complications compared to adult series may be due to lower doses and smaller fields of RT, but more detailed prospective studies regarding predictive factors for wound complications are needed.

In the ARST0332 study, postoperative RT was administered within six weeks after surgery with completion of post-surgical wound healing in the adjuvant RT arm and patients in the adjuvant CRT arm received RT four weeks after the 2nd course of ifosfamide plus doxorubicin CHT.[19] Patients in the preoperative CRT arm received two cycles of ifosfamide plus doxorubicin and two cycles of only ifosfamide concurrently with RT starting at the 4th week after the second cycle of CHT. If feasible, definitive resection was done at week 13, and a postoperative boost was applied to patients with residual tumors after the first cycle of adjuvant CHT.

In the EpSSG study protocol, as the value of CHT is unclear, it's recommended to start RT without delay. In patients with initial gross resection, RT is started after the third cycle of CHT which corresponds to the postoperative 9th week.[21] If second-look surgery will not be performed in patients with macroscopically residual disease (IRSG III), RT is started 8 weeks after surgery. In patients who underwent second-look surgery, RT starts in the 3rd week

unless there are postoperative complications. If preoperative RT is to be performed before second-look surgery, RT begins at week 9 after the first surgery, and the second surgery is performed at week 5 after RT.

Radiotherapy Technique

In the first and subsequent National Cancer Institute of Canada (NCIC) studies examining the organ preservation approach, only conventional two-dimensional (2D)-RT technique was used, including a large RT field and a sequential boost volume determined by surgical clips to reduce adjacent critical organ doses.[23] Although the standard RT technique today is three-dimensional (3D)-conformal RT (3D-CRT), several studies in adult patients have reported higher local control rates and lower toxicity rates with intensity modulated radiotherapy (IMRT) compared to 3D-CRT.[35,36]

The EpSSG protocol recommends 3D-CRT for all patients and includes megavoltage (MV) equipment, electrons, and brachytherapy (BRT).[21] Low energy (4 to 6 MV) photons are recommended for limb tumors and 6 to 20 MV photons for trunk tumors. While electrons can be used for superficial or slightly infiltrative tumors, BRT is generally reserved for incompletely resected tumors located in the vagina, perineum, prostate, bladder, and orbit. In a separate analysis of 56 patients with high-grade extremity tumors who underwent preoperative RT in the COG ARST0332 study, it was reported that target coverage increased with IMRT compared to 3D-CRT, at the same time, skin and adjacent joint doses decreased.[37] Reducing the skin dose is an essential advantage of IMRT, as clinicians' primary concern for preoperative RT is postoperative wound complications. However, it is especially important in pediatric cases that the low-dose areas with IMRT are higher than with 3D-CRT, which may increase the risk of secondary malignancies.[38,39] When deciding on the RT technique, evaluation should be made on a patient by patient basis. Insufficient immobilization, rapidly growing tumor or a large field size may be other reasons for preferring 3D-CRT over IMRT.

Image-guided RT (IGRT) improves the accuracy of RT delivery. With this technique, the safety margins given to the target volumes can be reduced and thus less toxicity is observed.[40] Therefore, it is recommended to perform IGRT regardless of the RT technique. Kilovoltage (KV) imaging is preferred over MV imaging for reducing the ionizing radiation exposure.

There are also promising results for proton beam therapy in Ewing sarcoma and RMS.[41,42] Proton beam therapy reduces normal tissue and organ doses with its Bragg peak feature. However, in a systematic review of 15 pediatric cancers, including sarcomas, it was concluded that clinical data supporting or rejecting proton beam therapy is insufficient, and high-quality clinical studies should be conducted on this subject.[43]

High doses of RT are often required for local control in NRSTS, resulting in increased normal tissue toxicity when external beam RT (EBRT) is administered. In many centers, single-fraction BRT or intraoperative RT (IORT) combined with lower-dose EBRT is applied to increase local control.[44,45] Local control rates are highest with BRT combined with EBRT, especially in positive surgical margins. However, it has been shown in the literature that the contribution of BRT is limited to high-grade tumors only.[46]

Simulation and Target Volumes of Radiotherapy

The use of appropriate immobilization devices during simulation is essential. Various devices such as limb masks, air or vacuum bags can be used for this purpose. Placing radiopaque markers on surgical scars before simulation facilitates target volume contouring. While contouring the target volumes, physical examination findings and radiological examinations should be used. When contouring the target volume in postoperative cases, performing fusion with the most appropriate

preoperative imaging technique is essential. MRI is superior to CT in terms of better soft tissue contrast. Contrast-enhanced T1 MRI images are frequently used in target volume delineation. The definitions of gross tumor volume (GTV) and clinical target volume (CTV) are summarized in (Table IV). The planning target volume (PTV) margin is usually 3-5 mm and differs from center to center depending on the treatment modality.

Although a craniocaudal safety margin of 4-5 cm is traditionally given when determining RT volumes in patients diagnosed with adult STS, narrower margins have been given in recent studies.[31] However, the optimal margin in pediatric cases is unknown. In a prospective study by Krasin et al., local RT fields were created by giving a 2 cm safety margin to the initial tumor volume. In the follow-up of 32 patients, local failure was observed in 4 patients. The mean dose at the site of local recurrence in all four patients was 97% of the prescribed radiation dose. The authors concluded that limited field RT is effective, but since failure occurs in the high-dose region, new treatment strategies are required to increase local control.[47]

In the COG ARST0332 study, CTV margins were created by giving 1.5 cm to the GTV, and PTV margins were created by giving 0.5 cm to the CTV.[19] In the EpSSG study, a 1 cm safety margin is given to GTV for CTV contouring[21]. A longitudinal safety margin of 2 cm is given for lesions located in the extremities. Biopsy or surgical scars and drain sites should also be included in the CTV. For PTV, a safety margin of 1 cm is given to the CTV, but a safety margin of 2 cm should be given for the chest wall localization. If high RT doses are to be administered, a new CT simulation should be performed after 50.4 Gy, and a PTVboost volume should be created by giving the residual tumor a 1-2 cm safety margin.

Table IV. Target volume definitions by ARST0332 study.[19]

Target Volumes	Definitions
GTV1	Defined as the visible and/or palpable disease defined by physical examination, CT, MRI or PET-CT, operative notes, and pathology reports.
	For patients with initial tumors that extend into body cavities (i.e., thorax, abdomen) the GTV1 may require modification. If the tumor has been resected or responded to CHT and the normal tissues have returned to their normal positions, the GTV1 excludes the volume which extends into the cavity. Examples include tumors which compress but not invade the lung, intestine or bladder that radiographically return to normal anatomic position following surgery or CHT.
	Include all infiltrative disease detected at initial presentation.
GTV2	For resected tumors, the GTV2 (volume reduction) is defined as the region of positive surgical margins, microscopic or gross residual disease determined by operative note, pathology report and imaging studies.
	For unresected tumors, the GTV2 is defined as the pre-treatment residual soft tissue disease following induction CHT.
	For partially resected tumors, the GTV2 is defined as the residual soft tissue disease following induction CHT and surgical debulking.
CTV1	Defined as GTV1 + 1.5 cm (but not extending outside of the patient).
	Also includes regional LN chains that are known to harbor pathologically involved nodes.
	For some sites, CTV1 is modified to account for specific anatomic barriers to tumor spread.
CTV2	Defined as the GTV2 + 1.0 cm (but not extending outside the patient).
	For some sites, CTV2 is modified to account for specific anatomic barriers to tumor spread.

CHT: chemotherapy, CT: computed tomography, CTV: clinically target volume, GTV: gross tumor volume, LN: lymph node, MRI: magnetic resonance imaging, PET: positron emission tomography.

In BRT, the target volume contains the surgical bed alone. Scar or drain sites are not included. Catheters should be placed 1-1.5 cm apart, parallel, or perpendicular to the incision scar. Although there is no clear consensus for the safety margin, a minimum of 2 cm craniocaudal and 1-2 cm radial are recommended by American Brachytherapy Society (ABS).[48] There is a relationship between catheter loading in the early postoperative period and postoperative wound complications. For this reason, loading is not recommended in the first five days postoperatively.[46] Removal of critical structures such as intestines, nerves, ureters, and main vessels from the RT field is important in terms of side effects. Target volumes and the RT plan of a patient with synovial sarcoma is shown in Figure 1 and Figure 2.

Radiotherapy Dose

RT approach in pediatric RMS, which is clearly a distinct entity, RT dose, fraction scheme and target volumes were clearly defined based on the results of randomized trials. However, there is no standard recommendation for details of RT in pediatric NRSTS. RT target volume definitions and administered doses differ in COG and EpSSG studies (Table V). Conventional RT is applied in daily fraction doses of 1.8 Gy, five days a week. In the presence of large treatment fields or cases <3 years of age, smaller fraction doses such as 1.2-1.5 Gy should be preferred. The total dose in high dose rate BRT is 34 Gy, twice a day at a fractional dose of 3.4 Gy. Due to toxicity, doses of <12 Gy should be administered in cases <6 years of age.[49]

For pediatric NRSTS, postoperative boost is still controversial. Although many centers apply postoperative boost to patients who cannot achieve R0 resection after neoadjuvant CRT, no study clearly shows the benefit of a postoperative boost in the pediatric population. There are also controversial results in adult series in the literature. A study of 216 adult patients with positive surgical margins showed that post-operative 16 Gy boost after neoadjuvant 50 Gy RT did not contribute to the prevention of local recurrence.[50]

It is very important to protect normal tissues during RT, especially in pediatric cases. The skin and subcutaneous tissues should be protected medially as a longitudinal strip, and at least 50% should receive a dose of <20 Gy to minimize the lymphedema risk. It is also recommended that <50% of normal weight-bearing bones receive 50 Gy to reduce fracture risk.[40] Epiphyseal growth plates should be preserved as much as possible because of the risk of asymmetrical growth and deformity in growing children.

Today, based on prospective COG ARST0332 and EpSSG studies, a risk-adapted multimodal

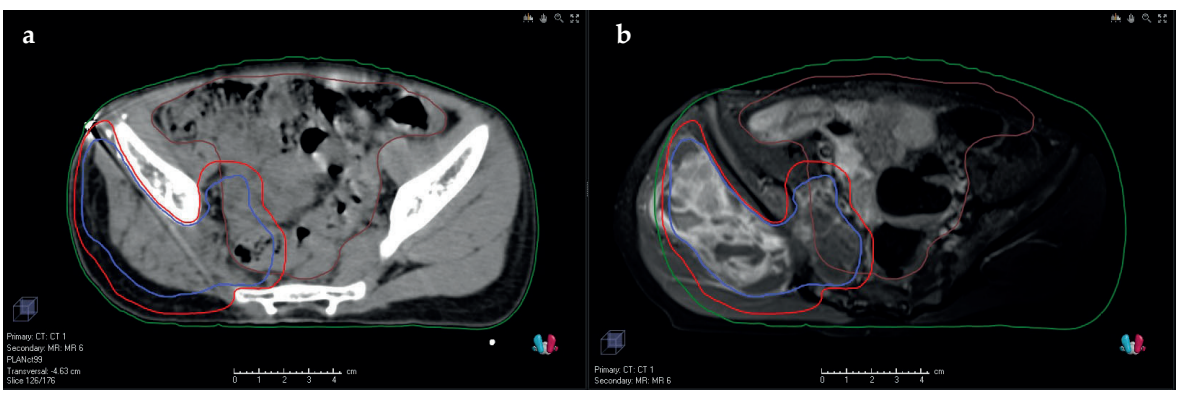


Fig. 1. Target volumes of radiotherapy in a patient with synovial sarcoma after surgery.

Fused images of simulation CT (a) and preoperative MRI (b). Blue contour is GTV-virtual (preoperative GTV). Red contour is CTV with 5 mm safety margin. Brown contour is bowel. Green contour is body.

CT: computed tomography; MRI: magnetic resonance imaging; GTV: gross tumor volume; CTV: clinically target volume

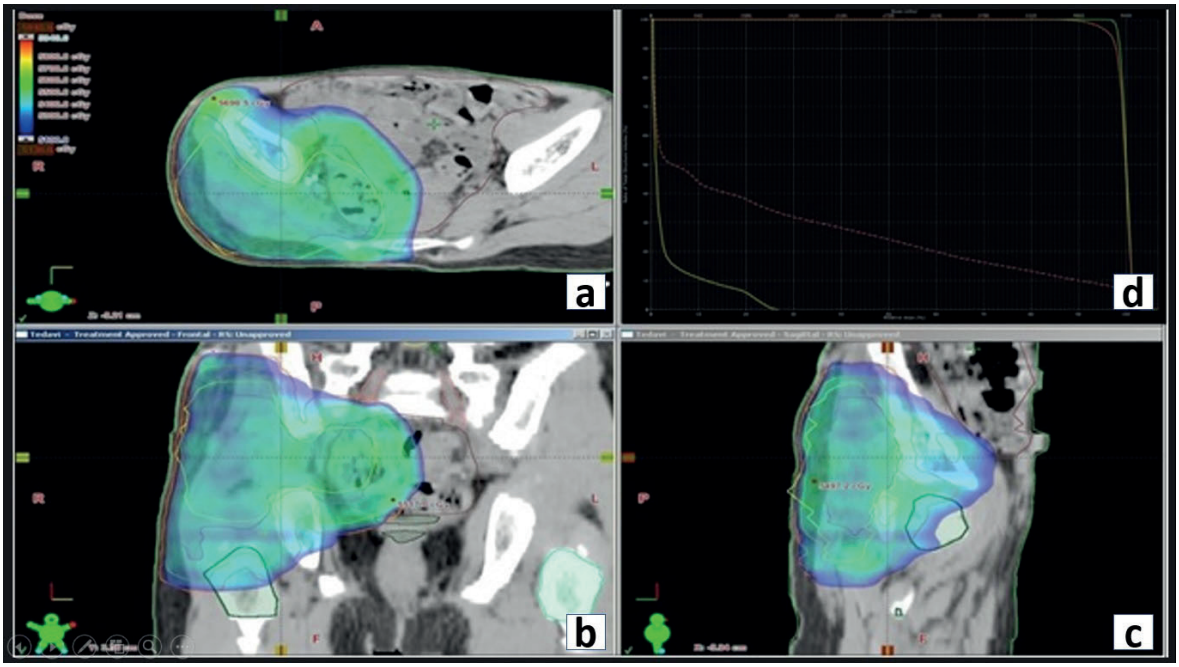


Fig. 2. Radiotherapy plan of a patient with synovial sarcoma.

Axial (a), coronal (b) and sagittal (c) simulation CT images of the RT plan and DVH (d). Blue dose-color wash is 95% isodose. At DVH, orange line is PTV, green line is CTV, dashed brown line is bowel, yellow line is spinal cord.

CT: computed tomography; RT: radiotherapy; DVH: dose-volume histogram; CTV: clinically target volume; PTV: planning target volume

Table V. Radiotherapy dose recommendations of the ARST0332 and EpSSG trials.[19,21]

COG ARST 0332	
High grade, ≤5 cm, R1 resection	Adjuvant RT (55.8 Gy)
High grade, >5 cm, R0/R1 resection	Adjuvant RT (55.8 Gy)
Initially unresectable	Neoadjuvant RT (45 Gy) ± postoperative boost (10.8 Gy for R1, 19.8 Gy for R2 resection or no surgery)
EpSSG – Synovial sarcoma	
R1 resection, ≤5 cm	Adjuvant RT (50.4 Gy)
R1 resection, >5 cm, axial site or resected N1	Adjuvant RT (54 Gy)
Unresected tumor or N1	Neoadjuvant RT (50.4 – 59.4 Gy)
EpSSG – Other NRSTS	
Grade 2, R0 resection, >5 cm	Adjuvant RT (50.4 Gy)
Grade 2-3, R1 resection, ≤5 cm	Adjuvant RT (54 Gy)
Grade 3, R0 or R1 resection, >5 cm or resected N1	Adjuvant RT (54 Gy)
Unresected tumor or N1	Neoadjuvant RT (50.4 – 59.4 Gy)

EpSSG: European pediatric Soft Tissue Sarcoma Study Group, NRSTS: non-rhabdomyosarcoma soft tissue sarcomas, R0: negative resection margins, R1: microscopic tumor infiltration, RT: radiotherapy, N1: nodal metastasis

treatment approach has become the standard in pediatric NRSTS. Surgery alone is sufficient in low-risk patients, and RT or CHT may safely be omitted. On the contrary, in intermediate- and high-risk patients, adjuvant treatment including RT, CHT, or both should be applied to reduce recurrence rates. In unresectable patients, the chance of surgery increases with

the neoadjuvant treatment approach and thus treatment results may improve. However, distant metastases are an important problem even in low-risk patients. In the risk-based treatment approach, limited numbers of studies have shown that the application of smaller target volumes and lower doses compared to the conventional RT approach may be effective and promising in order to reduce treatment-related morbidities in pediatric cases. In the future, better results can be obtained with a clearer clarification of molecular features and targeted therapies in such patients.

Ethical approval

Ethics committee approval was not sought because the manuscript did not contain any patient data.

Author contribution

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

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Milgrom SA, Million L, Mandeville H, Safwat A, Ermoian RP, Terezakis S. Non-rhabdomyosarcoma soft-tissue sarcoma. Pediatr Blood Cancer 2021; 68 Suppl 2: e28279. https://doi.org/10.1002/pbc.28279
2. Sultan I, Qaddoumi I, Yaser S, Rodriguez-Galindo C, Ferrari A. Comparing adult and pediatric rhabdomyosarcoma in the surveillance, epidemiology and end results program, 1973 to 2005: an analysis of 2,600 patients. J Clin Oncol 2009; 27: 3391-3397. https://doi.org/10.1200/JCO.2008.19.7483
3. Miller RW, Young JL Jr, Novakovic B. Childhood cancer. Cancer 1995; 75: 395-405. https://doi.org/10.1002/1097-0142(19950101)75:1+<395::aid-cncr2820751321>3.0.co;2-w
4. Spunt SL, Skapek SX, Coffin CM. Pediatric nonrhabdomyosarcoma soft tissue sarcomas. Oncologist 2008; 13: 668-678. https://doi.org/10.1634/theoncologist.2007-0182
5. deCou JM, Rao BN, Parham DM, et al. Malignant peripheral nerve sheath tumors: the St. Jude Children's Research Hospital experience. Ann Surg Oncol 1995; 2: 524-529. https://doi.org/10.1007/BF02307086
6. Chang F, Syrjänen S, Syrjänen K. Implications of the p53 tumor-suppressor gene in clinical oncology. J Clin Oncol 1995; 13: 1009-1022. https://doi.org/10.1200/JCO.1995.13.4.1009
7. Fong Y, Coit DG, Woodruff JM, Brennan MF. Lymph node metastasis from soft tissue sarcoma in adults. Analysis of data from a prospective database of 1772 sarcoma patients. Ann Surg 1993; 217: 72-77. https://doi.org/10.1097/00000658-199301000-00012
8. Pappo AS, Rao BN, Jenkins JJ, et al. Metastatic nonrhabdomyosarcomatous soft-tissue sarcomas in children and adolescents: The St. Jude Children's Research Hospital experience. Med Pediatr Oncol 1999; 33: 76-82. https://doi.org/10.1002/(sici)1096-911x(199908)33:2<76::aid-mpo3>3.0.co;2-b
9. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO Classification of Soft Tissue Tumours: news and perspectives. Pathologica 2021; 113: 70-84. https://doi.org/10.32074/1591-951X-213

10. Khoury JD, Coffin CM, Spunt SL, Anderson JR, Meyer WH, Parham DM. Grading of nonrhabdomyosarcoma soft tissue sarcoma in children and adolescents: a comparison of parameters used for the Fédération Nationale des Centers de Lutte Contre le Cancer and Pediatric Oncology Group Systems. Cancer 2010; 116: 2266-2274. https://doi.org/10.1002/cncr.24929

11. Parham DM, Webber BL, Jenkins JJ 3rd, Cantor AB, Maurer HM. Nonrhabdomyosarcomatous soft tissue sarcomas of childhood: formulation of a simplified system for grading. Mod Pathol 1995; 8: 705-710.

12. Rao BN. Nonrhabdomyosarcoma in children: prognostic factors influencing survival. Semin Surg Oncol 1993; 9: 524-531. https://doi.org/10.1002/ssu.2980090611

13. Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. J Clin Oncol 1997; 15: 350-362. https://doi.org/10.1200/JCO.1997.15.1.350

14. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474. https://doi.org/10.1245/s10434-010-0985-4

15. Maurer HM, Beltangady M, Gehan EA, et al. The Intergroup Rhabdomyosarcoma Study-I. A final report. Cancer 1988; 61: 209-220. https://doi.org/10.1002/1097-0142(19880115)61:2<209::aid-cncr2820610202>3.0.co;2-l

16. Spunt SL, Poquette CA, Hurt YS, et al. Prognostic factors for children and adolescents with surgically resected nonrhabdomyosarcoma soft tissue sarcoma: an analysis of 121 patients treated at St Jude Children's Research Hospital. J Clin Oncol 1999; 17: 3697-3705. https://doi.org/10.1200/JCO.1999.17.12.3697

17. Spunt SL, Hill DA, Motosue AM, et al. Clinical features and outcome of initially unresected nonmetastatic pediatric nonrhabdomyosarcoma soft tissue sarcoma. J Clin Oncol 2002; 20: 3225-3235. https://doi.org/10.1200/JCO.2002.06.066

18. Ferrari A, Miceli R, Rey A, et al. Non-metastatic unresected paediatric non-rhabdomyosarcoma soft tissue sarcomas: results of a pooled analysis from United States and European groups. Eur J Cancer 2011; 47: 724-731. https://doi.org/10.1016/j.ejca.2010.11.013

19. Spunt SL, Million L, Chi Y-Y, et al. A risk-based treatment strategy for non-rhabdomyosarcoma soft-tissue sarcomas in patients younger than 30 years (ARST0332): a Children's Oncology Group prospective study. Lancet Oncol 2020; 21: 145-161. https://doi.org/10.1016/S1470-2045(19)30672-2

20. Ferrari A, Casanova M, Collini P, et al. Adult-type soft tissue sarcomas in pediatric-age patients: experience at the Istituto Nazionale Tumori in Milan. J Clin Oncol 2005; 23: 4021-4030. https://doi.org/10.1200/JCO.2005.02.053

21. Ferrari A, van Noesel MM, Brennan B, et al. Paediatric non-rhabdomyosarcoma soft tissue sarcomas: the prospective NRSTS 2005 study by the European Pediatric Soft Tissue Sarcoma Study Group (EpSSG). Lancet Child Adolesc Health 2021; 5: 546-558. https://doi.org/10.1016/S2352-4642(21)00159-0

22. Ferrari A, Orbach D, Sparber-Sauer M, et al. The treatment approach to pediatric non-rhabdomyosarcoma soft tissue sarcomas: a critical review from the INternational Soft Tissue SaRcoma ConsorTium. Eur J Cancer 2022; 169: 10-19. https://doi.org/10.1016/j.ejca.2022.03.028

23. Rosenberg SA, Tepper J, Glatstein E, et al. The treatment of soft-tissue sarcomas of the extremities: prospective randomized evaluations of (1) limb-sparing surgery plus radiation therapy compared with amputation and (2) the role of adjuvant chemotherapy. Ann Surg 1982; 196: 305-315. https://doi.org/10.1097/00000658-198209000-00009

24. De Corti F, Dall'Igna P, Bisogno G, et al. Sentinel node biopsy in pediatric soft tissue sarcomas of extremities. Pediatr Blood Cancer 2009; 52: 51-54. https://doi.org/10.1002/pbc.21777

25. Ferrari A, Brecht IB, Koscielniak E, et al. The role of adjuvant chemotherapy in children and adolescents with surgically resected, high-risk adult-type soft tissue sarcomas. Pediatr Blood Cancer 2005; 45: 128-134. https://doi.org/10.1002/pbc.20376

26. Pratt CB, Pappo AS, Gieser P, et al. Role of adjuvant chemotherapy in the treatment of surgically resected pediatric nonrhabdomyosarcomatous soft tissue sarcomas: A Pediatric Oncology Group Study. J Clin Oncol 1999; 17: 1219. https://doi.org/10.1200/JCO.1999.17.4.1219

27. Weiss AR, Chen Y-L, Scharschmidt TJ, et al. Pathological response in children and adults with large unresected intermediate-grade or high-grade soft tissue sarcoma receiving preoperative chemoradiotherapy with or without pazopanib (ARST1321): a multicentre, randomised, open-label, phase 2 trial. Lancet Oncol 2020; 21: 1110-1122. https://doi.org/10.1016/S1470-2045(20)30325-9

28. Marcus RB Jr. Current controversies in pediatric radiation oncology. Orthop Clin North Am 1996; 27: 551-557.

29. Venkatramani R, Xue W, Randall RL, et al. Synovial sarcoma in children, adolescents, and young adults: a report from the Children's Oncology Group ARST0332 study. J Clin Oncol 2021; 39: 3927-3937. https://doi.org/10.1200/JCO.21.01628

30. Yang JC, Chang AE, Baker AR, et al. Randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. J Clin Oncol 1998; 16: 197-203. https://doi.org/10.1200/JCO.1998.16.1.197

31. O'Sullivan B, Davis AM, Turcotte R, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet 2002; 359: 2235-2241. https://doi.org/10.1016/S0140-6736(02)09292-9

32. Davis AM, O'Sullivan B, Turcotte R, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. Radiother Oncol 2005; 75: 48-53. https://doi.org/10.1016/j.radonc.2004.12.020

33. Kraybill WG, Harris J, Spiro IJ, et al. Phase II study of neoadjuvant chemotherapy and radiation therapy in the management of high-risk, high-grade, soft tissue sarcomas of the extremities and body wall: Radiation Therapy Oncology Group Trial 9514. J Clin Oncol 2006; 24: 619-625. https://doi.org/10.1200/JCO.2005.02.5577

34. Rivard JD, Puloski SS, Temple WJ, et al. Quality of life, functional outcomes, and wound complications in patients with soft tissue sarcomas treated with preoperative chemoradiation: a prospective study. Ann Surg Oncol 2015; 22: 2869-2875. https://doi.org/10.1245/s10434-015-4490-7

35. Alektiar KM, Brennan MF, Healey JH, Singer S. Impact of intensity-modulated radiation therapy on local control in primary soft-tissue sarcoma of the extremity. J Clin Oncol 2008; 26: 3440-3444. https://doi.org/10.1200/JCO.2008.16.6249

36. Folkert MR, Singer S, Brennan MF, et al. Comparison of local recurrence with conventional and intensity-modulated radiation therapy for primary soft-tissue sarcomas of the extremity. J Clin Oncol 2014; 32: 3236-3241. https://doi.org/10.1200/JCO.2013.53.9452

37. Rao AD, Chen Q, Million L, et al. Preoperative intensity modulated radiation therapy compared to three-dimensional conformal radiation therapy for high-grade extremity sarcomas in children: analysis of the Children's Oncology Group Study ARST0332. Int J Radiat Oncol Biol Phys 2019; 103: 38-44. https://doi.org/10.1016/j.ijrobp.2018.09.005

38. Lin C, Donaldson SS, Meza JL, et al. Effect of radiotherapy techniques (IMRT vs. 3D-CRT) on outcome in patients with intermediate-risk rhabdomyosarcoma enrolled in COG D9803-a report from the Children's Oncology Group. Int J Radiat Oncol Biol Phys 2012; 82: 1764-1770. https://doi.org/10.1016/j.ijrobp.2011.01.036

39. Sterzing F, Stoiber EM, Nill S, et al. Intensity modulated radiotherapy (IMRT) in the treatment of children and adolescents-a single institution's experience and a review of the literature. Radiat Oncol 2009; 4: 37. https://doi.org/10.1186/1748-717X-4-37

40. Wang D, Zhang Q, Eisenberg BL, et al. Significant reduction of late toxicities in patients with extremity sarcoma treated with image-guided radiation therapy to a reduced target volume: results of Radiation Therapy Oncology Group RTOG-0630 Trial. J Clin Oncol 2015; 33: 2231-2238. https://doi.org/10.1200/JCO.2014.58.5828

41. Childs SK, Kozak KR, Friedmann AM, et al. Proton radiotherapy for parameningeal rhabdomyosarcoma: clinical outcomes and late effects. Int J Radiat Oncol Biol Phys 2012; 82: 635-642. https://doi.org/10.1016/j.ijrobp.2010.11.048

42. Rombi B, DeLaney TF, MacDonald SM, et al. Proton radiotherapy for pediatric Ewing's sarcoma: initial clinical outcomes. Int J Radiat Oncol Biol Phys 2012; 82: 1142-1148. https://doi.org/10.1016/j.ijrobp.2011.03.038

43. Leroy R, Benahmed N, Hulstaert F, Van Damme N, De Ruysscher D. Proton therapy in children: a systematic review of clinical effectiveness in 15 pediatric cancers. Int J Radiat Oncol Biol Phys 2016; 95: 267-278. https://doi.org/10.1016/j.ijrobp.2015.10.025

44. Oertel S, Niethammer AG, Krempien R, et al. Combination of external-beam radiotherapy with intraoperative electron-beam therapy is effective in incompletely resected pediatric malignancies. Int J Radiat Oncol Biol Phys 2006; 64: 235-241. https://doi.org/10.1016/j.ijrobp.2005.06.038

45. Stauder MC, Laack NNI, Moir CR, Schomberg PJ. Excellent local control and survival after intraoperative and external beam radiotherapy for pediatric solid tumors: long-term follow-up of the Mayo Clinic experience. J Pediatr Hematol Oncol 2011; 33: 350-355. https://doi.org/10.1097/MPH.0b013e3182148dad

46. Pisters PW, Harrison LB, Leung DH, Woodruff JM, Casper ES, Brennan MF. Long-term results of a prospective randomized trial of adjuvant brachytherapy in soft tissue sarcoma. J Clin Oncol 1996; 14: 859-868. https://doi.org/10.1200/JCO.1996.14.3.859

47. Krasin MJ, Davidoff AM, Xiong X, et al. Preliminary results from a prospective study using limited margin radiotherapy in pediatric and young adult patients with high-grade nonrhabdomyosarcoma soft-tissue sarcoma. Int J Radiat Oncol Biol Phys 2010; 76: 874-878. https://doi.org/10.1016/j.ijrobp.2009.02.074

48. Holloway CL, Delaney TF, Alektiar KM, Devlin PM, O'Farrell DA, Demanes DJ. American Brachytherapy Society (ABS) consensus statement for sarcoma brachytherapy. Brachytherapy 2013; 12: 179-190. https://doi.org/10.1016/j.brachy.2012.12.002

49. Folkert MR, Tong WY, LaQuaglia MP, et al. 20-year experience with intraoperative high-dose-rate brachytherapy for pediatric sarcoma: outcomes, toxicity, and practice recommendations. Int J Radiat Oncol Biol Phys 2014; 90: 362-368. https://doi.org/10.1016/j.ijrobp.2014.06.016

50. Al Yami A, Griffin AM, Ferguson PC, et al. Positive surgical margins in soft tissue sarcoma treated with preoperative radiation: is a postoperative boost necessary? Int J Radiat Oncol Biol Phys 2010; 77: 1191-1197. https://doi.org/10.1016/j.ijrobp.2009.06.074

The Turkish Journal of Pediatrics 2023; 65: 377-386
https://doi.org/10.24953/turkjped.2022.1021

Original Article

Short-term outcomes of extremely low birth weight infants in a tertiary neonatal intensive care unit in Türkiye

Sumru Kavurt[1], Ahmet Yağmur Baş[2], Fatih İşleyen[1], Mehtap Durukan Tosun[1], Dilek Ulubaş Işık[1], Nihal Demirel[2]

[1]Department of Neonatology, Etlik Zubeyde Hanım Women's Health Training and Research Hospital, University of Health Sciences, Ankara; [2]Department of Neonatology, Ankara Yıldırım Beyazıt University, Ankara, Türkiye.

ABSTRACT

Background. Advances in neonatal care have led to increased survival of extremely preterm infants. Extremely low-birth-weight (ELBW) infants, defined as infants weighing less than 1000 g at birth, constitute a significant portion of neonatal intensive care unit (NICU) patients. The aim of this study is to determine the mortality and short-term morbidities of ELBW infants and assess the risk factors related to mortality.

Methods. The medical records of ELBW neonates hospitalized in the NICU of a tertiary-level hospital between January 2017 and December 2021 were evaluated retrospectively.

Results. 616 ELBW (289 females and 327 males) infants were admitted to the NICU during the study period. Mean birth weight (BW) and gestational age (GA) for the total cohort were 725 ± 134 g (range 420-980 g) and 26.3 ± 2.1 weeks (range 22-31), respectively. The rate of survival to discharge was 54.5% (336/616) [33% for the infants with ≤750 g BW, 76% for the infants with 750-1000 g BW], and 45.2% of survived infants had no major neonatal morbidity at discharge. Independent risk factors for mortality of ELBW infants were asphyxia at birth, birth weight, respiratory distress syndrome, pulmonary hemorrhage, severe intraventricular hemorrhage, and meningitis.

Conclusions. The incidence of mortality and morbidity was very high in ELBW infants, particularly for neonates born weighing less than 750 g in our study. We suggest that preventive and more effective treatment strategies are needed for improved outcomes in ELBW infants.

Key words: extremely low birth weight infant, survival, morbidity.

The improved survival of preterm infants due to recent advances in perinatal and neonatal care has produced a new population of infants at very high risk of developing neonatal mortality and morbidities. In current practice, extremely low-birth-weight (ELBW) infants, defined as infants weighing less than 1000g at birth, constitute a significant portion of patients in neonatal intensive care units (NICUs).[1-3] Gestational age is a significant predictor for the survival of ELBW infants. Extremely low-birth-weight infants constitute the highest risk group for mortality and morbidity. Being small for gestational age (SGA) with more advanced gestation represents different pathophysiologic processes. So, this distinction by gestational age is important.[4,5] Despite our knowledge and recognizing the vulnerability of these infants, care of ELBW newborns is challenging, they remain at high risk for mortality and major morbidities. As the survival of ELBW infants is increasing worldwide, mortality and neonatal morbidities of these infants need to be reported. Monitoring the outcomes in the NICU could guide the development of programs to improve preterm care. In this study, we aimed to evaluate the mortality and short-term morbidities of ELBW infants and assess the risk factors related to mortality.

✉ Sumru Kavurt
sumrukavurt@gmail.com

Received 29th November 2022, revised 24th January 2023, accepted 1st February 2023.

Material and Metods

Study Design and Subjects

This retrospective study was performed at Etlik Zubeyde Hanım Women's Health Training and Research Hospital. The medical records of ELBW infants hospitalized in the NICU between January 2017 and December 2021 were reviewed. The institutional ethics committee approved this study (17.06.22-08/24). Etlik Zubeyde Hanım Women's Health Training and Research Hospital is a reference center for high-risk pregnancies and preterm births and has one of the largest NICUs in Türkiye with 79 level III beds. An average of 350 preterm infants with birth weight (BW) <1500 g and GA <32 weeks are hospitalized annually.

Clinical Data and Definitions

Perinatal and neonatal characteristics of the infants and outcomes at discharge were collected from the medical records. The data recorded for each neonate included GA, BW, gender, mode of delivery, 5-minute Apgar score, singleton or multiple gestations, being SGA and antenatal steroid administration (two doses of 12 mg betamethasone given intramuscularly 24 hours apart before delivery). Clinical data of mothers including maternal age, preterm prelabor rupture of membranes (PPROM), chorioamnionitis (clinical or histopathological), gestational diabetes, preeclampsia, eclampsia, and maternal systemic diseases were recorded.

During the neonatal follow-up, respiratory distress syndrome (RDS), surfactant treatment, duration of respiratory support (noninvasive/invasive mechanical ventilation, and supplemental oxygen), duration of parenteral nutrition, and presence of complications related to prematurity; hemodynamically significant patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH) (according to Papile criteria)[6], late-onset sepsis, retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC) (≥ stage 2 NEC according to modified Bell staging criteria)[7], bronchopulmonary dysplasia (BPD) (oxygen requirement at 36th postmenstrual age)[8] and osteopenia of prematurity (OP) were recorded. Retinopathy of prematurity was defined according to Early Treatment for Retinopathy of Prematurity Cooperative Group criteria.[9] Osteopenia of prematurity was defined as hypophosphatemia (phosphorus levels <4 mg/dl), ALP levels >450 U/L, or signs of decreased mineralization in the x-ray of the forearm at four weeks of age.

Gestational age was calculated according to the date of the maternal last menstrual period, early pregnancy ultrasound examination findings, or the New Ballard score. SGA was defined as BW below the 10th percentile for their GA according to Fenton's growth curve.[10]

Outcome Measures

The primary outcome measure was the survival rate until discharge. Secondary outcome measures were major short-term morbidities of preterm birth. The major morbidities were defined as late-onset sepsis, severe IVH (>grade II IVH according to Papile criteria), NEC (≥ stage 2, according to the modified Bell's criteria), severe ROP requiring treatment, and BPD.

Statistical analysis

Statistical analyses were conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL). Categorical data are presented as numbers (n) and percentages (%). The Chi-square test was used to compare categorical variables. Variables were tested for normality using the Shapiro-Wilk test. The results for variables with normal distribution were reported as mean ± SD, while the non-normally distributed parameters were reported as median (interquartile range [IQR]). Mann-Whitney U test or independent samples t-test were used to compare the numerical variables, where appropriate. Statistical significance was accepted if the p-value ≤ 0.05.

Probable risk factors for mortality of ELBW infants were evaluated. The multivariate analysis of logistic regression was performed with mortality as a dependent variable and

clinical outcomes as independent variables. The risk factor with a p-value <0.1 in the univariate analysis was included in the multivariate analysis. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. Odds ratios and 95% confidence intervals for each risk factor were determined.

Results

During the study period 1549 preterm infants with GA <32 weeks, 616 of whom were ELBW, were admitted to the NICU. Medical records of 616 ELBW (289 females and 327 males) infants were reviewed. Mean BW and GA for the total cohort were 725 ± 134 g (range 420-980g) and 26.3 ± 2.1 weeks (range 22-31 weeks), respectively. The ratio of extremely preterm infants (≤28 weeks GA) was 85%. Of the total cohort 26 (3.4%) preterm infants were <500 g BW and ≤23 weeks GA. A total of 129 (21%) infants were SGA. The rate of resuscitation at the delivery room was 70.4%, and the median Apgar scores at 5 minutes were 6.

The most common perinatal complication was preeclampsia (29%), the others were; PPROM in 160 (26%), chorioamnionitis in 70 (11.4%), and gestational diabetes in 30 (5%) cases, respectively. The rate of cesarean section was 71%. When maternal characteristics were considered the mean maternal age was 29±5 years. Complete antenatal corticosteroid administration was performed in 42% of the mothers. Neonatal and maternal characteristics of the total cohort are detailed in Table I.

The rate of survival to discharge for the total cohort was 54.5% (336/616). None of the infants with <500 g BW and <23 weeks GA survived to discharge. The survival rate of infants with ≤750 g BW was 33%, whereas it increased to 76% with 751-1000 g BW. The same trend was observed in extremely preterm infants, where the survival rate was 21.2% (24/113) at 22-24 weeks. The survival rate of ELBW infants improved to 44% (84/192) after 24 weeks, and 78% (73/94) 28-32 weeks. The rate of survival to discharge without any major morbidity was 45%. Survival rates of infants by BW and GA are given in Table II.

Table I. Demographic characteristics of infants.

Infant characteristics	n= 616
Birth weight (g), mean ± SD	724.91 ± 133.65
≤ 500, n (%)	21 (3.4%)
501- 750, n (%)	270 (43.8%)
751-1000, n (%)	325 (52.7%)
Gestational age (weeks)*	26.31 ± 2.11
≤28 weeks, n (%)	
22-24	113 (18.3%)
25-26	192 (31.1%)
27-28	217 (35.2%)
>28 weeks, n (%)	94 (15.2%)
F/M	289/327
SGA, n (%)	129 (21%)
Multiple pregnancy, n (%)	
Twin	64 (10.3%)
Triplet	2 (1.9%)
Resuscitation, n (%)	441 (71.5%)
Apgar score, median (IQR)	6 (5-7)
Maternal characteristics	
Age yr, mean ± SD	29.4 ± 5.4
Cesarean section, n (%)	437 (71%)
IVF, n (%)	72 (11.8%)
Antenatal corticosteroid, n (%)	
Complete	259 (42%)
Incomplete	217 (35.2%)
None	140 (22.8%)
Preeclampsia, n (%)	178 (29%)
PPROM, n (%)	160 (26%)
Chorioamnionitis, n (%)	70 (11.4%)
Gestational diabetes, n (%)	30 (4.9%)

F/M: Female/Male, SGA: Small for gestation al age, IVF: In vitro fertilization, IQR: interquartile range, PPROM: Premature prelabor rupture of membranes.

Clinical outcomes of ELBW infants during hospitalization are given in Table III. Respiratory distress syndrome requiring surfactant therapy was present in 77% of the infants, and pulmonary hemorrhage occurred in 7.7% of them. Hemodynamically significant PDA requiring treatment was detected in 262 (51%) infants. Twelve of them (7 infants with BW 500-750 g, 5 infants with BW 751-1000 g)

Table II. Survival rates by birth weight and gestational age.

		Survival	Survival without morbidity*
	Overall	336/616 (54.5%)	151/336 (45.2%)
Birth weight (g)	≤ 500	0/21 (0)	-
	501- 750	89/270 (32.9%)	24/89 (26.9%)
	751-1000	247/325 (76%)	127/247 (51.4%)
Gestational age (weeks)	22-24	24/113 (21.2%)	4/24 (16.6%)
	25-26	84/192 (43.7%)	24/84 (28.2%)
	27-28	155/217 (71.4%)	70/155(45.1%)
	>28	73/94 (77.6%)	53/73 (72.6%)

*Rates of survival to discharge without major morbidity (severe intraventricular hemorrhage, bronchopulmonary dysplasia, necrotizing enterocolitis ≥ stage II, retinopathy of prematurity requiring treatment, culture proven sepsis) among infants who survived to discharge

Table III. Morbidities and mortality during hospitalization (Presented as number and percentages unless indicated otherwise).

	BW <500 g (n=21)	BW 501- 750 g (n=270)	BW 751-1000 g (n=325)	Overall (n=616)
RDS	21 (100%)	242 (89.8%)	265 (81.7%)	528 (85.7%)
Surfactant treatment	21 (100%)	224 (83%)	230 (70.7%)	475 (77.1%)
Pulmonary hemorrhage	3 (9.5%)	22 (8.1%)	9 (2.7%)	34 (5.5%)
Intraventricular hemorrhage*				
Grade 2	-	38 (18.1%)	28 (9.4%)	66 (31.5%)
Grade ≥ 3	4	26 (12.4%)	17 (5.7%)	43 (14.5%)
PDA requiring treatment**	5 (62.5%)	104 (50.2%)	156 (51.3%)	265 (50.7%)
Late-onset sepsis**	2 (50%)	63 (23.3%)	83(25.5%)	148 (24%)
Culture proven	-	52 (19.2%)	73 (22.4%)	125 (20.2%)
Meningitis	-	18 (9.6%)	21 (7%)	39 (7.9%)
NEC (≥ stage II)	-	14 (7.4%)	9 (3%)	23 (4.6%)
ROP ¥				
Any stage	-	54 (56.2%)	106 (41.5%)	160 (45.5%)
Severe ROP		19 (19.7%)	28 (10.9%)	47 (13.3%)
BPD Φ	-	51 (53.1%)	112 (43.9%)	163 (46.4%)
Osteopenia of prematurity ¥	-	47 (48.9%)	119 (46.6%)	166 (47.2%)
Duration of hospitalization, days, mean ± SD ¥		111 ± 33.95	82.95 ± 29.12	97.20±48.42
Postnatal time until death, days, PDA: patent ductus arteriosus				
<1	10/21	44/181	11/78	65/280
1-3	5/21	39/181	14/78	58/280
4-7	4/21	45/181	22/78	71/280
8-28	2/21	46/181	23/78	71/280
>28	0/21	7/181	8/78	15/280

BPD: bronchopulmonary dysplasia, BW: birth weight, NEC: necrotizing enterocolitis, RDS: respiratory distress syndrome, ROP: retinopathy of prematurity.
* Data available for 511 patients.
** Data available for 522 patients.
¥ Represents data among survivors at 28 days of life .
Φ Represents data among survivors at PMA of 36 weeks.

were referred for surgical closure. In general, the incidence of severe IVH was 7.6%, whereas it increased to 10.3% in infants with ≤ 750 g BW. A total of 166 neonates [67 (36%) with 500-750 g BW, 98 (33%) with 751-1000 g BW] developed culture-proven sepsis, and 39 (8%) of them had meningitis. Advanced NEC (stage 2 to 3) occurred in 23 infants [14 (7.4%) with 500-750 g BW, 9 (3%) with 751-1000 g BW]. Osteopenia of prematurity, ROP, and BPD were evaluated in infants who survived more than 28 days. Osteopenia of prematurity developed in 166 (42%) infants, 47 (49%) of them had 500-750 g BW. Any stage ROP was detected in 160 (46%) infants, in which 47(13%) of them had severe ROP requiring treatment. BPD developed in 163 (46%) infants. Postnatal corticosteroids were given to 56 infants for BPD. Two neonates with 500-750 g BW required tracheostomy for severe BPD. The mean duration of hospitalization of survived infants was 97.2±48.4 days. When all deaths were considered 65 (23%) deaths occurred within the first day of life and 194 (69%) occurred in the first week. Nearly half of the neonates with <500 g BW (48%) died within the first day of life.

Treatment methods during hospitalization are given in Table IV. The rate of resuscitation at the delivery room was 70.4%, and 235 (38%) infants required intubation. Noninvasive ventilation was the most common initial modality of respiratory support (n=381, 62%) in this cohort. The mean duration of invasive and noninvasive mechanical ventilation among survivors was 7.4±12.1 days and 23 ±13 days, respectively.

Gestational age, hemodynamically significant PDA, presence of culture-proven sepsis, meningitis, NEC (≥ stage 2, according to the modified Bell's criteria), RDS, pulmonary hemorrhage, asphyxia at birth, multiple pregnancies, gender, and severe IVH were put in the regression model. We found that asphyxia at birth, RDS, pulmonary hemorrhage, severe IVH, NEC (≥ stage 2, according to the modified Bell's criteria), and meningitis were independent risk factors for mortality among ELBW infants. Being born with ≤ 750 g BW was associated with a 5.7-fold increased risk for mortality (Table V).

Discussion

This report provided detailed documentation of short-term outcomes of ELBW infants in a tertiary NICU for 5 years. Although ELBW infants with extreme prematurity account for 5% of preterm births, they are at increased risk of mortality and morbidity. Management of ELBW infants, typically born at 27 weeks gestation or younger has great challenges for neonatologists.

Table IV. Treatment methods during hospitalization.

	BW 501- 750 g n=89	BW 751-1000 g n=247	Overall n=336
Duration of invasive MV* (days)	15.16 ±18.56	5.21±8.36	7.41 ±12.11
Duration of non-invasive MV* (days)	29.06 ±15.69	21.34 ±11.60	23.05 ±12.99
Duration of oxygen therapy* (days)	28.63 ±17.42	22.81±15.77	24.10 ±16.29
Duration of TPN* (days)	16.25±5.74	13.90±3.96	14.42 ± 4.51
Postnatal corticosteroids for BPD*, n (%)	22 (24.7%)	34 (13.7%)	56 (16.6%)
Tracheostomy for severe BPD*, n (%)	2 (2.2%)	0	2 (0.5%)
Surgical closure of PDA*, n (%)	7 (7.8%)	5 (2%)	12 (3.5%)
Laser therapy for ROP*, n (%)	13 (14.6%)	18 (7.2%)	31 (9.2%)

BPD: bronchopulmonary dysplasia, BW: birth weight, MV: mechanical ventilation, PDA: patent ductus arteriosus, ROP: retinopathy of prematurity, TPN: total parenteral nutrition.
*Represents data among survivors

Table V. Binary logistic regression analysis to identify independent risk factors for mortality.

	Odds ratio	95% CI	p value
Asphyxia at birth	2.03	1.264-3.276	0.003
Pulmonary hemorrhage	6.53	2.113-20.209	0.001
RDS	2.99	1.607- 5.576	0.001
NEC	2.68	0.904-7.947	0.075
Severe IVH	2.40	1.154-5.002	0.019
Meningitis	0.40	0.177-0.937	0.035
Birth weight*	5.70	3.898-8.349	0.000

CI: confidence interval, IVH: intraventricular hemorrhage, NEC: necrotizing enterocolitis (≥ stage II), RDS: respiratory distress syndrome.
*Odds ratio for birth weight <750 g

Developed centers reported increased survival rates for neonates born at 23-24 GA as survival to discharge was 33% at 23 weeks of GA and 65% at 24 weeks of GA[11] whereas significant variable rates of survival are observed in resource limited centers.[12,13] Trotman from a University Hospital of the West Indies reported the survival rate as 7% for the neonates with <27 weeks of GA.[13]

Our results indicated that the incidence of mortality and morbidity is still very high in these vulnerable infants. In the present study, the overall rate of survival to discharge was 54.5 % in ELBW infants with improved survival in higher birth weights. The rate of survival for infants with ≤750 g BW was 33%, whereas it increased to 76% for infants born weighing 751-1000 g. The short-term outcomes of very low birth weight (VLBW) infants have been reported by different NICUs in Türkiye[14,15], however, there is no recent documented data regarding the outcomes of ELBW infants. Turkish Neonatal Society publishes the mortality rates of preterm infants annually based on the data of the involved NICUs. In the last report, the mortality rate was 50.7% for preterm infants with 500-749 g BW in 2020.[16] The survival rate of ELBW infants in our cohort is less than that of developed centers where the survival rate was 33% for infants born at 23 weeks in the US.[11] Also, recent data from the UK reported improved survival for preterm infants, particularly at the lowest gestations. The rates of survival to discharge of infants born at 22 GA, 23 GA, 24 GA, and 25 GA were 17.9%, 35.9%, 58.6%, and 74%, respectively.[17] There is an important retrospective cohort study from our hospital analyzing the survival rates of periviable infants. In that cohort, the rate of survival to discharge was found to be 7.5% at 23 weeks, 29 % at 24 weeks, and 43.5% at 25 weeks.[18] Similarly in this report, survival rates for the infants born at 22-24 weeks of gestation remained at 21% most likely as an indication of the biological limit of viability.

According to the legal regulations in our country <20 weeks GA is considered abortion however performing resuscitation is proposed to each baby with any sign of vitality regardless of gestational age.[19] Twenty-one neonates with <500 g BW and <23 weeks GA were admitted to the NICU during this study period however, none of them survived to discharge. Nearly half of the infants at the limits of viability died within the first day of life and almost all of those remaining had died by the end of the first week. Likewise, according to the results of EPIPAGE 2 the chance of survival of neonates below 24 weeks did not change between the two study periods, none of the infants born alive at 22- 23 weeks survived to discharge.[20]

Perinatal characteristics such as older GA, higher BW, female gender, singleton birth, and antenatal steroid administration are known predictors for the survival of ELBW infants. Although in this study, ELBW infants who died in the NICU were found to be more premature,

singleton and male, have lower GA, and lower rates of antenatal corticosteroid administration. Only low BW, multiple births, and asphyxia at birth were found to be independent risk factors for mortality. According to our results being born with ≤ 750 g BW was associated with 5.7 times increased risk for mortality. When it was evaluated in terms of morbidities related to mortality in the NICU; RDS, pulmonary hemorrhage, severe IVH, advanced NEC, and meningitis were independent risk factors for mortality of ELBW infants. In a large cohort from China being SGA, being male, multiple births, low Apgar score, and being born to a mother with gestational diabetes were associated with a decreased chance of survival.[21]

As the survival of ELBW neonates increased, concerns have been raised regarding whether the same improvement was observed in morbidities. In this study severe IVH, BPD, advanced NEC, severe ROP requiring treatment and culture-proven sepsis were considered as major morbidities. We showed that 45.2% of the survived neonates had no major morbidity; the rate of survival without major morbidity was 27% in babies ≤750 g BW where it raised to 51% for the babies 751-1000 g BW. The same trend was observed when it was evaluated according to GA. The rate of survival without major morbidity was 28% for infants at 25-26 weeks GA, 45% at 27-28 weeks GA, and 73% between 28-32 weeks GA in this study. These rates are slightly lower than those in developed countries. According to the NICHD network data, 20% of babies at 25 weeks GA, 34% of babies at 26 weeks GA, and 44% of babies at 27 weeks GA had no morbidity at discharge.[22]

Intraventricular hemorrhage is the most common acute central nervous system complication of a preterm birth. The risk of IVH increases with decreasing GA and BW. In this study, the incidence of severe IVH was 12.4% in neonates ≤750 g BW, and 5.7% in neonates between 750-1000 g BW. The data of NICHD Neonatal Research reported an increased prevalence of severe IVH with rates of 38% and 36% for infants with 22 and 23 GA and 11% and 7% for infants with 27 and 28 GA, respectively.[22] Prematurity is the most closely related clinical condition to IVH. Other risk factors associated with the risk of IVH include lack of antenatal glucocorticoid therapy, neonatal transport, prolonged neonatal resuscitation, and respiratory distress requiring mechanical ventilation.[23] In this cohort, 61.2% of neonates with severe IVH had no antenatal glucocorticoid administration.

Sepsis is an important cause of morbidity and mortality among preterm neonates with increasing rates at low GA and BW. The estimated rate of culture-proven sepsis was reported as 43% among infants with ≤750 g BW and 28% among neonates with 750-1000 g BW.[11] In this study, late-onset sepsis was diagnosed in 24% of ELBW neonates, and the rate of culture-proven sepsis was 20.2%, which is comparable with developed centers.

Necrotizing enterocolitis (NEC) occurs in 2 to 10 percent of VLBW infants.[24] In accordance with the literature we found the overall incidence of advanced NEC as 4.6% in ELBW infants and 7.4% for the neonates with <750 g BW. Appropriate enteral feeding protocol and the high rate of breastfeeding in our unit are important factors to reduce NEC in preterm infants.

Retinopathy of prematurity affects a considerable number of preterm infants worldwide. The increased incidence and severity of ROP with decreasing GA have been demonstrated in previous studies.[25,26] In a multicenter study from the US, the incidence of ROP in preterm infants with <1251 g BW was 68%.[25] The overall incidence of severe ROP among infants born <32 weeks GA was reported as 10 % in a population-based cohort study from New Zealand and Australia, severe ROP increased from 3 to 34 % as GA decreased from 27 to 24 weeks, respectively.[26] A recent study from Türkiye (TR-ROP study) reported the incidence of any stage of ROP as 27% and severe ROP as 6.7%.[27] In our study any stage ROP was detected in 45.5% of ELBW infants, 13.3% of them were severe ROP requiring treatment. The

most important risk factor for developing ROP is prematurity however nearly all the morbidities in the NICU are thought to contribute. Better neonatal care and all preventive strategies to prevent neonatal morbidities may reduce the prevalence of ROP in preterm infants.

The rate of BPD varies among institutions, approximately a rate of 40% is reported for extremely preterm infants besides infants with <1250 g BW account for 97% of the cases of BPD.[28,29] In this study, the rate of BPD was 46.4% for the total cohort and 53% for infants with ≤750 g BW.

Pulmonary hemorrhage occurs most commonly in extremely preterm infants and it is associated with increased mortality. In a large cohort from the US, the incidence of pulmonary hemorrhage was reported as 9% among infants born at 24 weeks gestation with a higher mortality rate (41%) at seven days of age.[30] In our cohort, pulmonary hemorrhage occurred in 34 (5.5%) neonates with a peak incidence of 8% for infants with 500-750 g BW. In this study, we showed that pulmonary hemorrhage was an independent risk factor for the mortality of ELBW infants.

Major complications in NICUs have been associated with neurodevelopmental impairments in neonates. So, monitorization of outcomes of preterm infants is an essential component of patient management in the NICU. Defining patient characteristics and outcomes help achieve proper clinical management and policy making. Currently, the care of ELBW infants occupies an important part of duties in the NICU. Given the paucity of data regarding the outcomes of ELBW infants in Türkiye, we conducted a cohort study focusing on morbidities in ELBW infants. We are aware that our study has some limitations. First, this study is from a single center and the results may not reflect the other centers in our country. The retrospective nature and lack of long-term outcomes are the other limitations.

Nevertheless, the results of this study suggest that ELBW infants, particularly those with BW ≤ 750 g are at high risk of mortality. Major morbidities related to prematurity such as asphyxia at birth, RDS, pulmonary hemorrhage, and severe IVH are strongly related to mortality.

In conclusion, the short and long-term prognosis for preterm infants born weighing less than 750 g remain less favorable. Management of these infants requires high medical and nursing standards. General and institutional guidelines and more effective perinatal treatment strategies are needed to improve the outcomes of these neonates. Multicenter trials are necessary to define national data regarding the mortality and morbidity of ELBW infants.

Ethical approval

The institutional ethics committee at Etlik Zübeyde Hanım Women's Health Teaching and Research Hospital approved this study (17.06.22-08/24).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SK, AYB; data collection: SK, Fİ, MT, DUI; analysis and interpretation of results: SK, DUI; draft manuscript preparation: SK, AYB, ND. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Iams JD, Romero R, Culhane JF, Goldenberg RL. Primary, secondary, and tertiary interventions to reduce the morbidity and mortality of preterm birth. Lancet 2008; 371: 164-175. https://doi.org/10.1016/S0140-6736(08)60108-7
2. Bode MM, D'Eugenio DB, Forsyth N, Coleman J, Gross CR, Gross SJ. Outcome of extreme prematurity: a prospective comparison of 2 regional cohorts born 20 years apart. Pediatrics 2009; 124: 866-874. https://doi.org/10.1542/peds.2008-1669
3. Fanaroff A, Poole K, Duara S, et al. Micronates: 401-500 grams: the NICHD neonatal research network experience 1996-2001. Pediatr Res 2003; 53: 398A.
4. Doyle LW. Evaluation of neonatal intensive care for extremely-low-birth-weight infants. Semin Fetal Neonatal Med 2006; 11: 139-145. https://doi.org/10.1016/j.siny.2005.11.009
5. Bardin C, Zelkowitz P, Papageorgiou A. Outcome of small-for-gestational age and appropriate-for-gestational age infants born before 27 weeks of gestation. Pediatrics 1997; 100: E4. https://doi.org/10.1542/peds.100.2.e4
6. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr 1978; 92: 529-534. https://doi.org/10.1016/s0022-3476(78)80282-0
7. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986; 33: 179-201. https://doi.org/10.1016/s0031-3955(16)34975-6
8. Jobe AH. The new bronchopulmonary dysplasia. Curr Opin Pediatr 2011; 23: 167-172. https://doi.org/10.1097/MOP.0b013e3283423e6b
9. Early Treatment for Retinopathy of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. Arch Ophthalmol 2003; 121: 1684-1694. https://doi.org/10.1001/archopht.121.12.1684
10. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr 2013; 13: 59. https://doi.org/10.1186/1471-2431-13-59
11. Stoll BJ, Hansen NI, Bell EF, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993-2012. JAMA 2015; 314: 1039-1051. https://doi.org/10.1001/jama.2015.10244
12. Kalimba EM, Ballot D. Survival of extremely low-birth-weight infants. S Afr J CH 2013; 7: 13-16. https://doi.org/10.7196/sajch.488
13. Trotman H, Lord C. Outcome of extremely low birthweight infants at the University Hospital of the West Indies, Jamaica. West Indian Med J 2007; 56: 409-413.
14. Atasay B, Günlemez A, Unal S, Arsan S. Outcomes of very low birth weight infants in a newborn tertiary center in Turkey, 1997-2000. Turk J Pediatr 2003; 45: 283-289.
15. Güran O, Bülbül A, Uslu S, Dursun M, Zubarioğlu U, Nuhoğlu A. The change of morbidity and mortality rates in very low birth weight infants over time. Turk Arch Ped 2013; 48: 102-109. https://doi.org/10.4274/tpa.1062
16. Turkish Neonatal Society. Türkiye'deki yenidoğan merkezlerinde mortalite. Türk Neonatoloji Derneği Bülteni 2021; 33: 46-48.
17. Santhakumaran S, Statnikov Y, Gray D, et al. Survival of very preterm infants admitted to neonatal care in England 2008-2014: time trends and regional variation. Arch Dis Child Fetal Neonatal Ed 2018; 103: F208-F215. https://doi.org/10.1136/archdischild-2017-312748
18. Kulali F, Bas AY, Erol S, et al. Survival of periviable infants: 5-year experience at a single center. J Matern Fetal Neonatal Med 2020; 33: 3725-3731. https://doi.org/10.1080/14767058.2019.1583734
19. Oygür N, Önal EE, Zenciroğlu A. National guidelines for delivery room management. Turk Pediatri Ars 2018; 53: S3-S17. https://doi.org/10.5152/TurkPediatriArs.2018.01803
20. Ancel PY, Goffinet F; EPIPAGE-2 Writing Group, et al. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. JAMA Pediatr 2015; 169: 230-238. https://doi.org/10.1001/jamapediatrics.2014.3351
21. Zhu Z, Yuan L, Wang J, et al. Mortality and morbidity of infants born extremely preterm at tertiary medical centers in China from 2010 to 2019. JAMA Netw Open 2021; 4: e219382. https://doi.org/10.1001/jamanetworkopen.2021.9382
22. Stoll BJ, Hansen NI, Bell EF, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 2010; 126: 443-456. https://doi.org/10.1542/peds.2009-2959

23. Kenet G, Kuperman AA, Strauss T, Brenner B. Neonatal IVH-mechanisms and management. Thromb Res 2011; 127 Suppl 3: S120-2. https://doi.org/10.1016/S0049-3848(11)70032-9

24. Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. Arch Dis Child Fetal Neonatal Ed 2018; 103: F182-F189. https://doi.org/10.1136/archdischild-2017-313880

25. Good WV, Hardy RJ, Dobson V, et al. The incidence and course of retinopathy of prematurity: findings from the early treatment for retinopathy of prematurity study. Pediatrics 2005; 116: 15-23. https://doi.org/10.1542/peds.2004-1413

26. Darlow BA, Hutchinson JL, Henderson-Smart DJ, et al. Prenatal risk factors for severe retinopathy of prematurity among very preterm infants of the Australian and New Zealand Neonatal Network. Pediatrics 2005; 115: 990-996. https://doi.org/10.1542/peds.2004-1309

27. Bas AY, Demirel N, Koc E, et al. Incidence, risk factors and severity of retinopathy of prematurity in Turkey (TR-ROP study): a prospective, multicentre study in 69 neonatal intensive care units. Br J Ophthalmol 2018; 102: 1711-1716. https://doi.org/10.1136/bjophthalmol-2017-311789

28. Poindexter BB, Feng R, Schmidt B, et al. Comparisons and limitations of current definitions of bronchopulmonary dysplasia for the prematurity and respiratory outcomes program. Ann Am Thorac Soc 2015; 12: 1822-1830. https://doi.org/10.1513/AnnalsATS.201504-218OC

29. Walsh MC, Szefler S, Davis J, et al. Summary proceedings from the bronchopulmonary dysplasia group. Pediatrics 2006; 117: S52-S56. https://doi.org/10.1542/peds.2005-0620I

30. Ahmad KA, Bennett MM, Ahmad SF, Clark RH, Tolia VN. Morbidity and mortality with early pulmonary haemorrhage in preterm neonates. Arch Dis Child Fetal Neonatal Ed 2019; 104: F63-F68. https://doi.org/10.1136/archdischild-2017-314172

The Turkish Journal of Pediatrics 2023; 65: 387-397
https://doi.org/10.24953/turkjped.2022.748

Original Article

Twelve years of experience in the treatment of newborns with intrauterine gastrointestinal perforation

Olga Devrim Ayvaz, Sabri Cansaran, Ayşenur Celayir, Muhammed Hamidullah Çakmak

Department of Pediatric Surgery, University of Health Sciences, Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital, İstanbul, Türkiye.

ABSTRACT

Background. Meconium peritonitis occurs when meconium leaks into the peritoneal cavity as a result of intrauterine gastrointestinal perforation. In this study, we aimed to evaluate the results of newborn patients who were followed and treated due to intrauterine gastrointestinal perforation in the pediatric surgery clinic.

Methods. All newborn patients who were followed up and treated for intrauterine gastrointestinal perforation in our clinic between December 2009-2021 were analyzed retrospectively. Newborns who had no congenital gastrointestinal perforation were not included in our study. The data were analyzed using NCSS (Number Cruncher Statistical System) 2020 Statistical Software.

Results. Within twelve years, intrauterine gastrointestinal perforation was detected in 41 newborns, including 26 (63.4%) males, and 15 (36.6%) patients who were operated on in our pediatric surgery clinic. Surgical findings of 41 patients diagnosed with intrauterine gastrointestinal perforation revealed the presence of volvulus (n=21), meconium pseudocyst (n=18), jejunoileal atresia (n=17), malrotation-malfixation anomaly (n=6), volvulus due to internal hernia (n=6), Meckel's diverticulum (n=2), gastroschisis (n=2), perforated appendicitis (n=1), anal atresia (n=1), and gastric perforation (n=1). Eleven patients (26.8%) died. Total intubation time was significantly higher in deceased cases. Postoperatively, deceased cases passed their first stool significantly earlier than surviving newborns. Besides, ileal perforation was seen significantly more frequently in deceased cases. However, the frequency of jejunoileal atresia was significantly lower in the deceased patients.

Conclusions. Although sepsis has been held primarily responsible for the deaths in these infants from past to present, insufficiency in lung capacity necessitating intubation negatively affects their survival. Early passage of stool is not always an indicator of good prognosis after the operation, and patients may die due to malnutrition and dehydration, even after they are discharged after feeding, defecating and having weight gain.

Key words: intestinal perforation, newborns, peritonitis, prenatal diagnosis.

Meconium peritonitis, which is a sterile condition, occurs when meconium leaks into the peritoneal cavity as a result of perforation in the small intestine of the fetus during the intrauterine period.[1,2] Meconium peritonitis should be considered when obstetric ultrasound (US) performed in the second and third trimesters of pregnancy shows hyperechoic areas and pseudocyst-like structures in the fetal abdomen.[1] As is the case with all other gastrointestinal malformations, prenatal diagnosis of meconium peritonitis is of great importance in terms of ensuring that the mother gives birth in a well-equipped hospital with a neonatal intensive care unit and pediatric surgery clinic.[3]

In this retrospective study, we aimed to evaluate the outcomes of newborn patients who were followed up and treated due to intrauterine gastrointestinal perforation in the pediatric surgery clinic of a tertiary health center.

✉ Olga Devrim Ayvaz
olga_ozbay@yahoo.com

Received 4th August 2022, revised 19th October 2022, accepted 9th December 2022.

Material and Methods

The study was designed in accordance with the principles of the World Medical Association Declaration of Helsinki. Permission was obtained from the Clinical Research Ethics Committee of Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital (dated 19.01.2022, numbered 12).

Informed consent of the families was obtained preoperatively during the hospitalization of their infants. The parents of all cases had been informed about the disease, all procedures performed before the treatment and surgery, and their consent was obtained.

All newborn patients monitored and treated for intrauterine gastrointestinal perforation in the Pediatric Surgery Clinic of Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital between December 2009 and 2021 were analyzed retrospectively.

Newborns who had no intestinal perforation at birth but developed it later on during the postnatal period due to necrotizing enterocolitis, jejunoileal atresia or intestinal volvulus were not included in our study.

Descriptive characteristics including age at diagnosis, gender, presence of antenatal diagnosis, antenatal US findings, gestational age, birth weight, mode of delivery, presence of additional disease; clinical data including physical, radiographic and echocardiographic (ECHO) findings; surgery-related information including surgical indication on the day of birth, age at operation, presence and type of intraabdominal pathology, number and site of the perforation, intestinal circulation status, operation and ostomy types, resected bowel segment, presence of synchronous operation, and short bowel status; and outcome information including postpartum intubation status, total intubation time, initiation of oral feeding after birth, first day of post-operative defecation and feeding, the need for total parenteral nutrition (TPN), total number of days on TPN, early and late postoperative complications (wound infection, detachment, incisional hernia, anastomotic leak, adhesive intestinal obstruction, etc.), presence of sepsis, histopathological results, total hospitalization period, discharge or exitus status, outpatient follow-up findings and genetic test results (for cystic fibrosis) were retrieved retrospectively from the patient files and computer records.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2020 Statistical Software (Utah, USA) program was used for statistical analysis. While evaluating the study data, descriptive statistical methods (mean, standard deviation, median, frequency, ratio) as well as Shapiro- Wilk test and boxplot graphs were used to check the fitness of the variables to the normal distribution. Mann-Whitney U test was used for the comparisons between groups of non-normally distributed parameters. Pearson chi-square test, Fisher's exact test and Fisher-Freeman-Halton test were used to compare qualitative data. Kaplan- Meier survival analysis was also used. Statistical significance was evaluated at the $p<0.05$ level.

Results

In our pediatric surgery clinic, intrauterine bowel perforation was detected in 26 (63.4%) male, and 15 (36.6%) female newborns over a 12-year period. The patients were 0-4 days old (mean age: 0.78±0.93 days) when they were first evaluated by pediatric surgeons. The distribution of descriptive characteristics of the patients are shown in Table I.

The age of the patients at surgery varied between 1 and 5 days (mean age: 1.66±1.35 days). The general health condition of one patient who was operated on the fifth day after birth deteriorated, and required insertion of an intra-abdominal drain.

Oral feeding had been started in 12 (29.3%) cases without application of nasogastric tube drainage during their follow-up and treatment in the neonatal intensive care unit. Bowel dilatation and presence of intra-abdominal

Table I. Distribution of descriptive characteristics.

		n (%)*
Age (days)	Mean±SD	0.78±0.93
	Median (Min-Max)	1 (0-4)
Gender	Male	26 (63.4)
	Female	15 (36.6)
Antenatal Diagnosis	Yes	21 (51.2)
	No	20 (48.8)
Antenatal US findings (n=21)	Intestinal dilatation	16 (76.2)
	Intraabdominal cyst	4 (19)
	Intraabdominal mass	1 (4.8)
	Intraabdominal fluid	1 (4.8)
	Gastroschisis	2 (9.5)
	Double-bubble	1 (4.8)
Gestational age (weeks)	Mean±SD	34.9±3.51
	Median (Min-Max)	36 (25.39)
Birth weight (g)	Mean±SD	2646±701
	Median (Min-Max)	2730 (1250-3900)
Type of delivery	Spontaneous vaginal delivery	15 (36.6)
	Cesarean section	26 (63.4)
Comorbid diseases	None	24 (58.5)
	Cardiac pathologies	16 (39.0)
	Hirschsprung's disease	3 (7.3)
	Cystic fibrosis	2 (4.9)
	Urinary pathologies	2 (4.9)
	Esophageal atresia + TEF	1 (2.4)
	Phocomelia	1 (2.4)
	Vertebra anomalies	1 (2.4)
	Central hypothyroidism	1 (2.4)
	Hypoplasic thymus	1 (2.4)
	Polydactyly	1 (2.4)
	Trisomy 13	1 (2.4)
ECHO findings (n=16)	Patent ductus arteriosus	10 (24.4)
	Patent foramen ovale	8 (19.5)
	Pulmonary hypertension	4 (9.8)
	Atrial septal defect	4 (9.8)
	Ventricular septal defect	4 (9.8)
	Mitral insufficiency	3 (7.3)
	Tricuspid insufficiency	2 (4.9)
	Aortic hypoplasia	1 (2.4)
	Left ventricular hypoplasia	1 (2.4)
	Aortic insufficiency	1 (2.4)

ECHO: echocardiography, SD: standard deviation, TEF: tracheoesophageal fistula.
*Unless indicated otherwise.

Table II. Distribution of characteristics specific to disease states, and surgery.

		n (%)
Symptoms/Signs	Rectal bleeding	2 (4.9)
	Abdominal distension	33 (80.5)
	Inability to defecate	10 (24.4)
	Biliary vomiting / nasogastric drainage	21 (51.2)
	Abdominal skin lesion	3 (7.3)
	Poor intestinal blood circulation	2 (4.9)
	Palpable intra-abdominal mass	1 (2.4)
	Imperfore anus	1 (2.4)
Radiography findings	None	1 (2.4)
	Absence of meteorism	19 (46.3)
	Free air under diaphragm	12 (29.3)
	Air-fluid levels	8 (19.5)
	Calciifed cyst	8 (19.5)
Presence of abdominal pathology	No	3 (7.3)
	Yes	38 (92.7)
	Jejunoileal atresia	17 (41.5)
	Volvulus	21 (51.2)
	Malrotation	6 (14.6)
	Internal hernia	6 (14.6)
	Meconium pseudocyst	18 (43.9)
	Meckel's diverticulum	2 (4.9)
	Gastric perforation	1 (2.4)
	Transition zone pathology	2 (4.9)
	Gastroschisis	2 (4.9)
	Anal atresia	1 (2.4)
	Appendicitis	1 (2.4)
Locations of perforation	Terminal ileum	12 (29.3)
	Transvers colon	2 (4.9)
	Ileum	22 (53.7)
	Stomach	1 (2.4)
	Meckel's diverticulum	1 (2.4)
	Cecum	4 (9.8)
	Colon	2 (4.9)
	Appendix	1 (2.4)
Numbers of perforation	Single	25 (61.0)
	Multiple	16 (39.0)
Intestinal blood circulation	Good	32 (78.0)
	Poor	9 (22.0)
Type of surgery	Anastomosis	18 (43.9)
	Ostomy	14 (34.1)
	Both	9 (22.0)
Ostomy types (n=23)	Ileostomy	19 (46.3)
	Colostomy	3 (7.3)
	Cecostomy	1 (2.4)
Length of resected intestinal segment (cm)	Mean±SD	11.19±15.32
	Median (Min-Max)	7 (0-80)
Synchronous surgeries*	No	24 (58.5)
	Yes	17 (41.5)
Short bowel syndrome	Yes	9 (22.0)
	No	32 (78.0)

* Synchronous surgeries: appendectomy, adhesiolysis, central catheterization; excision of Meckel's divetticulum, ınsertion of a nephrostomy catheter, umbilical vein catheterization, rectal biopsy

Table III. Distribution of follow-up features of patients.

Surgery at birth, n (%)	Yes	7 (17.1)
	No	34 (82.9)
Timing of surgery, n (%)	At Birth	7 (17.1)
	Postnatal- Day 1	17 (41.5)
	Postnatal- Day 2	7 (17.1)
	Postnatal- Day 3	4 (9.8)
	Postnatal- Day 4	5 (12.2)
	Postnatal- Day 5	1 (2.4)
Intubation in the preoperative period, n (%)	Yes	18 (43.9)
	No	23 (56.1)
Total duration of intubation, days	Mean±SD	7.02±11.09
	Median (Min-Max)	2 (0-39)
Initiation of oral feeding after birth, n (%)	Yes	12 (29.3)
	No	29 (70.7)
Time to first passage of stool after surgery, days	Mean±SD	4.22±3.0
	Median (Min-Max)	4 (0-13)
Time to postoperative oral feeding, days	Mean±SD	5.17±3.59
	Median (Min-Max)	5 (0-13)
Treatment with TPN, n (%)	Yes	33 (80.5)
	No	8 (19.5)
Total duration of TPN, days	Mean±SD	12.66±15.22
	Median (Min-Max)	7 (0-48)
Total duration of hospitalization, days	Mean±SD	23.38±18.38
	Median (Min-Max)	19 (1-85)
Final outcome, n (%)	Died	11 (26.8)
	Survived	30 (73.2)

fluid were described prenatally in one of these cases. These 12 patients were consulted on the second postnatal day because they could not tolerate oral feeding.

The surgical findings of 41 patients diagnosed with intrauterine bowel perforation revealed the presence of volvulus in 21 (51.2%), meconium pseudocyst in 18 (43.9%), jejunoileal atresia in 17 (41.5%), malrotation malfixation anomaly in 6 (14.6%), internal hernia in 6 (14.6%), Meckel's diverticulum in 2 (4.9%) (including one case in invaginated bowel segment), gastroschisis in 2 (4.9%), appendix perforation in 1 (2.4%), anal atresia in 1 (2.4%), stomach perforation in 1 (2.4%), and Hirschsprung's disease in 3 (7.3%) patients. The distribution of disease states and their surgical characteristics are shown in Table II.

Postoperatively, 26 (63%) were intubated, and 15 extubated cases (37%) were followed up and treated in the intensive care unit. Seven patients who did not defecate post-operatively did not survive. The distribution of characteristics related to the follow-up of the patients are shown in Table III.

Wound infection, wound detachment, and incisional hernia were not observed in any of the patients in the early postoperative period. Postoperatively, 11 patients (26.8%) died before being discharged.

One patient (2.4%), who underwent an emergency operation due to postoperative umbilical catheter-induced liver hematoma and intra-abdominal bleeding, died on the 3rd postoperative day. One of two patients who were operated for the second time due to lack of a gastrointestinal passage, and the other two patients who were operated on for the third time died due to sepsis. Sepsis was the cause of exitus in 6 (54.5%) of 11 deceased cases. Seven (64%) deceased cases were intubated after delivery and could not be extubated until their death. The diagnosis of cystic fibrosis was confirmed based on genetic test results in 2 of 16 cases and, one of these cases died.

Table IV. Assessments based on mortality status.

		Deceased (n=11)	Survived (n=30)	p
Age, mo				
	Mean±SD	0.6±0.7	0.8±1	[a]0.762
	Median (Q1-Q3)	1 (0-1)	0.5 (0-2)	
Gender, n (%)				
	Female	5 (45.5)	10 (33.3)	[b]0.491
	Male	6 (54.5)	20 (66.7)	
Duration of intubation, days				
	Mean±SD	13.7±14.5	4.6±8.6	[a]0.005**
	Median (Q1-Q3)	6 (2-30)	0 (0-7)	
	None, n (%)	0	16 (53.3)	
	1-7 days, n (%)	7 (63.6)	8 (26.7)	
	8-14 days, n (%)	0	2 (6.7)	[c]0.004**
	15-30 days, n (%)	2 (18.2)	3 (10.0)	
	≥31 days, n (%)	2 (18.2)	1 (3.3)	
Time to surgery, n (%)				
	The first 24 hrs	9 (81.8)	15 (50)	[b]1.000
	>24 hrs	2 (18.2)	15 (50)	
Time to the first passage of stool, days				
	Mean±SD	2.5±2.8	4.9±3.4	[a]0.032*
	Median (Q1-Q3)	2 (0-5)	4 (2-6)	
Time to the first oral feeding, days				
	Mean±SD	3.5±4.1	5.8±3.2	[a]0.056
	Median (Q1-Q3)	2 (0-6)	5 (4-7)	
Treatment with TPN, n (%)				
	No	4 (36.4)	4 (13.3)	[b]0.178
	Yes	7 (63.6)	26 (86.7)	
Total duration of TPN, days				
	Mean±SD	15.4±18	11.7±14.3	[a]0.779
	Median (Q1-Q3)	5 (0-33)	7 (2-12)	
Gestational Week				
	Mean±SD	33.4±3.4	35.4±3.4	[a]0.065
	Median (Q1-Q3)	33 (30-36)	36 (35-38)	
Birth weight, g				
	Mean±SD	2243.2±735.7	2793.3±638.3	[a]0.068
	Median (Q1-Q3)	2600 (1500-2900)	2875 (2380-3110)	
Length of resected bowel segment, cm				
	Mean±SD	14.9±23.8	9.8±11.1	[a]0.836
	Median (Q1-Q3)	4 (2-20)	7 (1.5-15)	
	<10 cm, n (%)	7 (63.6)	18 (60.0)	[b]1.000
	≥10 cm, n (%)	4 (3.4)	12 (40.0)	
Short bowel syndrome, n (%)		3 (27.3)	6 (20.0)	[b]0.680
Rectal bleeding , n (%)		2 (18.2)	0	[b]0.067
Abdominal distension, n (%)		9 (81.8)	24 (80.0)	[b]1.000
Biliary vomiting / nasogastric drainage, n (%)		7 (63.6)	14 (46.7)	[d]0.335
Resection anastomosis, n (%)		5 (45.5)	22 (73.3)	[b]0.140
Ostomy, n (%)		8 (72.7)	15 (50.0)	[b]0.291
Perforation, n (%)				
	Single	6 (54.5)	19 (63.3)	[b]0.723
	Multiple	5 (44.5)	11 (36.7)	
Terminal ileum perforation, n (%)		6 (54.5)	6 (20.0)	[b]0.052
Transverse colon perforation, n (%)		1 (9.1)	1 (3.3)	[b]0.470
Ileum perforation, n (%)		3 (27.3)	19 (3.3)	[d]0.040*
Perforation of Meckel's diverticulum, n (%)		0	1 (3.3)	[b]1.000
Cecum perforation, n (%)		0	4 (13.3)	[b]0.559
Colon perforation, n (%)		1 (9.1)	1 (3.3)	[b]0.470
Jejunoileal atresia, n (%)		1 (9.1)	16 (53.3)	[b]0.014*
Meconium pseudocyst , n (%)		3 (27.3)	15 (50.0)	[b]0.291
Cardiac pathology, n (%)		3 (27.3)	13 (43.3)	[b]0.478
Total duration of hospitalization, days				
	Mean±SD	24.0±20.6	23.1±17.9	[a]0.743
	Median (Q1-Q3)	34 (3-44)	19 (13-30.5)	

[a]Mann Whitney U test, [b]Fisher's exact test, [c]Fisher-Freeman Halton test, [d]Pearson chi square test

*p<0.05, **p<0.01

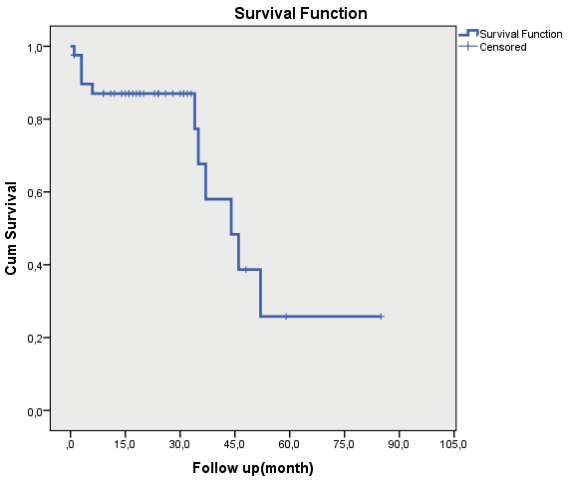


Fig. 1. Survival curve.

While ganglion positivity was reported in the histopathological examinations of intestinal specimens of 11 deceased patients, our diagnosis of Hirschsprung's disease was confirmed pathologically in 3 of our surviving cases.

From the outpatient follow-up information, it was learned that 5 (16.7%) of the 30 patients discharged died during follow-up period due to cardiac problems (n=1), food aspiration (n=1), and dehydration (n=3).

In our study; any statistically significant difference was not found between the mean age and gender distribution of the patients according to mortality rates ($p>0.05$).

Total intubation time was significantly longer in deceased cases ($p<0.01$). Postoperatively deceased patients had recovered their first bowel movements earlier than the other cases ($p<0.05$).

Ileal perforation was significantly associated with higher mortality, and jejunoileal atresia with lower mortality rates ($p<0.5$), but there were no statistically significant associations between mortality and the timing or type of feeding, length of the resected segment, or number of perforations, and other clinical parameters. Evaluations made according to mortality status are shown in Table IV.

Survival analysis

A total of 41 surgical operations were performed. Postoperatively 30 (73.2%) cases survived, and 11 cases did not. The mean, and median survival times were 47.987±6.791 (95% CI: 34.67-61.29), and 44.0 days, respectively. The latest death was seen on the postoperative 37th day; and the cumulative survival rate in one month was 25.6% with a standard error of 14.2%.

The survival curve of our patients is shown in Fig. 1.

Discussion

Meconium peritonitis was first described by Morgagni in 1761, but the first corrective surgery was successfully performed by Agerty in 1943.[4] Especially with the widespread use of US, prenatal diagnosis in fetuses with gastrointestinal anomalies has been increasing since 1975. With the referral of prenatally diagnosed cases to appropriate centers and the development of neonatal intensive care facilities, the survival rates are gradually increasing.

The underlying pathology in intrauterine gastrointestinal perforations affects the mortality and morbidity of infants. The main causes of intrauterine gastrointestinal perforation are jejunoileal atresia, intrauterine invagination, Hirschsprung's disease, meconium ileus (cystic fibrosis), segmental jejunoileal volvulus due to cystic fibrosis, midgut volvulus due to malrotation-malfixation anomalies, colonic atresia, Meckel's diverticulum and intrauterine mesenteric vascular insufficiency.[1,2,5] In our study covering a period of 12 years, 51.2% of 41 cases with intrauterine bowel perforation were antenatally diagnosed, and volvulus (51.2%), meconium pseudocyst (43.9%), and jejunoileal atresia (41.5%) were the most common etiologic factors. In our study, significantly lower mortality rates were detected in cases with jejunoileal atresia.

In some cases, intrauterine gastrointestinal perforation can resolve spontaneously and

these cases can continue their lives without any sequelae.[3] Successful results in a limited number of these patients followed with conservative treatment have been reported.[2] Surgery should be performed without delay when there are signs and symptoms of intestinal obstruction in these cases followed up with conservative treatment.[2]

In a study of 79 cases with meconium peritonitis, male patients constituted 60%, and 54% of the patients were operated on in the first 24 hours and afterwards.[6] In another study, male patients comprised 72.7% of 11 patients who had developed meconium peritonitis secondary to primary segmental volvulus.[7] In our study, male patients constituted 63.4% of the patients with meconium peritonitis. Although intrauterine gastrointestinal perforation was more common in our male patients as in many other studies[6-8], there was no significant difference between genders in terms of both incidence rates and prognosis of intrauterine gastrointestinal perforation.

Meconium peritonitis is rarely diagnosed prenatally before the 20th gestational week due to the lack of initiation of intestinal peristalsis.[9] The median gestational age at the first diagnosis of meconium peritonitis has been reported as 24 weeks.[9] In our study, the age at diagnosis of 17 prenatally diagnosed patients was found to be after 24 weeks (earliest: 25th week), which is consistent with the literature.

Ultrasonographic diagnosis of prenatal meconium peritonitis is made when ascites, calcified meconium and intestinal dilatation are seen together in the abdominal cavity of the fetus.[1] In a study of 15 cases with established prenatal diagnoses, meconium peritonitis had been observed in 73%, intestinal dilatation in 53%, ascites in 33%, pseudocyst in 13%, and polyhydramnios in all cases.[10] In another study, fetal ascites was the most common antenatal US finding.[9] In another study of 79 cases, the most common finding was bowel dilatation (78.6%).[6] Similar to the rates reported in the literature in our study, prenatal diagnosis was made in 51.2% of the cases and the most common antenatal US finding was intestinal dilatation (76.2%). With technical developments in US, the diagnosis of gastroschisis can be made at increasing rates, but it is difficult to diagnose antenatal intestinal perforation that rarely develops in cases with gastroschisis.[11] Although gastroschisis was diagnosed antenatally in our two cases, intrauterine bowel perforation could be diagnosed after birth.

The mean gestational ages at diagnosis of meconium peritonitis were reported as 37.2±2.3[6] and 37.2±2.3[7] weeks in two separate studies. In our study, the mean gestational age of the patients diagnosed as meconium peritonitis was 34.9±3.51 weeks. The mean birth weight of these patients was 3162±532 g in a study conducted regardless of the etiology of intestinal perforation[6] and as 3022±797[7] g in another study of cases with intestinal perforation due to primary segmental volvulus. In our study, the mean birth weight of these patients was 2646±701 g contrary to the literature findings. The mean gestational age of our patients with meconium peritonitis is lower compared to the literature findings, which can be naturally attributed to the lower average birth weight of our patients. Although elective preterm birth has been shown to be beneficial in improving surgical outcomes in prenatally diagnosed cases with gastroschisis, currently insufficient clinical evidence fails to support early prophylactic Caesarean delivery for all infants with gastroschisis.[11] However, the rate of Caesarian section (C/S) was high in many studies, as is the case with our study (63.4%).[6,7,12,13]

Newborns usually present with tense abdominal distension, visible veins on shiny abdominal skin, edematous abdominal wall, respiratory distress, biliary vomiting / nasogastric drainage, inability to pass meconium, and peritonitis.[2] Accordingly, abdominal distension (80.5%), biliary vomiting / nasogastric drainage (51.2%), and inability to defecate (24.4%) were the most common findings in our cases.

Intra-abdominal calcifications are observed in 86% of the cases with meconium peritonitis.[14] In our study, 19.5% of the patients had calcifications suggestive of meconium cysts. We think that our lower incidence of calcification compared to the literature is related to the etiology of the perforation and the time of occurrence of the event.

When meconium peritonitis occurs, early surgery is a valid way to prevent exacerbation of intra-abdominal inflammation, hyperemia and edema of the intestinal wall, and is important for reducing rates of intestinal adhesion, severe infection, and mortality.[6] In our study, the mean age at operation was 1.66±1.35 days, and the patients were operated within the first 24 hours after their admissions to our clinic.

The incidence of chromosomal abnormalities and genetic syndromes is low in cases with meconium peritonitis, but a relatively strong association with cystic fibrosis has been reported in 8-40% of the patients.[2] In a study, cystic fibrosis was reported in two cases diagnosed with intrauterine perforation due to prenatal volvulus.[5] Cystic fibrosis was suspected in the prenatal period in 2 patients in our study, and genetic test results confirmed this suspicion.

Recent studies do not provide clear guidelines on surgical strategies for the management of meconium peritonitis. Enterostomy, primary anastomosis, Bishop-Koop ileostomy and Santulli ileostomy are commonly used procedures.[15] Although the selection of the type of surgical procedure appears to depend on clinical signs, the patient's general condition and the technique preferred by the surgeon also matter , however few comparative studies have been conducted so far.[2] In one study, it was reported that peritoneal drainage was performed in 1.3%, intestinal resection-anastomosis in 43%, enterostomy in 54.5% of the patients, and the remaining mean bowel length was 105.3±42.3 cm.[6] In another study, segmental resection and ileostomy were performed in 63.6%, and segmental resection and primary anastomosis in 36.4% of patients.[7] In our study, resection-anastomosis was applied in 43.9%, ostomy in 34.1%, and resection-anastomosis and ostomy in 22% of the patients. The median length of the total resected bowel segment was 7 cm. In our study, dimensions of resected bowel segment and criteria of ICD-10-cm classification were also not statistically significantly correlated with mortality rates.

In the literature, it has been reported that the terminal ileum or ileum is the most common perforation site in the fetal period, and appendiceal perforation is very rare.[12] Two cases of perforated fetal appendix were presented in the study of Wang et al.[12] In our study, the perforation site was in the ileal segments in 53.7%, the terminal ileum in 29.3%, and appendix in 2.4% of the cases. In our study, rates of ileum perforation were significantly higher in deceased patients.

The prognosis of meconium peritonitis varies depending on its etiology.[1] If the diagnosis of meconium peritonitis can be made in the antenatal period, fetal morbidity and mortality can be reduced.[1] While the mortality rate was found to be 11-14% in cases diagnosed during pregnancy, mortality rates between 40 and 50% were reported in babies diagnosed after birth.[1] Recently, the survival rate for meconium peritonitis has exceeded 90%.[2] This higher rate of improvement is the result of advances in prenatal diagnostic techniques, timely intervention and the ability to provide intensive care services starting from birth of the infants.[2,10]

In one study, early surgery was shown to improve outcomes for severely affected patients by reducing intra-abdominal and systemic inflammation.[10] In a study of 79 patients, 95% of 40 patients who had been operated within the first 24 hours of life compared to only 79.5% of 39 patients who had been intervened later, survived.[6] In our study, 62.5% of the 24 patients who were operated within the first 24 hours of life compared to 88% of the 17 patients who were operated after 24 hours survived. In our study, the relationship between early surgery and prognosis was not statistically significant.

We thought that higher mortality rate in our study despite early surgery, compared to the literature was related to the greater number of premature cases with low birth weight and pulmonary dysfunction.

In a study of 20 cases, an average mortality rate of 60% was found in cases with short bowel syndrome. While these cases were lost due to sepsis in the early years of pediatric surgery practice, losses due to sepsis and liver failure due to TPN have come to the forefront in recent years.[8] Short bowel syndrome, defined as a type of malabsorption, occurs most commonly after massive bowel resections performed for the management of malrotation-volvulus, gastroschisis, intestinal atresia and necrotizing enterocolitis.[8] The prognosis in short bowel syndrome is closely related to the remaining bowel length, presence of ileocecal valve, intestinal motility and feeding tolerance and also to sepsis and liver failure, which are the complications of parenteral nutrition.[8] In our study, as in this above-mentioned study, sepsis was the primary cause of mortality. Besides sepsis, problems related to the patients' lung and intestinal motility were also other causes of mortality. In our study, 43.9% of our patients were intubated in the preoperative period. The prognosis of preoperatively intubated patients was statistically significantly deteriorated. Total intubation time was significantly longer in exited cases. They had problems regarding the insufficiency of their lung capacity. Prolonged intubation and long hospitalization also increased risk of sepsis. While our mortality rate was 33% (n=9) in patients with short bowel syndrome, our overall survival rate was 73.2%. The preservation of the ileocecal valve in all of our patients with short bowel syndrome may have contributed to the ineffectiveness of short bowel syndrome on mortality. Patients with short bowel syndrome may have passed their first stool in the early postoperative period because of their rapid bowel movements. The statement 'Switching to oral feeding' mistakenly considering that the patient has passed the critical postoperative follow-up period may delay and mask the recognition of secondary complications.

In conclusion, mortality and morbidity rates in newborns with complicated meconium peritonitis vary according to the etiology of intrauterine perforation. Although sepsis has been held primarily responsible for the deaths in these infants from past to present, the insufficiency of their lung capacity requiring intubation increases the mortality rates of these infants. Early passage of stool does not always result in satisfactory outcomes in these babies who have been operated on, and patients may be lost due to malnutrition and dehydration, even after they are discharged, despite oral intake, defecation and weight gain.

Ethical approval

Ethical approval was obtained from the Clinical Research Ethics Committee of Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital (dated 19.01.2022, numbered 12).

Author contribution

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

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Bilen E, Yüksel M, Oral HB, et al. Antenatal diagnosis of meconium peritonitis. SDU Journal of the Institute of Health Sciences. 2014; 5: 105-107.
2. Agrawal S, Verma A, Rajbhar S, et al. Meconium peritonitis: in utero diagnosis of a rare clinical entity and postnatal outcome. Obstet Gynecol Cases Rev 2020; 6: 1-7. https://doi.org/10.23937/2377-9004/1410180
3. Tutuş Ş, Yılmaz E, Yılmaz A, Turan C. Defining prenatal and postnatal ultrasonography findings in a patient who had meconium peritonitis and pseudocyst formation. J Ankara Univ Fac Med 2012; 65: 111-114. https://doi.org/10.1501/Tipfak_000000819
4. Gaddam SA, Tirunagari S. Meconium peritonitis - a rare cause of ascites. Journal of Case Reports 2018; 8: 190-193. https://doi.org/10.17659/01.2018.0051
5. Chouikh T, Mottet N, Cabrol C, Chaussy Y. Prenatal intestinal volvulus: look for cystic fibrosis. BMJ Case Rep 2016; 2016: bcr2016217003. https://doi.org/10.1136/bcr-2016-217003
6. Jiang Y, Pan W, Wu W, Wang W, Sun S, Wang J. Can early surgery improve the outcome of patients with meconium peritonitis? A single-center experience over 16 years. BMC Pediatr 2019; 19: 473. https://doi.org/10.1186/s12887-019-1844-5
7. Kim SH, Cho YH, Kim HY. Primary segmental volvulus of small intestine: surgical perspectives according to age at diagnosis. Front Pediatr 2019; 7: 146. https://doi.org/10.3389/fped.2019.00146
8. Celayir S, İlçe Z, Tekant GT, Sarımurat N, Erdoğan E, Yeker D. Çocukluk çağında kısa barsak sendromu ile ilgili deneyimlerimiz. Cerrahpasa Med J. 2001; 32: 100-104.
9. Ping LM, Rajadurai VS, Saffari SE, Chandran S. Meconium peritonitis: correlation of antenatal diagnosis and postnatal outcome - an institutional experience over 10 years. Fetal Diagn Ther 2017; 42: 57-62. https://doi.org/10.1159/000449380
10. Uchida K, Koike Y, Matsushita K, et al. Meconium peritonitis: prenatal diagnosis of a rare entity and postnatal management. Intractable Rare Dis Res 2015; 4: 93-97. https://doi.org/10.5582/irdr.2015.01011
11. Haberman S, Burgess T, Klass L, Cohn BD, Minkoff HL. Acute bowel perforation in a fetus with gastroschisis. Ultrasound Obstet Gynecol 2000; 15: 542-544. https://doi.org/10.1046/j.1469-0705.2000.00039.x
12. Wang Y, Wu Y, Guan W, et al. Meconium peritonitis due to fetal appendiceal perforation: two case reports and a brief review of the literature. BMC Pediatr 2018; 18: 162. https://doi.org/10.1186/s12887-018-1133-8
13. Gerçel G, Anadolulu Aİ. Intrauterine midgut volvulus as a rare cause of intestinal obstruction: a case report. J Med Case Rep 2021; 15: 239. https://doi.org/10.1186/s13256-021-02778-6
14. Barthel ER, Speer AL, Levin DE, Naik-Mathuria BJ, Grikscheit TC. Giant cystic meconium peritonitis presenting in a neonate with classic radiographic eggshell calcifications and treated with an elective surgical approach: a case report. J Med Case Rep 2012; 6: 229. https://doi.org/10.1186/1752-1947-6-229
15. Martynov I, Raedecke J, Klima-Frysch J, Kluwe W, Schoenberger J. The outcome of Bishop-Koop procedure compared to divided stoma in neonates with meconium ileus, congenital intestinal atresia and necrotizing enterocolitis. Medicine (Baltimore) 2019; 98: e16304. https://doi.org/10.1097/MD.0000000000016304

The Turkish Journal of Pediatrics 2023; 65: 398-405
https://doi.org/10.24953/turkjped.2022.843

Original Article

Retrospective validation of the postnatal growth and retinopathy of prematurity (G-ROP) and Colorado retinopathy of prematurity (CO-ROP) models in a Turkish cohort

Dilbade Yildiz Ekinci[1], Havvanur Bayraktar[1], Ozlem Leyla Sero[2], Nilufer Okur[3]

[1]*Department of Ophtalmology, University of Health Sciences , Gazi Yasargil Trainig and Research Hospital Diyarbakır;* [2]*Department of Pediatrics, University of Health Sciences , Gazi Yasargil Trainig and Research Hospital, Diyarbakır;* [3]*Department of Neonatology, University of Health Sciences, Gazi Yasargil Trainig and Research Hospital, Diyarbakır, Türkiye.*

ABSTRACT

Background. The aim of this study was to investigate the effectiveness of the Postnatal Growth and Retinopathy of Prematurity (G-ROP) and Colorado Retinopathy of Prematurity (CO-ROP) models in predicting the risk of Retinopathy of Prematurity (ROP) in preterm infants at a tertiary ROP diagnostic and treatment center.

Methods. The G-ROP and CO-ROP models were applied to the study group using the data obtained. The sensitivity and specificity of both models were then calculated.

Results. One hundred and twenty-six infants were included in the study. When the G-ROP model was applied to the study group, the model's sensitivity at detecting any stage ROP was 88.7%, while it was 93.3% for the treated group. The specificity of the model was 10.9% for any stage ROP, and 11.7% for the treated group. For the CO-ROP model in the same study group, the sensitivity at detecting any stage ROP was 87.3%, while it was 100% for the treated group. The CO-ROP model's specificity was 40% for any stage ROP, and 27.9% for the treated group. When cardiac pathology criteria were introduced to both models, the sensitivity of the G-ROP and CO-ROP models increased to 94.4% and 97.2%, respectively.

Conclusions. It was found that the G-ROP and CO-ROP models are simple and effective models for predicting any degree of ROP development, but that they are unable to be 100% accurate. When the models were modified by introducing cardiac pathology criteria, it was observed that they began to produce more accurate results. Studies with larger groups are needed in order to assess the applicability of the modified criteria.

Key words: retinopathy of prematurity, postnatal weight gain, G-ROP, CO-ROP.

Retinopathy of prematurity (ROP) is a proliferative vascular disorder of the immature retina characterized by the disruption of normal vascular development in retinal vessels and pathological retinal neovascularization in preterm infants.[1] This abnormal development of vascular structures may lead to severe visual impairment and blindness in infants if left untreated.[2] Timely screening to identify infants in need of treatment is therefore essential in preventing serious long-term visual consequences and even blindness.[1,2]

Currently, the recommended screening criteria for ROP are based on two commonly accepted risk factors for ROP, namely the gestational age (GA) and birth weight (BW) of the infant.[3] However, ROP may also affect preterm infants with normal BW or above.[4,5]

✉ Dilbade Yildiz Ekinci
dilbadeekinci@gmail.com

Received 6th September 2022, revised 13th January 2023, accepted 25th January 2023.

This study was presented as an oral presentation at the Turkish Ophthalmology Society, 33rd Summer Symposium in 25-26 September 2021 in Diyarbakır, Türkiye.

On the other hand, fundus examination for ROP is burdensome on infants, and about one-tenth of infants who develop ROP require treatment.[6] Therefore, many ROP prediction models have been proposed that attempt to reduce the number of unnecessary examinations in order to increase the efficiency of ROP screening, without overlooking severe forms of the disease that require treatment.[7-10]

Postnatal weight gain (WG) is considered an indirect indicator of the health and perinatal condition of an infant.[11] It is used as a common parameter by many ROP sampling algorithms.[7-10] The Postnatal Growth and Retinopathy of Prematurity (G-ROP) and the Colorado Retinopathy of Prematurity (CO-ROP) are models that use GA, BW, and postnatal WG data to estimate the risk of ROP. These two models have been applied across different cohorts and shown to be a simple way of increasing the efficiency of ROP screening without the need for complex calculations.[7,12]

The present study investigated the effectiveness of the G-ROP and CO-ROP models by applying them to a cohort of preterm infants in a tertiary neonatal intensive care unit in Türkiye.

Material and Methods

This was a retrospective study of preterm infants who underwent ROP screening at Gazi Yasargil Training and Research Hospital from January 2017 to July 2021, had a known ROP outcome, and whose weight data was available. The study was approved by the Institutional Review Board (IRB) of Gazi Yasargil Training and Research Hospital and followed the tenets of the Declaration of Helsinki.

ROP screening and classification

The screening criteria used at the study center were GA <32 weeks, BW <1500 g, or infants with an unstable clinical course who were determined as being at high risk by the neonatologist. Screening was conducted after pupillary dilation with 2.5% phenylephrine and 1% tropicamide using a binocular indirect ophthalmoscope. Infants included in the study were classified into three subsets in accordance with the Early Treatment for Retinopathy of Prematurity (ETROP) study.[2] The No ROP Group included infants who did not develop any form of ROP. Group 1 included infants with any ROP requiring treatment, such as Type 1 ROP and aggressive ROP (A-ROP); and Group 2 included infants with Type 2 ROP, which spontaneously regressed. ROP screening was continued until treatment was required or complete vascularization of the retina occurred. All treatments was conducted according to ETROP Study guidelines.[2]

Clinical data collection

The following clinical data was collected: the infants' demographics, GA, BW, serial weight measurements, age at the time of diagnosis (weeks), days of mechanical ventilation and oxygen administration, length of stay in neonatal intensive care unit (days), and details of systemic disease including intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), cardiac disease (atrial septal defect, ventricular septal defect, and patent ductus arteriosus), sepsis, and necrotizing enterocolitis (NEC). For diagnoses of ROP the following data was also collected: location of ROP, severity of ROP, vascular characteristics of ROP, treatment status, treatment modality, and retinal vascular development. Infants whose follow-up could not be completed because of incomplete data regarding GA, BW, weight measurements and ROP outcome were excluded from the study.

G-ROP model

The G-ROP model consists of six consecutive criteria used to make an ROP screening decision. The criteria in the model are checked sequentially, and infants who meet at least one of the criteria are tested for ROP. ROP examination is not performed if none of the criteria are met. The G-ROP model criteria are: 1) GA <28 weeks, 2) BW <1051 g, 3) WG between postnatal days

10 and 19 <120 g, 4) WG between postnatal days 20 and 29 <180 g, 5) WG between postnatal days 30 and 39 <170 g, and 6) hydrocephalus.[12]

CO-ROP model

To make a screening decision for ROP, the CO-ROP model requires all of the criteria for both BW and GA to be met, rather than one criterion, plus an additional WG measured at 4 weeks (28 days). The CO-ROP model calls for ROP examination in an infant to meet all of the following criteria: 1) GA ≤30 weeks, 2) BW ≤1500 g, and 3) WG between birth and postnatal 4 weeks ≤650 g.[7]

Study outcomes

The G-ROP and CO-ROP models were applied separately to the study group using the data obtained. The sensitivity and specificity of both models were then calculated.

Results

The study included 126 infants who underwent retinal examinations and had a known ROP outcome. All infants were eligible for G-ROP and CO-ROP analysis. Of 126 infants, 65 (51.6%) were male and 61 (48.4%) were female. The median GA was 28 weeks (range 23–35 weeks), and the median BW was 1050 g (range 550–2250 g). In 55 of the cases (43.7%) no degree of ROP was detected (No ROP Group), whereas in 15 (11.9%) infants, Type 1 ROP or A-ROP (ROP 1 Group) was detected and treatment was initiated. All infants included in the study completed their final ROP screening at follow-up. Table I shows the descriptive data for the infants.

When the G-ROP model was applied to the study group, it identified 112 out of 126 infants as high-risk and showed that they needed to be screened for ROP. The G-ROP model based 78 of the 126 infants on the criteria of GA <28 weeks, eight on the BW <1051 g, 12 on the WG between postnatal days 10 and 19 <120 g, 11 on the WG between postnatal days 20 and 29 <180 g, and three on the WG between postnatal days 30 and 39 <170 g criteria. The G-ROP algorithm correctly identified 63 of the 71 infants who developed any stage ROP, and 14 of the 15 infants in Group 1. The G-ROP algorithm was not able to identify eight infants who developed any stage ROP and one infant who developed ROP, requiring treatment. The model's sensitivity at detecting any stage ROP was 88.7%, while it was 93.3% for the treated group. The specificity of the model was 10.9% for any stage ROP, and 11.7% for the treated group. Application of the G-ROP model reduced the number of infants examined by 11.1%, based on current scanning criteria (Table II).

When the CO-ROP model was applied to the study group, 95 infants who met all of the CO-ROP criteria were shown as requiring screening for ROP. The model correctly recognized 62 of the 71 infants who developed any stage ROP, and all the 15 infants who developed ROP that required treatment. The CO-ROP algorithm missed nine infants who developed any stage ROP. With regard to the CO-ROP criteria, the model's sensitivity at detecting some degree of ROP was 87.3%, while it was 100% for the

Table I. Demographics of infants included in the study.

	All (n=126)	No ROP (n=55, 43.7%)	Group 2 (n=56, 44.4%)	Group 1 (n=15, 11.9%)
Female, n	61	28	27	6
Gestational age, wk, median (range)	28 (23-35)	29 (25-35)	27 (23-34)	25 (23-29)
Birth weight, gr, median (range)	1050 (550-2250)	1200 (750-2250)	1000 (550-2200)	740 (600-1360)
NICU stay, days, median (range)	56 (0-300)	49 (0-240)	60 (3-300)	110 (55-110)
Supplemental O_2, days, median (range)	38 (0-300)	49 (0-240)	48 (0-300)	101 (52-79)

NICU: neonatal intensive care unit, ROP: retinopathy of prematurity.

Table II. Sensitivity and specificity of G-ROP study criteria.

	ROP (+)	No ROP	Total	Group 1	Group 2	Total
G-ROP (+)	63	49	112	14	98	112
G-ROP (-)	8	6	14	1	13	14
Total	71	55	126	15	111	126
Sensitivity	63/71		88.7%	14/15		93.3%
Specificity		6/55	10.9%		13/111	11.7%

ROP: retinopathy of prematurity.

Table III. Sensitivity and specificity of CO-ROP study criteria.

	ROP (+)	No ROP	Total	Group 1	Group 2	Total
CO-ROP (+)	62	33	95	15	80	112
CO-ROP (-)	9	22	31	0	31	14
Total	71	55	126	15	111	126
Sensitivity	62/71		87.3%	15/15		100%
Specificity		22/55	40%		31/111	27.9%

ROP: retinopathy of prematurity.

treated group. The CO-ROP model's specificity was 40% for any stage ROP, and 27.9% for the treated group. Application of the CO-ROP model reduced the number of infants examined by 24.6%, based on current scanning criteria (Table III).

Additional risk factors for infants that could not be identified by either model are examined and presented in Table V. All of the infants were also in Group 2. Concomitant cardiac pathology that the G-ROP model was unable to identify was observed in four of the eight infants. Similarly, concomitant cardiac pathology that the CO-ROP was unable to identify was observed in seven of the nine infants (Table IV, Table V). When cardiac pathology criteria were introduced, the sensitivity of both the G-ROP and CO-ROP models for detecting any stage ROP increased to 94.4% and 97.2%, respectively. When the modified G-ROP and CO-ROP model were applied, four and two infants who did not require treatment would be missed, respectively.

Table IV. Demographics of infants undetected by G-ROP criteria.

ROP(+) G-ROP (-) Cases	ROP	GA (weeks)	BW (grams)	Stay in NICU (days)	Mechanical ventilation (days)	Oxygen administration (days)	Sepsis	IVH	NEC	BPD	Cardiac Pathology
1	Group 1	29	1100	55	23	52	+	−	−	−	+
2	Group 2	28	1100	48	10	15	−	−	−	−	−
3	Group 2	28	1100	38	8	35	+	−	−	−	+
4	Group 2	29	1150	41	3	5	−	−	−	−	−
5	Group 2	32	1200	42	3	5	+	−	−	−	+
6	Group 2	30	1200	37	3	36	−	−	−	−	−
7	Group 2	28	1250	40	9	33	−	−	−	−	+
8	Group 2	28	1300	55	23	50	−	−	−	+	−

ROP: retinopathy of prematurity, NICU: neonatal intensive care unit, IVH: cerebral intraventricular hemorrhages, NEC: necrotizing enterocolitis, BPD: bronchopulmonary dysplasia.

Table V. Demographics of infants undetected by CO-ROP criteria.

ROP (+) CO-ROP (-) Cases	ROP	GA (weeks)	BW (grams)	Stay in NICU (days)	Mechanical ventilation (days)	Oxygen administration (days)	Sepsis	IVH	NEC	BPD	Cardiac Pathology
1	Group 2	28	1000	33	12	33	−	+	−	+	+
2	Group 2	32	1006	40	17	35	+	−	−	−	+
3	Group 2	28	1100	38	8	35	+	−	−	−	+
4	Group 2	32	1150	37	0	10	−	−	−	−	+
5	Group 2	32	1200	42	3	5	+	−	−	−	+
6	Group 2	30	1200	37	3	36	−	−	−	−	−
7	Group 2	31	1300	49	7	11	−	−	−	−	−
8	Group 2	34	1600	37	10	30	−	+	−	+	+
9	Group 2	30	2200	93	4	16	+	−	−	−	+

ROP: retinopathy of prematurity, NICU: neonatal intensive care unit, IVH: cerebral intraventricular hemorrhages, NEC: necrotizing enterocolitis, BPD: bronchopulmonary dysplasia.

Discussion

Retinopathy of prematurity is one of the leading causes of childhood blindness worldwide. An effective screening program is needed to prevent blindness associated with ROP. An ideal screening program should reduce the number of stressful examinations for premature infants and the workload on healthcare personnel, while at the same time having high levels of sensitivity and specificity.

Vascular endothelial growth factor (VEGF) plays an important role in retinal vascularization. Insulin-like growth factor 1 (IGF-1) is critical for VEGF activation.[13] Low IGF-1 levels are an indirect indicator of decreased VEGF activity, resulting in poor retinal vessel development and ROP. Slow postpartum WG is accepted as a marker that serum IGF-1 levels are increasing more slowly than normal.[14,15] Models using postpartum WG as a screening criterion for ROP have therefore been proposed.[7,12] Two of these are the G-ROP and CO-ROP models. The G-ROP and CO-ROP models include criteria for GA, BW, and postnatal WG and are advantageous for clinical use as they do not involve calculations that require a nomogram or a computational program, or complex statistical algorithms. These two models have been applied to different populations from different countries in further studies, and different sensitivity and specificity results have been reported. For this retrospective study on Turkish premature infants, two different models were applied to the same cohort to determine which model has a higher sensitivity and specificity.

The G-ROP model was developed as a screening model that includes postnatal slow WG measurements using data from 7483 infants at 29 hospitals in the United States and Canada. In this study, it was shown that the G-ROP criteria had a 100% sensitivity rate when flagging 459 infants who developed Type 1 ROP, reducing the number of infants to be screened for ROP by 30%.[12] In a validation study for the G-ROP model, Binenbaum et al. reported that the model was 100% sensitive for a prospective validation cohort (G-ROP-2) and validated the criteria, concluding that when used clinically in the United States and Canada the model could reduce the number of infants receiving treatment.[16] Shiraki et al.[17] applied the G-ROP model to a Japanese cohort of 537 infants, reporting that the model had a sensitivity of 91.9% for any degree of ROP and 100% for Type 1 ROP. Similarly, a validation study conducted on an Egyptian and UK cohort of patients found that the model had a 100% sensitivity when detecting Type 1 ROP. In the same study, the sensitivity level for the detection of some degree of ROP was found to be 97.1% in the Egyptian cohort and 97.3% in the UK cohort.[18]

In a validation study conducted on the Turkish population, which included a cohort of 242 infants, the sensitivity of the G-ROP model at detecting some degree of ROP was 88.3%, and was 91.2% for the treated group.[19] In our cohort, the G-ROP model had a sensitivity rate of 88.7% when identifying infants at risk of developing some degree of ROP. When the model was applied to the treated group who were diagnosed with Type 1 ROP or A-ROP, the level of sensitivity rose to 93.3%. The different sensitivity levels in different populations may be due to differences in demographics, ethnic features, and postnatal care services, as well as variability in infants' oxygen requirements.

Despite the high sensitivity rates reported in these studies, specificity rates are low. Shiraki et al. [17] reported that the specificity of the G-ROP model for any degree of ROP and for the Type 1 ROP Group in the Japanese cohort was 28.9% and 45.3%, respectively. In the Turkish cohort, the specificity of the G-ROP model was 51.7% for any degree of ROP and 34.1% for ROP requiring treatment.[19] In our study, the specificity of the G-ROP model was 10.9% for any degree of ROP, while it was 11.7% for the treated group—lower than the rates reported in previous studies.

The CO-ROP model was originally developed at a tertiary center in Colorado to investigate the association between postpartum WG and the risk of ROP. In the first CO-ROP study with 499 infants, the sensitivity rate for detecting severe ROP was 100%, while the sensitivity rate for detecting any degree of ROP was 96.4%. The number of infants requiring screening decreased by 23.7%.[17] In a validation study that included 858 cases from four centers in the United States, the sensitivity of the CO-ROP algorithm was 98.1% for Type 1 ROP and 95% for detecting any degree of ROP.[20] A subsequent validation study in a large population of 7438 infants from different ethnic groups reported a sensitivity level of 96.9%, and a specificity level of 40.9% when detecting infants who developed ROP, reporting a 23.9% reduction in the number of infants screened for CO-ROP.[21] Similarly, in another study involving different ethnic groups, the CO-ROP model was applied to 374 premature infants and it was found to have a sensitivity level of 93.1% for Type 1 ROP as opposed to a sensitivity level of 84.8% when identifying any stage ROP.[22] When the CO-ROP model was applied to our cohort, the model's sensitivity at detecting any stage ROP was 87.3%, while it performed very well for the treated group with a 100% rate. In addition, application of the model reduced the number of infants examined by 24.6%, based on current scanning criteria. Meanwhile, the specificity of the CO-ROP model was 40% for any stage ROP, and 27.9% for the treated group.

When the risk of missing even a single infant who requires ROP treatment is so serious, it is imperative that an efficient screening algorithm of the highest sensitivity levels is developed. The G-ROP algorithm used in the present study was unable to identify eight infants who developed some degree of ROP and one infant who developed ROP that required treatment. Meanwhile, the CO-ROP algorithm missed nine infants who developed some degree of ROP. This highlights the need for further modifications to the models. Multiple studies show that many risk factors have been associated with developing ROP such as bronchopulmonary dysplasia, cardiac pathologies, intraventricular hemorrhage and sepsis.[23,24] A significant finding of the present study was that the infants the models failed to diagnose had sepsis and cardiac pathology. When the criteria in this study were modified and cardiac pathology was determined as a criterion, the sensitivity of the G-ROP model increased to 94.4%. The CO-ROP model's sensitivity at detecting any stage ROP increased to 97.2% when cardiac pathology criteria were added to the screening criteria. In addition to this, when applying the modified criteria the model was 100% successful at detecting all infants who developed ROP that required treatment.

This study has some limitations. Primarily, it is a retrospective study. However, despite its

retrospective design, the clinical data included in our analyses were obtained from reliable sources in neonatal intensive care units and routinely recorded. Secondly, it is a single-center study with a relatively small sample size compared to other validation studies. Despite these limitations, our study adds to the growing evidence that postpartum WG may be a predictor of ROP, which could be a useful detail to include in ROP screening guidelines.

To conclude, this study found that the G-ROP and CO-ROP models have a high degree of sensitivity when predicting the development of ROP, but that they are not 100% accurate. The CO-ROP model proved to be more efficient at detecting cases developing ROP that required treatment. It was observed that the sensitivity of both models was increased by adding cardiac pathology criteria to detect all cases requiring treatment. Our findings should be confirmed by multicenter studies with a larger cohort.

Ethical approval

The study was approved by the Institutional Review Board (IRB) of Gazi Yasargil Training and Research Hospital Ethics Committee (09.07.2021-Number: 851).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DYE, OLŞ, NO; data collection: DYE, OLŞ; analysis and interpretation of results: HB; draft manuscript preparation: DYE,OLŞ,HB,NO. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Terry TL. Fibroblastic overgrowth of persistent tunica vasculosa lentis in infants born prematurely: II. report of cases-clinical aspects. Trans Am Ophthalmol Soc 1942; 40: 262-284.
2. Good WV; Early treatment for retinopathy of prematurity cooperative group. Final results of the early treatment for retinopathy of prematurity (ETROP) randomized trial. Trans Am Ophthalmol Soc. 2004;102:233-250.
3. Fierson WM; American Academy of Pediatrics Section on Ophthalmology; American Academy of Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus; American Association of Certified Orthoptists. Screening examination of premature infants for retinopathy of prematurity. Pediatrics 2013; 131: 189-195. https://doi.org/10.1542/peds.2012-2996
4. Gunay M, Celik G, Tuten A, Karatekin G, Bardak H, Ovali F. Characteristics of severe retinopathy of prematurity in infants with birth weight above 1500 grams at a referral center in Turkey. PLoS One 2016; 11: e0161692. https://doi.org/10.1371/journal.pone.0161692
5. Tiryaki Demir S, Karapapak M, Uslu HS, et al. Retinopathy screening results of late-preterm infants born at 32-35 weeks of gestational age. Graefes Arch Clin Exp Ophthalmol 2019; 257: 1325-1329. https://doi.org/10.1007/s00417-019-04298-y
6. Early Treatment For Retinopathy of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. Arch Ophthalmol 2003; 121: 1684-1694. https://doi.org/10.1001/archopht.121.12.1684
7. Cao JH, Wagner BD, McCourt EA, et al. The Colorado-retinopathy of prematurity model (CO-ROP): postnatal weight gain screening algorithm. J AAPOS 2016; 20: 19-24. https://doi.org/10.1016/j.jaapos.2015.10.017
8. Ahmed IS, Badeeb AA. The Alexandria retinopathy of prematurity model (Alex-ROP): Postnatal weight gain screening algorithm application in a developing country. Inter J Ophthalmol 2019; 12: 296-301.
9. Binenbaum G, Ying G-S, Quinn GE, et al. The CHOP postnatal weight gain, birth weight, and gestational age retinopathy of prematurity risk model. Arch Ophthalmol 2012; 130: 1560-1565. https://doi.org/10.1001/archophthalmol.2012.2524

10. Wu C, Vanderveen DK, Hellström A, Löfqvist C, Smith LEH. Longitudinal postnatal weight measurements for the prediction of retinopathy of prematurity. Arch Ophthalmol 2010; 128: 443-447. https://doi.org/10.1001/archophthalmol.2010.31

11. Löfqvist C, Andersson E, Sigurdsson J, et al. Longitudinal postnatal weight and insulin-like growth factor I measurements in the prediction of retinopathy of prematurity. Arch Ophthalmol 2006; 124: 1711-1718. https://doi.org/10.1001/archopht.124.12.1711

12. Binenbaum G, Bell EF, Donohue P, et al. Development of modified screening criteria for retinopathy of prematurity: primary results from the postnatal growth and retinopathy of prematurity study. JAMA Ophthalmol 2018; 136: 1034-1040. https://doi.org/10.1001/jamaophthalmol.2018.2753

13. Smith LE, Shen W, Perruzzi C, et al. Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. Nat Med 1999; 5: 1390-1395. https://doi.org/10.1038/70963

14. Hellstrom A, Perruzzi C, Ju M, et al. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc Natl Acad Sci U S A 2001; 98: 5804-5808. https://doi.org/10.1073/pnas.101113998

15. Jensen AK, Ying GS, Huang J, Quinn GE, Binenbaum G. Postnatal serum insulin-like growth factor I and retinopathy of prematurity. Retina 2017; 37: 867-872. https://doi.org/10.1097/IAE.0000000000001247

16. Binenbaum G, Tomlinson LA, de Alba Campomanes AG, et al. Validation of the postnatal growth and retinopathy of prematurity screening criteria. JAMA Ophthalmol 2020; 138: 31-37. https://doi.org/10.1001/jamaophthalmol.2019.4517

17. Shiraki A, Fukushima Y, Kawasaki R, et al. Retrospective validation of the postnatal growth and retinopathy of prematurity (G-ROP) criteria in a Japanese cohort. Am J Ophthalmol 2019; 205: 50-53. https://doi.org/10.1016/j.ajo.2019.03.027

18. Ahmed ISH, Aclimandos W, Azad N, et al. The Postnatal growth and retinopathy of prematurity model: a multi-institutional validation study. Ophthalmic Epidemiol 2022; 29: 296-301. https://doi.org/10.1080/09286586.2021.1939885

19. Yabas Kiziloglu O, Coskun Y, Akman I. Assessment of the G-ROP study criteria for predicting retinopathy of prematurity: results from a tertiary centre in Turkey. Int Ophthalmol 2020; 40: 1647-1652. https://doi.org/10.1007/s10792-020-01332-5

20. Cao JH, Wagner BD, Cerda A, et al. Colorado retinopathy of prematurity model: a multi-institutional validation study. J AAPOS 2016; 20: 220-225. https://doi.org/10.1016/j.jaapos.2016.01.017

21. McCourt EA, Ying GS, Lynch AM, et al. Validation of the Colorado retinopathy of prematurity screening model. JAMA Ophthalmol 2018; 136: 409-416. https://doi.org/10.1001/jamaophthalmol.2018.0376

22. Huang JM, Lin X, He YG, Cao JH. Colorado retinopathy of prematurity screening algorithm (CO-ROP): a validation study at a tertiary care center. J AAPOS 2017; 21: 152-155. https://doi.org/10.1016/j.jaapos.2017.03.009

23. Kim SJ, Port AD, Swan R, Campbell JP, Chan RVP, Chiang MF. Retinopathy of prematurity: a review of risk factors and their clinical significance. Surv Ophthalmol 2018; 63: 618-637. https://doi.org/10.1016/j.survophthal.2018.04.002

24. Hadi AM, Hamdy IS. Correlation between risk factors during the neonatal period and appearance of retinopathy of prematurity in preterm infants in neonatal intensive care units in Alexandria, Egypt. Clin Ophthalmol 2013; 7: 8317.

The Turkish Journal of Pediatrics 2023; 65: 406-415
https://doi.org/10.24953/turkjped.2022.860

Original Article

Nutritional rickets in Turkish and refugee children aged 0-2: an increasing problem despite vitamin D prophylaxis

İsmail Dündar[1], Mehmet Akif Büyükavcı[2]

[1]Department of Pediatric Endocrinology, Faculty of Medicine, Inonu University, Malatya; [2]Department of Developmental Pediatrics, Faculty of Medicine, Inonu University, Malatya, Türkiye.

ABSTRACT

Background. Nutritional rickets (NR) is still a major problem and is exacerbated by an increasing influx of immigrants. In this study, Turkish and immigrant cases followed with the diagnosis of NR in our pediatric endocrinology clinic were retrospectively evaluated.

Methods. Detailed data of cases diagnosed with NR between 2013 and 2020 and followed for at least six months were reviewed.

Results. In the study period, 77 cases of NR were identified. Turkish children constituted 76.6% (n=59) while 18 (23.4%) were immigrant children. The mean age at diagnosis was 8.1±7.8 months, 32.5% (n=25) were female, and 67.5% (n=52) were male. The 25-hydroxyvitamin D3 was below normal in all patients, with a mean value of 4.3±2.6 ng/mL. Parathyroid hormone (PTH) was above normal in all and the mean value was 301.7±139.3 pg/mL. While there were 3.9 cases of NR in 10,000 endocrine clinic patients in 2013, this rate increased more than four-fold to 15.7 patients in 2019.

Conclusions. Despite the vitamin D prophylaxis program in Türkiye, NR is seen significantly more frequently in recent years, which may be associated with an increasing number of refugees. High PTH levels indicate the severity of NR cases admitted to our clinic. However, clinically significant NR is only the tip of the iceberg and the true burden of subclinical rickets is unknown. Increasing compliance with the vitamin D supplementation program in refugee and Turkish children is important for the prevention of nutritional rickets.

Key words: children, immigrant, nutritional rickets, vitamin D deficiency.

Despite inexpensive and effective treatment options, nutritional rickets (NR) remains a global public health problem.[1] NR is a disease characterized by defective chondrocyte differentiation and insufficient mineralization of the growth plate and osteoid tissue, that develops before the closure of the epiphyseal plates, due either to vitamin D deficiency or insufficient calcium intake. However, the most common cause of rickets is vitamin D deficiency.[1,2] In addition to various deformities in skeletal structure, serious findings such as tetany secondary to hypocalcemia, convulsions, laryngospasm, muscle weakness, and cardiomyopathy can also be seen in NR.[2]

NR remains an important public health problem in both developed and developing nations, such as Türkiye, but its incidence differs between countries and regions. In Türkiye, the frequency of NR has previously been reported to be between 1.67%-19%.[3,4] In recent years, the importance of vitamin D prophylaxis in preventing NR has been acknowledged and implemented in many countries.[2] For NR, a preventable disease, a five-year project (Prevention of Vitamin D Deficiency and Protection of Bone Health Project) was initiated in Türkiye in 2005, under the management of the Ministry of Health, General Directorate of Mother-Child Health and Family Planning.[3] The main aim of this project was to reduce the

✉ Mehmet Akif Büyükavcı
akifavci@yahoo.com

Received 22nd September 2022, revised 4th March 2023, 9th April 2023, accepted 18th April 2023.

frequency of NR by giving 400 IU of vitamin D daily to all children starting from birth. While the NR frequency before this project was 6% in a study conducted by Ozkan et al.[5] in 1998 in Erzurum for children aged 0-3 years, another study by Ozkan et al.[6] in 2008 showed that the NR frequency had decreased to 0.1%. This was evidence of the beneficial effect of the prophylaxis program.

However, and especially in recent years, NR cases have been encountered frequently in our clinic, and their medical histories revealed that prophylactic vitamin D was not used, or was only used sporadically, or for a very short time. This anecdotal evidence suggests a possible disruption (at least regionally) of the functioning of the prophylaxis program over time since the beginning of the project. Furthermore, immigrant children in Türkiye are at increased risk of NR due to poor living conditions, poor nutrition, and lack of access to health services.[7-9] With the civil war that started in Syria in 2011, a migrant crisis has emerged in various countries, especially in Türkiye. According to the latest figures obtained from the Presidency of Migration Management, there are 3.6 million Syrian refugees who have gained temporary protection status in Türkiye, and most of these people are not in the camps, but scattered throughout the country.[10] The distribution of Syrians under temporary protection by years is shown in Figure 1. In our country, Syrian migrant children are secured within the scope of preventive medicine. Therefore, this study aimed to retrospectively evaluate patients followed up with a diagnosis of NR in our pediatric endocrinology outpatient clinic between 2013 and 2020, with their clinical, laboratory, and radiological findings.

Material and Methods

Medical records of patients followed for NR in the Pediatric Endocrinology Clinic between October 2013 and July 2020, and the digital databank of the hospital were retrospectively reviewed. Patients with follow-up data for at least six months were included in the study. Age, sex, vitamin D prophylaxis status, symptoms at admission, anthropometric data, physical examination, laboratory evaluation, and imaging findings (wrist/knee X-rays) and treatments, and if any, length of stay in the

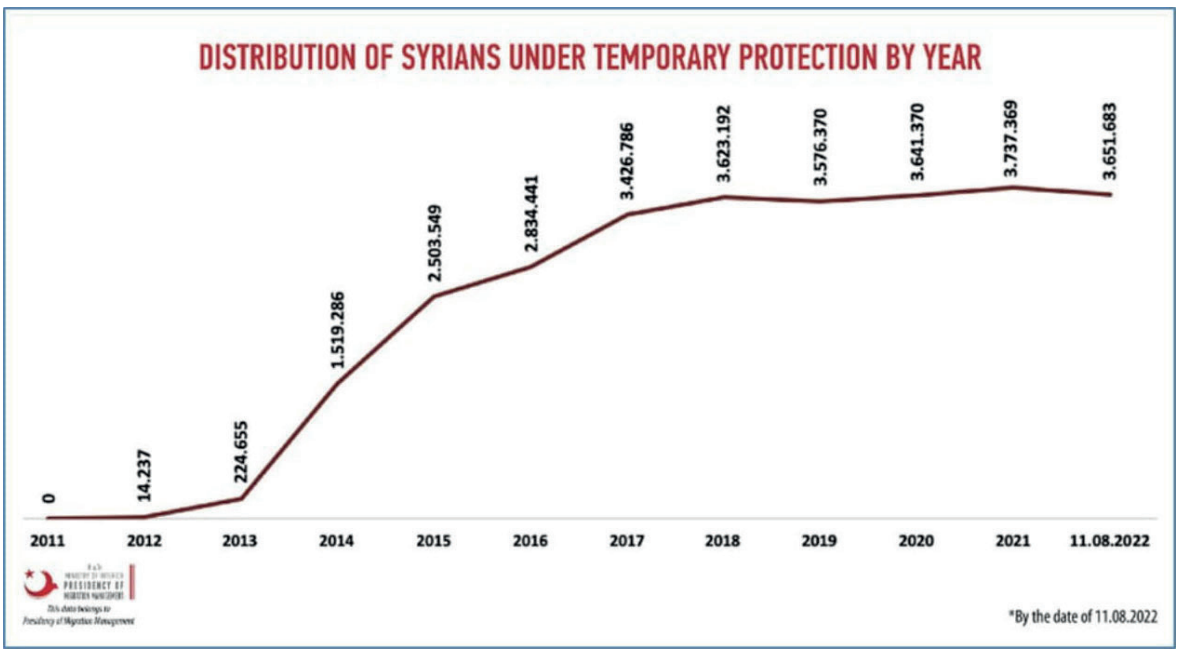


Fig. 1. Distribution of Syrians under temporary protection by year.[10]

hospital and recovery process information were recorded. Anthropometry included height, standard deviation score for height (height SDS), weight, weight SDS, body mass index (BMI), and BMI SDS. Laboratory results included serum calcium, phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH), and 25-hydroxy vitamin D (25(OH)D) levels.

The diagnosis of NR was made clinically (skeletal deformities and/or hypocalcemia) and by laboratory findings (hypocalcemia or normocalcemia, high ALP and PTH, low 25(OH)D) and supported by direct X-ray imaging. Anthropometric evaluations were made using the CHILD METRICS program[2], the anthropometric calculation program of the Turkish Society of Pediatric Endocrinology and Diabetes, according to the data of healthy Turkish children created by Neyzi et al.[11,12] The laboratory data of the patients were evaluated considering age-specific reference values of the laboratory. Reference ranges were 8.4-11 mg/dL for serum calcium, 134-350 mg/dL for ALP, and 14-72 pg/mL for PTH. The normal level of serum phosphorus in the first days of life is 4.8-8.2 mg/dL and then 3.8-6.5 mg/dL. A 25(OH)D level of <12 ng/mL was considered vitamin D deficiency, and a level of <5 ng/mL was considered severe vitamin D deficiency.[2] Defective mineralization appears radiographically as growth plate widening, as well as metaphyseal cupping and fraying, which confirm the diagnosis of NR.[2] Radiological evaluations were performed by a pediatric endocrinologist.

The study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (document number: E-23536505-000-3592, date:14.03.2022, Malatya Training and Research Hospital).

Informed consent was not obtained because of the retrospective nature of the study.

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 21.0, was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Categorical variables were analyzed using the chi-square test. Normal distribution of data was evaluated with the Kolmogorov-Smirnov test. Student t-test and Mann-Whitney U test were used to compare the groups for numerical data, as appropriate to distribution. Descriptive statistics for the data are given as median (minimum-maximum) for skewed parameters and mean±SD for normally-distributed parameters. A p-value of <0.05 was considered significant.

Results

The records of a total of 83 patients were accessed but six of the cases were excluded due to insufficient data. Finally, 77 patients with NR, aged 0-2 years, were included in the study. While 59 (76.6%) of the 77 cases were Turkish children, 18 (23.4%) were immigrants. All of the immigrants were of Syrian origin. The distribution of all patients admitted to the pediatric endocrinology outpatient clinic and patients with NR according to the years of diagnosis is shown in Figure 2. In addition, the number of patients with NR among the 10,000 pediatric endocrinology clinic patients per year is shown in Figure 2. While the number of NR patients per 10,000 pediatric endocrinology clinic patients was 3.8 in 2013, this rate increased more than four-fold to 15.7 in 2019 (Fig. 2). When distributed according to diagnosis months, March and January were the most common, and September and October were the least common (Fig. 3). All patients were under the age of two, and the mean age at diagnosis was 8.1±7.8 months (Median 5.9 months). In terms of sex, 32.5% (n=25) were female, and 67.5% (n=52) were male. Of the 77 cases whose birth weight and week were recorded, 11 were preterm. In nine patients, there was a history of small for gestational age (SGA). Anthropometric measurements by vitamin D level and ethnicity are shown in Table I.

Turkish and immigrant children were compared in terms of some clinical and laboratory findings,

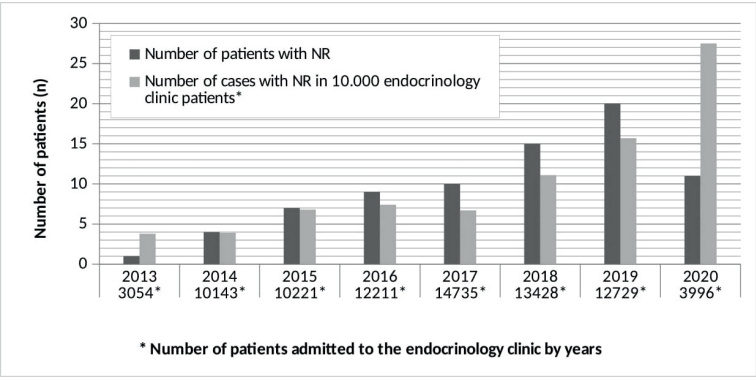


Fig. 2. Distribution of all patients admitted to the pediatric endocrinology outpatient clinic and patients with NR according to the years of diagnosis. In addition, the number of patients with NR per 10,000 pediatric endocrinology clinic patients per year (3 months in 2013 and 7 months in 2020).

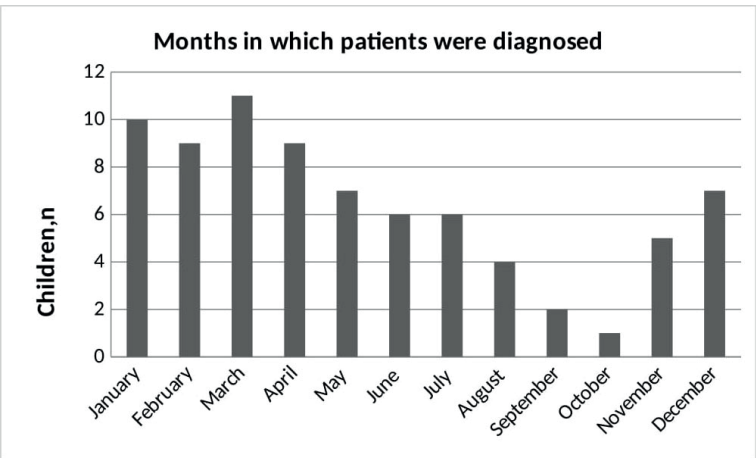


Fig. 3. Months in which patients were diagnosed.

Table I. Anthropometric measurements according to vitamin D level and ethnicity at the time of diagnosis (n=77).

	Full cohort n (%)	Turkish children (n=59)	Immigrant children (n=18)	*p*	25(OH)D ≤ 5 (n=47)	25(OH)D >5 (n=30)	*p*
Mean age, months	8.1±7.8	7.9±7.5	8.7±8.1	0.702	7.1±7.8	9.6±7.7	0.162
Sex (female/ male)	25/52 (32.5/67.5)	18/41 (30.5/69.5)	7/11 (38.9/61.1)	0.570	13/34 (27.2/73.2)	12/18 (40/60)	0.321
Birth weight (g)	3149±440	3115±63	3292±64	0.195	3077±48	3258±75	0.096
Height (cm)	67.6±11.2	66.7±11.0	70.3±2.8	0.230	64.4±11.2	72.6±9.2	**0.001**
Height SDS	-0.59±1.09	-0.58±1.0	-0.60	0.970	-0.60±1.2	-0.57±9.2	0.915
Body weight (kg)	7.6±3.0	7.5±3.1	8.1±2.8	0.442	6.7±3.0	9.1±2.3	**<0.001**
Body weight SDS	-0.46±1.1	-0.35±1.2	-0.8±0.8	0.148	-0.47±1.1	-0.43±1.1	0.876
BMI SDS	-0.01±1.1	0.04±1.2	-2.1±0.8	0.391	-0.10±1.1	0.12±1.1	**0.001**

SDS: standard deviation score, BMI: body mass index, 25(OH)D: 25-hydroxyvitamin D3 (ng/mL)

but no statistically significant difference was found. At presentation, short stature (height SDS <-2 SD) was found in 8 (13.5%) Turkish children, while this number was 2 (11.1%) in immigrant children (p=0.573). There were 2 (3.3%) Turkish and 1 (5.5%) immigrant child with BMI SDS <-2 SD (p=0.556). In our study, the number of children with 25(OH)D value <5 was 47 (61%) and 36 (61%) of them were Turkish and 11 (61%) were immigrants (p=0.610). Of the 55 children admitted with hypocalcemia (Ca<8.4 mg/dL), 44 (74.5%) were Turkish and 11 (61%) were immigrants (p=0.164). The number of Turkish children admitted with clinical findings only was 35 (59.3%) and the number of immigrant children was 11 (61%) (p=0.344). A total of 52 mothers had vitamin D levels (immigrant: 14). The number of Turkish mothers with 25(OH) D level <5 was 24 (63.1%) and the number of immigrant mothers was 9 (64.2%) (p=0.603).

The most common reasons for referral to the pediatric endocrinology outpatient clinic were incidentally-detected low calcium/vitamin D levels, bowed legs, and convulsions. Complaints at the time of diagnosis according to vitamin D level and ethnicity are shown in Table II. The most common findings on physical examination were rachitic rosary, enlarged wrists, and genu varum, in descending frequency. In cases of severe vitamin D deficiency; convulsions, rachitic rosary, and wrist enlargement were found to be significantly more common. When the laboratory data were evaluated, hypocalcemia was found in 68.8% (53/77) of the cases, hypophosphatemia was found in 61.0% (47/77), while ALP levels were high in 94.8% (73/77), and PTH levels were invariably high in all 77 cases examined. The 25(OH)D level was below normal in all cases. The mean vitamin D level was 4.3±2.6 ng/mL, and severe deficiency was found in 61.0% (47/77) of the patients. The level of vitamin D in the cases and the biochemical parameters according to ethnicity are shown in Table III.

Information about vitamin D prophylaxis was recorded in the records of 77 patients. It was noted that 55 (71.4%) patients never received vitamin D prophylaxis, whereas 22 patients

Table II. Complaints at the time of diagnosis according to vitamin D level and ethnicity (n=77).

	Full cohort n (%)	Turkish children	Immigrant children	*p*	25(OH)D≤5 (n:47)	25(OH)D>5 (n:30)	*p*
Low Ca/25(OH)D in incidental examination	29 (37.7)	23 (39.0)	6 (37.7)	0.784	14 (29.8)	15 (50.0)	0.062
Bowed legs	29 (37.7)	19 (32.2)	10 (55.6)	0.067	16 (34)	13 (43.3)	0.280
Convulsions	21 (27.3)	13 (22.0)	8 (44.4)	0.062	20 (42.6)	1 (3.3)	**<0.001**
Hand cramps, tremors	13 (16.9)	11 (18.6)	2 (11.1)	0.455	10 (21.3)	3 (23.1)	0.198
Anterior fontanelle width	9 (11.7)	7 (11.9)	2 (11.1)	0.931	6 (12.8)	3 (10.0)	0.507
Delay/problem in walking	5 (6.5)	3 (5.1)	2 (11.1)	0.332	2 (4.3)	3 (10.0)	0.072

ALP: alkaline phosphatase, Ca: calcium, 25(OH)D: 25-hydroxyvitamin D3 (ng/mL)

Table III. The level of vitamin D in the cases and the biochemical parameters according to ethnicity (n=77).

	Full cohort n (%)	Turkish children	Immigrant children	*p*	25(OH)D≤5	25(OH)D>5	*p*
Corrected serum calcium (mg/dL)	7.4±1.2	7.4±1.2	7.1±1.2	0.279	6.8±1.0	8.2±0.9	**<0.001**
Phosphorus (mg/dL)	3.4±0.7	3.4±0.7	3.4±0.7	0.788	3.2±0.6	3.6±0.8	**0.042**
ALP (IU/L)	914±401	905±644	943±489	0.903	1002±305	777±492	**0.016**
PTH (pg/mL)	301.7±139.3	302±146	298±114	0.922	146±21	116±21	**0.028**
25(OH)D (ng/mL)	4.3±2.6	4.0±2.5	5.3±2.8	0.097	2,6±1.3	6.9±1.7	**<0.001**

ALP: alkaline phosphatase, PTH: parathormone, 25(OH)D: 25-hydroxyvitamin D3 (ng/mL)

used it irregularly. Vitamin D levels of 52 mothers were measured, and the mean 25(OH) D level was 4.6 (range: 3-12) ng/mL.

There were no direct radiographic images available in 22 cases, whereas the imaging was suboptimal in five cases. In the 50 available records, the most common findings in the direct radiographs were cupping (84.0%; 42/50) and enlargement and fraying (76.0%; 38/50) in the wrist and/or knee metaphyses. In the patient with the most severe skeletal findings, a greenstick fracture and extensive osteopenia were detected in the bilateral radius diaphysis, except for metaphyseal findings. Stoss therapy with vitamin D (150,000 or 300,000 IU oral or IM) was administered in 16 cases (20.8%) of NR, long-term high-dose vitamin D (2,000 or 5,000 IU/day for six weeks) in fifty-five (71.4%), and 60,000 IU stoss + 5,000 IU/day in six cases (7.8%). Fifteen of the 22 (68.2%) patients who received stoss treatment were children of immigrants. Additional calcium was also added to the treatment in the form of intravenous and/or oral elemental calcium.

Discussion

This article draws attention to the problem of vitamin D deficiency and nutritional rickets in the light of the latest information in the literature and data in our country. Despite the vitamin D support programs, NR continues to be a significant and preventable public health problem worldwide. Vitamin D prophylaxis is recommended at 400 IU/day in the first year of life.[2,13] In studies conducted in Türkiye, the regular use of 400 IU/day of vitamin D was effective in preventing rickets.[14,15] NR cannot be completely eliminated in developed countries due to immigration from underdeveloped regions, and it has been reported that its frequency has increased recently.[7-9,16] The primary cause of NR is vitamin D deficiency worldwide. However, the inadequate calcium intake in underdeveloped regions is also seen as an important cause.[2,16] In Türkiye, the most common reason for NR has been shown to be vitamin D deficiency.[6,14,15,17] However, vitamin D supplementation was never used in more than 70% (n=55) of these patients who developed NR, and it was used irregularly by the remaining 22 cases. Inadequate and irregular use of vitamin D by our patients suggested that they did not know the importance of vitamin D and had problems in compliance with drug use.

Rickets due to vitamin D deficiency is most common in children under two years of age, with the highest incidence occurring between 3-18 months.[1,18] In children aged 0-3 years, NR is more common in boys.[19] In our study, the mean age at diagnosis was 8.1±7.8 months, and the male:female ratio was 2.08:1. While clinical findings in NR may include convulsions, tetany, respiratory problems, and heart failure due to hypocalcemia, skeletal findings, such as craniotabes, rachitic rosary, Harrison's groove, bowed legs, and enlargement of the joints can be seen due to insufficient mineralization in the fast-growing bones.[2,20] The most common causes of hospital admission in our patients in decreasing order were incidentally-detected low Ca or vitamin D, bowed legs, convulsions, and contraction and trembling in the hands, whereas the most common examination findings were rachitic rosary, enlargement of the wrists, and genu varum (bowlegs). In the study of Ward et al.[21], the most common reason for admission under one year of age was hypocalcemic convulsions, while skeletal deformities were observed in older children. NR may present with life-threatening findings and may also lead to permanent skeletal deformities.[2,21] However, no permanent skeletal deformity was detected in any of our patients.

Nutritional status, including vitamin D status, affects not only growth but also skeletal maturity in childhood.[1,20] Cesur et al.[17] found short stature in 34.4% of patients with NR between 0 and 2 years of age. NR has been associated with stunted growth, which is commonly observed in Afghan children (a country with one of the highest rates of malnutrition in the world).[22] In our study, short stature was found in 13% of the cases. Severe vitamin D deficiency was

associated with shorter height, lower weight and lower BMI SDS. In addition, the frequency of convulsions increased and metabolic parameters [lower calcium and phosphorus, higher ALP and PTH] were more impaired in severe vitamin D deficiency.

To maintain bone health in children, vitamin D levels must be sufficient. "Adequate vitamin D level" is defined as a serum 25(OH)D level >20 ng/mL. To maintain this level, the American Institute of Medicine (IOM) and the American Academy of Pediatrics (AAP) recommend 400 U/day of vitamin D be given to infants for the first 12 months.[23,24] Vitamin D levels were ≤ 11.9 ng/mL in all our patients and severe vitamin D deficiency was found in 61% of the cases.

Vitamin D supplementation for infants, adding vitamin D to various foods, and increasing sunlight exposure have been studied, and some protection strategies have been developed to prevent NR worldwide.[25,26] Additionally, sufficient calcium intake is advocated in underdeveloped countries.[27]

Considering the period of study starting in 2013, cases have increased over time, especially in 2019 and 2020 (Fig. 2). The dramatic increase in 2020 can be explained by the decrease in total hospital admissions due to the COVID-19 pandemic. This raises the question of whether there is a problem with the functioning of the prophylaxis program. In their incidence study in Canada, Ward et al.[21] detected 104 NR cases aged between 2 weeks and 6.3 years in a two-year period, and none of the patients had received prophylactic vitamin D supplementation. The authors argued that vitamin D prophylaxis is recommended in the current guidelines, but it is not applied consistently. We wish to highlight that refugee children are a much more vulnerable subpopulation than settled children and adults. For this reason, all countries should develop a public health policy that provides vitamin D supplementation and adequate calcium needs to protect refugee children, whose numbers have been increasing across the globe in recent years, as well as protecting indigenous children living in any high risk environment.

Although rickets prevention projects exist through prophylaxis programs and food-infused vitamin supplements in developed countries, the frequency of rickets is still increasing due to immigration. In Europe and America, most rickets cases are due to vitamin D deficiency and are frequently seen in children with dark skin coming from Asia, the Middle East, and Africa.[16,20] Asylum seekers are disadvantaged concerning preventive health services. This may be due to poor living conditions, nutritional problems, language barriers, financial difficulties, and lack of access to health services.[7,20] Türkiye received significant immigration from Syria in recent years (Fig. 1). Of the 77 cases identified in our study, 18 (23.4%) were immigrant children, suggesting a major contribution of immigration to NR frequencies. Thus, the implementation and maintenance of prophylaxis for immigrant babies will reduce the incidence of NR in Türkiye.

Immigrant health centers (IHCs) affiliated with community health centers have been established in areas with a high concentration of migrants in order to provide preventive health services and primary health care services more effectively and efficiently for Syrians in Türkiye and to increase access to health services. The costs related to the expansion and operation of IHCs are covered by the SIHHAT Project funded by the European Union.[28] These centers implement the same vaccination program for migrants as for Turkish citizens. All children receive 400 units/day of vitamin D supplementation until the age of one year. However, rickets can be seen not only in migrants or refugees but also in resident children. Therefore, all countries should consider vitamin D supplementation and adequate calcium intake as a public health policy to protect children from this preventable disease.

Our study found vitamin D deficiency in all 52 mothers whose vitamin D levels were checked. Severe vitamin D deficiency is common in pregnant women and newborns in low socioeconomic provinces of Türkiye.[29] Vitamin D deficiency in pregnant women can cause

congenital rickets in babies who do not receive adequate vitamin D support. When Paterson et al.[30] analyzed 24 cases diagnosed with rickets due to neonatal vitamin D deficiency, 11 of the 12 mothers with known serum vitamin D levels had a vitamin D measurement below 10 ng/mL, and symptomatic osteomalacia was found in 16 of the mothers. The risk factors for maternal vitamin D deficiency are low socioeconomic status, covered clothing for religious/cultural reasons, and low educational level, which are frequently observed in Türkiye.[31] In addition to these, language problems, inability to access preventive healthcare services and inability to continue follow-ups due to address changes increase the risk in migrant Syrian mothers.[7] The Vitamin D prophylaxis program for pregnant women, initiated by the Ministry of Health in 2011 is still ongoing.[32] This program should be followed and supported as well.

In the treatment of NR, oral vitamin D can be given as a single daily dose for three months or in high doses for patients >3 months in 1-3 divided doses. In addition, adequate calcium intake (500 mg/day) or oral calcium supplementation for 10-15 days is recommended.[2] Most (71.4%) of our patients were treated with a daily dose, while 20.8% received a single high dose and 7.8% received both a high dose and a daily dose. Compliance with treatment can be expected to be low among immigrants struggling with difficulties such as obtaining adequate food, shelter and the experience of leaving their homes. For these reasons, we believed that stoss treatment would be more appropriate, as we predicted that compliance with daily vitamin D use would be low in refugee families. NR patients with findings of severe hypocalcemia, such as convulsions, tetany, and respiratory distress, must be hospitalized. Hospitalization and intravenous calcium support were required in 32 of our patients (41.5%).

Some limitations of the study should be noted. The study was a retrospective file-based study and in some patients the symptoms may not have been systematically evaluated or tested. Secondly, there are two pediatric endocrinology centers in Malatya, and cases treated with NR in the other pediatric endocrinology center were not included. Therefore, our study does not provide incidence data for Malatya province. Thirdly, since we aimed to investigate issues related to the functioning of the prophylaxis program by revealing the frequency of cases seen in our center, the study did not include a control group. Fourthly, the very high PTH level in all our cases suggests that only the most severe cases of NR were seen during the study period. Therefore, the actual disease burden in Türkiye is likely to be much heavier and the true incidence of subclinical rickets is unknown. Finally, feeding pattern, time of sun exposure and other risk factors for NR were not assessed.

Despite the vitamin D prophylaxis available in Türkiye, there has been a trend towards an increasing incidence of NR in recent years. Since NR is completely preventable, universal vitamin D supplementation and adequate calcium should be provided, not only for the resident population but also for refugee and migrant children. Mothers should be encouraged to use vitamin D during breastfeeding. To reduce the frequency of NR it will be important to continue the vitamin D prophylaxis program for infants, which was initiated to prevent NR and was shown to be successful, and to carry out an ongoing clinical audit of the program to identify any problems and thus improve its effectiveness.

Ethical approval

The study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (document number: E-23536505-000-3592, date:14.03.2022). Informed consent was not obtained because of the retrospective nature of the study.

Author contribution

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

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Pettifor JM, Thandrayen K, Thacher TD. Vitamin D deficiency and nutritional rickets in children. In: Vitamin D. Elsevier; 2018: 179-201. https://doi.org/10.1016/b978-0-12-809963-6.00067-5
2. Munns CF, Shaw N, Kiely M, et al. Global consensus recommendations on prevention and management of nutritional rickets. Horm Res Paediatr 2016; 85: 83-106. https://doi.org/10.1159/000443136
3. Hatun Ş, Ozkan B, Bereket A. Vitamin D deficiency and prevention: Turkish experience. Acta Paediatr 2011; 100: 1195-1199. https://doi.org/10.1111/j.1651-2227.2011.02383.x
4. Ozkan B. Nutritional rickets. J Clin Res Pediatr Endocrinol 2010; 2: 137-143. https://doi.org/10.4274/jcrpe.v2i4.137
5. Ozkan B, Büyükavcı M, Aksoy H, Tan H, Akdağ R. Incidence of rickets among 0-3 year old children in Erzurum. Çocuk Sağlığı ve Hastalıkları Dergisi 1999; 42: 389-396.
6. Ozkan B, Doneray H, Karacan M, et al. Prevalence of vitamin D deficiency rickets in the eastern part of Turkey. Eur J Pediatr 2009; 168: 95-100. https://doi.org/10.1007/s00431-008-0821-z
7. Kurnaz E, Çetinkaya S, Elmaoğulları S, et al. A major health problem facing immigrant children: nutritional rickets. J Pediatr Endocrinol Metab 2021; 35: 223-229. https://doi.org/10.1515/jpem-2021-0420
8. Lane G, Farag M, White J, Nisbet C, Vatanparast H. Chronic health disparities among refugee and immigrant children in Canada. Appl Physiol Nutr Metab 2018; 43: 1043-1058. https://doi.org/10.1139/apnm-2017-0407
9. Pavlopoulou ID, Tanaka M, Dikalioti S, et al. Clinical and laboratory evaluation of new immigrant and refugee children arriving in Greece. BMC Pediatr 2017; 17: 132. https://doi.org/10.1186/s12887-017-0888-7
10. Republic of Turkiye Ministry of Interior Presidency of Migration Management. Temporary protection. Available at: https://en.goc.gov.tr/temporary-protection27 (Accessed on August 11, 2022).
11. Neyzi O, Bundak R, Gökçay G, et al. Reference values for weight, height, head circumference, and body mass index in Turkish children. J Clin Res Pediatr Endocrinol 2015; 7: 280-293. https://doi.org/10.4274/jcrpe.2183
12. Demir K, Özen S, Konakçı E, Aydın M, Darendeliler F. A comprehensive online calculator for pediatric endocrinologists: ÇEDD Çözüm/TPEDS metrics. J Clin Res Pediatr Endocrinol 2017; 9: 182-184. https://doi.org/10.4274/jcrpe.4526
13. Gartner LM, Greer FR, Section on Breastfeeding and Committee on Nutrition. American Academy of Pediatrics. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. Pediatrics 2003; 111: 908-910. https://doi.org/10.1542/peds.111.4.908
14. Hatun S, Bereket A, Ozkan B, Coşkun T, Köse R, Calýkoğlu AS. Free vitamin D supplementation for every infant in Turkey. Arch Dis Child 2007; 92: 373-374. https://doi.org/10.1136/adc.2006.113829
15. Mutlu GY, Kusdal Y, Ozsu E, Cizmecioglu FM, Hatun S. Prevention of Vitamin D deficiency in infancy: daily 400 IU vitamin D is sufficient. Int J Pediatr Endocrinol 2011; 2011: 4. https://doi.org/10.1186/1687-9856-2011-4
16. Creo AL, Thacher TD, Pettifor JM, Strand MA, Fischer PR. Nutritional rickets around the world: an update. Paediatr Int Child Health 2017; 37: 84-98. https://doi.org/10.1080/20469047.2016.1248170
17. Cesur Y, Doğan M, Ariyuca S, et al. Evaluation of children with nutritional rickets. J Pediatr Endocrinol Metab 2011; 24: 35-43. https://doi.org/10.1515/jpem.2011.108
18. el Hag AI, Karrar ZA. Nutritional vitamin D deficiency rickets in Sudanese children. Ann Trop Paediatr 1995; 15: 69-76. https://doi.org/10.1080/02724936.1995.11747751

19. Thirunagari R, Taha D. SAT-275 "Nutritional Rickets: Still A Problem In The 21StCentury." J Endocr Soc 2019; 3(Supplement_1): SAT-275. https://doi.org/10.1210/js.2019-sat-275

20. Vearing RM, Hart KH, Darling AL, et al. Global perspective of the Vitamin D status of African-Caribbean populations: a systematic review and meta-analysis. Eur J Clin Nutr 2022; 76: 516-526. https://doi.org/10.1038/s41430-021-00980-9

21. Ward LM, Gaboury I, Ladhani M, Zlotkin S. Vitamin D-deficiency rickets among children in Canada. CMAJ 2007; 177: 161-166. https://doi.org/10.1503/cmaj.061377

22. Walli NZ, Munubhi EK, Aboud S, Manji KP. Vitamin D levels in malnourished children under 5 years in a tertiary care center at Muhimbili National Hospital, Dar es Salaam, Tanzania-a cross-sectional study. J Trop Pediatr 2017; 63: 203-209. https://doi.org/10.1093/tropej/fmw081

23. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011; 96: 53-58. https://doi.org/10.1210/jc.2010-2704

24. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M; Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics 2008; 122: 398-417. https://doi.org/10.1542/peds.2007-1894

25. Gallo S, Comeau K, Vanstone C, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants: a randomized trial. JAMA 2013; 309: 1785-1792. https://doi.org/10.1001/jama.2013.3404

26. Aguiar M, Andronis L, Pallan M, Högler W, Frew E. The economic case for prevention of population vitamin D deficiency: a modelling study using data from England and Wales. Eur J Clin Nutr 2020; 74: 825-833. https://doi.org/10.1038/s41430-019-0486-x

27. Amrein K, Scherkl M, Hoffmann M, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr 2020; 74: 1498-1513. https://doi.org/10.1038/s41430-020-0558-y

28. Supporting Migrant Health Services in Turkey; SIHHAT project. Available at: http://www.sihhatproject.org/ (Accessed on March 1, 2023).

29. Parlak M, Kalay S, Kalay Z, Kirecci A, Guney O, Koklu E. Severe vitamin D deficiency among pregnant women and their newborns in Turkey. J Matern Fetal Neonatal Med 2015; 28: 548-551. https://doi.org/10.3109/14767058.2014.924103

30. Paterson CR, Ayoub D. Congenital rickets due to vitamin D deficiency in the mothers. Clin Nutr 2015; 34: 793-798. https://doi.org/10.1016/j.clnu.2014.12.006

31. Andiran N, Yordam N, Ozön A. Risk factors for vitamin D deficiency in breast-fed newborns and their mothers. Nutrition 2002; 18: 47-50. https://doi.org/10.1016/s0899-9007(01)00724-9

32. Republic of Turkey Ministry of Health, General Directorate of Public Health. Vitamin D Support Program Guide for Pregnant Women. Available at: http://www.saglik.gov.tr/TR/belge/1-12659/gebelere-d-vitamini-destek-programi-rehberi.html (Accessed on May 9, 2022).

The Turkish Journal of Pediatrics 2023; 65: 416-424
https://doi.org/10.24953/turkjped.2022.1039

Original Article

A histopathological view at the long-term effects of COVID-19 on the gastrointestinal system in children: a single center experience

Semih Sandal[1], Özlem Tanas[2], Şefika Karabulut[3], Muzaffer Çaydere[2], Sema Hücümendere[2]

[1]Department of Pediatric Gastroenterology, Ankara Training and Research Hospital, Ankara; [2]Department of Pathology, Ankara Training and Research Hospital, Ankara; [3]Department of Medical Microbiology-Virology, Gülhane Institute of Health Science, Ankara, Türkiye.

ABSTRACT

Background. Subacute and chronic long-term effects of coronavirus disease 2019 (COVID-19) in different organ systems have been studied in post-COVID patients recently. COVID-19 may cause gastrointestinal (GI) system findings due to the presence of its receptor, angiotensin converting enzyme type 2 (ACE2), which is extensively expressed in the GI tract. In this study, we aimed to evaluate the post-infectious histopathological alterations of COVID-19 in pediatric patients who had GI symptoms.

Methods. Fifty-six specimens of upper endoscopic biopsies (including esophagus, stomach, bulbus and duodenum) obtained from seven patients and 12 specimens of lower endoscopic biopsies obtained from one patient who had GI symptoms after having COVID-19 (proven by polymerase chain reaction [PCR]) were evaluated as the study group. Forty specimens from five patients presenting with similar complaints but without COVID-19 were selected as the control group. All biopsy materials were immunohistochemically stained with the anti-SARS-CoV-2S1 antibody.

Results. In all biopsies of the study group, anti-SARS-CoV-2S1 antibody was detected with moderate cytoplasmic positivity in epithelial cells and inflammatory cells in the lamina propria. No staining was observed in the control group. Epithelial damage, thrombus, or no other specific findings were detected in the GI tract biopsies of any of the patients.

Conclusions. The virus antigen was detected immunohistochemically in the stomach and duodenum, but not in the esophagus, even months after infection and causes gastritis and duodenitis. No specific histopathological finding was observed from non-COVID-19 gastritis/duodenitis. Therefore, the post-COVID-19 GI system involvement should be kept in mind in patients presenting with dyspeptic symptoms even if several months have passed.

Key words: COVID-19, gastrointestinal, child, endoscopy, immunohistochemistry, biopsy.

COVID-19, caused by the novel coronavirus named SARS-CoV-2, was announced as a pandemic by the World Health Organization (WHO) in March 2020. The first case in Türkiye was seen on March 11, 2020. According to WHO data, more than 750 million cases and more than 6.5 million deaths have been reported worldwide, to date.[1]

Although it is seen in all age groups, it differs between adults and children. While the most common clinical symptoms are fever and cough, the majority of the pediatric age group is asymptomatic and has mild to moderate disease. Moreover, leukocyte counts in children are generally normal compared to adults. The incidence of critical illness and vomiting

✉ Semih Sandal
sandal.semih@gmail.com

Received 9th December 2022, revised 20th January 2023, accepted 13th February 2023.

symptoms in children under 1 year of age was found to be higher than in the normal population. Although COVID-19 is typically an airborne infection, its receptor, angiotensin converting enzyme type 2 (ACE2) is extensively expressed along the GI tract. Diarrhea was reported in 8% of COVID-19 (+) patients, and vomiting in 7%.[2] Atypical clinical symptoms associated with the GI system and prolonged shedding of virus or viral fragments in the feces of infected individuals raised concerns about whether the virus can be transmitted by the oral-fecal route and the GI tract serves as a reservoir for reinfection.[2-7]

In this study, we aimed to evaluate the symptoms, histopathological alterations and endoscopic findings caused by COVID-19 in the GI tract of pediatric patients.

Material and Methods

In this retrospective study, fifty-six specimens of upper endoscopic biopsies (including esophagus, stomach, bulbous and duodenum) obtained from seven patients and 12 specimens of lower endoscopic biopsies obtained from one patient who had alarm GI symptoms after having COVID-19 infection (proven by PCR) were evaluated as the study group.

SARS-CoV-2 PCR positivity was checked from the Turkish Ministry of Health information system. Patients who had one or more positive results on the system were included as the study group.

Endoscopic evaluation decision was made according to the presence of alarm symptoms, according to American Academy of Pediatrics Subcommittee on Chronic Abdominal Pain as involuntary weight loss, difficulty swallowing (dysphagia) or painful swallowing (odynophagia), significant vomiting (bilious, protracted, projectile, or otherwise worrisome), chronic severe diarrhea (≥3 loose or watery stools per day for more than two weeks) or nocturnal diarrhea, unexplained fever, urinary symptoms (change in bladder function, dysuria, hematuria, flank pain), back pain, positive family history (inflammatory bowel disease (IBD), celiac disease, peptic ulcer disease, familial Mediterranean fever), bloody diarrhea or melena, skin changes (rash, eczema, hives), deceleration in linear growth (i.e., height gain <5 cm/year in a prepubertal child) and/or delayed puberty, oral aphthous ulcerations, localized right upper quadrant tenderness, localized pain, organomegaly, perianal abnormalities (i.e., skin tags, fissures, fistulae), and a stool sample positive for occult blood.[8]

None of the patients had a known chronic disease and their complaints emerged after the COVID-19 infection. The control group was selected as eight patients of the same age and gender, who presented with similar complaints and alarm symptoms and no positive PCR for SARS-CoV-2 at any time before or during onset.

All PCR results were negative during the endoscopy in both the study and control group.

Upper endoscopic biopsies were taken from the esophagus, stomach, bulbous and duodenum and lower endoscopic biopsies were taken from the ileum, colon and rectum and then were evaluated with hematoxylin and eosin (H&E) staining in both groups. Biopsies of seven patients in the study group and five patients in the control group were immunohistochemically stained with the anti-SARS-CoV-2 Spike Glycoprotein S1 (SARS-CoV-2S1) antibody.

Biopsy samples were fixed in %4 formaldehyde solution and embedded in paraffin with a routine procedure. Three millimeter-thick sections were cut from tissue blocks and stained with H&E. The SARS-CoV-2 Spike Glycoprotein S1 (rabbit polyclonal antibody, Abcam, Cambridge, UK, Cat# ab275759, gr3366181-3: 1:200 dilution) was used for the immunohistochemical staining and the procedure was held according to the manufacturer's instructions.

Verbal and written consent was taken from the patients and parents.

Ethics approval

The ethics committee of Ankara Training and Research Hospital approved the study protocol with the number E-21/724.

Results

Five girls and three boys were included and the median age was 16 (7-17) years in both groups included in the study. The mean time between the COVID-19 PCR positivity and biopsy was 5.8 months in the study group.

In the study group, the esophagus, stomach, bulbous and duodenum biopsies of seven patients and the ileum, colon and rectum biopsies of one patient were evaluated. There were no findings other than congestion in the esophageal biopsies and rare lymphocytes in the squamous epithelium in four biopsies (Fig. 1A). In gastric biopsies, two samples of each corpus and antrum were evaluated. All patients had active gastritis (Fig. 1B). In duodenum and bulbous biopsies, mild active inflammation was observed in six patients and chronic non-specific inflammation was observed in one patient (Fig. 1C). Biopsies of the ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum were examined from one patient. Flattening of the villi was noted in ileum biopsy. Edema was present in the colon biopsies. In the GI tract biopsies of eight patients, no epithelial damage, thrombus, or specific finding was found.

In the control group, the esophagus, stomach, bulbous and duodenum biopsies of seven patients and colon and rectal biopsies of one patient were evaluated. There were no findings other than congestion in esophageal biopsies and rare lymphocytes in the squamous epithelium in two biopsies. In gastric biopsies, two samples of each corpus and antrum were evaluated. All patients had active gastritis. In the duodenum and bulbous biopsies, mild active inflammation

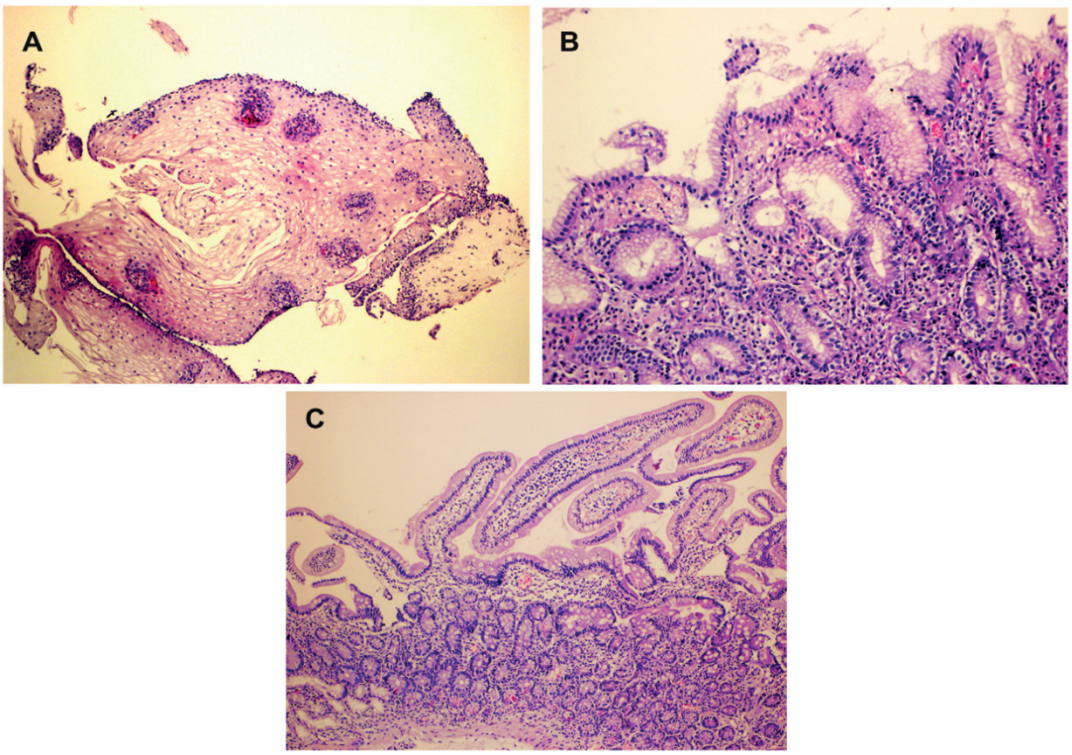


Fig. 1. Hematoxylin and eosin (H&E) staining.
Congestion in esophagus **(A)**, active chronic inflammation in gastric mucosa **(B)**, chronic inflammation in duodenum **(C)** of study group. (Magnification: X100 for **A** and **C**; X200 for **B**)

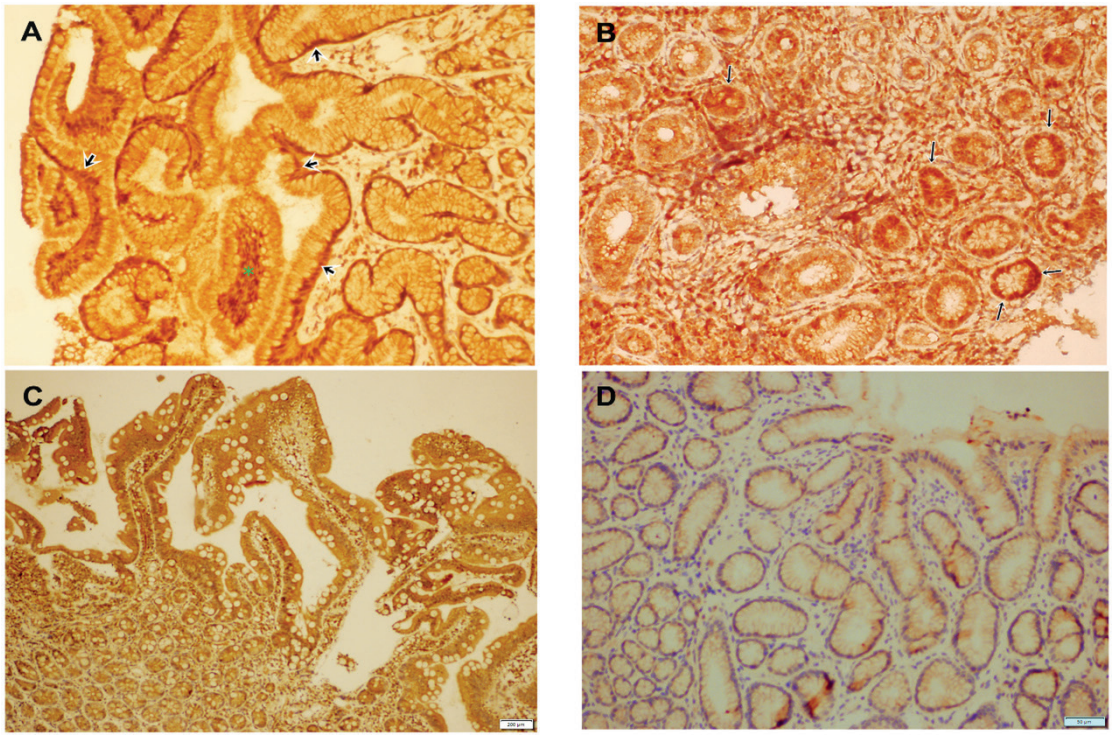


Fig. 2. Anti-SARS-CoV-2S1 antibody immunohistochemical staining.
Positive staining of anti-SARS-CoV-2S1 antibody in gastric mucosa **(A, B)**, duodenal mucosa **(C)** of study group. Negative staining of anti-SARS-CoV-2S1 antibody in gastric mucosa **(D)** of control group.
(Magnification: X200 for **A** and **D**; X100 for **B** and **C**)

was observed in two patients and chronic non-specific inflammation was observed in five patients. In one patient, the biopsies of the cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum were examined. Minimal active inflammation was observed in cecum and transverse colon biopsies, and non-specific chronic inflammation was observed in other biopsies.

Gastric (Fig. 2A, 2B) and duodenal biopsies (Fig. 2C) in seven cases of the study group, gastric and duodenal biopsies in five cases of the control group were evaluated by immunohistochemical staining with the anti-SARS-CoV-2S1 antibody. In all biopsies of the patient group, moderate cytoplasmic positivity was detected in epithelial cells and inflammatory cells in the lamina propria. No staining was observed in four cases in the control group (Fig. 2D), and weak cytoplasmic staining was observed in gastric epithelium and inflammatory cells in only one case.

Discussion

In the present study, moderate cytoplasmic positivity was detected in all biopsies with immunohistochemical staining with the anti-SARS-CoV-2S1 antibody in epithelial cells and inflammatory cells in the lamina propria in the study group, whereas no staining was observed in the control group. In addition, this finding may be evidence that the virus stays longer in the digestive system of the patients than the respiratory system and the inflammatory response lasts longer. To the best of our knowledge, this was one of the first studies evaluating the histopathological effects of COVID-19 on the GI tract in pediatric patients.

Although COVID-19 is typically an airborne infection, its receptor, ACE2 is extensively expressed along the GI tract.[3]

SARS-CoV-2 infection is manifested by respiratory symptoms but GI symptoms such

as nausea, vomiting, and diarrhea are also reported and common.[9,10]

SARS-CoV-2 consists of a spike (S), membrane (M), and envelope proteins that are embedded in the lipid bilayers and nucleic capsid (N) proteins covering single-stranded RNA. The S proteins are the key structures that attach to host cell receptor proteins ACE2 which is also present on the small intestinal epithelial cells in the GI tract in the duodenum, jejunum, and ileum but not the colon.[10,11]

ACE2 is considered as a homolog of ACE, but they have opposite actions in the body. ACE2 converts angiotensin 2 (Ang 2) to angiotensin 1-7 (Ang (1-7)). Ang (1-7) shows its effects via activation of its cognate G protein-coupled Mas receptor (MasR). ACE2/Ang (1e7)/ MasR pathway is considered anti-inflammatory and antifibrotic whereas ACE/AngII/AT1R pathway is proinflammatory and pro-fibrotic.[10,12]

Physiologically, ACE2/Ang (1e7) mediates neutral amino acid transport and facilitates the release of antimicrobial peptides to decrease gut dysbiosis and prevent inflammation in the GI tract. ACE2 functions decrease during SARS-Cov-2 infection and it may lead to an increase in Ang 2 and Ang (1-7). This may result in gut dysbiosis and inflammation in the GI tract.[10]

On the other hand, leaky gut can be mitigated or exacerbated with either the gain or loss of ACE2 expression as shown in animal models. Therefore, interaction of SARS-CoV-2 with ACE2 in the GI tract may lead to damage of the barrier function via disrupting barrier proteins ZO-1, occludin, and claudins, and increase in inflammatory cytokine production, which in turn may lead to dysbiosis and exacerbation of intestinal inflammation.[10,13,14]

It has been reported that 20% of children with COVID-19 were found to be asymptomatic, on the other hand, 7-8% of symptomatic cases presented with vomiting and diarrhea.[2] However, drug-induced GI symptoms such as nausea, abdominal pain, and diarrhea cannot be ignored, as many patients develop diarrhea after hospitalization or medication administration.[9] In the study group, two (28.5%) patients had vomiting, four (57.1%) patients had epigastric pain, four (57.1%) patients had regurgitation and five (%71.4) had anorexia. Three patients had received a proton pump inhibitor and/or antacid therapy before their admission. One patient had been hospitalized in the intensive care unit for 13 days with the diagnosis of multi-system inflammatory syndrome in children and adolescents (MIS-C) and received anakinra therapy four months before their admission.

A recent review estimated the prevalence of GI symptoms to be 17.6% in patients with COVID-19 [14]. The exact incidence of digestive symptoms is a matter of debate, with one study reporting anorexia as the most common symptom (39.9-50.2%), while other studies report diarrhea most commonly in both adult and pediatric populations (2-49.5%).[9,15] Fang et al. found that more than 50% of the cases with diarrhea presented after admission and initiation of antiviral therapy, and approximately 22.2% of them had loose stools before diagnosis of COVID-19.[16] There are reports of GI bleeding in addition to acute hemorrhagic colitis in the literature.[17] In our study, only one patient had diarrhea in the acute period of COVID-19, but there was no diarrhea at the time of admission to our clinic. Except for one patient, who received anakinra treatment, none of seven patients in study group received non-steroidal anti-inflammatory drugs (NSAID), steroids or antibiotics. No patient received specific antiviral treatment.

Except for nonspecific anorexia, approximately 20% of the patients develop GI symptoms. In our study, five (71.4%) patients had anorexia, but none of the patients had any complaints of weight loss leading to percentile loss. GI symptoms generally worsen with the progression of the disease and show a more insidious onset of the disease. In our study, all patients stated that dyspeptic complaints started after respiratory tract complaints. None of our patients had any complaints of GI bleeding.

SARS-CoV-2 binds to the ACE2 receptor and enters cells and multiplies. Although COVID-19 is typically an airborne infection, its receptor, ACE2, is present throughout the GI tract and virus has been identified in the surface epithelial cells of the stomach, small intestine and stomach, and in the colon. Atypical clinical symptoms associated with the GI system and evidence of prolonged shedding of virus or viral fragments in the feces of infected individuals have raised concerns over whether the virus can be transmitted by the oral-fecal route and whether the GI tract acts as a reservoir for reinfection.[18] According to Wang et al.[5], live virus from stool samples raised the possibility of fecal transmission of SARS-CoV-2, which might be resistant to the acidic environment in the human intestine and could be transmitted via the fecal route, as it was detected in the stools of approximately 32.8% of positive cases.[3,5]

However, while reverse transcription-polymerase chain reaction (RT-PCR) can detect viral fragments, but not the entire virus, stool cultures for SARS-CoV-2 are still incomplete or have low specificity.[19] Therefore, further studies are needed to understand the mechanisms of transmission and incubation times, along with contagiousness and clinical course duration.

The presence of viral nucleocapsid protein in COVID-19(+) patients has been confirmed in nearly the entire GI lumen, such as the stomach, duodenal, and rectal glandular epithelial cells, except for the esophagus. In our study, from a histological point of view, the GI epithelium shows plasmacytic and lymphocytic infiltration with interstitial edema mainly in the stomach, duodenum, and rectum, and patchy lymphocytic infiltration in the esophagus.

Therefore, COVID-19 can cause digestive symptoms through direct viral invasion in target cells and/or immune-mediated tissue and end-organ damage.[7] Consistent with the literature in our study, H&E examinations in the COVID-19 positive group revealed congestion in esophageal biopsies and rare lymphocytic infiltration in the squamous epithelium. Active inflammation with lymphocytic and plasmacytic infiltration was observed in all gastric biopsies, and in all duodenum and bulbous biopsies except one, and edema was observed in colon biopsies. However, interestingly, flattening of the villi was detected in the ileum biopsy, and as far as we can observe in the literature, such a finding has not been detected before. Congestion was detected in esophageal biopsies similar to colon biopsies.

It has been reported in the literature that extensive alveolar damage consisting of permanent and intracapillary thrombosis in alveolar epithelial cells and capillary endothelial cells was detected in lung biopsy of COVID-19(+) patients.[20] In contrast to the lung, no epithelial damage, thrombus, or specific finding was detected in any of the eight patients' GI tract biopsies in our study.

In the control group, gastric biopsies had similar findings, while chronic inflammation was found in all patients except two, in duodenal and bulbous biopsies. This supports the fact that the patients started to complain of diarrhea and abdominal pain after COVID-19, and that chronic inflammation had not yet developed. However, the fact that the current histopathological findings were also in our control group shows that it does not cause gastritis and/or duodenitis specific to SARS-CoV-2.

In our study, moderate cytoplasmic positivity was detected in all biopsies with immunohistochemical staining with anti-SARS-CoV-2S1 antibody in epithelial cells and inflammatory cells in the lamina propria in the study group, whereas, no staining was observed in the control group. In addition, this finding may be evidence that the SARS-CoV-2 stays longer in the digestive system of the patients than the respiratory system and that the inflammatory response lasts longer.

A higher incidence of critical illness and vomiting symptoms was found in children under one year of age, which may be a finding that the

virus replicates less in acidic environments. The acute character of the inflammation detected in our study revealed the reason why the patients presented with vomiting and it may also explain the higher incidence of vomiting in patients under 1 year of age, whose gastric pH value is more alkaline compared to older ages.

Entry of viruses into the cells is an important part of interspecies transmission. All coronaviruses encode a surface glycoprotein and spike protein that bind to host cell receptors and mediate virus entry.[21,22] A protein that binds to Spike1-glycoprotein was used in the immunohistochemical staining used in our study.

The distribution of ACE2 staining positivity is mainly in the cytoplasm of gastric and intestinal epithelial cells and in the cilia of glandular epithelial cells. This indicates that SARS-CoV-2 can invade target organs of the digestive tract via ACE2 receptors and cause primary damage.[23-25] In all biopsies of the patient group, moderate cytoplasmic positivity was detected in epithelial cells and inflammatory cells in the lamina propria.

ACE2 is a key enzyme in the renin-angiotensin system and is thought to play an important role in intestinal inflammation and regulating diarrhea.[26,27]

However, He et al.[28], pathological results of autopsies performed on patients who died from COVID-19 by ACE2, proinflammatory cytokines including interleukin (IL)-1β and IL-6 were highly expressed so it was reported that proinflammatory cytokine expression did not occur in cells that did not express ACE2.[15,28]

In our study, only one patient with the diagnosis of MIS-C received anakinra (IL-1 blocker) treatment, but no different findings were found in the pathology preparation examined, compared to other patients in the study group.

The strength of the present study is the long term histopathological evaluation of the GI tract after COVID-19. We are aware of the small number of patients, but 108 pathology specimens were evaluated. Each patient who underwent upper GI endoscopy had biopsies taken from the esophagus, stomach, and duodenum, and one patient had 12 colon biopsies taken from various segments of the colon.

The study combines both clinical and pathological evaluation and offers some recommendations for treatment. All preparations were examined using a special stain for SARS-CoV-2 with a special technique.

The limitations of our study were the low number of patients in the study and control groups, as it would be difficult to conduct such a study with a large number of patients in the pediatric population, and the failure to detect asymptomatic carriers prior to their admission.

In this study, we detected COVID-19 in the stomach and duodenum of pediatric patients even months after the infection which resulted in gastritis and duodenitis. For this reason, the history of COVID-19 should be questioned in patients who are admitted to pediatric outpatient clinics with dyspeptic complaints, and it should be kept in mind that SARS-CoV-2 may cause GI system complaints even several months after the infection.

The results obtained in our study need to be supported by more comprehensive studies in order to gain certainty. At this point, we think that our study will shed light on more comprehensive studies in the future.

Ethical approval

The ethics committee of Ankara Training and Research Hospital approved the study protocol with the number E-21/724. Verbal and written consent was taken from the patients and parents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SS; data collection: SS, ÖT, ŞK; analysis and

interpretation of results: SS, MÇ, SH; draft manuscript preparation: SS. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. World Health Organization (WHO). WHO Coronavirus (COVID-19) Dashboard. Available at: https://covid19.who.int/
2. Cui X, Zhao Z, Zhang T, et al. A systematic review and meta-analysis of children with coronavirus disease 2019 (COVID-19). J Med Virol 2021; 93: 1057-1069. https://doi.org/10.1002/jmv.26398
3. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat Med 2020; 26: 502-505. https://doi.org/10.1038/s41591-020-0817-4
4. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 2020; 158: 1831-1833.e3. https://doi.org/10.1053/j.gastro.2020.02.055
5. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020; 323: 1843-1844. https://doi.org/10.1001/jama.2020.3786
6. Gu J, Han B, Wang J. COVID-19: gastrointestinal manifestations and potential fecal-oral transmission. Gastroenterology 2020; 158: 1518-1519. https://doi.org/10.1053/j.gastro.2020.02.054
7. Ng SC, Tilg H. COVID-19 and the gastrointestinal tract: more than meets the eye. Gut 2020; 69: 973-974. https://doi.org/10.1136/gutjnl-2020-321195
8. Di Lorenzo C, Colletti RB, Lehmann HP, et al. Chronic abdominal pain in children: a technical report of the American Academy of Pediatrics and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2005; 40: 249-261. https://doi.org/10.1097/01.mpg.0000154661.39488.ac
9. Tian Y, Rong L, Nian W, He Y. Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. Aliment Pharmacol Ther 2020; 51: 843-851. https://doi.org/10.1111/apt.15731
10. Pola A, Murthy KS, Santhekadur PK. COVID-19 and gastrointestinal system: a brief review. Biomed J 2021; 44: 245-251. https://doi.org/10.1016/j.bj.2021.01.001
11. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004; 203: 631-637. https://doi.org/10.1002/path.1570
12. Sanchis-Gomar F, Lavie CJ, Perez-Quilis C, Henry BM, Lippi G. Angiotensin-Converting Enzyme 2 and Antihypertensives (Angiotensin Receptor Blockers and Angiotensin-Converting Enzyme Inhibitors) in Coronavirus Disease 2019. Mayo Clin Proc 2020; 95: 1222-1230. https://doi.org/10.1016/j.mayocp.2020.03.026
13. Chen L, Li L, Han Y, Lv B, Zou S, Yu Q. Tong-fu-li-fei decoction exerts a protective effect on intestinal barrier of sepsis in rats through upregulating ZO-1/occludin/claudin-1 expression. J Pharmacol Sci 2020; 143: 89-96. https://doi.org/10.1016/j.jphs.2020.02.009
14. Fernández-Blanco JA, Estévez J, Shea-Donohue T, Martínez V, Vergara P. Changes in epithelial barrier function in response to parasitic infection: implications for IBD pathogenesis. J Crohns Colitis 2015; 9: 463-476. https://doi.org/10.1093/ecco-jcc/jjv056
15. Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. Am J Pathol 2007; 170: 1136-1147. https://doi.org/10.2353/ajpath.2007.061088
16. Fang D, Ma J, Guan J, et al. Manifestations of digestive system of hospitalized patients with coronavirus disease 2019 in Wuhan, China: a single-center descriptive study. Chinese Journal of Digestion 2020;12:151-156.
17. Pan L, Mu M, Yang P, et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. Am J Gastroenterol 2020; 115: 766-773. https://doi.org/10.14309/ajg.0000000000000620
18. Galanopoulos M, Gkeros F, Doukatas A, et al. COVID-19 pandemic: pathophysiology and manifestations from the gastrointestinal tract. World J Gastroenterol 2020; 26: 4579-4588. https://doi.org/10.3748/wjg.v26.i31.4579

19. D'Amico F, Baumgart DC, Danese S, Peyrin-Biroulet L. Diarrhea during COVID-19 infection: pathogenesis, epidemiology, prevention, and management. Clin Gastroenterol Hepatol 2020; 18: 1663-1672. https://doi.org/10.1016/j.cgh.2020.04.001

20. Batah SS, Fabro AT. Pulmonary pathology of ARDS in COVID-19: A pathological review for clinicians. Respir Med 2021; 176: 106239. https://doi.org/10.1016/j.rmed.2020.106239

21. Li F. Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol 2016; 3: 237-261. https://doi.org/10.1146/annurev-virology-110615-042301

22. Ye Q, Wang B, Zhang T, Xu J, Shang S. The mechanism and treatment of gastrointestinal symptoms in patients with COVID-19. Am J Physiol Gastrointest Liver Physiol 2020; 319: G245-G252. https://doi.org/10.1152/ajpgi.00148.2020

23. Wang MK, Yue HY, Cai J, et al. COVID-19 and the digestive system: a comprehensive review. World J Clin Cases 2021; 9: 3796-3813. https://doi.org/10.12998/wjcc.v9.i16.3796

24. Zhang H, Liao Y-S, Gong J, Liu J, Xia X, Zhang H. Clinical characteristics of coronavirus disease (COVID-19) patients with gastrointestinal symptoms: a report of 164 cases. Dig Liver Dis 2020; 52: 1076-1079. https://doi.org/10.1016/j.dld.2020.04.034

25. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020; 382: 1708-1720. https://doi.org/10.1056/NEJMoa2002032

26. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002; 417: 822-828. https://doi.org/10.1038/nature00786

27. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005; 11: 875-879. https://doi.org/10.1038/nm1267

28. He L, Ding Y, Zhang Q, et al. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. J Pathol 2006; 210: 288-297. https://doi.org/10.1002/path.2067

The Turkish Journal of Pediatrics 2023; 65: 425-432
https://doi.org/10.24953/turkjped.2022.232

Original Article

Evaluation of ischemia-modified albumin in the diagnosis and the clinical severity of COVID-19 in children

Eda Karadağ Öncel[1], Ayşegül Elvan Tüz[1], Yıldız Ekemen Keleş[1], Aslıhan Şahin[1], Gülnihan Üstündağ[1], Selin Taşar[1], Tuba Kansu Altan[2], İnanç Karakoyun[2], Banu İşbilen Başok[2], Salim Neşelioğlu[3], Ahu Kara Aksay[1], Dilek Yılmaz[1,4], Özcan Erel[3]

[1]*Department of Pediatric Infectious Diseases, University of Health Sciences, Tepecik Training and Research Hospital, İzmir;* [2]*Department of Medical Biochemistry Clinic, University of Health Sciences, Tepecik Training and Research Hospital, İzmir;* [3]*Department of Medical Biochemistry Clinic, Yıldırım Beyazıt University, Ankara City Hospital, Ankara;* [4]*Department of Pediatric Infectious Diseases, İzmir Katip Çelebi University, İzmir, Türkiye.*

ABSTRACT

Background. There is no specific biomarker used in the diagnosis of COVID-19 and predicting its clinical severity. This study aimed to investigate the utility of ischemia-modified albumin (IMA) in diagnosing and predicting clinical severity in children with COVID-19.

Methods. Between October 2020 and March 2021, 41 cases constituted the COVID-19 group and 41 cases constituted the healthy control group. IMA levels were measured at admission (IMA-1) and 48-72 hours (IMA-2) in the COVID-19 group. In the control group, it was measured at admission. COVID-19 clinical severity was classified as asymptomatic infection, mild, moderate, severe, or critical disease. Patients were divided into two groups (asymptomatic/mild and moderate/severe) to evaluate IMA levels in terms of clinical severity.

Results. In the COVID-19 group, the mean IMA-1 level was 0.901±0.099, and the mean IMA-2 level was 0.866±0.090. The mean level of IMA-1 in the control group was 0.787±0.051. When IMA-1 levels of COVID-19 and control cases were compared, the difference was statistically significant ($p<0.001$). When clinical severity and laboratory data are compared, C-reactive protein, ferritin and ischemia-modified albumin ratio (IMAR) were statistically significantly higher in moderate-severe clinical cases ($p=0.034$, $p=0.034$, $p=0.037$ respectively). However, IMA-1 and IMA-2 levels were similar between the groups ($p=0.134$, $p=0.922$, respectively).

Conclusions. To date, no study has been conducted on IMA levels in children with COVID-19. The IMA level may be a new marker for the diagnosis of COVID-19 in children. Studies with a larger number of cases are needed to predict clinical severity.

Key words: ischemia-modified albumin, COVID-19, child, diagnosis, clinical severity.

The COVID-19 pandemic caused by SARS-CoV-2 is a major public health crisis threatening humanity all over the world. Although the disease seems to be milder in children, severe cases can be seen. The disease is associated with the laboratory and clinical features of a cytokine storm that triggers the pro-inflammatory state associated with severe tissue damage.[1] The SARS-CoV-2 infection leads to many abnormal laboratory indicators. Associated biomarkers include hematological, biochemical, coagulation-fibrinolysis system, and inflammatory markers.[1] There is no specific biomarker used in the diagnosis of COVID-19 or for predicting its clinical severity.

Ischemia-modified albumin (IMA), a new marker of oxidative stress, measured by

✉ Eda Karadağ Öncel
dredakaradag@gmail.com

Received 1st March 2022, revised 24th October 2022, 6th December 2022, 31st January 2023, accepted 2nd March 2023.

the albumin cobalt binding test is a marker whose level increases secondary to ischemia of myocardial and skeletal muscle.[2] In the literature, IMA levels have been investigated in inflammatory diseases such as obesity, asthma, appendicitis, irritable bowel syndrome, and nephrotic syndrome. IMA has additionally been evaluated in infectious diseases such as bacteremia, neonatal sepsis, and pneumonia.[3-5] Many biomarkers were evaluated in the diagnosis and for evaluating the clinical severity in children with COVID-19, predictors of a more severe course of the disease would help clinicians identify high-risk patients. The aim of the study was to establish the utility of IMA in the diagnosis and evaluation of clinical severity in children with COVID-19. To the best of our knowleadge, there was only one study published in the English literature including children with COVID-19.[3] However, our study is the first to examine the following IMA levels in children with COVID-19._

Material and Methods

Study Design and Definitions

This case-controlled study was conducted by the pediatric infectious diseases clinic of İzmir Tepecik Training and Research Hospital between October 2020 and March 2021. In the patient group, there were 41 cases of SARS-CoV-2 detected in the respiratory sample by reverse transcriptase-polymerase chain reaction method (Bio-speedy SARS CoV-2 double gene RT-qPCR kit, Bioeksen-Türkiye). The control group included 41 healthy children without any known chronic diseases.

Demographic characteristics, clinical findings, hemogram, biochemistry, coagulation, and cardiac enzyme levels of COVID-19 cases were recorded. IMA levels were measured at admission (IMA-1) and 48-72 hours (IMA-2) in the COVID-19 group, while in the control group they were measured at the time of admission. In accordance with the report of the WHO-China Joint Mission on COVID-19, patients with COVID-19 were divided into mild, moderate, severe, and critical disease.[6] Mild illness may have any of the various signs and symptoms of COVID-19, such as shortness of breath or abnormal chest imaging. The moderate disease was defined as pneumonia. Severe disease was defined by dyspnea, a respiratory rate greater than 30 per min, blood oxygen saturation (SpO2) of 93% or less, a PaO2/FiO2 ratio below 300, and/or lung infiltration in more than 50% of the lung field. Patients with critical diseases (respiratory failure requiring mechanical ventilation, septic shock, and/or organ failure requiring intensive care) were not included.[7] Patients were divided into two groups (asymptomatic/mild and moderate/severe) to evaluate IMA levels in terms of clinical severity.

The study protocol was approved by the Ethics Committee of Health Sciences University, İzmir Tepecik Training and Research Hospital (Decision number: 2021/01-36). Informed consent was obtained from the families of the participants for the study.

Laboratory analyses

Participants blood samples were collected into the clot-activating tubes containing gel separator (Ref No: 367955; BD Vacutainer® SST II Advance tube, 5 mL, 13x100 mm, NJ, USA). Serum samples were separated by centrifugation for 10 minutes at 1,500×g following blood sampling. Then, serum specimens were aliquoted and stored at −80°C until further analyses.

IMA levels were determined by the albumin cobalt binding test, a rapid colorimetric method developed by Bar-Or et al.[2] The method is based on the binding ability of reduced cobalt ions (Co^{2+}) in human serum albumin due to ischemia. Briefly, a known amount of exogenous Co ($CoCl_2$) was added to serum samples. Albumin, which is altered as a result of ischemic processes, binds to the Co (II) to a far lesser extent, and the excess (unbound) amount of Co^{2+} forms a colored complex with dithiothreitol which is measured spectrophotometrically at 480 nm.

Serum albumin levels were measured using the AU5800 autoanalyzer using the bromocresol green method (AU5800, Beckman Coulter Inc., USA). In an attempt to correct the IMA values for serum albumin levels, the following formula was applied: (individual serum albumin/mean albumin in the population) × IMA value.[8] This formula showed the albumin-adjusted IMA (adj-IMA). The ischemia-modified albumin ratio (IMAR) value was obtained by proportioning the IMA value to individual serum albumin.

Statistical Analysis

Statistical data were analyzed with IBM SPSS for Windows version 25.0 (Chicago, IL). Values for numerical variables were given as median (interquartile range) (IQR) or mean ± standard deviation, depending on the normality distribution. Categorical variables were presented as numbers and percentages. Continuous variables following normal distribution were compared using a one-way analysis of variance or t-tests. When distribution was not expected, the Kruskal-Wallis test was used. Categorical variables were compared using the chi-square test. Receiver operating characteristic (ROC) curve analyses were performed to determine diagnostic cut-off values and their sensitivity, specificity, and area under curve (AUC) values. In evaluating the accuracy of a diagnostic test, sensitivity and specificity were calculated at 95% confidence interval (CI). A p-value of <0.05 was considered statistically significant for all predictions.

Results

A total of 82 children were evaluated, 41 of them had COVID-19 and 41 were healthy subjects. There was no statistically significant difference in terms of ages and gender (p>0.05).

Twenty-two (53.7%) of the cases in the COVID-19 group were male and their median age was 123 (2-214) months. The most common symptoms were fever in 22 (53.7%) cases, cough in 15 (36.6%) cases, headache in 14 (34.1%) cases, nausea-vomiting in 9 (22%) cases, and diarrhea in 8 (19.5%) cases. When evaluated in terms of clinical severity; 35 (85.4%) showed asymptomatic or mild clinical severity; 6 (14.6%) presented with a moderate-severe clinic. The clinical and laboratory findings of the patients with COVID-19 are shown in Table I. In the COVID-19 group, the mean IMA-1 level was 0.901±0.099, and the mean IMA-2 level was 0.866±0.090. The mean level of IMA-1 in the control group was 0.787±0.051. When IMA-1 levels of COVID-19 and control cases were compared, the difference was statistically significant (p<0.001) (Fig. 1). In addition, IMA-1, IMA-2, albumin, IMAR, and adj-IMA values in the COVID-19 group are shown in Figure 2. However, there was no significant difference between IMA-1 and IMA-2 values in COVID-19 cases (p=0.241).

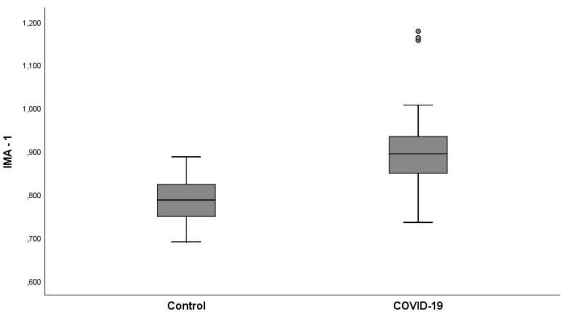


Fig. 1. Comparison of IMA-1 levels between COVID-19 group and control group (p<0.001).
IMA-1: Ischemia-modified albumin at admission

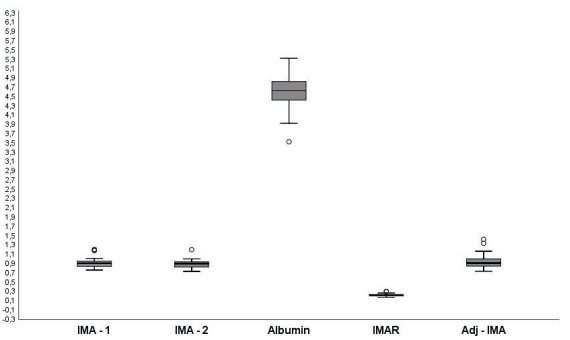


Fig. 2. IMA-1, IMA-2, albumin, IMAR and adj-IMA values in the COVID-19 group.
adj-IMA: Albumin-adjusted ischemia-modified albumin, IMA-1 and -2: IMA at admission and at 48-72 hours, IMAR: IMA ratio

Table I. Clinical and laboratory findings of patients.

	COVID-19 Group (n=41)	Control Group (n=41)	*P* Value
Age (months) , median (Q1-Q3)	131 (2-214)	138 (12-210)	>0.05
Gender (male), n (%)	22 (53.7)	26 (63.4)	>0.05
Clinical findings, n (%)			
Fever	22 (53.7)		
Cough	15 (36.6)		
Throat ache	7 (17.1)		
Rhinorrhea	4 (9.8)		
Nasal congestion	1 (2.4)		
Dyspnoea	4 (9.8)		
Nausea-vomiting	9 (22)		
Diarrhea	8 (19.5)		
Abdominal pain	5 (12.2)		
Headache	14 (34.1)		
Myalgia	2 (4.9)		
Anosmia-ageusia	5 (12.2)		
Clinical Severity, n (%)			
Asymptomatic/Mild	35 (85.4)		
Moderate/Severe	6 (14.6)		
Laboratory findings			
Total WBC (10^3/μL), median (Q1-Q3)	5700 (3200-15900)		
Platelet counts (10^3/μL), mean±SD	249317±84310		
Mean platelet volume (fL), mean±SD	8.2±1.01		
C-reactive protein (mg/L), median (Q1-Q3)	4.3 (0.1-157.4)		
Procalcitonin (μg/L), median (Q1-Q3)	0.05 (0.01-0.94)		
Ferritin (ng/mL), median (Q1-Q3)	59 (8-354)		
D-dimer (μg/L), median (Q1-Q3)	430 (190-4140)		
Fibrinogen (mg/dL), mean±SD	324±108		
IMA-1 (ABSU), mean±SD	0.901±0.099	0.787±0.051	***<0.001***
IMA-2 (ABSU), mean±SD	0.866±0.090		
Albumin (g/dL), mean±SD	4.41±0.43		
IMAR (ABSU), mean±SD	0.21±0.03		
Adj-IMA, median (Q1-Q3)	0.864 (0.709-1.398)		

ABSU: absorbance units, Adj-IMA: albumin-adjusted ischemia-modified albumin, IMA: ischemia-modified albumin, IMAR: ischemia-modified albumin ratio, SD: standart deviation, WBC: white blood cell.

When clinical severity and laboratory data were compared, C-reactive protein, ferritin and IMAR were statistically significantly higher in moderate-severe clinical cases (p=0.034, p=0.034, p=0.037 respectively). However, IMA-1 and IMA-2 levels were similar between the groups (p=0.134, p=0.922, respectively). There was also no difference between other inflammatory markers in terms of clinical severity. The laboratory data comparisons in both groups are summarized in Table II.

The optimal cut-off value for IMA-1 was identified by plotting ROC curves to predict the diagnosis of COVID-19. The cut-off value of IMA-1 to predict a diagnosis of a child with COVID-19 was 0.848 with a sensitivity

Table II. Comparison of laboratory data according to the clinical severity of COVID-19.

	Asymptomatic/Mild COVID-19 (n=35)	Moderate/Severe COVID-19 (n=6)	*P* Value
Age (months), median (Q1-Q3)	123 (2-214)	197 (60-214)	0.060
Gender, n (%)			0.280
Male	20 (57.1)	2 (33.3)	
Female	15 (42.9)	4 (66.7)	
Total WBC (10^3/μL), median (Q1-Q3)	5700 (3200-15900)	4800 (3300-10500)	0.293
Platelet counts (10^3/μL), mean±SD	254543±82793	218833±94588	0.344
Mean platelet volume (fL), mean±SD	8.2±1.06	8.38±0.7	0.691
C-reactive protein (mg/L), median (Q1-Q3)	3.4 (0.1-157.4)	35.35 (4.3-54.7)	***0.034***
Procalcitonin (μg/L), median (Q1-Q3)	0.05 (0.01-0.94)	0.05 (0.01-0.09)	0.852
Ferritin (ng/mL), median (Q1-Q3)	50 (8-354)	217 (54-256)	***0.034***
D-dimer (μg/L), median (Q1-Q3)	420 (190-4140)	685 (310-1740)	0.166
Fibrinogen (mg/dL), mean±SD	314±109	378±86	0.180
Troponin I (ng/L), median (Q1-Q3)	2.5 (2.5-1044)	2.5 (2.5-3.16)	0.284
IMA-1 (ABSU), mean±SD	0.891±0.096	0.958±0.109	0.134
IMA-2 (ABSU), mean±SD	0.867±0.091	0.861±0.096	0.922
Albumin (g/dL), mean±SD	4.45±0.45	4.15±0.23	0.116
IMAR (ABSU), mean±SD	0.2±0.03	0.23±0.03	***0.037***
Adj-IMA, median (Q1-Q3)	0.864 (0.709-1.398)	0.873 (0.802-1.128)	0.986

ABSU: absorbance units, Adj-IMA: albumin-adjusted ischemia-modified albumin, IMA: ischemia-modified albumin, IMAR: ischemia-modified albumin ratio, IQR: interquartile range, SD: standart deviation, WBC: white blood cell.

Table III. The area under the curve, cut-off levels, specifity, sensitivity, positive predictive values and negative predictive value of IMA-1.

	AUC	Cut-off level	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
IMA-1	0.867	0.848	90.2	75.6	88.5	78.7

AUC: area under the curve, IMA: ischemia-modified albumin, NPV: negative predictive value, PPV: positive predictive value

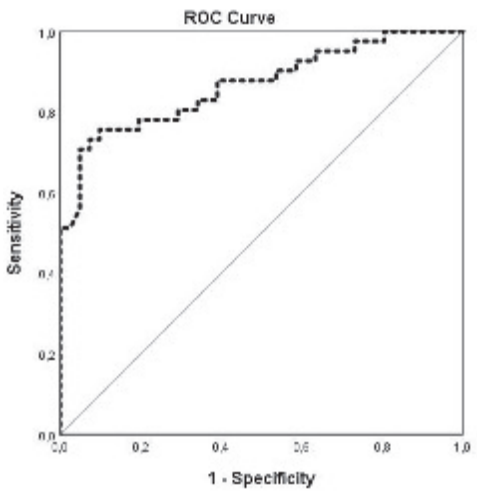


Fig. 3. Receiver operating characteristic (ROC) curves for patients with COVID-19 versus controls; ischemia-modified albumin (IMA).

of 75.6% and specificity of 90.2% (area under the curve 0.867, p<0.001) (95% CI=0.789-0.946). ROC curves of patients with COVID-19 versus controls are depicted in Figure 3 and Table III.

Discussion

The main result of this study was that IMA levels were higher in the COVID-19 group compared to the controls. However, there was no significant difference between groups in predicting clinical severity. In the study of Ducastel et al.[7], which included 160 cases of COVID-19, it was reported that the severity of the disease and the increase in the level of IMA were parallel. In a study on 74 adults and 79 children with COVID-19, IMA levels were found to be similar between patients

with COVID-19 and healthy controls. Similar to our study, IMA levels were not statistically significant between groups according to disease severity in children.[3] One of the remarkable results we obtained in the study for evaluating clinical severity was that IMAR was another marker that may be used. Studies with a larger number of cases are needed to investigate the importance of IMAR in assessing clinical severity. Laboratory findings including C-reactive protein (CRP) and procalsitonin have an irreplaceable role in the diagnosis and evaluation of COVID-19. Likewise, laboratory tests have an important role in evaluating disease severity and prognosis.

Significant changes in hematological values, which are among the basic biomarkers, include leukopenia and lymphopenia. However, in severe and critical COVID-19 disease, an increase in leukocyte, neutrophil and neutrophil-to-lymphocyte ratio values were detected.[9] Abnormal coagulation and fibrinolysis biomarkers are other important markers used in COVID-19 disease. It is associated with a poor prognosis and the most typical finding is the increased concentration of D-dimer. Because this disease causes an inflammatory storm, associated cytokines rise sharply, resulting in an increase in levels of certain inflammatory biomarkers such as CRP, ferritin, serum amyloid-A, and procalcitonin.[10] However, the search for new markers that can be used in the diagnosis and follow-up of children with COVID-19 continues worldwide. A study from Spain examined changes observed in adenosine deaminase and isoenzymes in saliva samples of individuals with COVID-19.[11] In another study from India, Viral Bacterial (VB10), a 10-gene serum-based biomarker, was discovered and investigated in COVID-19 cases.[12] This study aimed to identify the possible role of IMA as a new biomarker in the diagnosis of COVID-19.

IMA is a biomarker formed as a result of the modification of albumin by reactive oxygen species. Initially, although IMA levels were specific to ischemia, some factors such as acidosis, superoxide-radical damage, energy-dependent membrane degradation, and exposure to free iron and copper have been shown to cause IMA formation.[13] Depending on these factors, it has been found that IMA levels increase in inflammatory diseases in addition to ischemia-related diseases. Studies have shown that serum IMA levels increase in infectious diseases such as bacteremia, neonatal sepsis, and pneumonia in adults and children.[3-5] Based on the data in our study, IMA levels were statistically significantly higher in patients with COVID-19 than in the control group. ROC curve analysis revealed an IMA-1 value higher than 0.848 on presentation to have a sensitivity of 75.6%, a specificity of 90.2%, a positive predictive value (PPV) of 88.5%, and a negative predictive value (NPV) of 78.7% for COVID-19 diagnosis. In a study in which IMA levels were significantly higher in the patient group compared to healthy controls, the Area under the curve (AUC) value was found to be quite high in the ROC analysis, as in our study (0.937).[14] In our study, ROC analysis was found to be good. Based on this, evaluation of IMA levels in the diagnosis of COVID-19 may be considered.

Oxidative stress is defined as the disturbance of the balance between reactive oxygen species and antioxidants and is one of the well-established pathological conditions in various diseases. There is clinical evidence in the literature that oxidative stress is increased in COVID-19 patients and that this worsening redox state could potentially contribute to disease progression.[15] CRP has been previously reported as a reliable marker of oxidative stress and systemic inflammation. Specifically, CRP exhibits superior diagnostic value for bacterial infections with high plasma concentrations. However, CRP levels remain normal or increase only slightly during most viral infections.[16] Therefore, it can be thought that the role of CRP level in the evaluation of COVID-19 will be limited. This was not our main purpose, but in our study, by comparing CRP levels of COVID-19 patients according to clinical severity, we managed to demonstrate a statistically

significant difference between disease severity. Other biomarkers i.e., ferritin and IMA/albumin were determined to be statistically significantly higher in severe clinical course.

Free radicals formed due to tissue damage cause IMA levels to increase within minutes and remain high for 6-12 hours before returning to their normal value. In the study of Sinha et al.[17], it was shown that IMA levels increase early in ischemia and can be measured earlier than other markers. This situation is beneficial in the diagnosis of the disease when applied early.[17] However, our study observed that IMA-2 levels in the COVID-19 group did not decrease to similar levels as those of the control group. It may be thought that more than 48-72 hours are needed to re-evaluate the IMA-2 levels.

Hypoalbuminemia has been identified as a risk factor associated with poor clinical outcomes in the early stages of the disease. It has been reported that serum IMA levels can be misleading in patients with extremely low or high serum albumin levels.[18] In our study, the albumin levels of the participants were not within pathological limits. However, the corrected IMA level (IMAR) was also calculated for the data to be reliable. The formula was locally tested and verified in a cohort consisting of patients with autoimmune liver disease.[19] Our results also support this, and a significant relationship was found between the clinical severity of COVID-19 and IMAR. These data can be supported by large-scale studies.

Our study has several limitations. First, the number of patients with moderate/severe COVID-19 was low, so the comparison of the IMA according to disease severity may be affected. Also, as a control group, comparisons with non-COVID-19 patients with the same disease severity could not be made. Because, during the study, there were no previously healthy patients with similar severity due to other infectious factors. Second, IMA-2 was taken 48-72 hours after admission and it was noticed that the IMA-2 level did not decrease to the levels of the children in the control group. If we had taken the IMA-2 later than 48-72 hours, we might have seen lower levels similar to those of the control group. Finally, SARS CoV-2 IgM antibody levels could not be measured in the control group. Despite the limitations, our study has provided useful and significant insight into the new biomarkers of COVID-19 for diagnosis.

As studies on IMA's clinical benefits are still ongoing, we believe that future prospective studies may help elucidate whether IMA could be used in the diagnosis of COVID-19 patients, particularly for the evaluation of clinical severity. In our study, the sample size of the patient group may have been insufficient to reach general results. In addition, due to the limited number of patients in the moderate/severe group in clinical classification, the assessment may have been insufficient. Therefore, new studies including larger case numbers are required to analyze the usefulness of IMA and IMAR in the diagnosis and clinical severity of COVID-19.

Ethical approval

This study was approved by the Health Sciences University, İzmir Tepecik Training and Research Hospital Ethical Committee in accordance with the Helsinki Declaration (Decision number: 2020/14-70). Informed consent was obtained from the families of the participants for the study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EKO, AET, YEK, AS, GU; data collection: TKA, IK, BIB; analysis and interpretation of results: EKO, AKA, SN; draft manuscript preparation: DYC, OE. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Chen Z, Xu W, Ma W, et al. Clinical laboratory evaluation of COVID-19. Clin Chim Acta 2021; 519: 172-182. https://doi.org/10.1016/j.cca.2021.04.022
2. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000; 19: 311-315. https://doi.org/10.1016/s0736-4679(00)00255-9
3. Aykac K, Ozsurekci Y, Tanir Basaranoglu S, et al. Oxidant and antioxidant balance in children with bacteremia [published online ahead of print, 2020]. Minerva Pediatr. 2020. https://doi.org/10.23736/s0026-4946.20.05748-5
4. Bolatkale M, Duger M, Ülfer G, et al. A novel biochemical marker for community-acquired pneumonia: ischemia-modified albumin. Am J Emerg Med 2017; 35: 1121-1125. https://doi.org/10.1016/j.ajem.2017.03.018
5. Wang IK, Chang YC, Liang CC, et al. Bacteremia in hemodialysis and peritoneal dialysis patients. Intern Med 2012; 51: 1015-1021. https://doi.org/10.2169/internalmedicine.51.7111
6. World Health Organization (WHO). Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). 2020: 40. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019
7. Ducastel M, Chenevier-Gobeaux C, Ballaa Y, et al. Oxidative stress and ınflammatory biomarkers for the prediction of severity and ICU admission in unselected patients hospitalized with COVID-19. Int J Mol Sci 2021; 22: 7462. https://doi.org/10.3390/ijms22147462
8. Lippi G, Montagnana M, Salvagno GL, Guidi GC. Standardization of ischemia-modified albumin testing: adjustment for serum albumin. Clin Chem Lab Med 2007; 45: 261-262. https://doi.org/10.1515/CCLM.2007.039
9. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med 2020; 58: 1021-1028. https://doi.org/10.1515/cclm-2020-0369
10. Li H, Xiang X, Ren H, et al. Serum Amyloid A is a biomarker of severe Coronavirus Disease and poor prognosis. J Infect 2020; 80: 646-655. https://doi.org/10.1016/j.jinf.2020.03.035
11. Franco-Martínez L, Tecles F, Torres-Cantero A, et al. Analytical validation of an automated assay for the measurement of adenosine deaminase (ADA) and its isoenzymes in saliva and a pilot evaluation of their changes in patients with SARS-CoV-2 infection. Clin Chem Lab Med 2021; 59: 1592-1599. https://doi.org/10.1515/cclm-2021-0324
12. Ravichandran S, Banerjee U, Dr GD, et al. VB10, a new blood biomarker for differential diagnosis and recovery monitoring of acute viral and bacterial infections. EBioMedicine 2021; 67: 103352. https://doi.org/10.1016/j.ebiom.2021.103352
13. Sbarouni E, Georgiadou P, Voudris V. Ischemia modified albumin changes - review and clinical implications. Clin Chem Lab Med 2011; 49: 177-184. https://doi.org/10.1515/CCLM.2011.037
14. Yucel K, Fuat Gurbuz A. Hypoxia-inducible factor-1α and ischemia-modified albumin levels in intensive care COVID-19 patients. Horm Mol Biol Clin Investig 2022; 43: 415-420. https://doi.org/10.1515/hmbci-2022-0024
15. Zendelovska D, Atanasovska E, Petrushevska M, et al. Evaluation of oxidative stress markers in hospitalized patients with moderate and severe COVID-19. Rom J Intern Med 2021; 59: 375-383. https://doi.org/10.2478/rjim-2021-0014
16. Haran JP, Beaudoin FL, Suner S, Lu S. C-reactive protein as predictor of bacterial infection among patients with an influenza-like illness. Am J Emerg Med 2013; 31: 137-144. https://doi.org/10.1016/j.ajem.2012.06.026
17. Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC. Role of "Ischemia modified albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. Emerg Med J 2004; 21: 29-34. https://doi.org/10.1136/emj.2003.006007
18. Demir H, Topkaya BC, Erbay AR, Dogan M, Yücel D. Ischaemia-modified albumin elevation after percutaneous coronary intervention reflects albumin concentration rather than ischaemia. Ann Clin Biochem 2009; 46: 327-331. https://doi.org/10.1258/acb.2009.008238
19. Karakoyun I, Ulaşoğlu C, Arslan F, et al. Oxidative imbalance in autoimmune liver disease: evaluation of oxidant-antioxidant status and ischemia-modified albumin. SDÜ Tıp Fakültesi Derg. 2021; 28: 127-135. https://doi.org/10.17343/sdutfd.738119

The Turkish Journal of Pediatrics 2023; 65: 433-444
https://doi.org/10.24953/turkjped.2022.803

Original Article

Late-term cardiac magnetic resonance imaging in multisystem inflammatory syndrome in children

Mustafa Argun[1], Turgut Seber[2], Sevgi Yaşar Durmuş[3], İlhami Çelik[4], Onur Taşçı[5], Süleyman Sunkak[1], Serkan Özsoylu[6], Ferhan Elmalı[7]

[1]Department of Pediatric Cardiology, Health Sciences University School of Medicine, Kayseri City Training and Research Hospital, Kayseri; [2]Department of Pediatric Radiology, Health Sciences University School of Medicine, Kayseri City Training and Research Hospital, Kayseri; [3]Department of Pediatric Infectious Diseases, Health Sciences University School of Medicine, Kayseri City Training and Research Hospital, Kayseri; [4]Department of Infection Diseases and Clinical Microbiology, Health Sciences University School of Medicine, Kayseri City Training and Research Hospital, Kayseri; [5]Department of Pediatric Cardiology, Sivas Numune Hospital, Sivas; [6]Department of Pediatric Intensive Care, Health Sciences University School of Medicine, Kayseri City Training and Research Hospital, Kayseri; [7]Department of Biostatistics, İzmir Katip Çelebi University School of Medicine, İzmir, Türkiye.

ABSTRACT

Background. Cardiac involvement in multisystem inflammatory syndrome in children may have a spectrum ranging from mild disease to severe heart failure due to fulminant myocarditis. Cardiac involvement usually resolves after clinical recovery. However, the adverse effects of myocarditis on cardiac function after recovery are not fully known. This study aims to investigate cardiac involvement by performing cardiac magnetic resonance imaging (MRI) after the acute and recovery periods.

Methods. 21 patients with clinical and laboratory signs of myocarditis, including left ventricular systolic dysfunction, mitral regurgitation, elevated troponin T, elevated N-terminal pro-B-type natriuretic peptide and electrocardiographic changes, who had given consent for cardiac MRI, underwent cardiac MRI after completion of the acute and recovery phases.

Results. When compared to 16 patients with normal cardiac MRI, five patients with cardiac fibrosis on MRI were older, had greater body mass indexes, lower leucocyte counts, lower neutrophil counts, higher blood urea nitrogen levels and higher creatinine levels. Cardiac fibrosis on MRI was located in the posterior right ventricle insertion point and in mid ventricular septum.

Conclusions. Adolescence and obesity appear as risk factors for the development of fibrosis as a late-term sequela of myocarditis. Furthermore, future studies reporting the follow-up data of patients with fibrosis are necessary to predict and manage adverse outcomes.

Key words: multisystem inflammatory syndrome in children, cardiac involvement, myocarditis, cardiac magnetic resonance, fibrosis.

Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected millions of people around the world and caused around 7 million people to die since December 2019 when the first case was reported. COVID-19 has an asymptomatic or mild course in the majority of children. However, towards the end of April 2020, a life-threatening condition resembling severe Kawasaki disease and hyperinflammatory shock syndrome, were identified across Europe for the first time, led to the hospitalization of children which was thought to be associated with COVID-19. Most children affected by this condition had a negative real-time reverse transcription-polymerase chain reaction but a positive antibody test for the SARS-CoV-2, indicating past infection. The cause of this clinical syndrome was assumed to

✉ Mustafa Argun
dr.margun@hotmail.com

Received 23rd September 2022, revised 4th January 2023, 5th March 2023, accepted 6th March 2023.

be an inflammatory reaction following SARS-CoV-2. In May 2020, the Center for Disease Control (CDC) and World Health Organization denominated the disease as multisystem inflammatory syndrome in children (MIS-C).[1-10]

Compared to Kawasaki disease, left ventricular dysfunction and distributive shock are more common in MIS-C. Although MIS-C exhibits some features overlapping those of Kawasaki disease, evidences show that the two are distinct syndromes with differences in the degree of hyperinflammation and irregular immune reaction.[11,12] The cardiac signs of such a newly identified disease have been described only by short-term follow-up data, echocardiographic findings, limited cardiac magnetic resonance imaging (MRI) findings, and data obtained from biopsies and autopsy series. However, the adverse effects of the disease on cardiac function after its acute and recovery phases remain unclear.

The present study aimed to determine whether cardiac involvement occurred after the acute and recovery phases with cardiac MRI and its relationship with clinical and laboratory findings in patients with MIS-C.

Material and Methods

Ethical approval and consent to participate

This study was approved by Kayseri City Hospital Clinical Research Ethics Committee (March/2021; no.320). The study was conducted in accordance with the declaration of Helsinki. Informed consent was obtained from the parents of all patients and also from the adolescent patients themselves.

Subjects

Out of 25 patients diagnosed with MIS-C according to the CDC MIS-C diagnostic criteria between November 2020 and February 2021, at Health Sciences University School of Medicine, Kayseri City Training and Research Hospital Clinic of Pediatric Cardiology, 21 patients with clinical and laboratory signs of myocarditis including left ventricular systolic dysfunction, mitral valve insufficiency, elevated troponin T, elevated N-terminal pro–B-type natriuretic peptide, and electrocardiographic changes, who had given consent to undergo cardiac MRI, underwent cardiac MRI at outpatient follow-up after the completion of the acute and recovery phases. The demographic, clinical, laboratory, electrocardiogram, and echocardiographic data, treatment modalities, and disease course of the patients were recorded. The follow-up examinations of the patients after the acute phase were carried out and recorded by the same pediatric cardiology and infectious disease specialists.

Inclusion and exclusion criteria

Patients with clinical and laboratory findings that meet the MIS-C diagnostic criteria defined by the CDC (Table I) were included in the study.[13]

Patients with a microbial etiology of inflammation such as sepsis and septic shock, viral exanthematous diseases, alternative

Table I. Case definition of multisystem inflammatory syndrome in children.

Case definition of the CDC for multisystem inflammatory syndrome in children
(1) a person younger than 21 years of age, fever lasting for more than one day, laboratory evidence of inflammation, and evidence for clinically severe disease with multiorgan involvement requiring hospital admission;
(2) absence of alternative diagnoses; and
(3) with a temporal association with COVID-19 demonstrated by a positive current or recent SARS-CoV-2 reverse transcription-polymerase chain reaction, serology, or antigen test or known COVID-19 exposure within the four weeks before symptom onset

diagnoses such as anaphylaxis, chronic comorbid conditions, and antiinflammatory drug use were excluded.

Evidence for SARS-CoV-2 infection

The history of close contact with a person positive for SARS-CoV-2 infection until 4-6 weeks prior to symptom onset was questioned. The history of symptomatic or asymptomatic infection was recorded. A nasopharyngeal swab reverse transcription-polymerase chain reaction test was performed for SARS-CoV-2 in all patients during hospital presentation. Enzyme-Linked ImmunoSorbent Assay was used to determine serum SARS-CoV-2 IgG.

General laboratory tests

Complete blood count, routine biochemistry, C-reactive protein, sedimentation rate, and procalcitonin were studied at the time of diagnosis and follow-up.

Cardiac biomarkers, electrocardiogram and echocardiography

Troponin T and N-terminal pro–B-type natriuretic peptide were measured in all patients at the time of diagnosis and follow-up. At our clinical biochemistry laboratory, the normal level of troponin T and N-terminal pro–B-type natriuretic peptide are <14 ng/L and <300 ng/L, respectively. 12-lead standard electrocardiogram (ECG) was performed on all patients at the time of diagnosis. Two-dimensional, color Doppler and M-mode echocardiography were performed by the same pediatric cardiology specialist using Vivid 7 pro (GE Medical Systems) at the time of diagnosis and follow-up visits.

Cardiac MRI

Cardiac MRI was performed at pediatric cardiology outpatient follow-up visits after the completion of the acute and recovery phases in all MIS-C patients (3-6 months after diagnosis). Cardiac MRI was performed with a 1.5-T scanner (Magnetom Aera, Siemens Healthineers, Erlangen, Germany). Intravenous (IV) sedation was required for three children under the age of seven. Before administration of contrast agents, only native T1 maps were obtained (field of view, 340 x 340 mm; matrix, 256 x 256; slice thickness, 8 mm; TR/TE, 358/1.3 msn; flip angle, 35°; no interslice gap). After IV administration of contrast medium (gadobutrol [Gadovist, Bayer AG, Berlin] 0.1 mmol/kg of body weight), perfusion, cine, late gadolinium enhanced (LGE) and myocardial T1 maps were performed, respectively. Except for three sedated children whose images were obtained using free-breathing with motion correction, all other scannings (18 patients) were performed with breath-holding. Myocardial T1 maps (native and postcontrast) were obtained using modified look-locker inversion recovery sequence.

Four to five standard short-axis slices at the basal and mid-ventricular levels were acquired for myocardial T1 maps (native and postcontrast), perfusion and LGE images. Also, there were single 2-chamber and 4-chamber slices in all sequences except perfusion. LGE sequences were obtained 8 to 10 min after injection of the contrast agent (field of view, 340 x 340 mm; matrix, 256 x 256; slice thickness, 8 mm; TR/TE, 495/3 msn; flip angle, 25°; no interslice gap). Cine images were obtained in the 4-chamber, 2-chamber and short-axis heart views using steady-state free precession sequences.

Image analysis was performed by a radiologist with five years of experience in cardiac MRI. All cardiac MRI image analyses were performed on a Syngo Via (Software Version VA30A, Siemens AG, Germany) Workstation. Perfusion, LGE, and ventricular functions were evaluated qualitatively, and T1 maps were evaluated quantitatively due to 16-segment left ventricle (LV) model of the American Heart Association.[1] Noticeable high signal intensity in LGE was considered as fibrosis. Apical slices were not analysed because of motion artefacts.

Regions of interest were drawn global and focal on native and postcontrast short-axis T1 maps. From our previous experiences, we saw that the cutoff value of myocardial T1 relaxation time in 1.5 T MRI in normal patients was about 1060 msn, as determined by Burkhardt et al.[2] Focal (due to American Heart Association segmentation) or global, T1 relaxation time greater than 1060 msn was accepted as myocardial fibrosis. Extracellular volume was calculated in patients with fibrosis in LGE and native T1 maps. Slices of the T1 map with motion artefacts were not analysed. Endocardial contours of the left and right ventricle were manually traced on end-diastolic and end-systolic frames of each SSFP cine image.

Statistical analysis

The study data were analysed with IBM SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, New York, ABD) statistical software package. Descriptive statistics were reported as number of unit (*n*), percentage (%), mean ± standard deviation, median (*M*), minimum (*min*) and maximum (*max*). Normality of distribution of numerical data was tested with Shapiro Wilk normality test. Categoric variables were compared using Fisher's exact test. Bonferroni correction was applied in multiple comparisons. The significance values used in the statistical analyses are the values obtained by the exact method. A p value of less than 0.05 was considered statistically significant.

Results

Demographics

This study included 21 patients (8 males, 13 females) who underwent cardiac MRI examination out of 25 patients with MIS-C (9 males, 16 females) with a mean age of 8.8 ± 4.4 years (range: 1.8 –17.8 years). Patients with normal cardiac MRI and fibrosis were compared in terms of demographics, clinical and laboratory findings, and biomarkers (Table II-IV).

Evidence of SARS-CoV-2 infection

All patients had a history of being in close contact with a SARS-CoV-2 positive person until 4-6 weeks prior to symptom onset. The patients were either asymptomatic or had very mild symptoms. None of our patients had a history of moderate to severe infection. All but one patient had a negative reverse transcription-polymerase chain reaction test in nasopharyngeal swab test for SARS-CoV-2. SARS-CoV-2 IgG was positive in all patients.

Electrocardiogram

Six (28%) patients had T flattening and two (9.5%) patients had voltage depression in the electrocardiogram. None of the patients had tachyarrhythmias such as supraventricular tachycardia and ventricular tachycardia, nor had bradyarrhythmias such as atrioventricular block and sinus node dysfunction.

Table II. Demographic data of patients with normal cardiac MRI and cardiac MRI fibrosis.

	Cardiac MRI Normal (n=16)	Cardiac MRI Fibrosis (n=5)	P Values
Age, years	8.4±3.5	14.4±4.0	0.007*
Gender (male/female)	5 / 11	3 / 2	0.325
Body weight, kg	29.7±10.1	61.0±22.4	<0.001*
Height, cm	128.4±16.7	162.6±26.5	0.003*
Body surface area, m^2	1.07±0.25	1.67±0.42	0.002*
Body mass index, kg/m^2	17.73±3.15	22.68±4.48	0.012*

Data are expressed as mean ± standard deviation.
*= A p value of less than 0.05 was considered statistically significant.

Table III. Presenting symptoms, physical examination findings, complete blood count, acute phase reactants, biochemical parameters, urine analysis results at the time of diagnosis in patients with normal cardiac MRI and cardiac MRI fibrosis.

	Cardiac MRI Normal n=16	Cardiac MRI Fibrosis n=5	P Values
Symptoms			
Fever	16 (100%)	5 (100%)	-
Unrest	15 (93.7%)	5 (100%)	1
Anorexia	16 (100%)	5 (100%)	-
Myalgia	2 (12.5%)	1 (20%)	1
Redness in eyes	11 (68.7%)	4 (80%)	1
Sore throat	1 (6.3%)	1 (20%)	0.429
Nasal discharge	0 (0%)	0 (0%)	-
Cough	1 (6.3%)	0 (0%)	1
Strawberry tongue	9 (56.3%)	1 (20%)	0.311
Cervical lymphadenopathy	2 (12.5%)	2 (40%)	0.228
Skin rash	12 (87.5%)	2 (40%)	0.280
Hand and foot edema	4 (25%)	0 (0%)	0.532
Vomiting	10 (62.5%)	3 (60%)	1
Diarrhea	6 (37.5%)	4 (80%)	0.149
Abdominal pain	10 (62.5%)	4 (80%)	0.624
Physical examination			
Clinical severity (mild/severe)	16 (100%)	4 (80%)	-
Unrest	16 (100%)	5 (100%)	-
Conjunctivitis	11 (68.7%)	3 (60%)	1
Lip hyperemia	12 (87.5%)	3 (60%)	0.598
Strawberry tongue	9 (56.3%)	1 (20%)	0.311
Oropharyngeal hyperemia	4 (25%)	1 (20%)	1
Cervical lymphadenopathy	2 (12.5%)	2 (40%)	0.228
Skin rash	12 (12.5%)	2 (40%)	0.280
Hand and foot edema	4 (25%)	1 (20%)	1
Heart rate (beats per minute)	122±17	115±15	0.455
Pathological murmur	1 (6.3%)	1 (20%)	0.429
Respiration rate (per minute)	24±5	22±5	0.402
Respiratory system normal	16 (100%)	5 (100%)	-
Abdominal tenderness	9 (56.3%)	4 (80%)	0.606
Hepatomegaly	0 (0%)	0 (0%)	-
Splenomegaly	0 (0%)	0 (0%)	-
Complete blood count			
Leukocyte, 10^3/L	12065 (4210-21210)	5250 (3880-7790)	0.011*
Neutrophil, 10^3/μL	9875 (2540-18500)	4000 (2150-6560)	0.019*
Monocyte, 10^3/μL	325 (130-680)	320 (110-410)	0.494
Lymphocyte, 10^3/μL	1185 (560-2330)	680 (420-2480)	0.240
Hemoglobin, (gr/dL)	12.17±1.36	13.20±1.70	0.181
Hematocrit (%)	36.0±3.4	39.1±2.8	0.091
Platelet, 10^6/μL	183.5 (100-320)	163 (112-460)	0.905

Data are expressed as number (percentage), median (min-max) or mean ± standard deviation.
*= A p value of less than 0.05 was considered statistically significant.

Table III. Continued.

	Cardiac MRI Normal n=16	Cardiac MRI Fibrosis n=5	P Values
Acute phase reactants			
C-reactive protein, mg/L	170±68	172±90	0.952
Sedimentation, mm/hour	34.5 (18-101)	43 (16-65)	0.893
Procalcitonin, μg/L	3.93 (0.96-13.03)	0.58 (0.55-12.67)	0.362
Biochemical parameters			
Albumin, gr/L	28.9±5.8	30.5±1.7	0.372
Blood urea nitrogen, mg/dL	8.5 (4-18)	15 (11-26.8)	0.025*
Creatinine, mg/dL	0.45±0.14	0.61±0.07	0.024*
Uric acid, mg/dL	3.25 (1.70-6.60)	3.90 (3.38-5.50)	0.385
Sodium, mEq/L	133.2±4.4	137.8±2.5	0.015*
Potassium, mEq/L	3.86±0.51	4.07±0.33	0.409
Calcium, mg/dl	8.69±0.47	8.82±0.29	0.612
Alkaline phosphatase, U/L	126.5 (52-209)	152 (71-162)	1
γ-Glutamyltransferase, U/L	16 (9-94)	29 (27-119)	0.164
Aspartate transaminase, U/L	27.5 (15-111)	28 (10-101)	0.603
Alanine transaminase, U/L	18.5 (10-154)	24 (5-85)	0.905
Total bilirubin, mg/dL	0.3 (0.2-1.3)	0.6 (0.34-1.40)	0.152
Direct bilirubin, mg/dL	0.2 (0.1-.07)	0.25 (0.07-.0.80)	0.596
Amylase, U/L	38.5 (13-84)	73 (17-75)	0.509
Lipase, U/L	31 (11-104)	30 (8-98)	0.900
Ferritin, μg/L	452 (141-731)	276 (58-2011)	0.676
Urine examination			
Urine density	1019.8±11.7	1025.0±8.3	0.373
Pyuria	8 (50%)	0 (0%)	0.111

Data are expressed as number (percentage), median (min-max) or mean ± standard deviation.
*= A p value of less than 0.05 was considered statistically significant.

Table IV. Biomarker results of patients with normal cardiac MRI and cardiac MRI fibrosis.

Cardiac Biomarker	Cardiac MRI Normal (n=16)	Cardiac MRI Fibrosis (n=5)	P Values
Troponin, ng/L	16.09 (1.80-101)	29.1 (4.35-252.9)	0.240
NT proBNP, ng/L	5257 (57-35000)	1300 (158-6553)	0.320

Echocardiography

Five patients had a left ventricular ejection fraction of less than 55% on the echocardiographic examination. One patient had a left ventricular ejection fraction of 43% at the time of diagnosis which significantly deteriorated during follow-up. This patient had severe MIS-C and fibrosis was detected on MRI. Six patients had mild mitral insufficiency and five had moderate mitral insufficiency, making up 11 (54%) patients with mitral insufficiency. Six (30%) patients had slight pericardial effusion at the time of diagnosis, which completely disappeared during follow-up. No patient had coronary artery dilation and aneurysm on echocardiogram.

Table V. Echocardiography results of patients with normal cardiac MRI and cardiac MRI fibrosis.

Echocardiography	Cardiac MRI Normal n=16	Cardiac MRI Fibrosis n=5	P Values
Mitral valve regurgitation	Minimal (4 cases), First degree (3 cases)	Minimal (2 cases), First degree (1 case), Second degree (1 case)	0.174
Pericardial effusion	5 (31.3%)	1 (20%)	1
Normal coronary arteries	16 (100%)	5 (100%)	-
LVIDd z score	0.42±1.07	0.46±1.15	0.939
LVIDs z score	0.84±1.18	0.91±1.29	0.908
Ejection fraction (%)	60.8±7.5	59.8±6.3	0.790
Fractional shortening (%)	31.8±5.7	32.0±4.0	0.965

Data are expressed as number (percentage) or mean ± standard deviation. LVIDd: left ventricular internal diameter - end diastole, LVIDs: left ventricular internal diameter - end systole.

Patients with and without fibrosis on cardiac MRI had similar rates of mitral insufficiency and pericardial effusion, coronary arteries, left ventricular end-diastolic z scores, left ventricular end-systolic z scores, left ventricular ejection fraction, and fractional shortening on echocardiographic examination (Table V).

Treatment and outcome

According to the MIS-C clinical severity score developed by Jonat et al.[3], which is based on the vasoactive inotrope score, the degree of respiratory support, and the evidence of organ injury, twenty patients were in the mild group, and one patient was in the severe group. All patients were administered IV immunoglobulin at a dose of 2 gr/kg (maximum 100 gr). Only three patients received a second IV immunoglobulin dose because of unremitting fever 24 hours after the first IV immunoglobulin dose. In addition to IV immunoglobulin treatment, 11 patients were administered 2 mg/kg/day IV steroid and six patients 80-100 mg/kg/day p.o. acetylsalicylic acid. A patient with a serious clinical condition was administered 2 gram/kg IV immunoglobulin (twice), IV pulse steroid, and anakinra 2 mg/kg/dose single dose via subcutaneous route. This patient also received inotropic support, IV furosemide, and mechanical ventilation due to shock secondary to heart failure and severe respiratory distress due to pulmonary congestion. This patient had fibrosis on cardiac MRI. There was no significant difference between 12 patients that received IV immunoglobulin and steroid and six patients that received IV immunoglobulin and acetylsalicylic concerning cardiac fibrosis (p=>0.999). Three patients were administered only IV immunoglobulin treatment.

All of 21 patients who underwent cardiac MRI had their cardiac systolic function completely normalized by a 1-year of follow-up. Their mitral valve insufficiency recovered in time, with only three patients having remained to have mild mitral valve insufficiency at the end of 1 year. The echocardiographic evaluation of coronary arteries was normal in all patients at follow-up. No patient had tachyarrhythmia or bradyarrhythmia during follow-up. None of the patients died at the hospital or during follow-up.

Cardiac MRI

Cardiac MRI examination was performed at a mean of 123.2±62.7 days after the diagnosis in five patients with cardiac fibrosis and 117.7±35.8 days after the diagnosis in 16 patients with normal cardiac MRI (p=0.968). The cardiac MRI findings of each of the five patients with cardiac fibrosis are described in detail below.

In patient number 1, midmyocardial contrast enhanced (focal fibrosis) was observed at the posterior right ventricle insertion point at midventricular level. The regional mean native T1 relaxation time prolongation (1185 msec) at this level in T1 mapping and an increase in extracellular volume (36%) supported fibrosis. Since global mean myocardial native T1 relaxation time (995 msec) and extracellular volume (28%) were in normal limits, diffuse fibrosis was excluded. Regional wall motion and systolic function were normal in cine sequences.

In patient number 2, midmyocardial contrast enhanced (focal fibrosis) was observed at the posterior right ventricle insertion point at midventricular level in LGE. The regional mean native T1 relaxation time prolongation (1170 msec) at this level in T1 mapping and an increase in extracellular volume (33%) supported focal fibrosis. Since global mean myocardial native T1 relaxation time (995 msec) and extracellular volume (28%) were in normal limits, diffuse fibrosis was excluded. Cine sequences showed normal regional wall motion and systolic function.

In patient number 3, there was midmyocardial contrast enhancement (focal fibrosis) in midventricular anterior septum (American Heart Association segment 8), transmural contrast enhancement in anterolateral wall (segment 11), and midmyocardial contrast enhancement in posterolateral wall (segment 12) on LGE. In T1 mapping, regional mean native T1 relaxation times were prolonged (1080 msec at segment 8, 1083 msec at segment 11, and 1075 msec at segment 12), and extracellular volumes increased approximately 30%. These findings supported LGE findings. Since global mean myocardial native T1 relaxation time (1040 msec) and extracellular volume (28%) were in normal limits, diffuse fibrosis was excluded. Cine sequences showed normal regional wall motion and systolic function.

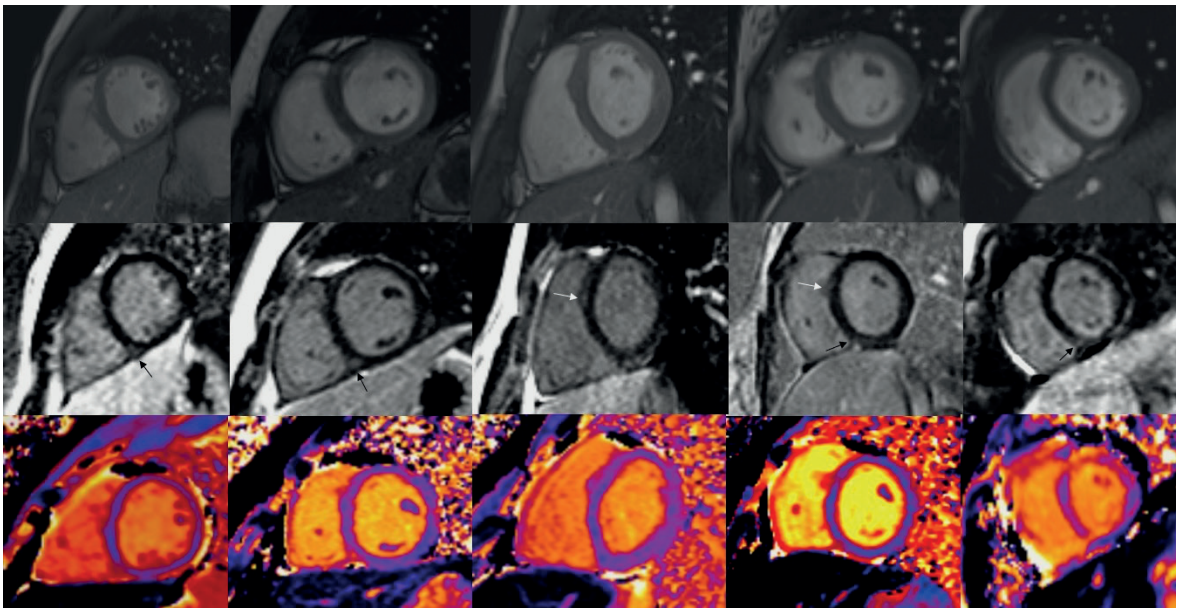


Fig. 1. Midventricular short axis cardiac MRI images in five children after a mean of 123.2±62.7 days of clinical diagnosis of MIS-C. First row shows no significant finding on cine images. Second row shows late gadolinium enhancement (LGE) confined to posterior right ventricular insertion points in patiens 1, 2, 4, 5 (black arrows) and anteroseptal wall adjacent to the septal papillary muscle attachment in patiens 3, 4 (white arrows). Third row demonstrates abnormal native T1 mapping which was greater than 1060 msec, on same localisations as LGE (second row) in all patients.

In patient number 4, midmyocardial contrast enhancement was detected in midventricular septum (segments 8 and 9) and posterior right ventricle insertion point, and subepicardial contrast enhancement in inferior wall (segment 10) (focal fibrosis) on LGE. T1 mapping showed prolonged regional mean native T1 relaxation times and increased extracellular volumes (1090 msec and 29%, respectively, at segment 8; 1100 msec and 30%, respectively, at segment 9; 1150 msec and 32%, respectively, at the posterior right ventricle insertion point, and 1097 msec and 30%, respectively, at segment 10). These findings supported LGE findings. Since global mean myocardial native T1 relaxation time (1027 msec) and extracellular volume (26%) were in normal limits, diffuse fibrosis was excluded. In cine sequences, normal regional wall motion and systolic function were observed.

In patient number 5, midventricular contrast enhancement was observed in inferior wall and inferior septum (segments 9 and 10), and transmural contrast enhancement in posterior right ventricle insertion point (focal fibrosis) in LGE. T1 mapping showed prolonged regional mean native T1 relaxation times and increased extracellular volumes (1100 msec and 44%, respectively, at segment 9; 1275 msec and 50%, respectively, at posterior right ventricle insertion point; and 1093 msec and 44%, respectively, at segment 10). These findings supported LGE findings. Since global mean myocardial native T1 relaxation time (1027 msec) and extracellular volume (26%) were in normal limits, diffuse fibrosis was excluded. In cine sequences, there was normal regional wall motion and systolic function (Fig. 1).

Discussion

In this study, we found that cardiac fibrosis, a sign of cardiac involvement, was more common in adolescents and obese children after the recovery of MIS-C. We also demonstrated that symptoms and physical examination findings were not effective in the development of fibrosis. Since we lacked a sufficient number of patients, we could not make any extrapolation as to the effect of clinical severity on fibrosis development. In patients who developed fibrosis, leucocyte and neutrophil counts were lower, while blood urea nitrogen, creatinine, and sodium level were higher. Cardiac fibrosis on MRI was located in the region of posterior right ventricle insertion point and midventricular septum. This study has a unique point as cardiac MRIs were obtained at a later stage compared to other literature published.

Hoste et al.[4], in a systematic review article, reported that overweight (having a body mass index > 25 kg/m^2 or being >85th percentile for age/gender) was present in 25.3% of patients. Other comorbidities were quite uncommon, with asthma being present in 4.1% of patients, chronic pulmonary disease in 1.5%, cardiovascular diseases in 1.3%, and immune deficiencies in 1%. They reported that obesity as a comorbidity was present in four out of eight (1.9%) patients who died. In a review article, Panigrahy et al.[5] reported that obesity (16.4%) and asthma (13.4%) were the most common comorbidities. In our study population, 2 (9.5%) patients had a body mass index > 25; these patients had cardiac fibrosis on cardiac MRI. None of our patients died.

In MIS-C, cardiac involvement, shock, and a complex inflammatory process affecting all systems is an exaggerated immune reaction involving overlapping mechanisms. This immune reaction and hemodynamic instability can cause tubular injury, podocytopathy, inflammatory process, and acute kidney injury secondary to vascular endothelial dysfunction.[6-8] Significantly higher BUN and creatinine levels in patients with cardiac fibrosis on MRI compared to those with a normal MRI suggest that the mechanisms causing renal injury may overlap with the mechanisms giving rise to cardiac fibrosis.

In many studies, it has been reported that troponin and N-terminal pro–B-type natriuretic

peptide levels were especially affected by cardiac function and increased, particularly in MIS-C patients with signs of shock.[9] In 61% of our patients, both troponin T and N-terminal pro–B-type natriuretic peptide were increased. There was no significant difference between patients with and without fibrosis regarding troponin T and N-terminal pro–B-type natriuretic peptide levels. NT-ProBNP is associated with left ventricular dilatation and heart failure. Troponin reflects myocardial damage. In addition to acute injury, many factors in the tissue healing process may be effective in the development of myocardial fibrosis, yet the small number of patients makes it difficult for us to comment on this issue.

MIS-C can cause many ECG changes. Sinus tachycardia is usually observed due to cardiac involvement and shock. Large case series have demonstrated PR prolongation, low QRS voltage, ST-segment, and T wave changes. Ventricular tachycardia, a malignant tachyarrhythmia form, rarely occurs due to myocarditis and electrolyte disorders.[11,12] In our case series, six patients had T wave flattening and 2 had low voltage as a pathological sign.

Reduced left ventricular ejection fraction is defined as EF<55%. In many studies, a decrease in left ventricular ejection fraction has been reported between 13-69% of patients.[3,14-16] Similar to the literature findings, 23% of our patients had a left ventricular ejection fraction < 55% on echocardiography. Varying degrees of atrioventricular valve insufficiency have been reported in patients with MIS-C. Valverde et al.[7] reported mild mitral insufficiency in 103 (38%) patients, moderate mitral insufficiency in 11 (4.2%) patients, and severe mitral insufficiency in one (0.3%) patient. Similarly, we detected six cases of mild mitral insufficiency, and five case of moderate mitral insufficiency, making a total of 54% of patients having mitral insufficiency. Pericardial effusion has been reported as "mild" in about 20% of patients at the time of diagnosis. Pericardial effusion usually improves over time, without causing any clinical problem.[16] Minimal pericardial effusion that was present in 28.5% of our patients completely disappeared over time.

In the literature, the reported rate of coronary artery involvement at the time of diagnosis is 14-28%. In a large-scale study, coronary dilation (Z score >2) in echocardiography was reported in 69 (24.1%) patients at the time of diagnosis.[7] However, it is well-known that coronary artery evaluation by echocardiography may be subjective. None of our patients had coronary artery dilation or aneurysm in echocardiography at the time of diagnosis.

Blondiaux et al.[17] performed cardiac MRI and showed diffuse myocardial edema in four patients, three of whom were in the acute phase and one in the recovery phase, when the first MIS-C cases started to be reported in April 2020. There was no evidence of replacement fibrosis or LGE indicating focal necrosis. These findings were interpreted as suggestive of post-infectious myocarditis among children and adolescents with COVID-19.[17] Theocharis et al.[18] performed cardiac MRI in 20 MIS-C patients they diagnosed in May 2020 after a median of 20 days. Cardiac MRI showed abnormal strain in 7 patients with global dysfunction (EF<55%), myocardial edema in 10 (50%) patients, and subendocardial infarction in one (5%) patient. Capone et al.[19] performed MRIs in 11 patients with left ventricular systolic dysfunction, troponin elevation and/or ECG changes 1-4 weeks after hospital discharge. None of the patients showed persistent edema or evidence of fibrosis. No coronary artery dilation or aneurysm was shown.

Aeschlimann et al.[20] published an article including 111 children with MIS-C (38% girls, median age ten years) from 17 centers, who had clinical cardiac involvement and underwent cardiac MRI. Sixty-five percent of patients had a depressed left ventricular ejection fraction at the time of diagnosis. Cardiac MRI was performed at a median of 28 (19-47) days after the symptom onset. Cardiac MRI diagnostic criteria for acute myocarditis were present in 20 patients; 18 of which also had evidence of myocardial necrosis

indicated by subepicardial LGE. A comparison of patients with and without myocarditis on cardiac MRI regarding clinical signs, laboratory data, and the applied treatment modalities revealed that the myocarditis positive group had a significantly greater rate of NYHA class IV disease, a significantly greater need for mechanical support, and a significantly higher lymphocyte count. In that study, LGE was identified in multiple different LV sites, most commonly in basal and mid inferolateral regions. In our research, on the other hand, late-term cardiac fibrosis on MRI was located in the region of posterior right ventricular insertion point and midventricular septum.

It has been reported that LGE is correlated with a worse composite endpoint of all-cause mortality, cardiac mortality, and/or major adverse cardiovascular events in adults with acute myocarditis.[21] It would be appropriate to follow MIS-C patients for such adverse outcomes in the long term.

The first limitation of the study is the relatively small number of patients. Therefore, no comparison could be made regarding the severity of the disease. The second limitation was that cardiac MRI was not available at the time of diagnosis, so late cardiac MRI findings could not be compared with cardiac MRI findings at the time of diagnosis. Another limitation is the absence of a healthy control group.

Clinically, MIS-C patients usually recover completely, irrespective of having cardiac fibrosis on MRI. Adolescence and obesity appear to be risk factors for cardiac fibrosis on MRI as a late sequela of myocarditis. We believe that the way the immune system reacts and other mechanisms causing acute kidney injury may be effective in the occurrence of cardiac fibrosis through complex overlapping mechanisms. Cardiac fibrosis on MRI was located in the region of posterior right ventricle insertion point and midventricular septum. However, this subject should be further clarified by more comprehensive studies. It can be evaluated whether cardiac fibrosis resolves over time. Future studies reporting the follow-up data of patients with fibrosis are necessary to predict and manage adverse outcomes.

Ethical approval

This study was approved by Kayseri City Hospital Clinical Research Ethics Committee (March/2021; no.320). Informed consent was obtained from the parents of all patients and also from the adolescent patients themselves.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MA; data collection: MA, TS, SYD, OT, SS, SÖ, FE; analysis and interpretation of results: MA, TS, İÇ, FE; draft manuscript preparation: MA, TS. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation 2002; 105: 539-542. https://doi.org/10.1161/hc0402.102975
2. Burkhardt BEU, Menghini C, Rücker B, Kellenberger CJ, Valsangiacomo Buechel ER. Normal myocardial native T1 values in children using single-point saturation recovery and modified look-locker inversion recovery (MOLLI). J Magn Reson Imaging 2020; 51: 897-903. https://doi.org/10.1002/jmri.26910

3. Jonat B, Gorelik M, Boneparth A, et al. Multisystem inflammatory syndrome in children associated with Coronavirus Disease 2019 in a children's hospital in New York City: patient characteristics and an institutional protocol for evaluation, management, and follow-up. Pediatr Crit Care Med. 2021; 22: e178-e191. https://doi.org/10.1097/pcc.0000000000002598

4. Hoste L, Van Paemel R, Haerynck F. Multisystem inflammatory syndrome in children related to COVID-19: a systematic review. Eur J Pediatr 2021; 180: 2019-2034. https://doi.org/10.1007/s00431-021-03993-5

5. Panigrahy N, Policarpio J, Ramanathan R. Multisystem inflammatory syndrome in children and SARS-CoV-2: a scoping review. J Pediatr Rehabil Med 2020; 13: 301-316. https://doi.org/10.3233/PRM-200794

6. Radia T, Williams N, Agrawal P, et al. Multi-system inflammatory syndrome in children & adolescents (MIS-C): a systematic review of clinical features and presentation. Paediatr Respir Rev 2021; 38: 51-57. https://doi.org/10.1016/j.prrv.2020.08.001

7. Valverde I, Singh Y, Sanchez-de-Toledo J, et al. Acute cardiovascular manifestations in 286 children with multisystem ınflammatory syndrome associated with COVID-19 infection in Europe. Circulation 2021; 143: 21-32. https://doi.org/10.1161/CIRCULATIONAHA.120.050065

8. Sethi SK, Rana A, Adnani H, et al. Kidney involvement in multisystem inflammatory syndrome in children: a pediatric nephrologist's perspective. Clin Kidney J 2021; 14: 2000-2011. https://doi.org/10.1093/ckj/sfab073

9. Whittaker E, Bamford A, Kenny J, et al. Clinical characteristics of 58 children with a pediatric ınflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA 2020; 324: 259-269. https://doi.org/10.1001/jama.2020.10369

10. Erol N, Sari E. Cardiac involvement in multisystem ınflammatory syndrome in children cases. Cardiol Young 2022; 32: 1780-1785. https://doi.org/10.1017/S1047951121004911

11. Wu EY, Campbell MJ. Cardiac manifestations of multisystem inflammatory syndrome in children (MIS-C) following COVID-19. Curr Cardiol Rep 2021; 23: 168. https://doi.org/10.1007/s11886-021-01602-3

12. Regan W, O'Byrne L, Stewart K, et al. Electrocardiographic changes in children with multisystem ınflammation associated with COVID-19: associated with coronavirus disease 2019. J Pediatr 2021; 234: 27-32.e2. https://doi.org/10.1016/j.jpeds.2020.12.033

13. Niaz T, Hope K, Fremed M, et al. Role of a pediatric cardiologist in the COVID-19 pandemic. Pediatr Cardiol 2021; 42: 19-35. https://doi.org/10.1007/s00246-020-02476-y

14. Sharma C, Ganigara M, Galeotti C, et al. Multisystem inflammatory syndrome in children and Kawasaki disease: a critical comparison. Nat Rev Rheumatol 2021; 17: 731-748. https://doi.org/10.1038/s41584-021-00709-9

15. Hejazi OI, Loke YH, Harahsheh AS. Short-term cardiovascular complications of multi-system ınflammatory syndrome in children (MIS-C) in adolescents and children. Curr Pediatr Rep 2021; 9: 93-103. https://doi.org/10.1007/s40124-021-00258-5

16. Lawrensia S, Henrina J, Wijaya E, Suciadi LP, Saboe A, Cool CJ. Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2: a new challenge amid the pandemic. SN Compr Clin Med 2020; 2: 2077-2085. https://doi.org/10.1007/s42399-020-00602-8

17. Blondiaux E, Parisot P, Redheuil A, et al. Cardiac MRI in children with multisystem ınflammatory syndrome associated with COVID-19. Radiology 2020; 297: E283-E288. https://doi.org/10.1148/radiol.2020202288

18. Theocharis P, Wong J, Pushparajah K, et al. Multimodality cardiac evaluation in children and young adults with multisystem inflammation associated with COVID-19. Eur Heart J Cardiovasc Imaging 2021; 22: 896-903. https://doi.org/10.1093/ehjci/jeaa212

19. Capone CA, Misra N, Ganigara M, et al. Six month follow-up of patients with multi-system ınflammatory syndrome in children. Pediatrics 2021; 148: e2021050973. https://doi.org/10.1542/peds.2021-050973

20. Aeschlimann FA, Misra N, Hussein T, et al. Myocardial involvement in children with post-COVID multisystem inflammatory syndrome: a cardiovascular magnetic resonance based multicenter international study-the CARDOVID registry. J Cardiovasc Magn Reson 2021; 23: 140. https://doi.org/10.1186/s12968-021-00841-1

21. Georgiopoulos G, Figliozzi S, Sanguineti F, et al. Prognostic ımpact of late gadolinium enhancement by cardiovascular magnetic resonance in myocarditis: a systematic review and meta-analysis. Circ Cardiovasc Imaging 2021; 14: e011492. https://doi.org/10.1161/CIRCIMAGING.120.011492

The Turkish Journal of Pediatrics 2023; 65: 445-455
https://doi.org/10.24953/turkjped.2022.1095

Original Article

Isolated hypogonadotropic hypogonadism in adolescence: Do we need to measure the pituitary, stalk or other imaging markers? A retrospective magnetic resonance imaging study

Ercan Ayaz[1], Ruken Yıldırım[2], Canan Çelebi[1], Şervan Özalkak[2]

[1]Department of Radiology, Diyarbakir Children's Hospital, Diyarbakır; [2]Department of Pediatric Endocrinology, Diyarbakir Children's Hospital, Diyarbakır, Türkiye.

ABSTRACT

Background. Rapid changes in the size of the pituitary gland occur during the pubertal period. Therefore, measuring and reporting magnetic resonance imaging (MRI) in adolescents with pituitary disorders can cause unease among radiologists. Our aim was to compare the size of the pituitary gland, stalk and other previously described imaging tools in patients with isolated hypogonadotropic hypogonadism (HH) versus adolescents with a normal pituitary gland.

Methods. Forty-one patients (22 female, 19 male, mean age 16.3 ±2.0 years) with HH who underwent MRI prior to starting hormone treatment were enrolled. Age, sex, and genetic mutations were noted. Pituitary height, width on the coronal plane, anteroposterior (AP) diameter on the sagittal plane, stalk thickness, pons ratio (PR), clivus canal angle (CCA) and Klaus index (KI) were measured by two radiologists twice with a one-month interval blinded to each other and patient information. Measurements were compared with the control group, including 83 subjects with normal hypothalamic-pituitary-gonadal axis and normal pituitary gland on MRI. Inter-rater and intra-rater agreements were also evaluated.

Results. No significant differences were found between the two groups regarding height, width or AP diameter ($p = 0.437$, 0.836, 0.681 respectively). No significant differences were found between the two groups regarding CCA and PR ($p = 0.890$, 0.412 respectively). The KI of the male patients was significantly higher than that of the female patients and the control group ($p < 0.001$). The interrater agreement was moderate for pituitary height and width, poor for pituitary AP diameter and stalk thickness, good for PR and KI, and excellent for CCA.

Conclusions. The measurements of the pituitary gland, stalk and posterior fossa structures were similar in adolescents with or without isolated HH. Consequently, pituitary gland, stalk or other posterior fossa measurements are unnecessary when evaluating a normal appearing pituitary gland on MRI.

Key words: hypogonadotropic hypogonadism, magnetic resonance imaging, delayed puberty, pons ratio, clivus canal angle.

Hypogonadotropic hypogonadism (HH), also known as secondary or central hypogonadism, reflects gonadal dysfunction resulting from hypothalamic-pituitary insufficiency.[1] It can be either congenital (called idiopathic) or due to an acquired cause. Isolated HH is more common in males, with prevalence rates of approximately 1:4000 to 1:10000 among males and 1:50000 among females.[2] Since the most common known genetic cause is Kallmann syndrome, isolated HH can be divided into three categories: Kallmann syndrome, isolated HH with normosmia, and complex non-Kallmann syndromes.[3] Although many tests are available to assess HH, most of them have limited accuracy in differentiating HH from

✉ Ercan Ayaz
ercan.ayaz1@gmail.com

Received 22nd December 2022, revised 23rd March 2023, accepted 18th April 2023.

This study was presented at the 57th Annual Meeting of European Society of Pediatric Radiology (ESPR 2023) in Belgrade, Serbia, June 05-09, 2023.

constitutional delay of growth and puberty in adolescents.[4,5] Since treatment can be considered at a younger age (up to 12 years old) for children with HH to maintain physiologic and psychosocial health, a definitive diagnosis is very important.[6]

Magnetic resonance imaging (MRI) of the pituitary gland is generally preferred before starting treatment for HH to exclude tumoral, infiltrative lesions, or malformative midline abnormalities affecting the hypothalamic-pituitary region that could damage GnRH neurons, the pituitary stalk, or pituitary gonadotrope cells and thereby prevent pubertal development.[7] MRI is also quite useful for evaluating the olfactory bulbs, tracts and sulci to differentiate Kallman syndrome from other causes of HH.[8] The pituitary size varies according to age, sex, and puberty, and the measures in patients with pituitary disorders can sometimes cause clinical and radiological confusion. There have been many studies defining the normal size and volume of the pituitary gland and stalk in children and adolescents.[9-11] However, there have been no comparative studies of the pituitary gland and stalk size between isolated HH patients and healthy adolescents in the literature.

In previous studies evaluating disorders of the hypothalamic-pituitary region, particularly for growth hormone deficiency, the pons ratio (PR) was described and suggested as an imaging biomarker. The PR was found to be significantly higher in patients with pituitary insufficiency than in healthy children.[12,13] The clivus canal angle (CCA) and Klaus index (KI) were also significantly different between those patients and the control group.[12] The first objective of our study was to assess the pituitary and stalk diameters of adolescents with isolated HH and compare them with those of healthy peers located in a similar region. Our second aim was to measure previously described imaging parameters (PR, CCA and KI) in our patients and compare them between the sexes and with healthy adolescents.

Material and Methods

Patients and Control Group

Ethics approval for this retrospective study was obtained from Diyarbakir Gazi Yaşargil Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 2021/947, date of approval: 03/12/2021). We evaluated the patients diagnosed with HH in our pediatric endocrinology department between January 2017 and June 2022. Clinical and laboratory data of the patients were obtained from their files in the archive of pediatric endocrinology. The diagnostic criteria for HH were absent or incomplete pubertal development (menarche and secondary sexual characters) for age, low gonadal volume of bilateral gonads without any organic gonadal lesion, low estradiol concentrations for female, low total testosterone for males and low or inappropriately normal serum gonadotropin concentrations, and normal results for prolactin, thyroid-stimulating hormone, growth hormone, cortisol and adrenocorticotropic hormone to exclude complete pituitary insufficiency.

We identified 46 patients (26 females and 20 males) with a clinical diagnosis of isolated HH, and 41 (22 females and 19 males) of those had available MRI examinations in our radiology archive. Age, sex, genetic mutations, age at MRI examination, and clinical findings were noted. We also formed a healthy control group for comparison with the patients instead of using normal reference values from the literature to prevent age, sex, and ethnicity discrepancies. We attempted to maintain an approximately 1:2 case/control ratio to increase the statistical power of the analysis. Subjects in the control group were selected for each patient of a similar age (± 12 months) at MRI examination. The control group consisted of patients who presented with various complaints, but laboratory data regarding the hypothalamic-pituitary axis were normal. We retrieved 100 pituitary MRI studies from our radiology archive to form the control group. We excluded seventeen patients who

had abnormal findings on pituitary MRI (six Rathke cleft cysts, five partial empty sella, three nonfunctioning adenomas, two suprasellar arachnoid cysts and one generalized atrophy of the brain and cerebellum). The remaining 83 patients constituted our control group. A flow chart of the sample selection for the study and control groups is presented in Fig. 1.

Imaging Evaluations

Pituitary MRI examinations in both groups were performed on a 1.5-T Signa HDxt scanner (General Electric Healthcare; Milwaukee, WI, USA) with an 8-channel, 8-element phased array head coil. The standard pituitary imaging protocol comprised coronal T1-weighted and T2-weighted images (slice thickness, 2 mm), mid-sagittal pre- and postcontrast T1-weighted images covering the area between the lateral wall of each cavernous sinus (slice thickness, 3 mm), and dynamic coronal T1-weighted images including 5 phases (1 precontrast and 4 postcontrast studies, 40-second interval between the phases and slice thickness, 2 mm).

For parametric data, pituitary height was measured from the postcontrast sagittal or coronal image for each patient, which revealed that the borders of the pituitary gland were more conspicuous than in the other sequences. Width was measured from the coronal images; anteroposterior (AP) diameter and pituitary stalk thickness were measured from the sagittal images (Fig. 2A,2B). The PR, CCA and KI were measured from mid-sagittal images. To measure the PR, a line from the tip of the dorsum sella to the fastigium of the fourth ventricle was drawn. The ratio of the height of the pons above this line to the total height of the pons on mid-sagittal images was defined as the PR (Fig. 2C). The CCA was the angle between the line coursing through the dorsal surface of the clivus and the posterior margin of the odontoid process of the C2 vertebra (Fig. 2D). The KI was the shortest distance between the tip of the odontoid process and Twining's line (the line between the tuberculum sella and torcula) (Fig. 2E).[12]

Images were reviewed by two radiologists with 7 (E.A.) and 5 (C.C.) years of experience in neuroimaging. Measurements were performed twice by the two radiologists independently, without knowledge of the patient's information, with a one-month interval between measurements. Measurements were performed on the best quality series, and these series were recorded. All of the measurements were performed utilizing RadiAnt DICOM Viewer software, version 2022.1.1 (Medixant, Poznan, Poland, https://www.radiantviewer.com).

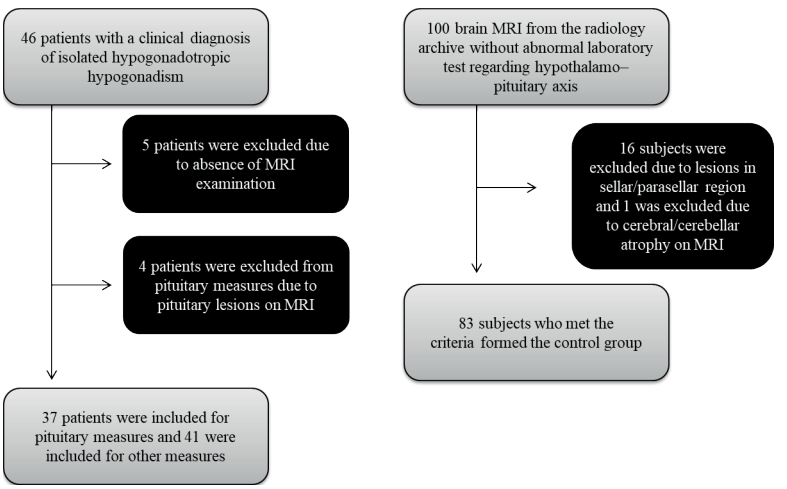


Fig. 1. Flow chart of the sample selection for the study and control groups.

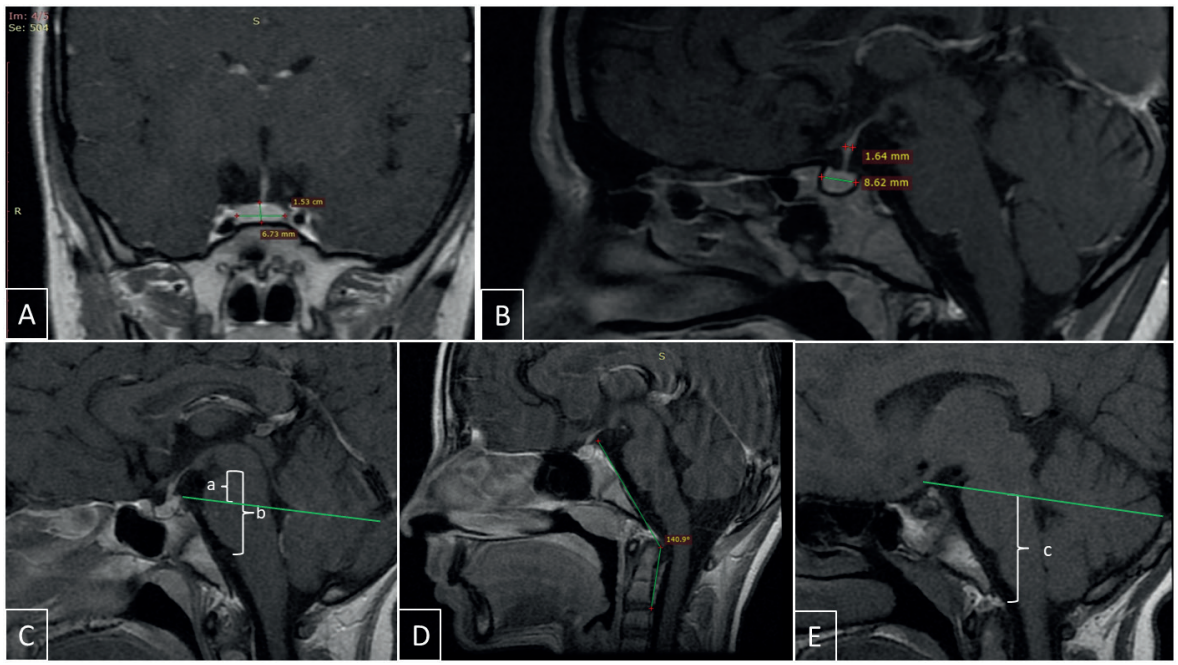


Fig. 2. Measurement methods used in the study. (A) Pituitary height and width measurements from coronal postcontrast T1-weighted images at the widest points of the pituitary gland. (B) Mid-sagittal T1-weighted image presents the anteroposterior diameter of the pituitary gland and the thickness of the stalk. (C) Mid-sagittal T1-weighted image demonstrates the pons ratio (a/b). (D) Mid-sagittal T1-weighted image represents measurement of the clivus canal angle, the angle between the dorsal surface of the clivus and the posterior margin of the odontoid process of the C2 vertebra. (E) Mid-sagittal T1-weighted image demonstrates the Klaus index (c) between the tip of the odontoid process and the line connecting the tuberculum sella and internal occipital protuberance.

Statistical analysis

Descriptive statistics were expressed as the mean ± standard deviation (SD) for continuous variables and as numbers and percentages for categorical variables. The median values of four measurements of distances, ratios and angles of the control and patient groups were used to prevent the effects of outliers. For continuous variables, the Kolmogorov-Smirnov test was used to test for the normal distribution of data. Pearson's chi-square test was used to compare categorical variables among groups. Student's t test was used for the comparison of two parametric variables that showed a normal distribution, and the Mann-Whitney U test was used for the comparison of two parametric variables that showed a nonnormal distribution.

To evaluate the reliability of inter-rater and intra-rater measurements, the intraclass correlation coefficient (ICC) was used with two-way random and two-way mixed effects models, respectively. When evaluating intra-rater reliability, average values of two measurements from each reviewer were used. The 95% confidence interval (CI) of the ICC estimate was used to evaluate the level of reliability, which was classified as excellent for values greater than 0.85, good for values between 0.70 and 0.85, moderate for values between 0.5 and 0.75, and poor for values less than 0.5.[14]

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 23.0 (SPSS Inc., Chicago, IL, USA). The level of significance was set at $p < 0.05$, and the significance level was adjusted according to Bonferroni's correction for multiple comparisons.

Results

The study group included 41 patients with isolated HH (22 female and 19 male, mean age at MRI = 16.3 ± 2 years old (range between 12.8 and 21.1 years)), and the control group included 83 healthy subjects (57 female and 26 male, mean age at MRI = 15.4 ± 3.6 years old (range between 12.1 and 22 years)). The demographic data of the patient and healthy control groups are presented in Table I. No significant difference was found between the two groups regarding age and sex (p = 0.166 and 0.122). Four patients (9.76%) had an organic lesion on pituitary MRI; three of those were Rathke cleft cysts (pars intermedia cysts), and one lesion was a partial empty sella. We excluded these patients from pituitary and stalk measures but included them in the CCA, PR, and KI measures. Therefore, 37 patients were included in the study group for pituitary height, width and AP diameter and for stalk thickness measurements, whereas 41 patients were included for PR, CCA and KI. Sixteen of the 41 (39%) patients were evaluated with next-generation sequencing of 38 targeted genes responsible for HH. Seven patients (43.8%) had a mutation in a particular gene (three had a mutation in *TACR3*, one had a mutation in *PROK2*, one had a mutation in *GNRHR*, one had a mutation in *WDR11* and one had a mutation in *SOX10*). No genetic mutations were identified in the remaining nine patients.

Pituitary gland, stalk and other measurements

In the comparison of the pituitary gland measurements, no significant differences were found between the two groups regarding height, width and AP diameter (p = 0.437, 0.836 and 0.681, respectively). Pituitary stalk thickness was significantly higher in patients than in the control group (p < 0.001). Additionally, no significant difference was found between the two groups regarding CCA and PR (p = 0.890, and 0.412). The KI was significantly higher in patients than in healthy controls (p = 0.027). The mean values and comparison of the measurements of the study group and the control group are presented in Table II and Fig. 3.

Table I. Demographic data of the study group, control group and their comparison.

	Central hypogonadism	Healthy subjects	P value
Number of cases	41	83	
Mean age ± SD (min/max) (years)	16.3±2.0 (12.8/21.1)	15.4±3.6 (12.1/22.0)	0.121
Gender (F/M)	22/19	57/26	0.122
Mean age ± SD of females (years)	16.5 ±2.1	15.9 ±3.8	0.386
Mean age ± SD of males (years)	16.1 ±1.9	14.2 ±2.7	0.033

F: female, M: male, max: maximum, min: minimum, SD: standard deviation

Table II. Mean distance and angle measurement parameters of the study group and the control group and comparison.

Measurement Parameters	Central hypogonadism (n=41) mean ± SD	Healthy subjects (n=83) mean ± SD	P value
Pituitary Height (mm)*	5.4 ± 1.01	5.6 ± 0.93	0.346**
Pituitary Width (mm)*	13.7 ± 2.18	13.9 ± 2.13	0.745**
Pituitary AP diameter (mm)*	9.5 ± 1.10	9.4 ± 1.22	0.781***
Pituitary stalk thickness*	1.9 ± 0.34	1.6 ± 0.25	<0.001***
Pons Ratio	0.32 ± 0.04	0.32 ± 0.04	0.827***
Clivus canal angle	149.8 ± 6.91	148.7 ± 8.68	0.406**
Klaus index (mm)	39.0 ± 3.66	37.7 ± 3.33	0.032**

AP: anteroposterior, n: number of subjects, SD: standard deviation . *Calculated in 37/41 patients with central hypogonadism **t test ***Mann-Whitney U test

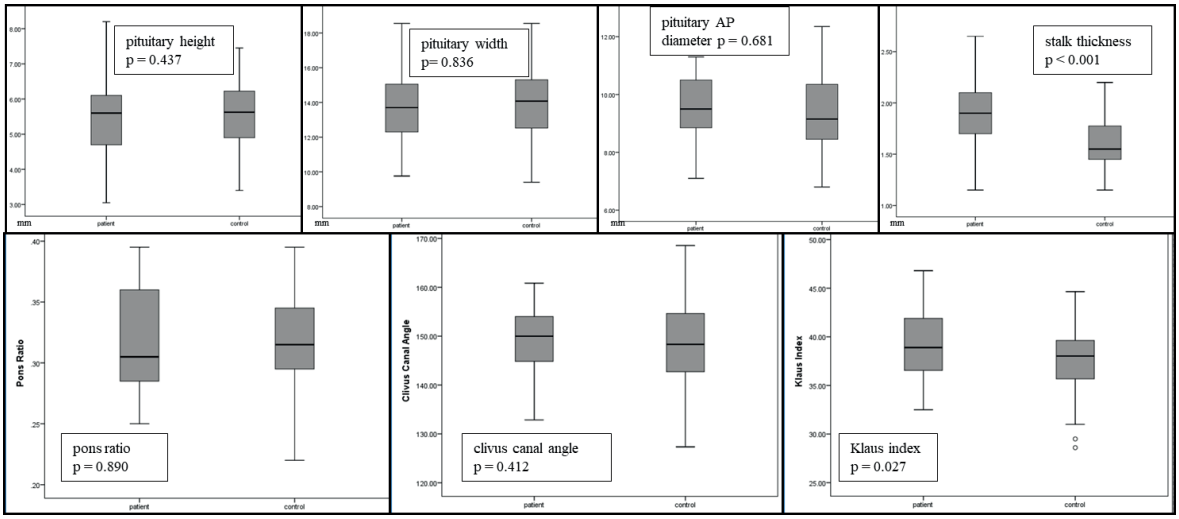


Fig. 3. Boxplot graphs showing the comparison of the diameter, index and angle measurements with p values between the adolescents with hypogonadotropic hypogonadism and the healthy subjects.

We also compared the measurements of the study group and the control group separately according to sex. In isolated HH patients, KI was significantly higher in males than in females (p = 0.001). No significant differences were found among the other measurements of the study group (p > 0.05). There were no significant differences between males and females in the control group among any of the measurement parameters (p > 0.05) (Table III).

The reliability analysis between the radiologists and the agreement between the two measurements of each individual radiologist are shown in Table IV. According to the 95% CIs of the ICC values, inter-rater agreement was moderate for pituitary height and width, poor for pituitary AP diameter and stalk thickness, good for PR and KI, and excellent for CCA. For reviewer 1 (E.A.), the inter-rater agreement was moderate for pituitary height and width, poor for pituitary AP diameter and stalk thickness, moderate for PR and excellent for CCA and KI. For reviewer 2 (C.Ç.), the inter-rater agreement was good for pituitary height, moderate for pituitary width, poor for pituitary AP diameter and stalk thickness, moderate for PR, excellent for CCA and good for KI.

Table III. Mean values of the study parameters according to sex in each group and comparison.

Measurement parameters	Central hypogonadism			Healthy subjects		
	Females mean ± SD (n = 22)	Males mean ± SD (n = 19)	*P* value	Females mean ± SD (n = 57)	Males mean ± SD (n = 26)	*P* value
Pituitary height (mm)	5.5 ± 1.1	5.3 ± 0.9	0.450*	5.7 ± 1.0	5.4 ± 0.8	0.175*
Pituitary width (mm)	13.6 ± 2.2	13.9 ± 2.2	0.652*	14.2 ± 2.2	13.2 ± 1.8	0.066*
Pituitary AP diameter (mm)	9.4 ± 1.2	9.5 ± 1.0	0.649*	9.5 ± 1.3	9.2 ± 1.0	0.271**
Pituitary stalk thickness	1.9 ± 0.3	1.9 ± 0.4	0.726*	1.6 ± 0.3	1.6 ± 0.2	0.246**
Pons ratio	0.31 ± 0.05	0.32 ± 0.04	0.791*	0.32 ± 0.04	0.32 ± 0.04	0.908*
Clivus canal angle	150.7 ± 6.2	149.7 ± 7.7	0.647*	149.8 ± 9.3	147.2 ± 6.6	0.203*
Klaus index (mm)	37.5 ± 2.9	41.1 ± 3.5	0.001*	37.3 ± 3.4	38.7 ± 3.1	0.078*

AP: anteroposterior, n: number of subjects, SD: standard deviation *t test **Mann-Whitney U test

Table IV. Inter-rater and intra-rater agreement intraclass coefficient (ICC) values with 95% confidence intervals.

		Pituitary height	Pituitary width	Pituitary AP length	Stalk thickness	Pons ratio	Clivus canal angle	Klaus index
		ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)
Inter-rater Agreement		0.790 (0.593–0.892)	0.831 (0.671–0.913)	0.648 (0.317–0.819)	0.584 (0.324–0.762)	0.884 (0.780–0.939)	0.948 (0.901–0.972)	0.914 (0.838–0.955)
Intra-rater Agreement	Rater 1	0.801 (0.613–0.897)	0.837 (0.683–0.916)	0.728 (0.471–0.860)	0.609 (0.358–0.777)	0.836 (0.689–0.913)	0.946 (0.898–0.972)	0.929 (0.867–0.963)
	Rater 2	0.891 (0.791–0.943)	0.824 (0.659–0.909)	0.737 (0.498–0.862)	0.652 (0.324–0.821)	0.785 (0.593–0.886)	0.931 (0.869–0.963)	0.916 (0.841–0.956)

* ICC: intraclass correlation coefficient, CI: confidence interval, AP: antero-posterior; all measurements had p values of < 0.005

Discussion

Based on the results of our study, there were no significant differences between the adolescents with isolated HH and healthy adolescents regarding the pituitary diameters, PR and CCA. Although the stalk thickness of the patient group was significantly higher than that of the control group, the reliability of this measurement was the lowest among all parameters of the study. The KI was found to be significantly higher than that of healthy subjects, and it was mainly based on the higher KI of males in the patient group. The KI is the craniocaudal distance of a particular part of the cranium, which might be affected by height differences among the subjects. Since we did not have the height information from both groups during the MRI examination, we were not able to correlate the height and KI values.

Pituitary MRI is generally included in the diagnostic investigation of pituitary endocrinopathies since it has the highest diagnostic accuracy among radiological modalities.[15,16] The gradual increase in normal pituitary gland volume during the pubertal period is a well-known phenomenon.[17] This increase in pituitary height begins at the age of 11 years old in females and 13 years old in males, reaches the maximum volume in the third decade of life and then gradually decreases.[9,17] Due to changing size in the pubertal period, the interpretation of pituitary MRI in adolescents can cause uncertainty among radiologists. Therefore, many articles have been published providing normative data for pituitary and stalk size and volume in the literature.[9,10,17,18] The abovementioned increase in pituitary volume during puberty has generally been attributed to the increase in gonadotropins (LH and FSH). However, our study showed that no significant difference was seen between hypogonadotropic and normogonadotropic adolescents in a similar age group regarding pituitary size. Although our study was based on a small number of subjects with a particular disorder, the findings can encourage researchers to revise this generally accepted hypothesis.

As a general opinion, the height and volume of the pituitary gland are larger in females than males during the pubertal period.[9,17] In the present study, although females had slightly larger height in both groups, there were no significant differences between males and females according to pituitary height, width and AP diameter in either the isolated HH group or the healthy subjects. Similar to our results, no significant difference was found between female and male subjects in the study by Naik et al.[18] performed on an Indian population between 10 and 19 years old according to pituitary height, length and width.

Pituitary stalk thickness also shows a correlation with pituitary gland enlargement during the pubertal period in healthy adolescents. Sari et al.[9] suggested using the pituitary stalk to basilar artery ratio on the same axial plane for evaluating stalk thickening. The maximum ratios were 0.73 and 0.70 for females and males, respectively, in adolescents, without significant differences between genders. However, the diameter of the arteries of the vertebrobasilar system is very variable, and the normal basilar artery diameter ranges from 3 to 7 mm.[19] Therefore, we believed that the aforementioned ratio was an unreliable tool, and we directly measured the stalk thickness on the sagittal plane. In our study, HH patients (mean 1.9 mm ± 0.34) had significantly thicker stalks than the control group (mean 1.6 mm ± 0.25). The most frequent cause of stalk thickening in adolescents is inflammatory and infectious etiologies such as lymphocytic hypophysitis, tuberculosis, or neurosarcoidosis. Also, the most common functional disorder is central diabetes insipitus, which is a posterior pituitary disorder.[20] However, recent studies showed that stalk thickening also indicates an anterior pituitary disorder or the combination of an anterior and posterior pituitary disorder.[20,21] Ling et al.[21] evaluated the etiology of stalk thickening in 325 patients with a median age of 30.5 years, and 277 of those had an anterior pituitary disorder. The most common cause of the anterior pituitary disorder was hypogonadism (31%), followed by growth hormone deficiency (25.3%) and hypothyroidism (6.8%), which is concordant with our study.[21] Nevertheless, poor interrater and intrarater ICC of the pituitary stalk and nonnormal distributions of the values of both groups decreased the reliability of our stalk measurements.

Four of 41 patients (9.76%) in our study group and 16 of 100 (16%) subjects with a normal hypothalamic-pituitary-gonadal axis forming the control group had non-adenomatous pituitary lesions on MRI. The most common lesion was a Rathke cleft cyst, followed by a partially empty sella. Accordingly, a partially empty sella is found in 20% of the population, and Rathke cleft cysts are found in 11-33% of autopsy series.[22,23] In the study by Tang et al.[2], empty sella were found in 7.1% of isolated HHs. These results suggest that organic lesions of the pituitary gland can be seen in isolated HH but not more frequently than in the normal population.

The low pituitary gland height in adolescents with isolated or multiple pituitary hormone deficiencies was reported to be between 25% and 62%.[13,24,25] This broad variation renders height measurement unreliable; therefore, other imaging tools have been described, such as PR, CCA and KI.[12,13] Although pituitary diameter measurements vary greatly during puberty, these imaging methods are not affected by pubertal status. In patients with growth hormone deficiency, endochondral ossification and the development of spheno-occipital synchondrosis and the skull base cause delays due to reduced production of insulin-like growth factor 1, also called somatomedin C.[26] Underdevelopment of the skull base, particularly the posterior fossa, can cause a superior shift of the brain stem along with the hypothalamus, leading to traction in

the pituitary stalk.[13] In previous studies, PR was significantly higher in children with growth hormone deficiency, likely due to upward displacement of the pons.[12,13] Additionally, CCA and KI were significantly lower in those patients, probably due to underdevelopment of the posterior fossa skull base.[12] Other pituitary hormones, such as gonadotropin, TSH and ACTH, have been suggested to potentiate this effect together with growth hormone deficiency , thereby leading to a higher PR.[13] However, we did not find a significant difference between the isolated HH patients and the control group according to PR or CCA. In contrast to previous studies, KI was significantly higher in males with isolated HH than in females and healthy controls. However, we had only 19 males with isolated HH, and we were not able to correlate with the height of the subjects, which might have led to bias in our data.

This study has several limitations. First, due to the retrospective nature of the study, we were not able to arrange the imaging protocol according to our parameters, but we had to use the available examinations instead. 3D T1 or axial thin section steady-state free procession (SSFP) with a higher magnetic field would be more accurate for evaluating tiny anatomical structures, particularly the pituitary stalk. We were not able to control the positions of the patients during MRI scanning, which might have affected the CCA measurements. Second, we could not compare the different types of isolated HH, such as Kallmann syndrome, which is diagnosed with the detection of genetic mutations or objective smell identification tests.[27] Finally, the number of included patients might not be sufficient to attain a definitive conclusion. Further multicenter, prospective studies and comparisons with other types of central hypogonadism are required to validate our results. Since the time of examination could not be organized, no correlation between the hormonal levels and MRI measurements was established.

In conclusion, this study revealed that pituitary measurements on pituitary MRI did not contribute any additional value when evaluating isolated HH in adolescents other than entailing a loss of time. Therefore, only evaluating structural abnormalities and lesions seems to be sufficient in these patients. Further studies of similar parameters in other pituitary disorders in the same age group would be beneficial to expand on our results.

Ethical approval

Ethics approval for the retrospective study was obtained from Diyarbakir Gazi Yasargil Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 2021/947, date of approval: 03/12/2021).

Author contribution

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

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Rey RA. Recent advancement in the treatment of boys and adolescents with hypogonadism. Ther Adv Endocrinol Metab 2022; 13: 20420188211065660. https://doi.org/10.1177/20420188211065660
2. Tang RY, Chen R, Ma M, Lin SQ, Zhang YW, Wang YP. Clinical characteristics of 138 Chinese female patients with idiopathic hypogonadotropic hypogonadism. Endocr Connect 2017; 6: 800-810. https://doi.org/10.1530/EC-17-0251
3. Cho CY, Tsai WY, Lee CT, et al. Clinical and molecular features of idiopathic hypogonadotropic hypogonadism in Taiwan: a single center experience. J Formos Med Assoc 2022; 121: 218-226. https://doi.org/10.1016/j.jfma.2021.03.010
4. Klein KO, Phillips SA. Review of hormone replacement therapy in girls and adolescents with hypogonadism. J Pediatr Adolesc Gynecol 2019; 32: 460-468. https://doi.org/10.1016/j.jpag.2019.04.010
5. Dwyer AA, Phan-Hug F, Hauschild M, Elowe-Gruau E, Pitteloud N. Transition in endocrinology: hypogonadism in adolescence. Eur J Endocrinol 2015; 173: R15-24. https://doi.org/10.1530/EJE-14-0947
6. Sbardella E, Pozza C, Isidori AM, Grossman AB. Endocrinology and adolescence: dealing with transition in young patients with pituitary disorders. Eur J Endocrinol 2019; 181: R155-R171. https://doi.org/10.1530/EJE-19-0298
7. Young J. Approach to the male patient with congenital hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2012; 97: 707-718. https://doi.org/10.1210/jc.2011-1664
8. Jagtap VS, Sarathi V, Lila AR, et al. An objective olfactory evaluation and its correlation with magnetic resonance imaging findings in Asian Indian patients with idiopathic hypogonadotropic hypogonadism. Endocr Pract 2013; 19: 669-674. https://doi.org/10.4158/EP13008.OR
9. Sari S, Sari E, Akgun V, et al. Measures of pituitary gland and stalk: from neonate to adolescence. J Pediatr Endocrinol Metab 2014; 27: 1071-1076. https://doi.org/10.1515/jpem-2014-0054
10. Fink AM, Vidmar S, Kumbla S, et al. Age-related pituitary volumes in prepubertal children with normal endocrine function: volumetric magnetic resonance data. J Clin Endocrinol Metab 2005; 90: 3274-3278. https://doi.org/10.1210/jc.2004-1558
11. Marziali S, Gaudiello F, Bozzao A, et al. Evaluation of anterior pituitary gland volume in childhood using three-dimensional MRI. Pediatr Radiol 2004; 34: 547-551. https://doi.org/10.1007/s00247-004-1208-6
12. Tunçyürek Ö, Turgut M, Ünüvar T, Tubbs RS, Özsunar Y. Pons ratio as a potential diagnostic biomarker for the detection of growth hormone deficiency in children. Neurol India 2018; 66: 1680-1684. https://doi.org/10.4103/0028-3886.246236
13. Demiral M, Salih Karaca M, Unal E, et al. A novel diagnostic tool for the evaluation of hypothalamic-pituitary region and diagnosis of growth hormone deficiency: pons ratio. J Pediatr Endocrinol Metab 2020; 33: 735-742. https://doi.org/10.1515/jpem-2019-0321
14. Koo TK, Li MY. A Guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 2016; 15: 155-163. https://doi.org/10.1016/j.jcm.2016.02.012
15. Dalvi M, Walker BR, Strachan MWJ, Zammitt NN, Gibb FW. The prevalence of structural pituitary abnormalities by MRI scanning in men presenting with isolated hypogonadotrophic hypogonadism. Clin Endocrinol (Oxf) 2016; 84: 858-861. https://doi.org/10.1111/cen.13015
16. Chaudhary V, Bano S. Imaging of pediatric pituitary endocrinopathies. Indian J Endocrinol Metab 2012; 16: 682-691. https://doi.org/10.4103/2230-8210.100635
17. Yadav P, Singhal S, Chauhan S, Harit S. MRI evaluation of size and shape of normal pituitary gland: age and sex related changes. J Clin Diagn Res 2017; 11: 1-4. https://doi.org/10.7860/jcdr/2017/31034.10933
18. Naik D, Reddy DP, Srinath MG, Ashok Kumar A. Pituitary gland assessment by MR volumetry in the normal Indian adolescent population. International Journal of Medical Imaging 2015; 3: 105-109. https://doi.org/10.11648/j.ijmi.20150306.11
19. Pai BS, Varma RG, Kulkarni RN, Nirmala S, Manjunath LC, Rakshith S. Microsurgical anatomy of the posterior circulation. Neurol India 2007; 55: 31-41. https://doi.org/10.4103/0028-3886.30424
20. Moszczyńska E, Baszyńska-Wilk M, Zasada K, Majak D, Szaniawska M, Szalecki M. Pituitary stalk thickening in patients under 18 years of age - the most common causes and diagnostic procedures. Pediatr Endocrinol Diabetes Metab 2022; 28: 213-227. https://doi.org/10.5114/pedm.2022.115202
21. Ling SY, Zhao ZY, Tao B, et al. Pituitary stalk thickening in a large cohort: toward more accurate predictors of pituitary dysfunction and etiology. Endocr Pract 2019; 25: 534-544. https://doi.org/10.4158/EP-2018-0550
22. Gaillard F, Weerakkody Y, Deng F, et al. Empty sella. Reference article, Radiopaedia.org. Available at: https://radiopaedia.org/articles/17774 (Accessed on Nov 17, 2022).

23. Trifanescu R, Ansorge O, Wass JAH, Grossman AB, Karavitaki N. Rathke's cleft cysts. Clin Endocrinol (Oxf) 2012; 76: 151-160. https://doi.org/10.1111/j.1365-2265.2011.04235.x

24. Nakaguma M, Correa FA, Santana LS, et al. Genetic diagnosis of congenital hypopituitarism by a target gene panel: novel pathogenic variants in GLI2, OTX2 and GHRHR. Endocr Connect 2019; 8: 590-595. https://doi.org/10.1530/EC-19-0085

25. Acharya SV, Gopal RA, Lila A, et al. Phenotype and radiological correlation in patients with growth hormone deficiency. Indian J Pediatr 2011; 78: 49-54. https://doi.org/10.1007/s12098-010-0211-1

26. de Faria MEJ, Carvalho LR, Rossetto SM, et al. Analysis of craniofacial and extremity growth in patients with growth hormone deficiency during growth hormone therapy. Horm Res 2009; 71: 173-177. https://doi.org/10.1159/000197875

27. Maione L, Cantone E, Nettore IC, et al. Flavor perception test: evaluation in patients with Kallmann syndrome. Endocrine 2016; 52: 236-243. https://doi.org/10.1007/s12020-015-0690-y

The Turkish Journal of Pediatrics 2023; 65: 456-468
https://doi.org/10.24953/turkjped.2022.735

Original Article

The outcomes of renin-angiotensin-aldosterone system inhibition and immunosuppressive therapy in children with X-linked Alport syndrome

Gülşah Özdemir[1], Bora Gülhan[1], Eda Didem Kurt Şükür[1], Emine Atayar[2], Raziye Atan[3], İsmail Dursun[4], Zeynep Birsin Özçakar[5], Seha Saygılı[6], Alper Soylu[7], Oğuz Söylemezoğlu[8], Alev Yılmaz[9], Aysun Karabay Bayazıt[10], Fehime Kara Eroğlu[11], Belde Kasap Demir[12], Selçuk Yüksel[13], Yılmaz Tabel[14], Ayşe Ağbaş[15], Ali Düzova[1], Mutlu Hayran[16], Fatih Özaltın[1,2], Rezan Topaloğlu[1]

[1]Division of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara; [2]Division of Pediatric Nephrology, Nephrogenetics Laboratory, Hacettepe University Faculty of Medicine, Ankara; [3]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara; [4]Division of Pediatric Nephrology, Erciyes University Faculty of Medicine, Kayseri; [5]Division of Pediatric Nephrology, Ankara University Faculty of Medicine, Ankara; [6]Division of Pediatric Nephrology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul; [7]Division of Pediatric Nephrology, Dokuz Eylül University Faculty of Medicine, İzmir; [8]Division of Pediatric Nephrology, Gazi University Faculty of Medicine, Ankara; [9]Division of Pediatric Nephrology, İstanbul University Çapa Faculty of Medicine, Istanbul; [10]Division of Pediatric Nephrology, Çukurova University Faculty of Medicine, Adana; [11]Division of Pediatric Nephrology, Dr. Sami Ulus Maternity and Children's Health Hospital, Ankara; [12]Division of Pediatric Nephrology, İzmir Katip Çelebi University, Tepecik Research and Training Hospital, İzmir; [13]Division of Pediatric Nephrology, Pamukkale University Faculty of Medicine, Denizli; [14]Division of Pediatric Nephrology, İnönü University Faculty of Medicine, Malatya; [15]Division of Pediatric Nephrology, Haseki Training and Research Hospital, İstanbul; [16]Department of Preventive Oncology, Hacettepe University Faculty of Medicine, Ankara, Türkiye.

ABSTRACT

Background. Alport syndrome (AS) is characterized by progressive kidney disease. There is increasing evidence that renin-angiotensin-aldosterone system (RAAS) inhibition delays chronic kidney disease (CKD) while the effectiveness of immunosuppressive (IS) therapy in AS is still uncertain. In this study, we aimed to analyze the outcomes of pediatric patients with X-linked AS (XLAS) who received RAAS inhibitors and IS therapy.

Methods. Seventy-four children with XLAS were included in this multicenter study. Demographic features, clinical and laboratory data, treatments, histopathological examinations, and genetic analyses were analyzed retrospectively.

Results. Among 74 children, 52 (70.2%) received RAAS inhibitors, 11 (14.9%) received RAAS inhibitors and IS, and 11 (14.9%) were followed up without treatment. During follow-up, glomerular filtration rate (GFR) decreased <60 ml/min/1.73 m^2 in 7 (9.5%) of 74 patients (M/F=6/1). In male patients with XLAS, kidney survival was not different between RAAS and RAAS+IS groups (p=0.42). The rate of progression to CKD was significantly higher in patients with nephrotic range proteinuria and nephrotic syndrome (NS), respectively (p=0.006, p=0.05). The median age at the onset of RAAS inhibitors was significantly higher in male patients who progressed to CKD (13.9 vs 8.1 years, p=0.003).

Conclusions. RAAS inhibitors have beneficial effects on proteinuria and early initiation of therapy may delay the progression to CKD in children with XLAS. There was no significant difference between the RAAS and RAAS+IS groups in kidney survival. AS patients presenting with NS or nephrotic range proteinuria should be followed up more carefully considering the risk of early progression to CKD.

Key words: Alport syndrome, cyclosporin A, immunosuppressive therapy, nephrotic syndrome, RAAS inhibitors.

✉ Rezan Topaloğlu
rezantopaloglu@hacettepe.edu.tr

Received 9th September 2022, revised 19th January 2023, accepted 13th February 2023.

The study has been presented at the 53rd Annual Scientific Meeting of the European Society for Paediatric Nephrology, 16-19 September 2021, Amsterdam, the Netherlands.

Alport syndrome (AS) is characterized by progressive kidney disease.[1,2] There are three types of inheritance patterns; X-linked AS (XLAS) caused by *COL4A5* gene mutations, autosomal recessive AS (ARAS) caused by homozygous or compound heterozygous mutations in the *COL4A3*/*COL4A4* genes, and autosomal dominant AS (ADAS) caused by heterozygous mutations in the *COL4A3*/*COL4A4* genes.[3] XLAS has a severe clinical course in males and approximately 50% of patients develop kidney failure by the age of 20 years.[4] There is no specific curative therapy for AS. The treatment aims to delay the progression to chronic kidney disease (CKD). There is increasing evidence that this goal can be achieved with renin-angiotensin-aldosterone system (RAAS) inhibition and optimum results are gathered when treatment is initiated before glomerular filtration rate (GFR) begins to decline.[5,6] On the other hand, studies evaluating the effectiveness of immunosuppressives (IS) in AS are limited. Studies on the use of cyclosporin A (CSA) in AS have shown that it can slow down disease progression by reducing proteinuria via its effects on the podocyte cytoskeleton and collagen IV cycle. However, an important number of studies do not recommend the routine use of CSA due to its nephrotoxicity.[7,8]

In this retrospective study, we aimed to analyze the baseline characteristics and outcomes of pediatric patients with XLAS, evaluate the effects of treatment modalities (reduction of proteinuria and changes in GFR), and obtain more information about the potential positive effects of RAAS inhibitors and IS treatment on the clinical course of the disease.

Material and Methods

Patients and data collection

Seventy-four children with XLAS from 13 pediatric nephrology centers in Türkiye, admitted between 2002-2019, were included. Inclusion criteria were XLAS diagnosis based on family history and/or pathological findings and/or genetic analysis and being under 18 years of age at disease presentation. Patients who were followed up for less than 6 months were excluded. All centers were requested to fill out a standard questionnaire, which included patients' demographic features, clinical and laboratory data (i.e., serum creatinine, serum albumin, spot urine protein to creatinine ratio or 24-hour urine protein quantification, and estimated GFR [eGFR] at first presentation, the onset of treatment, 6^{th} -12^{th} -24^{th} months after treatment, and the last visit), histopathological examinations and genetic test results. Data from medical records were analyzed retrospectively.

The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University (KA 19073) and written informed consents were obtained from the parents and patients older than 10 years of age. The study was conducted in accordance with the principles of the Helsinki Declaration.

Definitions

XLAS diagnosis was suspected in the presence of persistent hematuria and/or kidney failure and/or hearing loss. The diagnosis was made based on family history and/or pathological findings of AS and/or heterozygous (in females) or hemizygous (in males) pathogenic variants found in *COL4A5*. Nephrotic-range proteinuria was defined as spot urine protein/creatinine ratio of >2 mg/mg or 24-hour urine protein >40 mg/m^2/h. Nephrotic syndrome (NS) was defined by nephrotic range proteinuria, hypoalbuminemia (serum albumin <2.5 g/dl), edema, and hyperlipidemia. CKD classification was defined by the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline and the latest KDIGO Consensus Conference.[9,10] For evaluating kidney prognosis, progression to CKD was defined as a GFR value equal to or less than 60 ml/min/1.73m^2. eGFR was calculated using the original Schwartz formula.[11] eGFR change per year (ΔeGFR/year) was calculated as [eGFR at the last visit – eGFR at the first visit)] / Follow-up duration. Genetic variations detected were categorized

into two groups as missense and non-missense mutations (i.e., deletion, duplication, splice site, and non-sense). Patients were divided into three groups in terms of treatment regimens; RAAS inhibitors (RAAS), IS therapy with RAAS inhibitors (RAAS+IS), and the no treatment (NT) group.

Statistical analysis

All data were analyzed using IBM SPSS Statistics for Windows v.21 (IBM Corp., Armonk, NY, USA). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk's test) to determine whether they were normally distributed. Descriptive statistical analysis methods were used to evaluate demographic and clinical data. Where appropriate, mean, standard deviation (SD), median, and interquartile range (IQR) were calculated for numeric variables. Frequency tables were used to describe categorical data. Categorical variables were compared by Chi-square test and Fisher's exact test where appropriate. Friedman test was conducted to test whether there is a significant change in 24-hour urine protein and eGFR variables due to violation of parametric test assumptions (i.e., non-normal distribution). The nonparametric paired Wilcoxon test was used to assess the differences from the start of treatment to each follow-up time point. Mann–Whitney U test or independent samples t-test was used to compare two independent samples. One-way ANOVA or Kruskal-Wallis tests were used for 3-group comparisons. Survival analysis was performed using the Kaplan–Meier analysis with overall log-rank testing. p values <0.05 in two-tailed tests were considered statistically significant in all analyses. The study had 80% power, with a 5% type-1 error level to detect differences corresponding to a large effect size (a maximum of f=0.80 with a minimum coefficient of variation of 40%) among the treatment modalities as statistically significant given the distribution of patients into the three groups.

Results

Patient characteristics

The study included 74 children with XLAS (41 males, 33 females). The median age at first presentation was 6.0 years (IQR 3.4 – 9.9). The median follow-up duration was 4.0 years (IQR 1.7–7.3). Sixty-three (85.1%) patients had proteinuria at first presentation, 11 (14.8%) had nephrotic range proteinuria, and 4 patients (5.4%) presented with NS. Kidney biopsy was performed in 46 (62%) patients, 25 (25/46; 54.3%) of which also had electron microscopic (EM) examination. Histopathological findings were consistent with AS in 22 (47.8%) patients while focal segmental glomerulosclerosis (FSGS) was detected in 9 (%19.6) of the patients. XLAS diagnosis was confirmed with genetic analysis in 56 (75.7%) patients. Of the remaining 18 patients, XLAS was diagnosed with family history and kidney biopsy in 12 (16.2%), and clinical presentation and family history in 6 patients (8.1%). During the follow-up, 7 (9.5%) of the 74 patients progressed to CKD [Stage 3 (n=6), Stage 5 (n=1)]. Demographic and clinical features of male and female patients with XLAS are given in Table I.

Renal outcomes after RAAS inhibitors and IS treatment

Among 74 patients with XLAS, 52 (70.2%) received only RAAS inhibitors (RAAS group), 11 (14.9%) patients received RAAS inhibitors and IS treatment (RAAS+IS group), and 11 (14.9%) patients were followed up without any treatment (NT group). The median age for onset of RAAS inhibition was 9.1 years (IQR 5.0 – 11.7) which did not differ significantly between males and females (8.2 years, IQR 5.0 – 12.3; vs. 9.3 years, IQR 4.5 – 11.4; p = 0.62]. In the RAAS group (n=52), 39 (75%) received either angiotensin-converting enzyme inhibitor (ACEI) (n=34) or angiotensin receptor blocker (ARB) (n=5) treatment (monotherapy); while 13 (25%) received both ACEI and ARB treatments (dual therapy). In patients treated with dual therapy, ARB was added to ACEIs if proteinuria

Table I. Demographic and clinical characteristics of X-linked Alport syndrome (XLAS) patients.

	Overall (n=74)	Males (n=41) (55.4%)	Females (n=33) (44.6%)
Age at first presentation, median (IQR), yr	6.0 (3.4 – 9.9)	5.3 (3.2 – 10.5)	7.0 (3.4 – 9.6)
Family history, n (%)			
Yes	56 (75.6)	28 (68.3)	28 (84.8)
No	15 (20.3)	12 (29.3)	3 (9.1)
Unknown	3 (4.1)	1 (2.4)	2 (6.1)
Follow-up duration, median (IQR), yr	4.0 (1.7 – 7.3)	4.1 (1.7 – 6.7)	3.9 (1.6 – 8.3)
Urinalysis at first presentation, n (%)			
Hematuria and proteinuria	61 (82.4)	34 (82.9)	27 (81.8)
Hematuria	11 (14.9)	5 (12.2)	6 (18.2)
Proteinuria	2 (2.7)	2 (4.9)	0 (0.0)
Nephrotic syndrome at first presentation, n (%)	4 (5.4)	3 (7.3)	1 (3.0)
Histopathology, n (%)			
Biopsy performed	46 (62)	29 (70.7)	17 (51.5)
Alport	22/46 (47.8)	14/29 (48.3)	8/17 (47.1)
FSGS	9/46(19.6)	7/29 (24.1)	2/17 (11.8)
Mesengial proliferation	8/46 (17.4)	5/29 (17.2)	3/17 (17.6)
Normal	6/46 (13.0)	3/29 (10.4)	3/17 (17.6)
Postinfectious glomerulonephritis	1/46 (2.2)	0/29 (0.0)	1/17 (5.9)
Mutation type, n (%)			
Genetic test performed	56 (75.6)	31 (75.6)	25 (75.7)
Missense	26/56 (46.4)	13/31 (41.9)	13/25 (52.0)
Deletion	16/56 (28.6)	11/31 (35.5)	5/25 (20.0)
Splice-site	10/56 (17.8)	4/31 (12.9)	6/25 (24.0)
Duplication	2/56 (3.6)	2 (6.5)	0/25 (0.0)
Nonsense	2/56 (3.6)	1 (3.2)	1/25 (4.0)
Treatment, n (%)			
No treatment	11 (14.9)	4 (9.8)	7 (21.2)
RAAS inhibitor	52 (70.2)	28 (68.2)	24 (72.7)
RAAS inhibitor + IS	11 (14.9)	9 (22.0)	2 (6.1)
Progression to CKD, n (%)	7 (9.5)	6 (14.6)	1 (3.0)
Age at the onset of CKD, median (IQR), yr	16.2 (15.2 – 16.6)	15.9 (14.5 – 16.7)	16.7 †

† The age at the onset of CKD of the female patient progressed to CKD (n=1) is given.
CKD: chronic kidney disease, FSGS: focal segmental glomerulosclerosis, IS: immunosuppressive, IQR: interquartile range, RAAS: renin-angiotensin-aldosterone system, XLAS: X-linked Alport syndrome.

persisted despite ACEI treatment. The median age at the onset of ACEI and ARB treatment was 8.6 years (IQR 4.6 – 11.8) and 11.3 years (IQR 10.2 – 13.0), respectively. The median time interval between the onset of ACEI and ARB treatment was 1.5 years (IQR 0.65 – 3.41).

In male patients treated with RAAS inhibitors (n=28), the median proteinuria levels decreased by about 50% at the end of the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively (p=0.08 and p=0.08). Also, the median eGFR levels did not show any significant difference at the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively (p=0.23 and p=0.60). Female patients had lower proteinuria levels than male patients

at the onset of RAAS inhibitor treatment (12.0 vs 27.7 mg/m^2/h, p=0.06). In female patients treated with RAAS inhibitors (n=24), the median proteinuria levels decreased at the end of the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively (p=0.04 and p=0.59). Similar to male patients, the median eGFR levels did not differ significantly at the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively (p=0.73 and p=0.71). Male and female proteinuria and eGFR values at the onset, 6th, 12th, and 24th of RAAS inhibitor treatment are given in Table II.

IS treatment was given to 11 patients (M/F=9/2) with the diagnosis of NS in 4 patients, rapid progression of post-infectious glomerulonephritis (PIGN) in 1 patient, and AS in 6 patients. Of 4 patients who presented with NS, 2 received steroids only, 1 received steroids and mycophenolate mofetil (MMF) and 1 received steroids and tacrolimus (TAC) before the genetic diagnosis of XLAS. Kidney biopsy findings were consistent with FSGS in all NS patients. Six patients received CSA with the diagnosis of XLAS. CSA treatment was initiated at a median of 1.0 year (IQR 0.3 – 7.1) after the first presentation and was stopped after 0.5, 1.6, 2.1, and 3.3 years due to elevated serum creatinine in four patients and the other 2 patients were still receiving CSA at last visit. In patients with IS treatment, by the end of treatment, while proteinuria was decreased except for one patient (Patient 5), the decline in GFR continued in all except one patient (Patient 7). Features of patients treated with IS are given in Table III.

Median 24-hour urine protein level at first presentation was 40.1 mg/m^2/h in the RAAS+IS group, 12 mg/m^2/h in the RAAS group and 7.8 mg/m^2/h in the NT group (p=0.016). Mean eGFR at first presentation was similar between treatment groups (p=0.42). Characteristics of patients according to the treatment regimens are given in Table IV.

Progression to CKD and renal survival

After the median follow-up duration of 4.0 years (IQR 1.7 – 7.3), 7 (9.5%) of the 74 patients progressed to CKD [CKD stage 3 (n = 6) and CKD stage 5 (n = 1)]. Six out of 41 (14.6%) male patients and 1 out of 33 (3%) female patients progressed to CKD (p=0.09). The median age at the onset of CKD was 15.9 years (IQR 14.5 – 16.7) in male patients. The age of the female patient was 16.7 years at the onset of CKD. Since there was only one female patient who progressed to CKD, male patients were evaluated in terms of progression to CKD and renal survival. Among male patients with XLAS, the median age at first presentation was higher in patients with CKD than those without (12.4 vs. 5.1 years, p=0.07) and the median follow-up duration did not differ significantly between patients with and without CKD (5.0 vs 3.9 years, p=0.60). The rate of progression to CKD was significantly higher in patients who had nephrotic range proteinuria and/or NS at first presentation (p=0.006 and p=0.05, respectively). Median 24-hour urine protein levels at first presentation and the onset of RAAS inhibitor treatment were significantly higher in patients who progressed to CKD than those who did not, respectively (p=0.001 and p<0.001). The median eGFR level at first presentation was significantly lower in patients who progressed to CKD than those who did not (p=0.002). Among the 6 patients who progressed to CKD, 4 were treated with only RAAS inhibitors, and 2 were treated with RAAS + IS (p=0.57). The patients who were followed up without treatment did not progress to CKD, however, their follow-up period was significantly shorter compared to other groups (p=0.02). Furthermore, among male patients who received RAAS or RAAS+IS therapy, the median age at the onset of RAAS inhibitors was significantly higher in patients who progressed to CKD than those who did not (13.9 vs 8.1 years, p=0.003).

Kidney survival analysis was performed in male patients. The overall median kidney survival rate without CKD was 11.6 years (95% CI 9.7–13.6). There was no significant difference

Table II. Proteinuria and eGFR values of patients with RAAS inhibitor treatment, expressed as medians (IQR).

	RAAS inhibitor treatment						
Parameters	Onset	6th month	p-value*	12th month	p-value*	24th month	p-value*
Proteinuria (mg/m^2/h)							
Males							
ACEI or ARB (n=19)	24.8 (13.0 – 46.0)	12.0 (6.7 – 36.3)	0.09	16.8 (5.1 – 34.3)	0.31	11.7 (5.1 – 27.2)	0.08
ACEI+ARB (n=9)	26.0 (17.0 – 117.0)	24.8 (10.0 – 98.0)	0.60	15.1 (7.8 – 93.1)	0.04	13.2 (6.1 – 64.5)	0.08
Females							
ACEI or ARB (n=20)	12.4 (7.7 – 15.2)	12.3 (4.9 – 33.2)	0.93	9.8 (5.1 – 13.0)	0.34	6.3 (5.2 – 12.4)	0.04
ACEI+ARB (n=4)	12.0 (9.3 – 63.8)	10.8 (5.3 – 22.7)	0.28	13.7 (8.0 – 15.1)	0.59	10.9 (1.8 – 16.6)	0.59
eGFR (ml/min/1.73m^2)							
Males							
ACEI or ARB (n=19)	156.0 (117.0 – 180.0)	130.0 (98.5 – 187.5)	0.22	141.0 (94.0 – 175.0)	0.21	140.0 (122.2 – 182.5)	0.23
ACEI+ARB (n=9)	140.0 (117.5 – 163.0)	139.5 (130.0 – 154.7)	0.72	142.0 (104.0 – 159.0)	0.49	149.0 (74.5 – 155.7)	0.60
Females							
ACEI or ARB (n=20)	154.0 (132.0 – 174.0)	149.5 (118.7 – 170.0)	0.65	144.5 (110.0 – 166.7)	0.90	146.0 (116.5 – 170.0)	0.73
ACEI+ARB (n=4)	155.0 (122.7 – 186.0)	150.0 (110.5 – 180.7)	0.46	144.0 (106.0 – 176.0)	0.60	158.0 (96.7 – 173.7)	0.71

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker: eGFR: estimated glomerular filtration rate, IQR: interquartile range
(Friedman test, followed by pairwise comparisons with Wilcoxon test were applied. *The p-values are obtained from the Wilcoxon test and show the difference between the onset of treatment and the specified time.)

Table III. Characteristics of patients treated with immunosuppressives.

Patient	Sex	Age at first presentation (years)	Mutation type	Proteinuria	NS	Histopathology	IS	Onset of treatment			End of treatment			The last visit		
								Age (years)	Proteinuria ($mg/m^2/h$)	eGFR (ml/min/ $1.73m^2$)	Age (years)	Proteinuria ($mg/m^2/h$)	eGFR (ml/min/ $1.73m^2$)	Age (years)	Proteinuria ($mg/m^2/h$)	eGFR (ml/min/ $1.73m^2$)
1	M	4.3	Deletion	Nephrotic	No	-	CSA	5.8	1.6 †	202	continued	continued	continued	10.1	0.54 †	180
2	M	8.3	Deletion	Non-nephrotic	No	FSGS	CSA	8.3	0.34 †	145	continued	continued	continued	14.1	1.4 †	72
3	M	15.7	Duplication	Nephrotic	Yes	FSGS	Steroid + MMF	15.7	224	106	16.2	135	60	16.3	31.0	44
4	M	15.8	Splice site	Nephrotic	Yes	FSGS	Steroid + TAC	16.1	148	74	16.9	9.6	44	17.3	80.2	57
5	M	14.7	Missense	Nephrotic	No	Alport	CSA	15.3	9.2	134	15.8	10.6	73	17.1	135	107
6	M	0.9	Missense	Non-nephrotic	No	Alport	CSA	10.6	97	163	12.8	36	129	13.0	130	190
7	F	10.8	Deletion	Non-nephrotic	No	PIGN	Steroid	10.8	22	83	10.9	12	188	11.4	NA	188
8	M	2.4	Deletion	Non-nephrotic	No	Alport	CSA	2.9	18	162	6.2	12	116	6.8	23.6	147
9	F	10.2	Deletion	Nephrotic	Yes	FSGS	Steroid	10.2	4.5 †	150	10.8	0.24 †	133	22.9	0.28 †	131
10	M	5.1	Splice site	Non-nephrotic	No	Alport	CSA	11.4	76	186	13.1	61	75	15.7	109	154
11	M	12.2	Missense	Nephrotic	Yes	FSGS	Steroid	12.7	134	189	13.2	74	165	13.3	78	141

CSA: cyclosporin A, eGFR: estimated glomerular filtration rate, IS: immunosuppressive, FSGS: focal segmental glomerulosclerosis, MMF: mycophenolate mofetil, NA: not available, NS: nephrotic syndrome, PIGN: post-infectious glomerulonephritis, TAC: tacrolimus

† Spot urine protein/creatinine (mg/mg) values are given due to the lack of data on 24-h protein levels

Table IV. Characteristics of patients according to treatment modalities.

Characteristics	No treatment (n=11)	RAAS (n=52)	RAAS + IS (n=11)	p-value
Gender, n (%)				
Male (n=41)	4 (36.3)	28 (53.8)	9 (81.8)	0.92
Female (n=33)	7 (63.7)	24 (46.2)	2 (18.2)	
Follow-up duration, yr, median (IQR)	1.7 (0.7 – 2.5)	5.2 (2.6 – 8.2)	4.3 (1.1 – 10.6)	0.02
Age at first presentation, yr, mean ± SD	5.5 ± 3.5	6.5 ± 3.8	9.1 ± 5.3	0.08
Number of patients with missense mutations, n (%)	3 (33.3) †	20 (55.5) †	3 (27.2) †	0.17
24-hour urine protein at first presentation, mg/m^2/h, median (IQR)	7.8 (4.0 – 17.8)	12.0 (5.5 – 24.0)	40.1 (22.0 – 98.0)	0.016
eGFR at first presentation, ml/min/1.73m^2, mean ± SD	156.9 ± 39.7	147.7 ± 45.5	132.7 ± 40.0	0.42
24-hour urine protein at last visit, mg/m^2/h, median (IQR)	6.2 (3.1 – 12.8)	16.8 (10.3 – 34.5)	79.1 (25.4 – 124.7)	0.001
eGFR at last visit, ml/min/1.73m^2, mean ± SD	185.8 ± 25.9	135.7 ± 42.7	128.2 ± 51.9	0.002
Number of patients who developed CKD (GFR <60 ml/min/1.73 m^2), n (%)	0 (0)	5 (9.6)	2 (18.1)	0.34
Age at onset of CKD, yr, mean ± SD	-	15.3 ± 1.6	16.5 ± 0.4	0.33
ΔeGFR / year, ml/min/1.73 m^2, median (IQR) ‡	12.1 (0.6 – 30.7)	- 2.1 (- 9.0 – 4.4)	- 3.4 (- 12.5 – 3.1)	0.01

CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate, IS: immunosuppressive, IQR: interquartile range, RAAS: renin-angiotensin-aldosterone system, SD: standard deviation,
† % values are given as the ratio of the number of patients with missense mutations to the patients with the genetic testing in each group. Genetic testing was performed in 9, 36, and 11 patients in NT, RAAS, and RAAS+IS groups, respectively.
‡ ΔeGFR / year = (eGFR at the last visit − eGFR at the first visit) / Follow-up duration
One way ANOVA or Kruskal-Wallis tests were used where appropriate.

in kidney survival rates between the RAAS and RAAS+IS groups (Fig. 1). After the first presentation, the 10-year cumulative risk of CKD was 12.3% in patients who received RAAS and 22.2% in patients who received RAAS+IS (p=0.42). There was no significant difference between patients who received ACEI/ARB monotherapy and ACEI+ARB dual therapy in terms of progression to CKD and kidney survival, respectively (p=0.31 and p=0.25). After the first presentation, the 10-year cumulative risk of CKD was 23.6% in patients who received ACEI/ARB monotherapy and 8.3% in patients who received ACEI+ARB dual therapy (p=0.25). Progression to CKD did not significantly differ between patients with missense mutations and non-missense mutations (p=0.52) and no significant difference was found in terms of mutation types (i.e., missense, or non-missense) between NT, RAAS, and RAAS+IS groups (p=0.17). The characteristics of male patients with and without CKD are summarized in Table V. Kidney survival analysis in male patients showed that only patients with NS had worse kidney survival than those without (Fig. 1).

Discussion

This is the first multicenter retrospective study analyzing the outcomes of RAAS inhibitors and immunosuppressives in Turkish children with XLAS. We observed that RAAS inhibitors had beneficial effects on proteinuria. Also, the median age at the onset of RAAS inhibitor treatment was significantly higher in children who progressed to CKD. Nephrotic range proteinuria and/or NS at presentation were associated with poor prognosis and in the kidney survival analysis of male patients, no significant difference was found in patients who received immunosuppressive therapy or not.

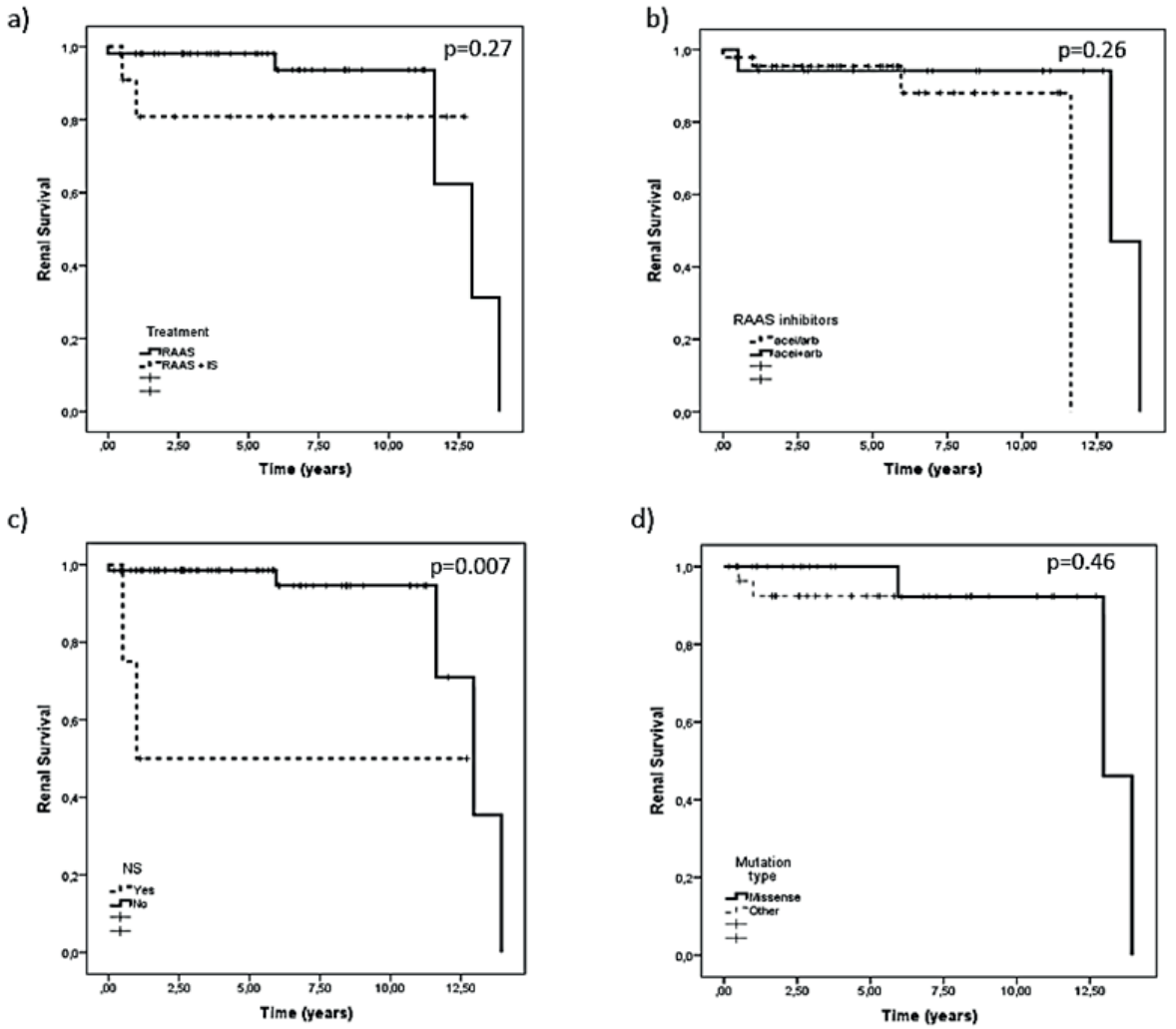


Fig. 1. Time to chronic kidney disease in male XLAS patients. Kidney survival in (a) patients who received RAAS vs. RAAS+IS; (b) patients on ACEI or ARB (monotherapy) vs. ACEI+ARB (dual therapy); (c) patients with or without nephrotic syndrome; and (d) patients with missense vs. other mutations. Note that patients with nephrotic syndrome (NS) at presentation progressed to chronic kidney disease earlier than those without. ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker, IS: immunesuppression, RAAS: renin-angiotensin-aldosterone system inhibition, XLAS: X-linked Alport syndrome.

In the literature, it was shown that RAAS inhibition has nephroprotective effects and may delay progression to CKD in patients with AS.[5,12-14] Firstly, in 2004, Proesmans et al.[15] reported the 5 years results of enalapril treatment in a group of ten children with AS and stated that enalapril reduces urinary protein excretion and preserves glomerular filtration. Subsequently, Webb et al.[16] demonstrated that losartan reduced proteinuria significantly and was well tolerated after 12 weeks of treatment in a group of 15 children with AS. Zhang et al.[13] analyzed the long-term efficacy and safety of ACEI and ACEI + ARB treatments in a cohort of 79 children with AS and showed that proteinuria decreased significantly during the first 2 years of treatment. There was no significant difference in anti-proteinuric effects of ACEI and ACEI + ARB treatments in patients with severe or less severe mutations after 1 year of therapy. Webb et al.[17] also analyzed the effects of ACEI versus ARB treatments and reported that enalapril

Table V. The characteristics of male XLAS patients with and without CKD.

Characteristics	Patients with CKD (n=6)	Patients without CKD (n=35)	p value
Age at first presentation, yr, median (IQR)	12.4 (2.3 – 15.7)	5.1 (3.5 – 9.2)	0.07
Follow up duration, yr, median (IQR)	5.0 (1.3 – 12.5)	3.9 (1.7 – 6.7)	0.60
Age at the last visit, yr, median (IQR)	16.6 (15.2 – 18.0)	11.4 (6.9 – 14.0)	0.009
Proteinuria, n (%)			
None (n=5)	0 (0)	5 (14.3)	
Non-nephrotic (n=28)	2 (33.3)	26 (74.3)	0.006
Nephrotic (n=8)	4 (66.7)	4 (11.4)	
NS at first presentation, n (%)			
Yes (n=3)	2 (33.3)	1 (2.9)	0.05
No (n=38)	4 (66.7)	34 (97.1)	
24h urine protein at first presentation, mg/m^2/h, median (IQR)	114.5 (54.0 – 163.7)	15.2 (4.6 – 29.2)	0.001
eGFR at first presentation, ml/min/1.73m^2, median (IQR)	84.5 (58.0 – 135.0)	149.0 (115.0 – 171.0)	0.002
Age at onset of RAAS inhibitor treatment †, yr, mean ± SD	13.9 ± 2.6 †	8.1 ± 4.1 †	0.003
24h urine protein at onset of RAAS inhibitor treatment †, mg/m^2/h, median (IQR)	149.0 (97.2 – 182.7) †	24.4 (14.4 – 34.2) †	<0.001
Mutation type, n (%)			
Missense (n=13)	2 (33.3)	11 (31.4)	0.46
Other (n=28)	4 (66.7)	24 (68.6)	
Treatment, n (%)			
No treatment (n=4)	0 (0)	4 (11.4)	
RAAS (n=28)	4 (66.7)	24 (68.6)	0.57
RAAS+IS (n=9)	2 (33.3)	7 (20.0)	
RAAS inhibitors, n (%)			
No treatment (n=4)	0 (0)	4 (11.4)	
ACEI/ARB (n=25)	4 (66.7)	21 (60.0)	0.65
ACEI+ARB (n=12)	2 (33.3)	10 (28.6)	

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker, CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate, IS: immunosuppressive, IQR interquartile range, NS: nephrotic syndrome, RAAS: renin-angiotensin-aldosterone system, SD standard deviation, XLAS: X-linked Alport syndrome.
† Male patients without treatment (n=4) were not included at this analysis.
Mann–Whitney U test or independent samples t-test was used where appropriate.

and losartan were comparable in reducing proteinuria in children with AS. In agreement with previous studies, we showed that proteinuria levels decreased after both ACEI/ARB and ACEI+ARB treatments while eGFR remained relatively stable. We also observed that there was no significant difference between patients who received monotherapy and dual therapy in terms of kidney survival.

When the effect of RAAS inhibitors on the progression to CKD is concerning; Gross et al.[5] showed that ACEIs delayed the progression of kidney failure for 3 years in a patient group with impaired renal functions and 18 years in the patient group with proteinuria, while none of the patients in the hematuria and microalbuminuria group progressed to kidney failure. Also, among patients with heterozygous mutations in *COL4A3*, *COL4A4*,

or *COL4A5*, those treated with ACEIs showed a lower and delayed incidence of kidney failure and improved survival than untreated patients.[18] In a study investigating treatment response by genotype, ACEI/ARBs delayed CKD for 17 years in patients with *COL4A5* variants without truncating and 12 years in patients with truncating *COL4A5* variants.[14] The latest guidelines recommend initiating ACEIs at the time of diagnosis in males and when microalbuminuria develops in females with XLAS.[6] Similarly, among male patients who received RAAS or RAAS+IS therapy in our cohort, we demonstrated that the median age at the onset of RAAS inhibitor therapy was significantly higher in patients who progressed to CKD than those who did not. On the other hand, the median age at disease presentation was higher in patients who progressed to CKD (statistically nonsignificant) and median eGFR at presentation was found to be significantly lower. These findings and our observations suggest that evaluating patients before their eGFR starts to decline and initiating RAAS inhibitor therapy early may help to delay the progression of CKD.

Previously, it has been reported that phenocopy-causing mutations, including *COL4A* mutations, were detected in 3.7% of patients with steroid-resistant NS (SRNS).[19] Also, it has been shown that *COL4A* mutations can be detected in patients with familial or sporadic FSGS.[20-22] The association between AS and FSGS is not clear yet but it is thought that FSGS phenocopy may be more likely than the development of secondary FSGS in AS patients.[22] In our cohort, 4 patients (5.4%) presented with NS and all of them had FSGS histopathologically. It is well known that proteinuria is a risk factor for the development and progression of CKD. However, data on the outcome of AS patients with significant proteinuria at first presentation are limited. In agreement with the study by Ozdemir et al.[23], in this study patients who presented with NS and/or nephrotic range proteinuria progressed to CKD earlier. Taken together, AS should be included in the differential diagnosis of patients presenting with NS and/or nephrotic range proteinuria, and those AS patients who presented with NS and/or nephrotic range proteinuria should be followed up more carefully.

The effects of immunosuppressives, especially that of CSA, have been investigated in patients with AS. Some animal and human studies reported that CSA reduced proteinuria and slowed progression to CKD, however, due to its nephrotoxic effects, CSA is no longer recommended for routine use.[7,8,24,25] Petrova et al.[26] compared the effects of MMF and placebo in *COL4A3*-deficient (*COL4A3*$^{-/-}$) mice and showed that MMF improved kidney function, probably by inhibition of tubulointerstitial fibrosis. There is no human study examining the effects of MMF, tacrolimus, or other immunosuppressives in AS patients. In our cohort, 11 patients were treated with IS. We observed that patients who received IS treatment remained relatively stable except for 2 patients who progressed to CKD Stage 3, and it should be noted that one of these patients had a baseline GFR <90 ml/min/1.73m^2. In kidney survival analysis, there was no significant difference between the RAAS and RAAS+IS groups. Possibly due to the small size of the cohort, our study did not demonstrate an ameliorating or worsening effect of immunosuppressives. With these results, we think that the effects of immunosuppressives in AS are still unclear, therefore studies with larger patient cohorts are needed to reach an evidence-based conclusion.

It has been reported that patients with missense mutations have a better prognosis and progress to CKD later.[4,27] We observed that there was no difference in kidney survival rates between patients with missense and non-missense variations. This issue remains to be confirmed with larger studies focusing more on the genetic background of the disease.

The strength of our study is that it is the first study that reveals the outcomes of the patients who received RAAS inhibitors and IS therapy in the Turkish pediatric XLAS cohort. The limitations of our study are its retrospective

design, relatively short follow-up duration (i.e., 4.0 years), and relatively small-sized study group. Since we included pediatric patients and the follow-up period was relatively short, the rate of progression to CKD might be lower than in the previous studies. Progression to CKD and kidney survival analysis couldn't be performed in female patients due to the small number of patients who progressed to CKD. Due to the limited size, we are aware that some clinically significant differences might not come out as statistically significant but given the rarity of the disease, we believe that the descriptive statistics we present will be informative for specialists in this field.

Our data showed that RAAS inhibitors have beneficial effects on proteinuria in children with XLAS and early initiation of therapy may delay the progression to CKD. In the kidney survival analysis of males, there was no significant difference between the RAAS and RAAS+IS groups, however, larger scaled prospective studies are still needed to make a definite conclusion regarding kidney survival in AS patients receiving IS therapy. AS should be in the differential diagnosis of patients with NS and/or nephrotic range proteinuria and AS patients presenting with NS or nephrotic range proteinuria should be followed up more carefully due to the risk of early progression to CKD. Early diagnosis and prompt intervention are of high importance to provide appropriate care in children with an AS diagnosis.

Ethical approval

The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University (KA 19073).

Author contribution

The authors confirm contribution to the paper as follows: research formulation and study design: RT, BG, EDKS, FO and AD; data acquisition: RT, GO, RA, ID, ZBO, SS, AS, OS, AY, AKB, FKE, BKD, SY, YT, AA; genetic analysis: FO, EA; statistical analysis: MH and GO; data analysis/interpretation: RT, GO, BG, EDKS, FO, AD and MH. All authors contributed important intellectual content during manuscript drafting and/or revision and approved the final version. Furthermore, they all accept responsibility for the overall work, including the accuracy and integrity of all portions of the work.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Alport AC. Hereditary familial congenital haemorrhagic nephritis. Br Med J 1927; 1: 504-506. https://doi.org/10.1136/bmj.1.3454.504
2. Kashtan CE. Alport syndrome. An inherited disorder of renal, ocular, and cochlear basement membranes. Medicine (Baltimore) 1999; 78: 338-360. https://doi.org/10.1097/00005792-199909000-00005
3. Nozu K, Nakanishi K, Abe Y, et al. A review of clinical characteristics and genetic backgrounds in Alport syndrome. Clin Exp Nephrol 2019; 23: 158-168. https://doi.org/10.1007/s10157-018-1629-4
4. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history in 195 families and genotype- phenotype correlations in males. J Am Soc Nephrol 2000; 11: 649-657. https://doi.org/10.1681/ASN.V114649
5. Gross O, Licht C, Anders HJ, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. Kidney Int 2012; 81: 494-501. https://doi.org/10.1038/ki.2011.407
6. Kashtan CE, Gross O. Clinical practice recommendations for the diagnosis and management of Alport syndrome in children, adolescents, and young adults-an update for 2020. Pediatr Nephrol 2021; 36: 711-719. https://doi.org/10.1007/s00467-020-04819-6
7. Gross O, Kashtan CE. Treatment of Alport syndrome: beyond animal models. Kidney Int 2009; 76: 599-603. https://doi.org/10.1038/ki.2009.223

8. Heidet L, Gubler MC. Alport syndrome: hereditary nephropathy associated with mutations in genes coding for type IV collagen chains. Nephrol Ther 2016; 12: 544-551. https://doi.org/10.1016/j.nephro.2016.09.001

9. Andrassy KM. Comments on "KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease". Kidney Int 2013; 84: 622-623. https://doi.org/10.1038/ki.2013.243

10. Levey AS, Eckardt K-U, Dorman NM, et al. Nomenclature for Kidney Function and Disease: Executive Summary and Glossary From a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference. Kidney Int Rep 2020; 5: 965-972. https://doi.org/10.1016/j.ekir.2020.03.027

11. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976; 58: 259-263

12. Kashtan CE, Ding J, Gregory M, et al. Clinical practice recommendations for the treatment of Alport syndrome: a statement of the Alport Syndrome Research Collaborative. Pediatr Nephrol 2013; 28: 5-11. https://doi.org/10.1007/s00467-012-2138-4

13. Zhang Y, Wang F, Ding J, et al. Long-term treatment by ACE inhibitors and angiotensin receptor blockers in children with Alport syndrome. Pediatr Nephrol 2016; 31: 67-72. https://doi.org/10.1007/s00467-015-3184-5

14. Yamamura T, Horinouchi T, Nagano C, et al. Genotype-phenotype correlations influence the response to angiotensin-targeting drugs in Japanese patients with male X-linked Alport syndrome. Kidney Int 2020; 98: 1605-1614. https://doi.org/10.1016/j.kint.2020.06.038

15. Proesmans W, Van Dyck M. Enalapril in children with Alport syndrome. Pediatr Nephrol 2004; 19: 271-275. https://doi.org/10.1007/s00467-003-1366-z

16. Webb NJA, Lam C, Shahinfar S, et al. Efficacy and safety of losartan in children with Alport syndrome-results from a subgroup analysis of a prospective, randomized, placebo- or amlodipine-controlled trial. Nephrol Dial Transplant 2011; 26: 2521-2526. https://doi.org/10.1093/ndt/gfq797

17. Webb NJA, Shahinfar S, Wells TG, et al. Losartan and enalapril are comparable in reducing proteinuria in children with Alport syndrome. Pediatr Nephrol 2013; 28: 737-743. https://doi.org/10.1007/s00467-012-2372-9

18. Temme J, Peters F, Lange K, et al. Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-chromosomal and autosomal recessive Alport mutations. Kidney Int 2012; 81: 779-783. https://doi.org/10.1038/ki.2011.452

19. Warejko JK, Tan W, Daga A, et al. Whole Exome sequencing of patients with steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2018; 13: 53-62. https://doi.org/10.2215/CJN.04120417

20. Malone AF, Phelan PJ, Hall G, et al. Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis. Kidney Int 2014; 86: 1253-1259. https://doi.org/10.1038/ki.2014.305

21. Xie J, Wu X, Ren H, et al. COL4A3 mutations cause focal segmental glomerulosclerosis. J Mol Cell Biol 2014; 6: 498-505. https://doi.org/10.1093/jmcb/mju040

22. Gast C, Pengelly RJ, Lyon M, et al. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. Nephrol Dial Transplant 2016; 31: 961-970. https://doi.org/10.1093/ndt/gfv325

23. Ozdemir G, Gulhan B, Atayar E, et al. COL4A3 mutation is an independent risk factor for poor prognosis in children with Alport syndrome. Pediatr Nephrol 2020; 35: 1941-1952. https://doi.org/10.1007/s00467-020-04574-8

24. Charbit M, Gubler MC, Dechaux M, Gagnadoux MF, Grünfeld JP, Niaudet P. Cyclosporin therapy in patients with Alport syndrome. Pediatr Nephrol 2007; 22: 57-63. https://doi.org/10.1007/s00467-006-0227-y

25. Massella L, Muda AO, Legato A, Di Zazzo G, Giannakakis K, Emma F. Cyclosporine a treatment in patients with Alport syndrome: a single-center experience. Pediatr Nephrol 2010; 25: 1269-1275. https://doi.org/10.1007/s00467-010-1484-3

26. Petrova DT, Schultze FC, Brandhorst G, et al. Effects of mycophenolate mofetil on kidney function and phosphorylation status of renal proteins in Alport COL4A3-deficient mice. Proteome Sci 2014; 12: 56. https://doi.org/10.1186/s12953-014-0056-z

27. Bekheirnia MR, Reed B, Gregory MC, et al. Genotype-phenotype correlation in X-linked Alport syndrome. J Am Soc Nephrol 2010; 21: 876-883. https://doi.org/10.1681/ASN.2009070784

The Turkish Journal of Pediatrics 2023; 65: 469-478
https://doi.org/10.24953/turkjped.2021.4583

Original Article

Community-acquired *S. aureus* infection in childhood: a multi-center study

Gülsüm İclal Bayhan[1], Ayşe Kaman[2], Esra Çakmak Taşkın[3], Fatma Nur Öz[4], Zeynep Gökçe Gayretli Aydın[5], Halil Özdemir[6], Fatih Ocak[7], Özden Türel[8], Ümmühan Çay[9], Ergin Çiftçi[10], Özge Metin Akcan[11], Türkan Aydın Teke[12], Burcu Bursal Duramaz[13], Metin Doğan[14], Erdal İnce[15], Gönül Tanır[16], Ateş Kara[17]

[1]Department of Pediatric Infectious Disease, Yıldırım Beyazıt University Faculty of Medicine, Yenimahalle Educational and Training Hospital, Ankara; [2]Department of Pediatric Infectious Diseases, Dr. Sami Ulus Children's Health and Diseases Training and Research Hospital, Ankara; [3]Department of Pediatric Infectious Diseases, Ankara University Faculty of Medicine, Ankara; [4]Department of Pediatric Infectious Diseases, Dr. Sami Ulus Children's Health and Diseases Training and Research Hospital, Ankara; [5]Department of Pediatric Infectious Diseases, Karadeniz Technical University Faculty of Medicine, Trabzon; [6]Department of Pediatric Infectious Diseases, Ankara University, Faculty of Medicine, Ankara; [7]Department of Microbiology, Yıldırım Beyazıt University, Faculty of Medicine, Yenimahalle Educational and Training Hospital, Ankara; [8]Departments of Pediatric Infectious Diseases, Bezmialem Vakıf University, İstanbul; [9]Departments of Pediatric Infectious Diseases, Trabzon Kanuni Training and Research Hospital, Trabzon; [10]Department of Pediatric Infectious Diseases, Ankara University, Faculty of Medicine, Ankara, [11]Department of Pediatric Infectious Diseases, Necmettin Erbakan University Meram Faculty of Medicine, Konya; [12]Department of Pediatric Infectious Diseases, Dr. Sami Ulus Children's Health and Diseases Training and Research Hospital, Ankara; [13]Departments of Pediatric Infectious Diseases, Bezmialem Vakıf University, İstanbul; [14]Department of Pediatric Infectious Diseases, Necmettin Erbakan University Meram Faculty of Medicine, Konya; [15]Department of Pediatric Infectious Diseases, Ankara University Faculty of Medicine, Ankara; [16]Department of Pediatric Infectious Diseases, Dr. Sami Ulus Children's Health and Diseases Training and Research Hospital, Ankara; [17]Department of Pediatric Infectious Diseases, Hacettepe University, Faculty of Medicine, Ankara, Türkiye.

ABSTRACT

Background. The prevalence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has been increasing worldwide. We aimed to investigate the prevalence of MRSA in community-acquired *S. aureus* infections, the risk factors for CA-MRSA infection and the clinical features of CA-MRSA.

Methods. A multi-center study with prospective and retrospective sections was conducted. Patients ≥ 3 months old and ≤18 years of age who were diagnosed with community-acquired *S. aureus* infections were included in this study and the patients' information were reviewed from the medical and microbiological database of the hospital. A standard question form about living conditions and exposure risk factors was administered to the parents of patients. The CA-MRSA infections were compared with the methicillin-susceptible *S. aureus* (CA-MSSA) infections in terms of the queried risk factors and clinical variables.

Results. We identified 334 pediatric patients with *S. aureus* infection, 58 (17.4%) had an infection with CA-MRSA. The refugee rate was higher in the CA-MRSA group. There was no significant difference regarding the exposure risk. The treatment modalities and outcomes were similar.

Conclusions. The study was not able to show reliable clinical variables or epidemiological risk factors except for being a refugee for CA-MRSA infections. Empirical antibiotic treatment should therefore be determined according to the local CA-MRSA prevalence in patients presenting with a possible staphylococcus infection.

Key words: cellulitis, bacteremia, sulbactam ampicillin, refugee, immigrant.

✉ Gülsüm İclal Bayhan
gibayhan@gmail.com

Received 21st September 2021, revised 14th January 2023, accepted 6th March 2023.

Staphylococcus aureus is an important etiologic agent of community-acquired infections, mainly involving the skin and soft tissue but also including bacteremia, endocarditis, osteomyelitis, pyomyositis, and necrotizing pneumonia. Less frequently, it can cause staphylococcal toxic shock syndrome, sepsis, and meningitis.[1] The most important issue when planning the treatment of *S. aureus* infections is whether the isolate is sensitive or resistant to methicillin, because beta lactam antibiotics are not effective on methicillin resistant *S. aureus*.

Methicillin-resistant *S. aureus* (MRSA) isolates produce penicillin-binding protein 2A (PBP2A) which is encoded by the *mecA* gene. PBP2A has a low affinity for methicillin and other β-lactam drugs, and MRSA isolates are therefore intrinsically resistant to almost all β-lactam antibiotics.[2] MRSA isolates were first described as hospital-acquired pathogens, and community-acquired *S. aureus* isolates (CA-MRSA) appeared later on.[2] CA-MRSA is susceptible to several antimicrobial agents, unlike hospital acquired MRSA (HA-MRSA). The prevalence of CA-MRSA varies from country to country and has been increasing for a long time. In the United States, the percentage of CA-MRSA has increased to 76%.[3] There is no active surveillance programme for CA-MRSA in Türkiye and the data on the frequency of CA-MRSA in the country is inadequate. The methicillin resistance rate for community-acquired *S. aureus* needs to be considered when planning empirical treatment of *S. aureus* infections. We therefore conducted this three-year multi-center surveillance study aiming to investigate the prevalence of CA-MRSA, the resistance patterns of community-acquired *S. aureus* isolates, and the risk factors for the acquisition of CA-MRSA infection.

Material and Methods

A multi-center study with prospective and retrospective sections was conducted. The study was conducted at the pediatric infectious disease departments of 7 regional pediatric referral hospitals from 4 cities (Ankara, İstanbul, Konya, Trabzon). Cases of laboratory-confirmed community-acquired *S. aureus* infections were included.

Patients ≥3 months old and ≤18 years of age who presented with an infectious disease complaint to the outpatient clinics and whose clinical isolates grew *S. aureus*, or hospitalized patients with clinical presentation compatible with infectious disease where *S. aureus* grew in the clinical isolates within 72 hours of hospitalization, who did not have a history of hospital admission in the last 3 months were included in the study. Patients diagnosed with *S. aureus* infection between January 1st, 2017 and February 28th, 2018 were retrospectively evaluated from hospital medical data and national health registration data screened and included in the study while patients diagnosed with *S. aureus* infection between March 1st, 2018 and January 1st, 2020 were prospectively included.

Patients who had a history of catheter insertion or surgery within the last year, patients on dialysis, and patients with an acquired *S. aureus* infection after an intramuscular injection were not included in the study. The *S. aureus* infections related to foreign material, such as grafts and implants, and ventriculoperitoneal shunt-associated infections were also excluded from the study, as were patients with *S. aureus* growth in the urine culture.

The demographic features of the patients, medical history especially antibiotic usage, underlying skin disorders, infection type, anatomical localization of the infection, treatment information, whether the treatment was as an outpatient or inpatient, whether drainage was used, the antibiotic regimens, and the antibiogram results of the *S. aureus* isolates were reviewed from the medical and microbiological database of the hospitals for retrospectively included patients. The same information was recorded by the physician during the prospective phase. Patients included in the study prospectively were contacted

directly at the time of the diagnosis and a standard question form about living conditions and exposure risk factors was administered to the parents. The number of people living in the house, the number of showers per week, the history of attending a nursery or school, history of living with a health care worker, history of household contact with a person suffering from any cutaneous infection, the travel history, antibiotic usage in the last 3 months, the presence of an underlying skin disorder, and hospitalization in the last year were queried. The CA-MRSA infections were then compared with the methicillin-susceptible *S. aureus* (CA-MSSA) infections in terms of the queried risk factors.

The infections were classified as skin and soft tissue infections (SSTI) and invasive infections (II). Skin abscesses, furuncles, and cellulitis were classified as SSTI. Lymphadenitis/deep neck infection, osteomyelitis/septic arthritis, and bacteremia were classified as II.[4] Pyomyositis/ileopsoas abscess, otitis media, mastoiditis, thyroiditis, pneumonia, empyema, sepsis/staphylococcal toxic shock syndrome, and meningitis were also classified as II. The infection was also classified as II if the primary site of infection was the skin and soft tissue but *S. aureus* grew from blood cultures.

The samples for SSTI were retrieved by spontaneously draining material from the infection site or by needle puncture or surgical drainage of the infection site. The samples for lymphadenitis/deep neck infection were retrieved by needle puncture or surgical drainage. The samples for osteomyelitis/septic arthritis, pyomyositis/ileopsoas abscess, thyroiditis, and mastoiditis were retrieved surgically. *S. aureus* were isolated from the pus draining from the ear in otitis media, from the pleural fluid in empyema, from the sputum in pneumonia cases, from blood cultures in patients with sepsis/staphylococcal toxic shock syndrome, and from the cerebrospinal fluid in meningitis patients.

Antimicrobial susceptibility was determined using the disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing protocol. Isolates that were methicillin resistant and also those that were resistant to ≥3 antibiotic classes (tetracycline, erythromycin, trimethoprim-sulfamethoxazole, fusidic acid, mupirocin, ciprofloxacin, and gentamicin) were evaluated as multi-drug resistant strains.

Ethics committee approval was obtained from Yıldırım Beyazıt University Yenimahalle Training and Research Hospital Ethics Committee (approval reference number: 2018/30).

Results

We identified 334 pediatric patients infected with *S. aureus*. 276 (82.6%) of these 334 patients were infected with CA-MSSA, while 58 (17.4%) were infected with CA-MRSA.

The median age was 1 year (IQR 1-18 years). There were 163 (39%) girls and 171 (61%) boys. Patient demographic characteristics and their comparison between the MSSA and MRSA groups are presented in Table I. Age and gender distribution were similar in the CA-MSSA and CA-MRSA groups. The refugee rate was higher in the CA-MRSA group.

Table I. Demographic characteristics of patients with CA-MSSA and CA-MRSA infection.

Characteristics	Total N=334	MSSA N=276	MRSA N=58	*p* value
Age (y), median (IQR)	1 (0-18)	1 (0-18)	1 (0-17)	0.69
Gender, Female/Total	163/334	132/276	31/58	0.715
Refugee, n (%)	22 (6.6)	14 (5.1)	8 (13.8)	0.01

CA-MSSA: community-acquired methicillin-susceptible *S. aureus*, CA-MRSA community-acquired methicillin-resistant *S. aureus*, IQR: interquartile range

The comparison of living conditions and possible exposure to risk factors for MRSA between the patients with CA-MRSA and CA-MSSA infections is shown in Table II. There was no significant difference regarding the exposure risk. No difference was found between the two groups in terms of underlying skin disease.

SSTI (198 patients [59.6%]) cases were more common than invasive infections (134 patients [40.4%]), both in the entire study population and in the CA-MSSA and CA- MRSA groups. The most common invasive infection types in both the CA-MSSA and CA-MRSA groups were lymphadenitis/deep neck infection, musculoskeletal infection, and bacteremia. Table III shows the clinical presentations of the patients with MRSA and MSSA infections. According to age, there was no difference between SSTI and II (p= 0.33).

The treatments administered to the patients are shown in Table IV. A history of hospitalization was present in 50.5% of the patients. There was no statistically significant difference between the MRSA and MSSA groups in terms of the hospitalization rate, duration of antibiotic treatment, or drainage application rate. When looking for SSTT, hospitalization rate, duration of antibiotic treatment, or drainage application rate were similar in MRSA and MSSA groups (p=0.72, p=0.47, p=0.26); these were also similar between groups in II (p=0.22, p=0.40, p=0.36).

Systemic (intravenous or peroral) antibiotics were used for the treatment of 88.3% of the patients. Anti-MRSA antibiotic treatment was started empirically at admission for 66 (19.8%) patients. Of these 66 patients, 18 had SSTT and 48 had II. The most commonly started parenteral antibiotics at admission were sulbactam ampicillin/amoxicillin clavulanate and third generation cephalosporins. A total of 103 patients had initially received IV sulbactam ampicillin / amoxicillin clavulanate while 75 patients received sulbactam ampicillin / amoxicillin clavulanate alone; in the other patients, the antibiotic used in combination with an anti-MRSA antibiotic was clindamycin in 17 patients, teicoplanin in 4 patients, and vancomycin in 2 patients. A total of 60 patients had received ceftriaxone/

Table II. Risk factors/exposures associated with community-onset methicillin resistance among patients with CA-MSSA and CA-MRSA infection.

	Total N=334	MSSA N=276	MRSA N=58	*p* value
Number of people living in the house, mean ± SD	4.3 ± 1.6	4.2 ± 1.6	4.6 ± 1.6	0.19
Number of showers per week, median (IQR)	2 (1-7)	2 (1-7)	2 (1-4)	0.44
Currently attending nursery or school, n (%)	83 (24.8)	68 (24.6)	15 (25.8)	0.86
Living with a health care worker, n (%)	3 (0.9)	3 (1)	0 (0)	
Being in prison, n (%)	1 (0.3)	0 (0)	1 (1.7)	
Household contact with person with cutaneous infections, n (%)	11 (3.3)	10 (3.6)	1 (1.7)	
Travel history, n (%)	29 (8.7)	22 (8)	7 (12.1)	0.21
Antibiotic usage in the last 3 months, n (%)	7 (2.1)	7 (2.5)	0 (0)	0.31
Underlying skin disorder, n (%)	73 (21.9)	64 (23.2)	9 (15.5)	0.19
Eczema/ Atopic dermatitis/urticaria, n (%)	16 (4.8)	13 (4.7)	3 (5.2)	
Trauma/Abrasion/varicella, enteroviral rash, herpes simplex virus, n (%)	53 (15.9)	47 (17)	6 (10.3)	
Insect bite, n (%)	4 (1.2)	4 (1.4)	0 (0)	
Hospitalization in the last year, n (%)	36 (10.8)	30 (10.9)	6 (10.3)	0.22

CA-MSSA: community-acquired methicillin-susceptible *S. aureus*, CA-MRSA community-acquired methicillin-resistant *S. aureus*, IQR: interquartile range, SD: standard deviation

Table III. Duration, type, and localization of infections in the MSSA and MRSA groups.

	Total N=334	MSSA N=276	MRSA N=58	*p* value
Duration of symptoms (days), Mean ± SD	14 ± 56.5	15.3 ± 61.5	7.6 ± 10.2	0.38
Type of infection				
SSTI, n (%)	198 (59.6)	166 (60.1)	34 (58.6)	0.86
Invasive infection, n (%)	134 (40.1)	110 (39.8)	24 (41.4)	
Localization of SSTI				
Head and neck, n (%)	41 (12.2)	35 (12.6)	6 (10.3)	
Trunk, n (%)	65 (19.5)	49 (17.7)	16 (27.6)	
Genitalia, n (%)	7 (2.1)	7 (2.5)	0 (0)	
Perianal, n (%)	3 (0.9)	2 (0.7)	1 (1.7)	
Foot-leg, n (%)	34 (10.2)	29 (10.5)	5 (8.6)	
Hand-arm, n (%)	36 (10.8)	32 (11.6)	4 (6.9)	
Gluteus, n (%)	12 (3.6)	10 (3.6)	2 (3.4)	
Type of invasive infection				
Lymphadenitis / deep neck infection, n (%)	51 (15.3)	41 (14.9)	10 (17.2)	
Bacteremia, n (%)	44 (13.2)	40 (14.5)	4 (6.9)	
Osteomyelitis / septic arthritis, n (%)	19 (5.7)	15 (5.4)	4 (6.9)	
Otitis media, n (%)	14 (4.2)	11 (4)	3 (5.2)	
Sepsis/staphylococcal toxic shock syndrome, n (%)	4 (1.2)	4 (1.4)	0 (0)	
Pneumonia ± empyema, n (%)	4 (1.2)	3 (0.01)	1 (1.7)	
Mastoiditis, n (%)	1 (0.3)	1 (0.4)	0 (0)	
Thyroiditis, n (%)	1 (0.3)	1 (0.4)	0 (0)	
Pyomyositis / iliopsoas abscess, n (%)	2 (0.6)	2 (0.6)	0 (0)	
Infective endocarditis, n (%)	1 (0.3)	1 (0.3)	0 (0)	
Meningitis, n (%)	1 (0.0)	0 (0)	1 (1.7)	

CA-MSSA: community-acquired methicillin-susceptible *S. aureus*, CA-MRSA community-acquired methicillin-resistant *S. aureus*, SD: standard deviation, SSTI: skin and soft tissue infections

Table IV. Rate of hospitalization, route of antibiotic administered, duration of antibiotic treatment and drainage application, change of antibiotics according to the culture result in CA-MSSA and CA-MRSA groups.

	Total N=334	MSSA N=276	MRSA N=58	*p* value
Hospitalization and intravenous antibiotic treatment, n (%)	169 (50.5)	135 (48.9)	34 (58.6)	0.33
Local antibiotic treatment, n (%)	68 (20.4)	60 (21.7)	8 (13.7)	0.32
Drainage, n (%)	166 (49.7)	131 (47.5)	35 (60.3)	0.18
Intravenous antibiotic treatment duration (days), median (IQR)	9 (6-13)	9 (6-12)	8 (5-14)	0.9
Total treatment duration (days), median (IQR)	10 (2-77)	10 (2-77)	10 (4-36)	0.35
Antibiotic change after culture result, n (%)	50 (15)	37 (13.4)	13 (22.4)	**0.02**
Escalation, n (%)	25 (7.4)	15 (5.4)	10 (17.2)	
De-escalation, n (%)	25 (7.4)	22 (7.9)	3 (5.1)	

CA-MSSA: community-acquired methicillin-susceptible *S. aureus*, CA-MRSA community-acquired methicillin-resistant *S. aureus*, IQR: interquartile range

cefotaxime, 3 of these with vancomycin and 3 with clindamycin. A total of 68 patients had initially been given oral antibiotics, consisting of 60 patients receiving amoxicillin-clavulanate, 2 patients receiving amoxicillin, 1 patient receiving trimethoprim-sulfamethoxazole, 1 patient receiving clarithromycin, 2 patients receiving cefuroxime-axetil, and 2 patients receiving cefixime. One patient with CA-MSSA bacteremia and one patient with necrotizing pneumonia accompanying the bacteremia due to CA-MRSA died. All other patients recovered completely.

The antibiotic resistance profiles of CA-MRSA and CA-MSSA isolates are shown in Table V. The tests showed resistance to penicillin in 85.8% of the CA-MSSA isolates, to erythromycin in 2.7%, and to clindamycin in 3.8%. All of the CA-MRSA isolates were resistant to penicillin, while 42% were resistant to erythromycin, and 20% to clindamycin. All CA-MRSA and CA-MSSA isolates were sensitive to teicoplanin and vancomycin. There were 4 (6.9%) MRSA isolates (out of 58) that were resistant to three or more antibiotic classes.

Discussion

We found the MRSA prevalence among community-acquired *S. aureus* infections to be 17.4%, 17.2% in SSTI and 17.8% in II. The refugee rate was significantly higher in the CA-MRSA group, and we could not find any other exposure risk factor for CA-MRSA infection. When the patients infected with CA-MSSA and CA-MRSA were compared, we did not find any difference regarding the invasive infection rate, hospitalization rate, drainage rate, and antibiotic treatment duration. CA-MRSA isolates were frequently susceptible to non-beta-lactam antibiotics, especially clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, and gentamycin.

A Turkish study conducted in 1997 found a prevalence of 26.4% for CA-MRSA infections during that year at a single medical center.[5] Another study conducted in Türkiye between 2014 and 2018 in a single center in the province of Izmir found that 31.25% of S.aureus-caused SSTI infections were caused by CA-MRSA.[6] When evaluated together with the results of our study, it seems that the frequency of MRSA has not increased in Türkiye since then while an increase is reported in several countries around the world. The reason for the lack of an increase in Türkiye can be clarified by illuminating the risk factors that play a role in the development of MRSA infections, which have been extensively studied. There are conflicting results in this regard. Some studies have reported antibiotic use in the last 6 months as a risk factor for MRSA infection while others report no such

Table V. Antibiotic susceptibilities (% susceptible) of the CA-MSSA and CA-MRSA isolates.

	MSSA N:276			MRSA N:58		
	Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
PEN	3.5	0.7	85.8	0	0	100
CLI	87.2	3.8	9	76.3	3.7	20
ERY	81	2.7	16.3	56	2	42
SXT	98.4	0	1.6	78.4	4	17.6
CIP	95.4	1	3.6	84.5	2.2	13.3
TET	94.4	0	5.6	58.6	0	44.4
TEC	100	0	0	100	0	0
VAN	100	0	0	100	0	0
GEN	98.9	0.4	0.7	89.7	1.7	8.6

CA-MSSA: community-acquired methicillin-susceptible *S. aureus*, CA-MRSA community-acquired methicillin-resistant *S. aureus*, CIP: ciprofloxacin, CLI: clindamycin, ERY: erythromycin, GEN: gentamicin, SXT: trimethoprim/sulfamethoxazole, PEN: penicillin, TEC: teicoplanin, TET: tetracycline, VAN: vancomycin

relationship.[7-9] Similarly, some studies report day care attendance to be associated with MRSA transmission while others have found similar rates of day care attendance among those with MSSA and MRSA infections.[7,10,11] No relationship has been found between CA-MRSA infection and close contact with healthcare professionals, steroid use, underlying skin disease, sports activities, and travel abroad.[7,10] Current or previous homeless status, current or prior incarceration, and alcoholism have each been found to be more common in patients with MRSA infection compared to those with MSSA infection.[9] In the current study, the MRSA and MSSA groups were found to be similar in terms of the exposure risk factors. We had one imprisoned subject who was infected with MRSA.

CA-MRSA has been reported to be more common in minority groups. In a study conducted in Israel, Arab ethnicity was found to be a risk factor for CA-MRSA infection.[12] CA-MRSA has similarly been reported to be more common in Aborigines and those of Polynesian ethnicity in Australia.[13,14] In the USA, a higher proportion of CA-MRSA infections are found in African American patients than in white or Hispanic patients.[7,9] A study from Denmark has reported a higher incidence of CA-MRSA infections in those of non-Danish origin.[10] The CA-MRSA clone, which is predominant in Spain, has similarly been found to be more common in immigrants from South America than in the native Spanish population.[15] High rate of CA-MRSA colonization has been reported in the refugee population.[16] In the current study, being a refugee was found to be a risk factor for CA-MRSA infection but other exposure risks were similar between the groups. Although minority groups are reported as a risk factor for CA-MRSA in the literature, we are not aware of any other publication reporting being a refugee as such a risk factor. It is possible that a combination/interaction of many factors and not a single factor plays a role in minority groups and immigrants as regards development of MRSA infections. The long journeys during migration, accommodation in multiple locations, the difficult conditions of the migration routes, crowded home conditions, and lower hygiene conditions due to socioeconomic problems may be contributing factors for colonization of MRSA, thereby CA-MRSA infection. Considering the higher prevalence of CA-MRSA among the homeless and those with alcoholism, we believe that the residential environment is a very important factor in determining the risk of CA-MRSA infection.

Results from studies investigating the clinical picture caused by CA-MSSA and CA-MRSA in the literature are conflicting. CA-MSSA has been reported to be associated with a higher rate of invasive infections in some studies while CA-MRSA patients have been reported to have higher morbidity, mortality, and hospitalization rates than those with CA-MSSA infection in another study.[10,17,18] Some studies report no difference in severity between MSSA and MRSA infections.19 The most common type of infection with both MSSA and MRSA has been reported to be those of the skin and soft tissues.[10] The most common invasive infections caused by CA-MRSA isolates have been found to be musculoskeletal and pulmonary infections, while the most common ones caused by CA-MSSA have been reported as septic arthritis, bacteremia, osteomyelitis, and lymphadenitis.[17] Skin and soft tissue infections were most common with both CA-MSSA and CA-MRSA isolates in the current study, and the frequency of invasive infections was similar in the two groups, with the most common type of invasive infection caused by both CA-MSSA and CA-MRSA isolates found to be lymphadenitis/deep neck infection and bacteremia.

The optimal treatment for skin and soft tissue infections is drainage if the lesion is suitable. No difference has been reported between MSSA and MRSA infections in terms of drainage rates.[19] Drainage was used in half of the patients in the current study and most had received systemic antibiotic treatment. The rates of invasive infection, needing drainage,

and hospitalization, as well as the treatment duration were similar in our groups, indicating that methicillin resistance was not a factor that increased virulence. It was reported that the *S. aureus* virulence increases with virulence factors such as PVL, regardless of the methicillin resistance.[19]

CA-MRSA isolates have been reported to be highly susceptible to non-beta-lactam antibiotics, unlike hospital-acquired MRSA.[20] Clindamycin resistance has been increasing over the years in both CA-MRSA and CA-MSSA isolates.[17] The sensitivity rates to erythromycin and clindamycin, trimethoprim/sulfamethoxazole, and ciprofloxacin was higher in the current study than those reported in the literature.[10,17,21,22] Tetracycline sensitivity rates vary greatly in the literature (24.2-100%).[9,21] The CA-MSSA isolates were highly susceptible to tetracycline while half of the CA-MRSA isolates were resistant in the current study. CA-MSSA was found to be more sensitive to all the antibiotics tested when compared to CA-MRSA.

The strong points of our study are the prospective inclusion of patients who were queried about exposure risk factors, and evaluating both inpatients and outpatients for community-acquired *S. aureus* infection. Our study was conducted at seven health care centers in four cities and we therefore believe it is able to highly represent findings that may be generalized to our country. One of the limitations of this study is that we did not screen for the carrier state by nasal culture. We also did not investigate the living conditions and exposure risks in the retrospective part of our study. We found being a refugee to be a significant risk factor for CA-MRSA infection but we did not investigate the refugees' time of immigration, place of immigration, and living conditions in more detail. Therefore, our results cannot be generalized to all refugees. While our study was conducted in multiple centers, we did not include centers from all of Türkiye's geographical regions, which have varying climatic conditions, so our results may not be representative of other regions.

In conclusion, we found no reliable epidemiological risk factor except for being a refugee for CA-MRSA infections. We also did not find clinical variables that could differentiate between CA-MRSA and CA-MSSA infections at admission, before the culture results were available. Empirical antibiotic treatment should therefore be determined according to the local CA-MRSA prevalence in patients presenting with a possible staphylococcus infection. Further studies that focus on why CA-MRSA is more common in refugees are needed.

Ethical approval

Ethics committee approval was obtained (approval reference number: 2018/30) from Yıldırım Beyazıt University Yenimahalle Training and Research Hospital Ethics Committee for the study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: GİB, HA, FNO, OMA, BBD, ZGGA, data collection: GİB, UÇ, EÇT, FO; analysis and interpretation of results: UÇ, AK, OT, EÇ; draft manuscript preparation: AK, TAT, Eİ, GT. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Katz SE, Williams DJ. Pediatric community-acquired pneumonia in the United States: changing epidemiology, diagnostic and therapeutic challenges, and areas for future research. Infect Dis Clin North Am 2018; 32: 47-63. https://doi.org/10.1016/j.idc.2017.11.002
2. Que YA, Moreillon P. Staphylococcus aureus (Including Staphylococcal Toxic Shock Syndrome). In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 9th ed. Philadelphia: Elsevier; 2020: 2293-2431.
3. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired Staphylococcus aureus infections in children. Clin Infect Dis 2005; 40: 1785-1791. https://doi.org/10.1086/430312
4. Penteado FD, Tubero TZ, Hein N, Gilio AE. Frequency of community-acquired methicillin-resistant staphylococcus aureus in pediatric population in a general hospital in São Paulo, Brazil, over 5 years. Pediatr Infect Dis J 2019; 38: e87-e89. https://doi.org/10.1097/INF.0000000000002158
5. Durmaz B, Durmaz R, Sahin K. Methicillin-resistance among Turkish isolates of Staphylococcus aureus strains from nosocomial and community infections and their resistance patterns using various antimicrobial agents. J Hosp Infect 1997; 37: 325-329. https://doi.org/10.1016/s0195-6701(97)90149-3
6. Böncüoğlu E, Kıymet E, Çağlar İ, et al. Upward trend in the frequency of community-acquired methicillin-resistant Staphylococcus aureus as a cause of pediatric skin and soft tissue infections over five years: a cross-sectional study. Turk J Pediatr 2021; 63: 200-205. https://doi.org/10.24953/turkjped.2021.02.003
7. Sattler CA, Mason EO Jr, Kaplan SL. Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible Staphylococcus aureus infection in children. Pediatr Infect Dis J 2002; 21: 910-917. https://doi.org/10.1097/00006454-200210000-00005
8. Baggett HC, Hennessy TW, Leman R, et al. An outbreak of community-onset methicillin-resistant Staphylococcus aureus skin infections in southwestern Alaska. Infect Control Hosp Epidemiol 2003; 24: 397-402. https://doi.org/10.1086/502221
9. Skiest DJ, Brown K, Cooper TW, Hoffman-Roberts H, Mussa HR, Elliott AC. Prospective comparison of methicillin-susceptible and methicillin-resistant community-associated Staphylococcus aureus infections in hospitalized patients. J Infect 2007; 54: 427-434. https://doi.org/10.1016/j.jinf.2006.09.012
10. Böcher S, Gervelmeyer A, Monnet DL, Mølbak K, Skov RL; Danish CA-MRSA Study Group. Methicillin-resistant Staphylococcus aureus: risk factors associated with community-onset infections in Denmark. Clin Microbiol Infect 2008; 14: 942-948. https://doi.org/10.1111/j.1469-0691.2008.02055.x
11. Shahin R, Johnson IL, Jamieson F, McGeer A, Tolkin J, Ford-Jones EL. Methicillin-resistant Staphylococcus aureus carriage in a child care center following a case of disease. Toronto Child Care Center Study Group. Arch Pediatr Adolesc Med 1999; 153: 864-868. https://doi.org/10.1001/archpedi.153.8.864
12. Galper E, Bdolah-Abram T, Megged O. Assessment of infections rate due to community-acquired methicillin-resistant Staphylococcus aureus and evaluation of risk factors in the paediatric population. Acta Paediatr 2021; 110: 1579-1584. https://doi.org/10.1111/apa.15698
13. Nimmo GR, Coombs GW. Community-associated methicillin-resistant Staphylococcus aureus (MRSA) in Australia. Int J Antimicrob Agents 2008; 31: 401-410. https://doi.org/10.1016/j.ijantimicag.2007.08.011
14. Gosbell IB, Mercer JL, Neville SA, Chant KG, Munro R. Community-acquired, non-multiresistant oxacillin-resistant Staphylococcus aureus (NORSA) in South Western Sydney. Pathology 2001; 33: 206-210. https://doi.org/10.1080/00313020123439
15. Manzur A, Dominguez AM, Pujol M, et al. Community-acquired methicillin-resistant Staphylococcus aureus infections: an emerging threat in Spain. Clin Microbiol Infect 2008; 14: 377-380. https://doi.org/10.1111/j.1469-0691.2007.01934.x
16. Aro T, Kantele A. High rates of meticillin-resistant Staphylococcus aureus among asylum seekers and refugees admitted to Helsinki University Hospital, 2010 to 2017. Euro Surveill 2018; 23: 1700797. https://doi.org/10.2807/1560-7917.ES.2018.23.45.1700797
17. Hultén KG, Kaplan SL, Gonzalez BE, et al. Three-year surveillance of community onset health care-associated staphylococcus aureus infections in children. Pediatr Infect Dis J 2006; 25: 349-353. https://doi.org/10.1097/01.inf.0000207404.50143.1e
18. Gomes RT, Lyra TG, Alves NN, Caldas RM, Barberino MG, Nascimento-Carvalho CM. Methicillin-resistant and methicillin-susceptible community-acquired Staphylococcus aureus infection among children. Braz J Infect Dis 2013; 17: 573-578. https://doi.org/10.1016/j.bjid.2013.02.010
19. Bennett CM, Coombs GW, Wood GM, et al. Community-onset Staphylococcus aureus infections presenting to general practices in South-eastern Australia. Epidemiol Infect 2014; 142: 501-511. https://doi.org/10.1017/S0950268813001581

20. Groom AV, Wolsey DH, Naimi TS, et al. Community-acquired methicillin-resistant Staphylococcus aureus in a rural American Indian community. JAMA 2001; 286: 1201-1205. https://doi.org/10.1001/jama.286.10.1201

21. Wu D, Wang Q, Yang Y, et al. Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible Staphylococcus aureus from skin/soft tissue infections in a children's hospital in Beijing, China. Diagn Microbiol Infect Dis 2010; 67: 1-8. https://doi.org/10.1016/j.diagmicrobio.2009.12.006

22. Liu Y, Xu Z, Yang Z, Sun J, Ma L. Characterization of community-associated Staphylococcus aureus from skin and soft-tissue infections: a multicenter study in China. Emerg Microbes Infect 2016; 5: e127. https://doi.org/10.1038/emi.2016.128

The Turkish Journal of Pediatrics 2023; 65: 479-488
https://doi.org/10.24953/turkjped.2022.922

Original Article

Cardiac rhabdomyomas: clinical progression, efficacy and safety of everolimus treatment

Saygın Yıldırım[1], Ebru Aypar[2], Burça Aydın[3], Canan Akyüz[3], Hayrettin Hakan Aykan[2], İlker Ertuğrul[2], Tevfik Karagöz[2], Dursun Alehan[2]

[1]Department of Pediatrics, Hacettepe University İhsan Doğramacı Children's Hospital, Ankara; [2]Department of Pediatric Cardiology, Hacettepe University İhsan Doğramacı Children's Hospital, Ankara; [3]Department of Pediatric Oncology, Hacettepe University Oncology Institute, Ankara, Türkiye.

ABSTRACT

Background. Primary cardiac tumors are extremely rare. Cardiac rhabdomyoma is the most common primary cardiac tumor. 50-80% of solitary rhabdomyomas and all multiple rhabdomyomas are associated with tuberous sclerosis complex. Due to spontaneous regression, surgery is necessary only in severe hemodynamic compromise and persistent arrhythmias. Everolimus, a mechanistic target of rapamycin (mTOR) inhibitor, can be used in the treatment of rhabdomyomas seen in tuberous sclerosis complex. We aimed to evaluate the clinical progression of rhabdomyomas followed-up in our center between the years 2014-2019 and evaluate the efficacy and safety of everolimus treatment on tumor regression.

Methods. Clinical features, prenatal diagnosis, clinical findings, tuberous sclerosis complex presence, treatment and follow-up results were evaluated retrospectively.

Results. Among 56 children with primary cardiac tumors, 47 were diagnosed as rhabdomyomas, 28/47 patients (59.6%) had prenatal diagnosis, 85.1% were diagnosed before one year of age and 42/47 patients (89.3%) were asymptomatic. Multiple rhabdomyomas were present in 51% and median diameter of tumors was 16mm (4.5 - 52 mm). In 29/47 patients (61.7%) no medical or surgical treatment were necessary while 34% of these had spontaneous regression. Surgery was necessary in 6/47 patients (12.7%). Everolimus was used in 14/47 patients (29.8%). Indications were seizures (2 patients) and cardiac dysfunction (12 patients). Regression in size of rhabdomyomas was achieved in 10/12 patients (83%). Although, in the long-term, the amount of tumor mass shrinkage was not significantly different between patients who received everolimus and untreated patients (p=0.139), the rate of mass reduction was 12.4 times higher in patients who received everolimus. Leukopenia was not detected in any of the patients, but, hyperlipidemia was noted in 3/14 patients (21.4%).

Conclusions. According to our results, everolimus accelerates tumor mass reduction, but not amount of mass regression in the long term. Everolimus may be considered for treatment of rhabdomyomas which cause hemodynamic compromise or life-threatening arrhythmias before surgical intervention.

Key words: cardiac tumor, everolimus, rhabdomyoma, tuberous sclerosis complex.

Primary cardiac tumors in children are extremely rare. Cardiac rhabdomyoma is the most common primary cardiac tumor.[1-4] Most rhabdomyomas are related with tuberous sclerosis complex (TSC), but they may also occur as isolated lesions. Patients with rhabdomyomas are mostly asymptomatic. However, depending on their location, rhabdomyomas may obstruct inflow or outflow of blood, affect conduction pathways, sinus or atrioventricular nodes and cause congestive heart failure, arrhythmia, cyanosis, respiratory distress, and murmur. Large or multiple rhabdomyomas can cause stillbirths or sudden death due to impairment in myocardial function and arrhythmias. Echocardiography has a primary role for both diagnosis and follow-up of rhabdomyomas.

✉ Saygın Yıldırım
sygnyldrm@gmail.com

Received 12th October 2022, revised 28th January 2023, accepted 9th April 2023.

Magnetic resonance imaging can be used as a complementary tool when diagnosis is not clear or for preoperative planning, and, cardiac catheterization is rarely required. Although histological evaluation with biopsy is the gold standard in confirming the diagnosis, it is rarely required.[1]

Since spontaneous regression of rhabdomyomas have been well documented, surgical or therapeutic interventions are only recommended for cases with severe hemodynamic compromise or persistent arrhythmias.[1-9] There is no relationship between the spontaneous regression rate or size of the tumor and its number or location.[10] The survival rate of the disease is between 81-92%.[10-12]

As tuberous sclerosis complex has autosomal dominant inheritance caused by mutations in the TSC1 (Tuberous sclerosis Complex) and TSC2 genes in the mTOR pathway responsible for the regulation of cell growth, mechanistic target of rapamycin (mTOR) inhibitors, mostly everolimus, have been used in the treatment of rhabdomyomas.[13-16] Everolimus is effective for the reduction of tumor size, and, is even life-saving for complications such as arrhythmias and ventricular outflow obstruction.[17] It is also used for treatment of tuberous sclerosis associated giant cell astrocytomas or angiomyolipomas. In addition to simple gastrointestinal system side effects, side effects such as leukopenia, susceptibility to infections, stomatitis, skin rash, hypercholesterolemia, hypertriglyceridemia, hyperglycemia, hypophosphatemia, hyperuricemia and bone marrow suppression have also been reported.[18]

We aimed to evaluate patients diagnosed with cardiac rhabdomyomas and followed-up in Hacettepe University İhsan Doğramacı Children's Hospital Pediatric Cardiology and Pediatric Oncology Departments between January 1, 2014 and June 30, 2019. We also aimed to observe the efficacy and safety of everolimus for the treatment of rhabdomyomas.

Material and Methods

Among 56 patients aged between 0-18 years who were admitted to our institution and who were found to have a cardiac mass by echocardiography, 47 patients were diagnosed as cardiac rhabdomyomas. During echocardiographic examination, intracardiac masses were classified and diagnosed as rhabdomyomas when they were highly echogenic, homogeneous, especially multiple, well-circumscribed, intramural or intracavitary nodules with a finely speckled pattern occurring anywhere within the heart. Differentiation from intracardiac thrombi, myxomas, and hemangiomas were made by the absence of circumscribed echolucent areas as a result of hemorrhage formation. Differentiation from fibromas was made by the absence of calcification and cystic degeneration in rhabdomyomas.[19]

The demographic characteristics, diagnosis, presence of intrauterine diagnosis, clinical complaints, comorbidities, clinical follow-up, examinations, treatment details and outcome of patients who were diagnosed as rhabdomyomas by echocardiography, radiological investigations or pathologic examinations were evaluated retrospectively. Data were collected from electronic records and patient files. Echocardiographic data consisted of results from both our hospital's and other referral center's data. Records of patients who received everolimus were also evaluated. Everolimus was started at a dose of 4.5 mg/m^2/week (two days of the week, twice daily) and dose adjustment was made according to serum everolimus levels. Everolimus treatment indications, treatment durations, responses to treatment and side effects were analyzed. Echocardiographic data of patients who received everolimus and who did not receive everolimus were compared to analyze everolimus efficacy.

The study was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Committee (GO-19/690).

Statistical analysis

All statistical analyzes were performed using IBM SPSS Statistics for Windows Version 23.0 package program. Numerical variables were summarized as mean±standard deviation, median (minimum - maximum) values. Categorical variables were shown with numbers and percentages. The Mann-Whitney U test was used to compare the regression in mass sizes of tumors of patients who received and did not receive everolimus because it did not comply with the normal distribution. Statistical significance was accepted as $p<0.05$.

Results

The study included 22 female and 25 male patients. Out of 47 patients, 28 patients (59.6%) had prenatal diagnosis and 12 patients (25.5%) were diagnosed before one year of age. Totally, 85.1% of the patients had a diagnosis of rhabdomyoma before the age of one.

The median age of the patients with postnatal diagnosis was 0.5 years (1 day-16.1 years). Single cardiac rhabdomyomas were present in 23 patients (49%), and multiple rhabdomyomas in 24 patients (51%). Demographic characteristics, referral reasons, clinical findings, tumor locations, and associated diseases are summarized in Table I.

Twenty two patients (46.8%) did not have any clinical findings. Arrhythmias were present in 12 patients (25.5%), murmur in nine patients (19.1%), valve insufficiency in eight patients (17%) and pericardial effusion in two patients (4.3%). Three patients had respiratory distress and one patient had bruising. Of the 23 patients with a single rhabdomyoma, five patients (21.7%) had arrhythmias, two (8.7%) had valve insufficiency, two (8.7%) had both valve insufficiency and arrhythmias. Of the 24 patients with multiple rhabdomyomas, six patients (25%) had murmur, five (20.8%) had arrhythmias, and three (12.5%) had valve insufficiency.

Table I. Demographic and clinical characteristics of all patients with cardiac rhabdomyomas.

Gender, n (%)	
Male	25 (53%)
Female	22 (47%)
Prenatal diagnosis	
Yes	28 (59.6%)
No	19 (40.4%)
Age of patients with postnatal diagnosis	
Mean (years)	2.7±4.9
Median (years) (range)	0.5 (1 day-16.1 years)
Referral reasons, n (%)	
Prenatal diagnosis	28 (59.6%)
Prematurity	1 (2.1%)
Murmur	7 (14.9%)
Respiratory distress	2 (4.3%)
Bruising	1 (2.1%)
Palpitations	1 (2.1%)
Symptoms	
Asymptomatic	42 (89.3%)
Respiratory distress	3 (6.4%)
Bruising	1 (2.1%)
Palpitations	1 (2.1%)
Number of tumors, n (%)	
Single	23 (49%)
Multiple	24 (51%)
Tumor size (mm)	
Median largest diameter at diagnosis	16
Minimum diameter	4.5
Maximum diameter	52
Tumor location, n (%)	
Left ventricle	17 (36.2%)
Left and right ventricle	16 (34%)
Right ventricle	2 (4.3%)
Interventricular septum	1 (2.1%)
Additional disease, n (%)	
Tuberous sclerosis complex	33 (70.2%)
Cleft palate and lip	1 (2.1%)
No treatment, n (%)	29 (61.7%)
Follow-up results without any treatment	
Complete regression (n)	2
Partial regression (n)	14
Total regression, n (%)	16 (34%)
Everolimus, n (%)	14 (29.8%)
Treatment response, n (%)	12 (85.7%)
Surgery, n (%)	6 (12.7 %)

Median diameter of tumors at the time of diagnosis was 16 mm (minimum: 4.5 mm, maximum: 52 mm). Thirty-three patients (70.2%) with rhabdomyomas were also diagnosed as TSC. Of these patients, 20/33 patients (60.6%) had epileptic seizures and were on antiepileptic treatment. Cranial imaging was performed in 18/33 patients with tuberous sclerosis complex. 14/18 had a history of epileptic seizures. All 18 patients with cranial imaging had findings consistent with TSC. Giant cell astrocytomas were detected in three patients (9.1%) and angiomyolipomas were documented in four patients (12.1%).

Treatment

In 29/47 patients (61.7%), no medical or surgical treatment was necessary, during the follow-up, 34% of these had spontaneous regression. Everolimus treatment were necessary in fourteen patients. Patient characteristics of those given everolimus and treatment results are shown in Table II. Surgery were necessary in six patients (12.7%), four patients due to severe left ventricular outflow tract obstruction, one patient due to severe right ventricular outflow tract obstruction, one patient because of a giant mass in left ventricle, decreased left ventricular functions and pericardial effusion.

Indications for everolimus treatment were cardiac dysfunction in 12/14 patients (85.7%) (valve dysfunction in seven (58.3%), cardiological inflow or outflow obstruction in three (25%), life-threatening arrhythmias (one patient had frequent ventricular premature contractions causing syncope, one patient had ventricular tachycardia) in two patients (16.7%)) and epileptic seizures in 2/14 patients (14.3%). Antiepileptic therapy was also used in two patients whom everolimus was started for neurological complications, and seizures were under control.

Regression in size of rhabdomyomas were achieved in 10/12 patients (83%). Considering the two patients with seizures, everolimus treatment was successfully achieved in 12/14 patients (86%).

The target serum everolimus levels were between 3-8 ng/ml. The target serum levels were reached in all nine patients whose data could be reached, and dose adjustments were made according to the serum levels. Serum everolimus level data were not available in five patients. Most common side effect was hyperlipidemia, which was observed in 3/14 patients (21.4%). However, in only one patient, hyperlipidemia reached a level that required treatment cessation. Leukopenia was not detected in any of the 14 patients who received everolimus treatment.

To evaluate the efficacy of everolimus on tumor regression, we compared 14 patients who received everolimus with 33 untreated patients. However, two patients were excluded because of surgery, one patient, because of spontaneous regression during the follow-up and ten patients for insufficient echocardiographic data, finally 20 patients were included in the untreated patients group.

The median amount of tumor mass shrinkage was -5.75 mm (-42mm-+10mm) in patients who received everolimus, and -2mm (-18mm-+11.5mm) in untreated patients. There was no statistically significant difference between the groups (p=0.139). However, when the rate of tumor size reduction was evaluated, the rate of reduction was 39.3% in patients who received everolimus and 3.2% in untreated patients within the first six months of treatment. In conclusion, the rate of tumor mass reduction was 12.4 times higher in patients with everolimus treatment compared to untreated patients.

Everolimus treatment was given to a patient with a 52x36 mm giant cardiac rhabdomyoma surrounding the left ventricle who was diagnosed prenatally and without a family history of tuberous sclerosis complex (Figs 1 and 2). The average drug blood level of everolimus achieved was 5.4 ng/ml. The mass regressed to 26x26 mm after two months of everolimus treatment, and to 12x4 mm after four months of treatment so everolimus dose was decreased (Figs 3 and 4). However, at 6 months the size

Table II. Clinical characteristics of patients who had everolimus treatment.

Patient No	Age	CR	Diagnosis of TSC	Everolimus indication	Treatment duration (months)	Everolimus serum level (ng /ml) (min-max (median))	Target serum level (3-8 ng /ml)	Pre-treatment size (mm)	Post-treatment size (mm)	Regression (%)	Complications
1	1 y 5 m	S	-	RVOT obstruction	4	1-3.6 (2)	Yes	20x15	22x15	none	Hyperlipidemia
2	2 y 2 m	S	-	Giant mass	13	0.6-47 (2.6)	Yes	52x36	26x26	50	Hyperlipidemia
3	15 y 5 m	S	+	Arrhythmias	11	2-5.1 (3.2)	Yes	18x10	0	100	-
4	17 y	S	+	Seizures	21	0-4.9 (0.7)	Yes	7x7	7x6,5	none	-
5	10 y	S	+	Arrhythmias, MR	11	No data	No data	29x22	39x33	none	Hyperlipidemia
6	1 y 1 m	S	+	Giant mass	6	0.9-4.6 (1.8)	Yes	50x36	8x6	84	-
7	6 y 6 m	S	-	Arrhythmias	No data	No data	No data	23x10	7x7	69	-
8	2 y 10 m	M	+	MR	8	No data	No data	26x17	20x6	23	-
9	5 y 4 m	M	+	LVOT obstruction	9	0.4-10.1 (3.6)	Yes	12x9 (9 masses)	13x7 (2 masses)	0	-
10	5 y 8 m	M	+	LVOT obstruction	7	0.6-14.4 (4.3)	Yes	8x7	6x5	26	-
11	7 y 4 m	M	+	TR	6	No data	No data	20x15	15x9	27	-
12	3y 7 m	M	+	LVOT obstruction	6	0-14.5 (1.7)	Yes	29x20	25x11	12	-
13	2 y 11 m	M	+	Seizures	Treatment is ongoing	3.8-9.1 (7.4)	Yes	10x5	0	100	-
14	8 y 1 m	M	+	Treatment started in another center	26	No data	No data	21x19	15x8	28	-

CR: cardiac rhabdomyoma, LVOT: left ventricular outflow tract, m: months, M: multiple, MR: mitral regurgitation, RVOT: right ventricular outflow tract, S: single, TR: tricuspid regurgitation, TSC: tuberous sclerosis complex, y: years.

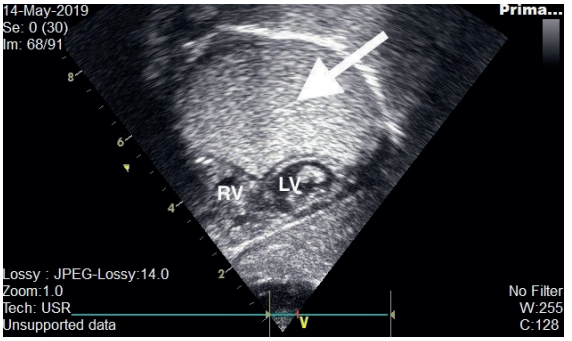


Fig. 1. Transthoracic subcostal view showing 52x36 mm giant cardiac rhabdomyoma (arrow) surrounding the left ventricle (LV) and extending to the right ventricular (RV) outflow tract and interventricular septum.

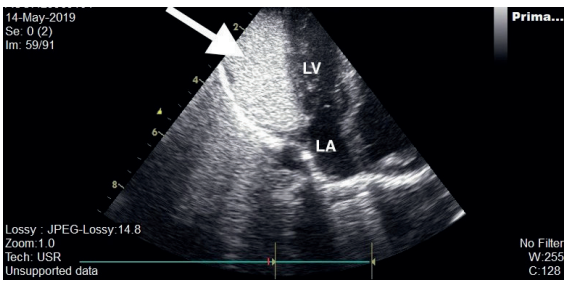


Fig. 2. Transthoracic echocardiography apical 4-chamber view showing cardiac rhabdomyoma (arrow) in the left ventricle (LV). LA: left atrium.

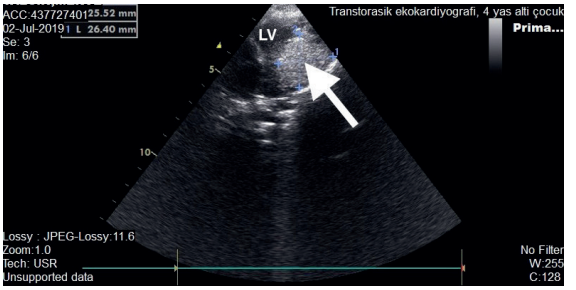


Fig. 3. Two months after everolimus treatment, transthoracic echocardiographic view. Cardiac rhabdomyoma (arrow) surrounding the left ventricle (LV) regressed from 52x36 mm to 26x26 mm.

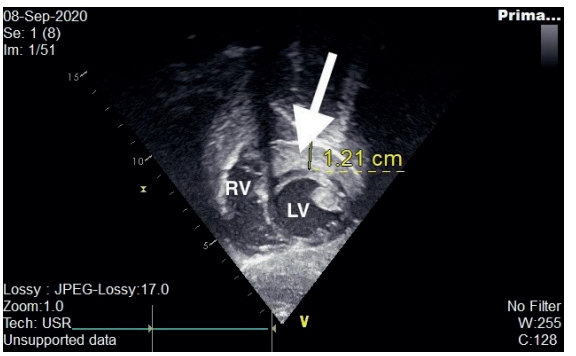


Fig. 4. Four months after everolimus treatment, transthoracic echocardiographic subcostal view. Cardiac rhabdomyoma regressed from 52x36 mm to 12x4 mm in size. LV: Left ventricle, RV: Right ventricle.

of the tumor had doubled once again. During the follow-up, the patient had hyperlipidemia (total cholesterol: 217 mg/dl, LDL: 145 mg/dl, triglyceride: 211 mg/dl), so the treatment was discontinued. Everolimus treatment was given to another patient without signs of tuberous sclerosis complex because of mass-related ventricular tachycardia. Approximately 60% regression was achieved after two months, and the patient did not require ablation or implantable cardiac defibrillator during everolimus treatment.

Discussion

Cardiac tumors are extremely rare in children and the most common primary cardiac tumor is cardiac rhabdomyoma.[2] Cardiac rhabdomyomas can present with tuberous sclerosis complex with a rate of 40-100% and they may be the first signs of the disease even from the prenatal period.[20-22] Jozwiak et al.[23] reported that 66% of patients with cardiac rhabdomyomas were younger than two years of age. In accordance with this research, in our study, 59.6% of the patients were diagnosed prenatally, 25.5% were younger than one year of age. In total, the percentage of patients diagnosed under one year of age was 85.1%.

Forty two of the 47 patients (89.3%) with rhabdomyomas were asymptomatic. This rate is very high when compared to the literature. In the pediatric cardiac tumor series which consisted of 255 articles, the rate of asymptomatic patients was 7.2%. The most common symptoms were respiratory distress

(14.2%), cyanosis (6.9%), chest pain (2.8%). The most common clinical findings were murmur (28.9%), heart failure (19.1%), and arrhythmias (14.9%).[15] However, since this series consisted of patients who had undergone surgery, it is not unusual that serious symptoms such as heart failure and arrhythmias leading to surgery were present in these patients. In another study, 24.7% of the 166 pediatric cardiac tumor patients were asymptomatic and 12% were detected prenatally. The most common clinical manifestations were murmur (32.5%), respiratory distress (7.8%), arrhythmias (6.6%) and pericardial effusion (5.4%).[24] The high rate of asymptomatic patients in our study can be explained by the high number of patients detected in the prenatal period.

In literature, succesful and rapid regression of rhabdomyomas with everolimus treatment have been reported as case reports[25-28] and as case series[14,29]. Çetin et al.[25] showed that 4 months of everolimus treatment reduced rhabdomyomas, arrhythmias and outflow tract stenosis in a 3 month-old patient with multiple cardiac rhabdomyomas causing left ventricular outflow tract obstruction, tuberous sclerosis and Wolff-Parkinson-White syndrome. Öztunç et al.[28] reported that arrhythmias were reduced after everolimus treatment in a patient with tuberous sclerosis and supraventricular tachycardia due to a cardiac rhabdomyoma. Davis et al.[30] reported that everolimus was started in a patient who was diagnosed as cardiac rhabdomyoma and without diagnosis of tuberous sclerosis for polymorphic ventricular tachycardia at the age of 10 months and at the end of the six months of treatment, ventricular tachycardia was not detected. They stated that everolimus can be considered in the treatment of not only patients with cardiac rhabdomyoma accompanying tuberous sclerosis, but also in patients without tuberous sclerosis. Also in our study, although patients 2 and 7 (Table II) were not diagnosed as tuberous sclerosis, size of rhabdomyomas were reduced with everolimus treatment. Dhulipudi et al.[14] also showed that everolimus treatment regressed cardiac rhabdomyomas 11.8 times faster compared to spontaneous regression treatment in their five patient series. However, it is noteworthy that one patient had sudden cardiac death in the fourth month of treatment. The results of Aw et al.[13] were also similar in terms of regression rate of everolimus treatment. Martínez-García et al.[17] summarized the mass and arrhythmia reducing effects of everolimus in the article in which they compiled 17 cases and case series. In the literature, there are reports that were successfully treated with a daily dose of 1.5-2 mg/m^2 of everolimus, with serum levels targeted to be between 5-15 ng/ml[27,31-33]. Chang et al.[29] gave everolimus treatment to three newborns with cardiac rhabdomyomas, starting from a dose of 0.3-0.67 mg/m^2/day, with target serum levels between 3-7 ng/ml, which is lower than reported in the literature. Rhabdomyomas were regressed after two months without any side effects. In our center, our initial treatment dose of 4.5 mg/m^2/week is similar as a total weekly dose and supports findings of Chang et al.[29] Doğan et al.[34] also showed the efficacy of everolimus treatment by keeping serum everolimus between 3.6-7.8 ng/ml.

In accordance with the literature, our study showed that there was no difference in the amount of reduction in the size of the tumors with everolimus treatment in the long term. However, the rate of mass reduction was found to be 12.4 times higher in everolimus patients compared to untreated patients.[13,14,35-37] Our results have shown everolimus should be used only to rapidly shrink rhabdomyomas that disrupt hemodynamics or cause life-threatening arrhythmias.

Due to spontaneous regression, rhabdomyomas that do not cause hemodynamic compromise or life-threatening arrhythmias can be followed-up without medical treatment or surgical intervention.[1-9] Echocardiography is essential to evaluate tumor size and cardiac function. In addition, we also suggest to perform electrocardiogram and 24-hour rhythm monitoring during the follow-up of rhabdomyoma patients, due to high rates of arrhythmias in our study (26%).

The fact that no serious complications with everolimus other than hyperlipidemia in one patient, supports the notion that everolimus treatment is also safe.

In accordance with the literature, the relationship of tuberous sclerosis complex with multiple cardiac rhabdomyomas were very high in our study.[38] Therefore, patients who do not have a family history of tuberous sclerosis complex but who have multiple cardiac rhabdomyomas in the prenatal period, should be closely followed up for signs of postnatal tuberous sclerosis complex.

As a limitation, our study was a retrospective study and it was not a randomized controlled study.

According to our study results, everolimus accelerates tumor mass reduction, but not the amount of mass regression in the long term. Everolimus may be considered for treatment of rhabdomyomas that cause hemodynamic compromise or life-threatening arrhythmias before surgical intervention.

Acknowledgement

We would like to thank all Hacettepe University Hospital staff who contributed to our study during the diagnosis, follow-up and treatment of the patients and storage of the data.

Ethical approval

This study does not include human and/or animal experimentation and it was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Committee on July 2, 2019 (Number: GO-19/690).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SY, DA, EA; data collection: SY, EA, BA, CA, HHA, IE, TK, DA; analysis and interpretation of results: SY, EA, BA, CA, DA; draft manuscript preparation: SY, EA, BA, DA. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Beghetti M, Gow RM, Haney I, Mawson J, Williams WG, Freedom RM. Pediatric primary benign cardiac tumors: a 15-year review. Am Heart J 1997; 134: 1107-1114. https://doi.org/10.1016/s0002-8703(97)70032-2
2. Von Recklinghausen F. Ein Hertz von einem Neugeborenen welches mehrere theils nach aussen, theils nach den Hoehlen prominirende Tumoren (Myomen) trug. Verb Ges Geburtsh 25 Marz, Monatsschr Gebur 1862; 20: 1-2.
3. McAllister HA Jr. Primary tumors and cysts of the heart and pericardium. Pathol Annu 1979; 14: 325-355.
4. Nadas AS, Ellison RC. Cardiac tumors in infancy. Am J Cardiol 1968; 21: 363-366. https://doi.org/10.1016/0002-9149(68)90140-9
5. Arciniegas E, Hakimi M, Farooki ZQ, Truccone NJ, Green EW. Primary cardiac tumors in children. J Thorac Cardiovasc Surg 1980; 79: 582-591
6. Chan HS, Sonley MJ, Moës CA, Daneman A, Smith CR, Martin DJ. Primary and secondary tumors of childhood involving the heart, pericardium, and great vessels. A report of 75 cases and review of the literature. Cancer 1985; 56: 825-836. https://doi.org/10.1002/1097-0142(19850815)56:4<825::aid-cncr2820560421>3.0.co;2-7

7. Isaacs H Jr. Fetal and neonatal cardiac tumors. Pediatr Cardiol 2004; 25: 252-273. https://doi.org/10.1007/s00246-003-0590-4

8. Van der Hauwaert LG. Cardiac tumours in infancy and childhood. Br Heart J 1971; 33: 125-132. https://doi.org/10.1136/hrt.33.1.125

9. Smythe JF, Dyck JD, Smallhorn JF, Freedom RM. Natural history of cardiac rhabdomyoma in infancy and childhood. Am J Cardiol 1990; 66: 1247-1249. https://doi.org/10.1016/0002-9149(90)91109-j

10. D'Addario V, Pinto V, Di Naro E, Del Bianco A, Di Cagno L, Volpe P. Prenatal diagnosis and postnatal outcome of cardiac rhabdomyomas. J Perinat Med 2002; 30: 170-175. https://doi.org/10.1515/JPM.2002.022

11. Bosi G, Lintermans JP, Pellegrino PA, Svaluto-Moreolo G, Vliers A. The natural history of cardiac rhabdomyoma with and without tuberous sclerosis. Acta Paediatr 1996; 85: 928-931. https://doi.org/10.1111/j.1651-2227.1996.tb14188.x

12. Holley DG, Martin GR, Brenner JI, et al. Diagnosis and management of fetal cardiac tumors: a multicenter experience and review of published reports. J Am Coll Cardiol 1995; 26: 516-520. https://doi.org/10.1016/0735-1097(95)80031-b

13. Aw F, Goyer I, Raboisson M-J, Boutin C, Major P, Dahdah N. Accelerated cardiac rhabdomyoma regression with everolimus in infants with tuberous sclerosis complex. Pediatr Cardiol 2017; 38: 394-400. https://doi.org/10.1007/s00246-016-1528-y

14. Dhulipudi B, Bhakru S, Rajan S, Doraiswamy V, Koneti NR. Symptomatic improvement using everolimus in infants with cardiac rhabdomyoma. Ann Pediatr Cardiol 2019; 12: 45-48. https://doi.org/10.4103/apc.APC_79_18

15. Tzani A, Doulamis IP, Mylonas KS, Avgerinos DV, Nasioudis D. Cardiac Tumors in Pediatric Patients: A Systematic Review. World J Pediatr Congenit Heart Surg 2017; 8: 624-632. https://doi.org/10.1177/2150135117723904

16. Uzun O, Wilson DG, Vujanic GM, Parsons JM, De Giovanni JV. Cardiac tumours in children. Orphanet J Rare Dis 2007; 2: 11. https://doi.org/10.1186/1750-1172-2-11

17. Martínez-García A, Michel-Macías C, Cordero-González G, et al. Giant left ventricular rhabdomyoma treated successfully with everolimus: case report and review of literature. Cardiol Young 2018; 28: 903-909. https://doi.org/10.1017/S1047951118000598

18. Davies M, Saxena A, Kingswood JC. Management of everolimus-associated adverse events in patients with tuberous sclerosis complex: a practical guide. Orphanet J Rare Dis 2017; 12: 35. https://doi.org/10.1186/s13023-017-0581-9

19. Marx GR, Moran AM. Cardiac Tumors. In: Shaddy RE, Penny DJ, Feltes TF, Cetta F, Mital S, editors. Moss & Adams' Heart Disease in infants, children, and adolescents (10th ed). Lippincott Williams & Wilkins; 2021: 48584910.

20. Bader RS, Chitayat D, Kelly E, et al. Fetal rhabdomyoma: prenatal diagnosis, clinical outcome, and incidence of associated tuberous sclerosis complex. J Pediatr 2003; 143: 620-624. https://doi.org/10.1067/S0022-3476(03)00494-3

21. Harding CO, Pagon RA. Incidence of tuberous sclerosis in patients with cardiac rhabdomyoma. Am J Med Genet 1990; 37: 443-446. https://doi.org/10.1002/ajmg.1320370402

22. Sciacca P, Giacchi V, Mattia C, et al. Rhabdomyomas and tuberous sclerosis complex: our experience in 33 cases. BMC Cardiovasc Disord 2014; 14: 66. https://doi.org/10.1186/1471-2261-14-66

23. Jóźwiak S, Kotulska K, Kasprzyk-Obara J, et al. Clinical and genotype studies of cardiac tumors in 154 patients with tuberous sclerosis complex. Pediatrics 2006; 118: e1146-51. https://doi.org/10.1542/peds.2006-0504

24. Shi L, Wu L, Fang H, et al. Identification and clinical course of 166 pediatric cardiac tumors. Eur J Pediatr 2017; 176: 253-260. https://doi.org/10.1007/s00431-016-2833-4

25. Çetin M, Aydın AA, Karaman K. Everolimus treatment in a 3-month-old infant with tuberous sclerosis complex cardiac rhabdomyoma, severe left ventricular outflow tract obstruction, and hearing loss. Cardiol Young 2021; 31: 1359-1362. https://doi.org/10.1017/S1047951121000639

26. Çetiner N, Kavas N, Küçükosmanoğlu O. Rapid regression everolimus therapy in a neonate with cardiac rhabdomyoma. Pediatr Int 2022; 64: e15188. https://doi.org/10.1111/ped.15188

27. Demir HA, Ekici F, Yazal Erdem A, Emir S, Tunç B. Everolimus: a challenging drug in the treatment of multifocal inoperable cardiac rhabdomyoma. Pediatrics 2012; 130: e243-7. https://doi.org/10.1542/peds.2011-3476

28. Öztunç F, Atik SU, Güneş AO. Everolimus treatment of a newborn with rhabdomyoma causing severe arrhythmia. Cardiol Young 2015; 25: 1411-1414. https://doi.org/10.1017/S1047951114002261

29. Chang JS, Chiou PY, Yao SH, Chou IC, Lin CY. Regression of neonatal cardiac rhabdomyoma in two months through low-dose everolimus therapy: a report of three cases. Pediatr Cardiol 2017; 38: 1478-1484. https://doi.org/10.1007/s00246-017-1688-4

30. Davis KA, Dodeja AK, Clark A, et al. Use of cardiac MRI to assess antitumor efficacy of everolimus in sporadic cardiac rhabdomyoma. Pediatrics 2019; 143: e20182495. https://doi.org/10.1542/peds.2018-2495

31. Mohamed I, Ethier G, Goyer I, Major P, Dahdah N. Oral everolimus treatment in a preterm infant with multifocal inoperable cardiac rhabdomyoma associated with tuberous sclerosis complex and a structural heart defect. BMJ Case Rep 2014; 2014: bcr2014205138. https://doi.org/10.1136/bcr-2014-205138

32. Tiberio D, Franz DN, Phillips JR. Regression of a cardiac rhabdomyoma in a patient receiving everolimus. Pediatrics 2011; 127: e1335-7. https://doi.org/10.1542/peds.2010-2910

33. Wagner R, Riede FT, Seki H, et al. Oral everolimus for treatment of a giant left ventricular rhabdomyoma in a neonate-rapid tumor regression documented by real time 3D echocardiography. Echocardiography 2015; 32: 1876-1879. https://doi.org/10.1111/echo.13015

34. Doğan V, Yeşil Ş, Kayalı Ş, et al. Regression of symptomatic multiple cardiac rhabdomyomas associated with tuberous sclerosis complex in a newborn receiving everolimus. J Trop Pediatr 2015; 61: 74-77. https://doi.org/10.1093/tropej/fmu056

35. Dahdah N. Everolimus for the treatment of tuberous sclerosis complex-related cardiac rhabdomyomas in pediatric patients. J Pediatr 2017; 190: 21-26.e7. https://doi.org/10.1016/j.jpeds.2017.06.076

36. Garg A, Gorla SR, Kardon RE, Swaminathan S. Rapid involution of large cardiac rhabdomyomas with everolimus therapy. World J Pediatr Congenit Heart Surg 2021; 12: 426-429. https://doi.org/10.1177/2150135118822711

37. Saffari A, Brösse I, Wiemer-Kruel A, et al. Safety and efficacy of mTOR inhibitor treatment in patients with tuberous sclerosis complex under 2 years of age - a multicenter retrospective study. Orphanet J Rare Dis 2019; 14: 96. https://doi.org/10.1186/s13023-019-1077-6

38. Mariscal-Mendizábal LF, Sevilla-Montoya R, Martínez-García AJ, et al. Clinical and genetic description of patients with prenatally identified cardiac tumors. Prenat Diagn 2019; 39: 998-1004. https://doi.org/10.1002/pd.5521

The Turkish Journal of Pediatrics 2023; 65: 489-499
https://doi.org/10.24953/turkjped.2022.743

Original Article

Early period intensive care follow-up after liver transplantation in children: a single center experience

Edin Botan[1], Emrah Gün[1], Setenay Akyüzlüer Güneş[8], Anar Gurbanov[1], Hasan Özen[1], Zarife Kuloglu[2], Ceyda Kırsaçlıoğlu[2], Elvan Onur Kırımker[4], Özlem Can Selvi[7], Ergin Çiftçi[3], Suat Fitöz[6], Meltem Koloğlu[5], Aydan Kansu[2], Deniz Balcı[4], Tanıl Kendirli[1]

[1]Division of Pediatric Critical Care Medicine, Ankara University Faculty of Medicine, Ankara; [2]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Ankara University Faculty of Medicine, Ankara; [3]Division of Pediatric Infectious Diseases, Ankara University Faculty of Medicine, Ankara; [4]Department of General Surgery and Liver Transplantation, Ankara University Faculty of Medicine, Ankara; [5]Department of Pediatric Surgery, Ankara University Faculty of Medicine, Ankara; [6]Department of Radiology, Ankara University Faculty of Medicine, Ankara, [7]Department of Anaesthesia and Intensive Care, Ankara University Faculty of Medicine, Ankara; [8]Department of Pediatrics Ankara University Faculty of Medicine, Ankara, Türkiye.

ABSTRACT

Background. Liver transplantation (LT) is a well-established, life-saving treatment for children with irreversible acute and chronic liver failure (LF). We aimed to evaluate the factors associated with morbidity and mortality in the early period of LT in children by reviewing our pediatric intensive care unit (PICU) experience.

Methods. We reviewed children's medical records followed in the PICU after LT between May 2015-August 2021, including demographic parameters, indications for LT, operative variables, respiratory and circulatory support requirements, LT-related complications and survival.

Results. During this period, 40 pediatric patients who underwent LT were evaluated. LT was performed in 35 (87.5%) cases of chronic liver disease and 5 (12.5%) cases of acute liver failure. Twenty-four patients had chronic liver failure due to cholestatic liver disease. The patients' Pediatric Risk of Mortality (PRISM) III score was 18.82±SD (2-58) at PICU admission. 1-year survival was 87.5%, and overall survival was 85%. Younger age, low body weight, preoperative pediatric end-stage liver disease (PELD), and model for end-stage liver disease (MELD) values of 20 and higher were important risk factors for unfavorable outcomes after living donor liver transplantation (LDLT). These risk factors are both associated with technically more challenging vascular and bile duct reconstruction and higher complication rates, and increased mortality during the early period after LT.

Conclusions. The early period of optimum PICU management in pediatric LT recipients is crucial for successful outcomes, which is also related to the patients' characteristics, disease severity scores, and surgical procedures.

Key words: liver transplantation, pediatric intensive care, children.

Liver transplantation (LT) is the standard of treatment with excellent outcomes for many end-stage pediatric liver disorders. It can provide a long and healthy life, especially for pediatric patients following recovery from the early period. The classical indication for LT is liver failure causing a life-threatening situation resulting in a mortality risk higher than 90% at one year. End-stage liver disease (ESLD) from biliary atresia remains the most common cause of liver disease leading to transplantation. Progressive familial intrahepatic cholestasis (PFIC), metabolic diseases, fulminant liver failure, and cryptogenic cirrhosis are the other causes of end-stage liver disease leading to LT.[1,2]

✉ Edin Botan
edinbotan65@hotmail.com

Received 3rd August 2022, revised 8th January 2023, 21st February 2023, accepted 20th March 2023.

One of the main limitations of pediatric LT is the scarcity of size-matched pediatric deceased donors. As a result, living donor liver transplantation (LDLT) was performed to reduce mortality in children who could not receive timely LT due to a lack of deceased size-matched donors. Despite all efforts, organ donation is not widespread in our country because of cultural, social, and historical background and religious beliefs; thus, LDLT has become the first option for pediatric patients with ESLD.

Pediatric intensive care unit (PICU) management of the patients following LT is crucial for preventing morbidity and mortality.[3] In these patients, effective and sufficient early respiratory and circulatory support, maintenance of fluid and electrolyte balance, neurological assessment monitoring of surgical complications, initiation of immunosuppressive therapy, and prevention of infection are the basic concepts of PICU management.[4] This study aimed to evaluate the factors associated with morbidity and mortality in the early period of LT in children by reviewing our PICU experience.

Material and Methods

Patients and data collection

In this study, all pediatric LT recipients aged one month to 18 years old who had undergone LT between May 2015 and December 2021 and followed in our PICU during the early period of LT were identified. Each patient's demographic and clinical data (preoperative, perioperative, and early postoperative) were recorded from a prospectively designed database. The following data were collected: age, sex, anthropometric data (weight, height, body-mass index), the underlying disease, presence of another disease, type of liver failure, presence and stage of hepatic encephalopathy, preoperative pediatric end-stage liver disease (PELD) (<12 years)[5], and model for end-stage liver disease (MELD) (>12 years)[6] scores. The following intraoperative details were documented: the donor type, graft type (living, reduced or whole); graft weight/recipient weight (GWRW) ratio and graft volume, number of bile ducts reconstructed, type of bile duct reconstruction, blood loss, cold and warm ischemia time, operative time, type of abdominal wall closure. The following early postoperative data were noted: duration of intensive care, need for respiratory, renal, and circulatory support, including mechanical ventilation, non-invasive mechanical ventilation (NIMV), high-flow nasal cannula (HFNC) treatment, continuous renal replacement therapy (CRRT), plasma exchange (PEX), and blood product requirement, complications, re-operation, and mortality. The mortality score [Pediatric Risk of Mortality III (PRISM III)][7] and Pediatric Logistic Organ dysfunction score (PELOD)[5] were calculated within 24 hours of admission to the PICU. The impact of the duration on mechanical ventilation, postoperative bleeding, vascular complications, length of stay in the PICU, and mortality were investigated.

Postoperative management

All children were admitted to the PICU after LT and were closely monitored for 3-5 days. Standard patient management was performed according to a written protocol. Methylprednisolone + tacrolimus ± mycophenolate mofetil-based immune-suppression were initiated. The first dose of methylprednisolone (10 mg/kg/day) is intravenously administered intraoperatively, and then the steroid dose is gradually reduced to 0.3 mg/kg/day on the sixth day. Tacrolimus was started orally 6-12 hours after LT at 0.1 mg/kg/day. Tacrolimus trough levels are adjusted according to the targeted drug level. Mycophenolate mofetil was added to 600 mg/m^2/day within the first five days. Maintenance fluid therapy was given with non-hypotonic fluids, and was aimed at keeping electrolytes and minerals within normal limits. To maintain optimal serum albumin level (>3 g/dL) and intravascular volume, 5% albumin was given to all patients for at least 5 or 7 days post-

operation, according to the amount of fluid in the drainage tubes. Fluid balance was monitored by measuring central venous pressure (CVP). Portal system Doppler ultrasound (USG) was performed twice in the first 3-5 days to check the patency of the vascular anastomosis. Prostaglandin E1 infusion in the first five days, low-molecular-weight heparin (if INR is <2), and aspirin (if the platelet count is >100 000/ mm^3) treatment were used to prevent vascular complications. Blood product transfusion and hemostatic management were performed according to a protocol for LT, which has a restrictive transfusion policy.

Transfusion policy:

The aim is to keep Hct between 25-30% and Hb between 8-10 g/dl. When the Hct falls below 25% and Hb 7 g/dl, if dilution due to fluid load is not considered or in the presence of bleeding, replacement with erythrocyte suspension (10 mL/kg/4 hours) is applied.

Cytomegalovirus (CMV) prophylaxis in patients

If the recipient is CMV (-) and the donor is CMV (+) then intravenous (IV) gancyclovir is given at 10 mg/kg/day (2 doses) for 14 days, then continued with per-oral (PO) valgancyclovir.

If the recipient is CMV (+) and the donor is CMV (+) then IV ganciclovir is given at 10 mg/kg/day (2 doses) and continued with PO valgancyclovir when the patient is eligible for oral intake (+) / Donor (-)

If the recipient is CMV (-) and the donor is CMV (+) then intravenous (IV) gancyclovir is given at 10 mg/kg/day (2 doses) for 14 days, then continued with per-oral (PO) valgancyclovir.

Rejection therapy

For rejection IV ganciclovir 10 mg/kg/day 2 doses was administered and increased during dosing of immunosuppression.

Antimicrobial, antifungal, CMV, and *Pneumocystis jiroveci* prophylaxis were administered to all LT recipients. In addition, the CMV and Epstein-Barr virus (EBV) PCR of the patients were periodically monitored.

Patients were discharged from the PICU if they had stable vital signs, alert and oriented mental status, and stable laboratory and ultrasound findings.

Because LT is performed at Ankara University Children's Hospital and the surgical team is not comfortable moving the adult donor to the adult hospital in the early postoperative period, the living donors were monitored in our PICU for one night after the transplant surgery. A complication requiring surgical intervention related to the donor was recorded.

Ethical approval for the research was obtained from the Ethics Committee of Ankara University, Medical Faculty (Dated 2021, number: 2021000074, decision number: 74), and the study was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

Numerical data from the findings were expressed as arithmetic mean ± standard deviation (minimum-maximum), while categorical data were expressed as number (n) and percentage (%). Survived and non-survived patients Mann-Whitney U analysis was performed. Kaplan method was used in the analysis. Spearman's rho test was used in the correlation analysis of continuous variables. The statistical significance value (p) was accepted as <0.05.

Results

A total of 43 patients underwent LT in our hospital within six years, and 40 were followed in our unit. Twenty-five (62.5%) of the patients were male, and 15 (37.5) were female. The median age was 61.2±65 (3-197) months, the median body weight was 20.3±16.4 (4.2-61) kg, and the median height was 98.6±36.7 (56-167) cm. The median PELD score in patients under the age of 12 group (n=33) was 24±9.8 (4-54),

and the median MELD score in the age of 12 and up group (n=7) was 29.7±4 (23-35). Hepatic encephalopathy leading to emergent LT was present in 13 (32.5%) patients, and 4 (10%) of these patients required mechanical ventilation before LT.

LT was performed in 35 (87.5%) of the recipients due to chronic liver disease and 5 (12.5%) of the patients due to acute liver failure. Twenty-four (60%) patients had chronic liver failure due to cholestatic liver disease. The demographic data and clinical findings of the patients are also shown in Table I.

Surgical technique

Of the 40 pediatric patients who underwent LT, 39 (97.5%) patients received liver grafts from living donors, and one (2.5%) patient received a liver from a pediatric deceased donor. Left lateral section (LLS) and reduced LLS grafts were used in 20 (53.8%) patients, left lobe grafts in 11 (28.2%) patients, right lobe and right posterior sector grafts in 4 (10.3%), and mono segment grafts in 4 (10.3%) patients. Preoperative portal vein (PV) thrombosis was present in 7 (17.5) patients. PV resection and placement of the PV anastomosis to the level of superior mesenteric artery (SMA) and splenic vein (SV) confluence were done in 4 patients with portal vein thrombosis (PVT). PV reconstructions either by jump graft or patch plasty were done in 3 patients (one for thrombosis and two for narrow and fibrotic PV), inferior vena cava (IVC) reconstruction was done in 3 patients for narrow IVC (n=2), and tumor thrombosis (n=1). The mean graft weight was 318±144gram, mean graft weight/body weight ratio was 2.2±1. Mean cold ischemia time, warm ischemia time, and mean operative time were 48±27, 38±9, and 412±91minutes, respectively. Mean operative blood loss was 275±82 ml. A duct-duct anastomosis was done in 23 (57.5%) patients, and Roux-en-Y-hepaticojejunostomy was done in 17 (42.5%). In 13 (44.8%) patients, initially, the abdomen was closed with either a Bogota bag or Gortex mesh. Complete closure of the abdomen was done in 15-30 days.

Post-transplant follow-up

The patients' PRISM III score was 18.82 (2-58) at PICU admission. All of the patients were admitted to the PICU while being intubated, and the average time spent on mechanical ventilation was 86.17±139.5 (10-720) hours, the mean duration of stay at the PICU was 172±143.3 (72-809) hours [7.6±5.07 (3-34) days]. The average usage time of epinephrine in 40 patients was 19.78±32 (0-124) hours; the average usage time of norepinephrine in 40 patients was 16.1±25.4 (0-98) hours, and the average usage time of dopamine in 14 patients was 20.01±30.4 (0-120) hours. The duration of stay in the PICU and mechanical ventilator according to age and body weight are given in Figures 1 and 2.

A significant negative correlation was found between age groups and the duration of mechanical ventilation, and the duration of stay

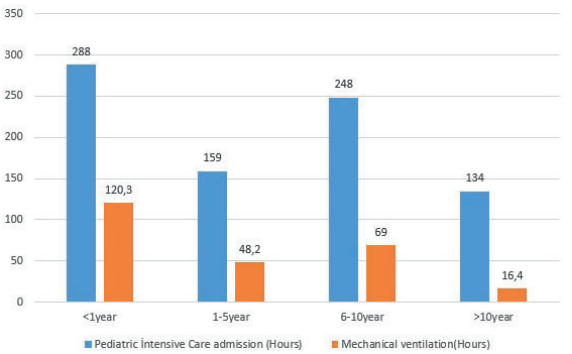


Fig. 1. Patients' length stay of in the pediatric intensive care unit and mechanical ventilation time distributions according to age.

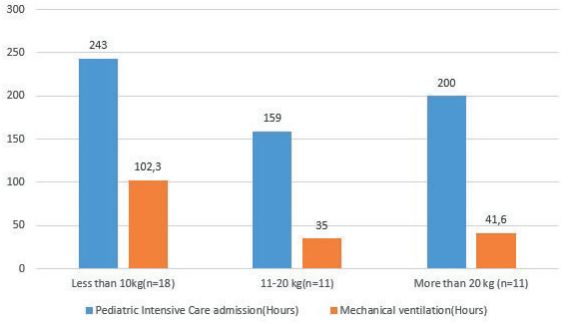


Fig. 2. Patients's length of stay in the pediatric intensive care unit and mechanical ventilation time distributions according to body weight.

Table I. Demographic and clinical findings of patients.

Parameters	n (%)
Gender	
Female	15 (37.5)
Male	25 (62.5)
Age groups	
<1 year	12 (30)
1-5 years	9 (22.5)
6-10 years	10 (25)
11-15 years	5 (12.5)
16-18 years	4 (10)
Anthropometric measurements, median (range)	
Body weight (kg)	20.3 (4.2-61)
Height (cm)	98.6 (56-182)
BMI	16.3 (11.7-20.8)
Scores, median (range)	
PELD	24 (4-54)
MELD	29.7 (23-35)
PRISM III, median (range)	18.82 (2-58)
Concomitant chronic disease	
No	35 (87.5)
Yes	5 (12.5)
Citrullinemia type 1	1
CVID (GMAP5 deficiency), HCC +EBV related lenfoma, hepatopulmonary syndrome	1
Hyperinsulinemic hypoglycaemia (KCNJ 11 mutation)+choledochal cyst	1
Inflammatory myofibroblastic tumor	1
DOCK8 deficiency+ cholestatic cirrhosis	1
Etiology	
❖ Acute liver failure	5 (12.5)
➢ Toxic hepatitis	2 (5)
➢ Idiopathic	3 (7.5)
❖ Chronic liver failure	35 (87.5)
➢ Cholestatic	24(60)
▪ Biliary atresia	10 (25)
▪ Genetic cholestatic disorders	12(30)
• PFIC	6 (15)
• Bile acid synthesis defects	2 (5)
• Mitochondrial depletion syndromes	2(5)
• Alfa-1 antitrypsin deficiency	1 (2.5)
• DOCK8 deficiency+ cholestatic cirrhosis	1 (2.5)
▪ Secondary biliary cirrhosis	2(5)
• Inflammatory myofibroblastic tumor+ Secondary biliary cirrhosis	1(2.5)
• Choledochal cyst +secondary biliary cirrhosis	1 (2.5)
➢ Inherited metabolic disorders	5(12.5)
▪ Wilson's disease	3(7.5)
▪ Tyrosinemia	1 (2.5)
▪ Citrullinemia type 1	1 (2.5)
➢ Chronic viral hepatitis	1 (2.5)
▪ HCV related cirrhosis	1 (2.5)
➢ Cryptogenic	4(10)
▪ Cryptogenic +HCC	1(2.5)
▪ Unknown	3(7.5)
➢ CVID (GMAP5 deficiency), HCC +EBV related lenfoma, hepatopulmonary syndrome	1(2.5)
Clinical and laboratory findings before transplantation	
Hepatic encephalopathy	13 (35.1)
Mechanical ventilation	4 (10.8)

BMI: body Mass Index, CVID: common variable immunodeficiency, EBV: Epstein-Barr virus, HCC: hepatocellular carcinoma, GMAP5: "GTPase of immunity-associated proteins" protein 5, HCV: Hepatitis C virus, KCNJ 11: potassium inwardly rectifying channel, subfamily J, member 11, PELD: Pediatric end-stage liver disease, MELD: Model for end-stage liver disease, PFIC: progressive familial intrahepatic cholestasis, PRISM: Pediatric Risk of Mortality

in the PICU (p=0.011, p=0.047). While there was a significant negative correlation between body weight and duration of mechanical ventilation, there was no significant correlation between body weight and length of PICU stay (p=0.006, p=0.130). In the first days of transplantation, 37 of 40 patients received erythrocyte suspension, 30 patients received fresh frozen plasma (FFP), and six patients received one unit of platelet suspension. The mean duration of alprostadil infusion was 5.2±2.2 (1-11) days.

Methylprednisolone, tacrolimus, and mycophenolate mofetil were given in appropriate doses to all patients according to the standard treatment protocol. Blood plasma tacrolimus trough level >20 was detected between 2 and 3 days in 5 patients. Hyperglycemia was observed in 2 of these patients, and seizures and sedative status were observed in 2. The average age of these patients was 25.6±32.3 (3-85) months. Severe diarrhea was observed in one patient after the use of mycophenolate mofetil. Apart from routine postoperative antibiotics, 13 patients were treated with meropenem.

We were able to start enteral nutrition in 24 of 40 patients during the PICU course, and their enteral feeding start time was 2.5 (1-8) days. Total parenteral nutrition (TPN) was started in 6 patients. TPN start time was 6 (4-5-9) days. The blood parameters of the patients on the day of transplantation and the first day after transplantation are shown in Table II. The complications that develop during the PICU follow-up and the distribution of these complications among the patients who survived

Table II. Laboratory findings at admission to PICU, and one day after LT.

Parameters	Transplantation Day (Mean ± SD)	First Day After Transplantation (Mean ± SD)	Normal Range
WBC (x10^3/mm^3)	10.70 (14 ± 3.4)	12.79 (18 ± 4.2)	4.5-12.5
Hb (g/dL)	8.6 (12 ± 5.4)	7.2 (11 ± 5.1)	12.5 - 16.2
Platelets (x10^3/mm^3)	115 (158 ± 62)	83 (110 ± 55)	150 – 450
AST (U/L)	1625 (2870 ± 840,4)	1040 (1642 ± 442)	0-50
ALT (U/L)	925 (1720 ± 640)	768 (1400 ± 452)	0-50
GGT (U/L)	68 ((214 ± 34)	38 (89 ± 14)	0-55
ALP (U/L)	205 (284 ± 87)	125 (198 ± 94)	109-449
LDH (U/L)	1345 (2650 ± 1840)	1125 (1804 ± 895)	0-248
Total bilirubin (mg/dL)	7.47 (14 ± 3.4)	6.32 (12 ± 3.1)	0.3- 1.2
Direct bilirubin (mg/dL)	4.06 (10 ± 2.4)	2.67 (8 ± 1.8)	0- 0.2
APTT (sec)	51.9 (87 ± 27)	59.72 (97 ± 34.4)	25.1-36.5
PT (sec)	34.8 (42,8 ± 17.2)	38.2 (68.1 ± 23.4)	9.4-12.5
INR	3.4 (5.1 ± 1.9)	3.11 (4.9 ± 1.4)	0.82-1.09
Albumin (g/dL)	3.1 (3.5 ± 2.2)	4.4 (5.4 ± 2.4)	3.5- 5.2
Glucose (mg/dL)	161 (244 ± 142,4)	143 (213 ± 134)	74-100
BUN (mg/dL)	10.5 (14 ± 3.4)	18.7 (24.1 ± 5.2)	5-18
Creatinine (mg/dL)	0.24 (1,4 ± 0,15)	0.14 (1.2 ± 0.1)	0.57-0.87
Sodium (mmol/L)	137 (155 ± 124)	140 (165 ± 133)	136-146
Potassium (mmol/L)	3.9 (5.9 ± 2.4)	3.8 (5.2 ± 2.5)	3.5-5.1
Magnesium (mg/dL)	2.13 (3.8 ± 1.4)	2.02 (3.4 ± 1.2)	1.7-2.2
Phosphorus (mg/dL)	4.85 (8.31 ± 3.4)	4.3 (7.65 ± 2.4)	3.4-6.2

ALP: alkaline phosphatase, ALT: alanine aminotransferase, APTT: activated partial thromboplastin time, AST: aspartate aminotransferase, BUN: blood urea nitrogen, Hb: hemoglobin, GGT: gamma-glutamyl transferase, INR: international normalized ratio, LDH: lactate dehydrogenase, LT: liver transplantation, PICU: pediatric intensive care unit, PT: prothrombin time, SD: standard deviation, WBC: white blood cells

and who did not survive are given in Table III. Four patients received both CRRT and PEX therapy. In addition, one patient received only CRRT.

Graft loss leading to mortality occurred in the initial four patients due to insufficient portal flow (n:3) and insufficient graft outflow (n:1). These patients were less than 6 kg and had hypoplastic and fibrotic PV with less than 3mm diameter and insufficient portal flow. These four patients were listed for retransplantation, but they could not receive a timely liver graft from a deceased donor. After experiencing portal inflow issues in these patients, the surgical team changed their approach to removing hypoplastic and fibrotic portal veins in small pediatric recipients and placed the PV anastomosis at the level of SMVa and SV confluence.

Acute antibody-related rejection was observed in only one LT recipient with PFIC2 one week after LT. Even though aggressive immune suppressive and supportive therapies were applied, she died due to hemodynamic instability developed during PEX therapy. No graft or patient loss occurred in the latest 24 consecutive pediatric LDLT recipients. One patient with a mitochondrial disease died of pulmonary hypertension related to his primary disease 13 months after LDLT.

Table III. Complications after liver transplantation and comparison between survived and non-survived patients.

	Complications	n (%)	Survived (n)	Non-survived (n)	p
Operative	Portal flow issues	3 (7.5)	0	3	0.001
	Hepatic outflow issues	1 (2.5)	0	1	
	Postoperative bleeding	4 (10)	4	0	
	Bile leak (from parenchymal dissection site and Roux limb stump)	2 (5)	2	0	
Respiratory system	Atelectasis	12 (30)	11	0	0.023
	Pleural effusion	4 (10)	3	1	0.560
	Pneumothorax	4 (10)	3	1	0.790
Gastrointestinal tract	Hematemesis	2 (5)	0	2	0.008
	Melena	2 (5)	0	2	0.007
	Intestinal perforation (terminal ileum)	1(2.5)	0	1	0,067
Circulatory system	Hypertension	9 (22.5)	7	2	0.836
	Inotropic need after 24 hours	14 (35)	10	4	0.148
	Cardiac arrest	5 (12.5)	0	5	0.001
Infection	Blood infection	7 (17.5)	6	1	0.790
	VAP	2 (5)	1	1	0.319
Renal system	UTI	1 (2.5)	1	0	0.499
	Hematuria	4 (10)	3	1	0.024
	CRRT	5 (12.5)	3	2	0.142
Central nervous system	Intracranial bleeding	1 (2.7)	0	1	0.065
	Brain death	1 (2.7)	0	1	0.499
	Seizures	2 (5)	1	1	0.324
Central nervous system	Hyperglycemia	8 (20)	6	2	0.679
Acute humoral rejection		1 (2.5)	0	1	0.459
Re-operation		10(25)	6	4	0.012

CRRT: continuous renal replacement therapy, UTI: urinary tract infection, VAP: ventilator associated pneumonia

At discharge from the PICU, neurological examinations of the patients were normal, and there was no patient who required respiratory support.

One year and overall Kaplan Meier survival curves of pediatric LDLT recipients are shown in Fig. 3. 1- year survival was 87.5%, and overall survival was 85%.

Discussion

This study reports the early postoperative experience after LT in children admitted to our PICU. The main indication for LT was cholestatic liver disease, including biliary atresia (60%), which was in accordance with previous reports in children.[8] The majority (97.3%) of the LTs were LDLT in our series due to the lack of available deceased donors in our country. LDLT is technically more challenging than deceased donor LT and requires the highest level of technical expertise in pediatric patients, especially those of younger age and lower body weight. Meticulous surgery and tailoring the graft to each recipient by providing sufficient and balanced inflow and outflow dynamics are mandatory for a successful LDLT in children.

Young age, low body weight, PELD, and MELD values of 20 and higher are important risk factors for unfavorable outcomes after LDLT.[5,6] These risk factors are both associated with technically more challenging vascular and bile duct reconstruction and higher complication rates, and increased mortality during the early period after LT. LDLT is even more challenging in pediatric patients with low body weight given that often there is a big graft for the patient's size, which needs to be reduced to provide sufficient portal flow, and there are short and smaller vascular structures, and bile ducts, which is more difficult to reconstruct.

Our recipients' mean PELD (24) /MELD (29.7) scores were higher than the literature. Haseli et al.[9] reported 392 patients with a mean age of 102±68.4 months and a mean PELD/MELD score of 20.3±8.9. They reported an increased risk of post-transplant complications and death when the PELD/MELD score was more than 20. In accordance with this report, the mean PELD/MELD score of patients who died was 25.3±7.5, suggesting that a high PELD score is associated with higher mortality. Similarly, Kukreti et al.[1] reported the mean PRISM III score of the patients as 7 (4-12) in a study of 145 children who underwent LT between 1988 and 2011. In another study, 17 LT patients in the pediatric intensive care unit were shown to have a PRISM III score of 5.7 (0-14).[10] In our study, the PRISM III score was 18.82 (2-58) higher than the literature, which correlated with our mortality.

The short mechanical ventilation time after LT in children positively affects the prognosis.[11] In the literature, the mean duration of mechanical ventilation in pediatric patients varies between 1-10 days.[12] In the study conducted by Qian et al.[13], the mean duration of mechanical ventilation for 81 LT patients with a median age of 8.2 months and a mean body weight of 8.4±4.6 kg was 120 hours. Alaçakır et al.[14] published that

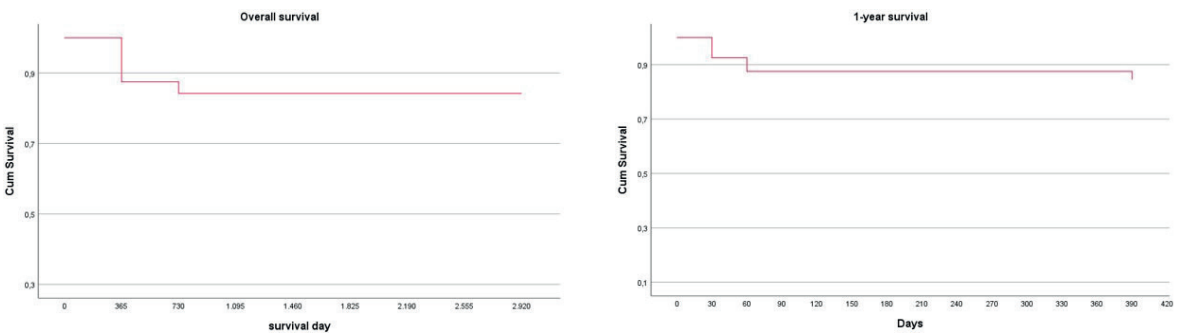


Fig. 3. One year and overall Kaplan Meier survival curves of pediatric LDLT recipients.

the mean mechanical ventilation time of 31 LT recipients with a mean body weight of 14 ± 3.01 kg was 18.4 hours. In agreement with previous reports, in our series, the time on mechanical ventilation was longer when the recipient was younger and smaller.

During PICU admission, the most common complications in our patients were respiratory system complications in 51.3% (n=19), followed by circulatory system complications in 43.2% (n=16). Atelectasis was observed to be frequent in patients with long mechanical ventilation days. Patients with pneumothorax and pleural effusion did not have long durations of mechanical ventilation.

We believe that early mobilization has positive effects on both the respiratory and musculoskeletal systems and is very important in reducing complications. Our average mobilization time in older children was 2-3 days postoperatively. McDiarmid et al.[15] reported that complications such as vascular thrombosis, intestinal perforation, septicemia, and retransplantation were the primary risks for mortality in pediatric LT recipients. A higher incidence of pulmonary complications has been reported among pediatric LT recipients.[16] Similarly, the frequency of pulmonary complications (atelectasis n=14, pneumothorax n=4, pleural effusion n=3) was high in the early period of our series. The higher incidence of respiratory system complications in pediatric patients can be attributed to a diaphragmatic malfunction related to dissection of the bare area of the recipient's native liver from the diaphragm, phrenic nerve injuries during recipient hepatectomy and ligation of the diaphragmatic veins during IVC cross-clamping and graft implantation. Moreover, in LDLT for small infants, liver grafts can be too big for small infants depending on the small space of the recipients' abdomen or graft thickness. In most infants undergoing LDLT, staged abdominal closure is preferred to avoid large for size syndrome and graft inflow and outflow problems. When full-thickness abdominal closure cannot be achieved, respiratory issues are induced in small infants due to the lack of abdominal wall support.

Five early graft losses and recipient mortality occurred in this series. Thus, the early mortality rate within the first 28 days was 12.5%, higher than other data provided in the literature.[1] Four of these patients were small infants with hypoplastic portal veins (portal vein diameter <3mm) and high PELD and PRISM III scores. Retransplantation is not a rare condition in pediatric liver transplants, it is reported in the literature at a rate of approximately 10% to 20%.[17] In another study, this rate was 9% in 167 LT patients.[18] Unfortunately, we could not retransplant these patients with vascular issues due to the lack of deceased donors. Even though these patients were listed in the first place for more than a week, they could not get a suitable graft from a deceased donor. If these patients had had a chance of retransplantation, our mortality rate might have been lower.

Also, donor-specific antibodies (DSA) cause antibody-mediated rejection (AMR); however, their pathogenic role after LT has not yet been adequately investigated. Kovandova et al.[19] reported that preformed complement-binding DSA to HLA Class I antigens is associated with an increased risk of acute antibody-mediated rejection, while chronic AMR is more common in patients with de novo-produced antibodies to HLA Class II antigens after liver transplantation. In our study, acute antibody-associated rejection was observed in only one patient, followed one week later. HLA Class I antigens were detected in the liver biopsy.

In conclusion, careful pediatric intensive care monitoring according to established protocols and timely dealing with complications are essential to decreasing morbidity and mortality after LT in children. Small children and patients with high disease severity scores such as PRISM III, PELD, and MELD have a higher risk of morbidity and mortality.

Ethical approval

This study was approved by the local Institutional Ethics Committee of Ankara University, Medical Faculty (Dated 2021, number: 2021000074, decision number: 74).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TK,EB; data collection: AG, EB, SAG; analysis and interpretation of results: HÖ, ZK, EB, TK, EG, EÇ; draft manuscript preparation: CK, EOK, ÖCS, SF, MK, AK, DB, TK. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding. Data were extracted from the hospital database founded by Ankara University Hospitals and a specified Children's Hospital research database.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Kukreti V, Daoud H, Bola SS, Singh RN, Atkison P, Kornecki A. Early critical care course in children after liver transplant. Crit Care Res Pract 2014; 2014: 725748. https://doi.org/10.1155/2014/725748
2. Cantez MS, Durmaz Ö. Acute liver failure in children: questions and answers. J Child 2012; 12: 1-5. https://doi.org/10.5222/j.child.2012.001
3. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. Am J Transplant 2010; 10: 1420-1427. https://doi.org/10.1111/j.1600-6143.2010.03126.x
4. Alonso EM, Emerick K, Whitington PF, Busuttil RW, Klintmalm GB. General citeria for pediatric transplantation. In: Transplantation of The Liver. Philadelphia: Elsevier; 2005: 287-302.
5. Leteurtre S, Martinot A, Duhamel A, et al. Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study. Lancet 2003; 362: 192-197. https://doi.org/10.1016/S0140-6736(03)13908-6
6. Kamath PS, Kim WR; Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). Hepatology 2007; 45: 797-805. https://doi.org/10.1002/hep.21563
7. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996; 24: 743-752. https://doi.org/10.1097/00003246-199605000-00004
8. Rawal N, Yazigi N. Pediatric liver transplantation. Pediatr Clin North Am 2017; 64: 677-684. https://doi.org/10.1016/j.pcl.2017.02.003
9. Haseli N, Hassanzadeh J, Dehghani SM, Bahador A, Malek Hosseini SA. Long-term survival and its related factors in pediatric liver transplant recipients of shiraz transplant center, shiraz, iran in 2012. Hepat Mon 2013; 13: e10257. https://doi.org/10.5812/hepatmon.10257
10. Kalayoglu M, Sollinger HW, Stratta RJ, et al. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1: 617-619. https://doi.org/10.1016/s0140-6736(88)91416-x
11. Ulukaya S, Arikan C, Aydogdu S, Ayanoglu HO, Tokat Y. Immediate tracheal extubation of pediatric liver transplant recipients in the operating room. Pediatr Transplant 2003; 7: 381-384. https://doi.org/10.1034/j.1399-3046.2003.00072.x
12. Garcia S, Ruza F, Gonzalez M, et al. Evolution and complications in the immediate postoperative period after pediatric liver transplantation: our experience with 176 transplantations. Transplant Proc 1999; 31: 1691-1695. https://doi.org/10.1016/s0041-1345(99)00066-4
13. Qian J, Zhou T, Qiu B-J, et al. Postoperative risk factors and outcome of patients with liver transplantation who were admitted to pediatric intensive care unit: a 10-year single-center review in China. J Intensive Care Med 2020; 35: 1241-1249. https://doi.org/10.1177/0885066619849558
14. Alaçakır N, Tekgüç H, Keçeli M, et al. After liver transplant pediatric intensive care follow-up: 5 years of experience. J Pediatr Emerg Intens Care Med 2015; 2: 115-120. https://doi.org/10.5505/cayb.2015.97269

15. McDiarmid SV, Anand R, Martz K, Millis MJ, Mazariegos G. A multivariate analysis of pre-, peri-, and post-transplant factors affecting outcome after pediatric liver transplantation. Ann Surg 2011; 254: 145-154. https://doi.org/10.1097/SLA.0b013e31821ad86a

16. Levesque E, Hoti E, Azoulay D, et al. Pulmonary complications after elective liver transplantation-incidence, risk factors, and outcome. Transplantation 2012; 94: 532-538. https://doi.org/10.1097/TP.0b013e31825c1d41

17. Uribe M, Alba A, Hunter B, et al. Liver transplantation in children weighing less than 10 kg: Chilean experience. Transplant Proc 2013; 45: 3731-3733. https://doi.org/10.1016/j.transproceed.2013.08.092

18. Selimoğlu MA, Kaya S, Güngör Ş, Varol Fİ, Gözükara-Bağ HG, Yılmaz S. Infection risk after paediatric liver transplantation. Turk J Pediatr 2020; 62: 46-52. https://doi.org/10.24953/turkjped.2020.01.007

19. Kovandova B, Slavcev A, Honsova E, et al. De novo HLA Class II antibodies are associated with the development of chronic but not acute antibody-mediated rejection after liver transplantation - a retrospective study. Transpl Int 2020; 33: 1799-1806. https://doi.org/10.1111/tri.13763

The Turkish Journal of Pediatrics 2023; 65: 500-511
https://doi.org/10.24953/turkjped.2022.56

Original Article

Sleep problems in adolescents with epilepsy and their caregivers: associations with behavioural difficulties

İpek Dokurel Çetin[1], Birsen Şentürk[2], Sezen Köse[2], Gül Aktan[3], Hasan Tekgül[3], Seda Kanmaz[3], Mine Serin[3], Sanem Yılmaz[3], Sarenur Gökben[3]

[1]Department of Pediatric Neurology, Ataturk City Hospital, Balıkesir; [2]Department of Child and Adolescent Psychiatry, Ege University Faculty of Medicine, İzmir; [3]Department of Pediatric Neurology, Ege University Faculty of Medicine, İzmir, Türkiye.

ABSTRACT

Background. The aim of this study was to investigate the frequency of sleep problems in adolescents with epilepsy and their caregivers. We also examined the behavioural difficulties in adolescents with epilepsy and compared these behaviors with healthy controls.

Methods. This observational case-control study included 37 adolescents with epilepsy and their caregivers, and 43 healthy age-matched adolescents and their caregivers. The Children's Sleep Habits Questionnaire (CSHQ), DSM-5 Level 2 Sleep Disorders Scale for Children, and Strengths & Difficulties Questionnaire (SDQ) were used to evaluate sleep habits, sleep problems, and behavioural difficulties in adolescents. The DSM-5 sleep disorder scale for adults was used to evaluate the caregivers' sleep problems.

Results. Adolescents with epilepsy had higher sleep problem scores such as daytime sleepiness and overall sleep problems compared with healthy controls. The psychopathological symptoms such as conduct problems, hyperactivity/inattention, and total behavior were also more frequent in adolescents with epilepsy. There was a nonsignificant increase in DSM-5 sleep disturbance score in caregivers of adolescents with epilepsy. Sleep onset delay had a significant negative correlation with total behavioral difficulties ($r = -0.44$, $p<0.01$), and emotional problems ($r = -0.47$, $p < 0.05$) in adolescents with epilepsy. Sleep duration was negatively correlated with conduct problems ($r = -0.33$, $p<0.05$), but positively correlated with prosocial score ($r = 0.46$, $p < 0.01$) in adolescents with epilepsy. Night waking was positively correlated with total behavioral difficulties ($r = 0.35$, $p<0.05$) and hyperactivity score ($r = 0.38$, $p<0.05$) in adolescents with epilepsy.

Conclusions. Adolescents with epilepsy have more frequent sleep disturbances and maladaptive behaviors such as hyperactivity/inattention, and conduct problems compared with healthy controls, and their caregivers are more vulnerable to sleep problems. Moreover, we also demonstrated a strong association between sleep disturbances and behavioral problems in adolescents with epilepsy.

Key words: adolescent, epilepsy, caregivers, sleep.

Epilepsy is a common chronic neurological disorder that afflicts up to 1% of the pediatric population.[1-4] Sleep problems are frequently reported in children with epilepsy as in healthy children.[2-4] The caregivers of adolescents with epilepsy also reveal poor sleep quality and emotional disturbances.

✉ İpek Dokurel Çetin
dripekdokurel@gmail.com

Received 21st January 2022, revised 9th July 2022, 26th August 2022, accepted 13th February 2023.

The characteristics of sleep reflect the degree of neurocognitive development in children.[5] Epileptic discharges interrupt the organization of sleep.[4] Poor sleep quality is linked with a decline in children's physical growth, behavior and cognitive functions, such as memory, attention, and learning.[1,6,7] Available evidence suggests that sleep disturbances, particularly poor sleep quality, are associated with behavioral difficulties and neurocognitive impairments in pediatric epilepsy.[8] The relationship between behavioral assessment and sleep disturbances has only been evaluated in a few studies,

which were predominantly based on parental statements about their children's sleep.[9-13] Family observations may beincoherent with reality and these studies have revealed several inconsistencies.[9,12] Foremost, families may tend to exaggerate their children's sleep problems. We planned to evaluate sleep problems and behavioral difficulties of epileptic adolescents by using both self- and parent reports. For this purpose, we assessed adolescents with the Children's Sleep Habits Questionnaire and Diagnostic and Statistical Manual of Mental Disorders (DSM) 5 Sleep Disturbance - Child Report. These are validated tools used for assessing sleep disorders and enables us to compare adolescents with epilepsy to healthy controls.[3,8,14] The caregivers' sleep problems were investigated with DSM−5 Sleep Disturbance -Adult Report. Furthermore, the Strength and Difficulties Questionnaire (SDQ) was used as a reliable behavioral screening tool also used in clinical trials and can be administered as a self- or parent report.[15]

The aim of this study was to investigate the frequency of sleep disorders in adolescents with epilepsy and their caregivers and compare them with healthy controls. We also studied the relationship between maladaptive behavior and sleep disorders in adolescents with epilepsy.

Material and Methods

Study population

The World Health Organization defines adolescents as those between the ages of 10 and 19.[16] A total of 120 adolescents (adolescents with epilepsy: 50, healthy controls: 70) aged 11-17 years who had attended the pediatric neurology outpatient clinic of Ege University Medical Faculty between February and June 2020 were recruited for this observational case-control study. In the patient group, 13 adolescents with epilepsy were excluded due to incomplete questionnaires and the exclusion criteria given below. In the control group, 23 healthy adolescents with incomplete forms and 4 adolescents with the presence of a depressive or an anxiety disorder were excluded. The study cohort consisted of 80 cases: 37 who made up the study group, were adolescents with epilepsyand 43 who were similar aged healthy adolescents, made up the control group. The control group was matched in terms of their age, gender, and caregiver's educational level. The caregiver was described as the person who spent most of their time caring for the adolescents' needs. All the caregivers of adolescents with epilepsy (n=37) and healthy controls (n=43) were recruited to the study.

Subjects were excluded from the study if they had : (1) an IQ ≤80, as measured by the Wechsler Intelligence Scale for Children-Revised Form (WISC-R) or below-average academic performance documented by last year's final school grade; (2) a history of psychiatric, developmental disorder, or psychosis; (3) a history of a progressive neurological disorder other than epilepsy; (4) any complaint (e.g., behavior problems, depression, or anxiety) that could interfere with sleep; and (5) their caregiver had any medical or psychiatric condition (e.g., depression or anxiety) that could interfere with sleep.

All epileptic adolescents were diagnosed based on the International League Against Epilepsy (ILAE) operational clinical definition criteria for epilepsy and pediatric neurologists made the diagnosis.[17] The study grouphad been receiving treatment for at least one year. Adolescents with epilepsy were grouped into idiopathic generalized or self-limiting focal epilepsy syndromes according to the ILAE criteria for epileptic seizures and syndromes and guidelines for epidemiological studies for diagnosis and classification.[18,19] All of the adolescents with epilepsy were using antiseizure medications and categorized into the monotherapy group (levetiracetam, valproic acid, carbamazepine) and polytherapy group (valproic acid plus levetiracetam combination, and levetiracetam plus carbamazepine combination).

This study was approved by the local institutional ethics committee of Ege University (24.6.20-E.148753) and was registered at ClinicalTrials.gov (No. NCT05191576). The groups' caregivers agreed to participate in the study by signing the relevant informed consent form.

Measures

The study instruments include two parts. First, data on socio-demographics, height, weight, sleep duration, the caregiver's level of education (illiterate, elementary school, high school, university), caregiver sleep problems, and medical background were noted. Anthropometric measures such as height and weight were used to estimate the Body Mass Index (BMI) obtained by dividing weight in kilograms by the square of height in meters. Body Mass Indexis a common metric for defining height/weight characteristics It is used as a marker for chronic health problems, and is used to determine social issues as well.

Clinical data regarding epilepsy (the type of seizure, etiology, the frequency of seizures, current and previous antiseizure medications) were collected from interviews with participants/parents and from examinations.

Second, psychometric assessment tools were used to evaluate all participants and caregivers;

Children's Sleep Habits Questionnaire (CSHQ)

The CSHQ is a validated 33-item parent questionnaire designed to screen sleep behavior in children's assessments of pediatric sleep problems.[20] The eight subscales of CSHQ are bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, sleep-disordered breathing, parasomnias, and daytime sleepiness. The frequency of each item on a three-point Likert scale: ''usually'' (5-7 times per week), ''sometimes'' (2-4 times per week), and ''rarely'' (0-1 time per week). The CSHQ yields a total sleep disturbance score and eight subscale scores. Higher scores indicate more severe sleep disruptions. The Turkish validity and reliability study of this questionnaire was conducted by Perdahlı Fiş et al.[21]

Diagnostic and Statistical Manual of Mental Disorders (DSM) −5 Level 2 Sleep Disturbance - Child Report

The DSM-5 Child Report is a retrospective, 8-item self-report used to assess the pure domain of sleep disturbances in adolescents aged 11-17 years in the past seven days. The frequency of each item on a five-point Likert scale is rated: "Always" (5), "Often" (4), "Sometimes" (3), "Rarely" (2), and "Never" (1). The total sleep disturbance score ranges from 8 to 40. The Turkish validity and reliability study of this questionnaire was conducted by Erkuran et al.[22] Higher scores indicate more severe sleep disorders. To the best of our knowledge, no cut-off score for DSM-5-level 2 is available for children and adolescents. High scores on a particular domain might indicate significant and problematic areas for the child that may warrant further assessment.

Diagnostic and Statistical Manual of Mental Disorders (DSM) −5 Sleep Disturbance -Adult Report

The DSM-5 Adult Report is a retrospective, 8-item self-report used to assess the pure domain of sleep disturbances in adults for the past seven days. The frequency of each item on a five-point Likert scale is rated: ''Always'' (5), ''Often'' (4), ''Sometimes'' (3), ''Rarely'' (2), and ''Never'' (1). The total sleep disturbance score ranges from 8 to 40. The Turkish validity and reliability study of this questionnaire was conducted by Yüzeren et al.[23] Higher scores indicate more severe sleep disorders. DSM-5-level 2 scores of <54 points indicate a lack of sleep disturbance. A total score of ≥ 55 is considered as poor sleeper.

Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a 25-item report used to screen for emotional and behavioral problems in adolescents. Strength and difficulties questionnaire can be administered as a self-

report, or to parents of adolescents. It was developed by Goodman in 1997.[24] Each of the five subscales of the SDQ has five questions; emotional problems, hyperactivity/inattention, behavioral problems, peer problems and social behavior. Whereas, high social behavior scores demonstrate the strengths of the individual in the social field; high scores in the other four areas reveal that the problem areas are severe. The Turkish validity and reliability study of this questionnaire for both parent and adolescent forms was conducted.[25]

Statistical analysis

Statistical analyses were performed using the SPSS software version 25.0 (SPSS Statistics for Windows, IBM, 2017). The variables were investigated using visual (histogram, probability plots) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk's test) to determine whether they are normally distributed. Descriptive analyses were presented (using frequencies for ordinal variables) and using mean and standard deviation. Continuous variables were compared among the two groups by using an independent t-test. If the variable did not have a normal distribution, the Mann-Whitney U test was applied for comparing two groups, and the Kruskal-Wallis test for comparing more than two groups. A chi-square test was used to examine the association between categorical variables. As both parameters were normally distributed, the correlation coefficients and their significance were calculated using the Pearson test. The Spearman correlation analysis was used to define the correlation of non-normally distributed variables. Linear regression analysis was used to investigate whether sleep problems (CSHQ total scores) were associated with behavioral difficulties (SDQ total scores) for epileptic adolescents. A 5% type-I error level was used to infer statistical significance.

Results

The characteristics of the participants

The demographic characteristics of the participants and their caregivers are summarized in Table I. The education level and body mass index (BMI) of caregivers between the groups revealed nonsignificant differences. Epileptic syndromes in adolescents were identified as follows: Juvenile myoclonic epilepsy (n = 12), self-limited epilepsy with centrotemporal spikes (n = 10), generalized tonic-clonic seizures alone (n = 5), late-onset self-limited occipital epilepsy of childhood (n = 6), juvenile absence

Table I. Demographic characteristics of the participants of the study.

	Adolescents with epilepsy (n= 37)	Healthy controls (n= 43)	p value
Age, years (mean ± SD)	13.7 ± 2	14.5± 2	.096[1]
Gender, n (%)			
Female	13 (36.1)	23 (63.9)	.100[2]
Male	24 (54.5)	20 (45.5)	
BMI, kg/m² (mean ± SD)	20.7 ± 4	22.5± 4.6	.086[1]
Sleep duration, hours (mean ± SD)	8.83 ± 1.04	8.81± 1.05	.919[1]
Caregiver's age, years (mean ± SD)	41.3 ± 5.21	41.7 ± 5.7	.751[1]
Education level of caregiver, n (%)			
Illiterate	3 (3.8)	3 (3.8)	.798[2]
Preliminary school	14 (17.5)	20 (24.9)	
High school	11 (13.8)	9 (11.2)	
University	9 (11.2)	11 (13.8)	

BMI: Body mass index, SD: standard deviation.
[1]Student's t-test, [2]Chi-square test.

epilepsy (n = 3), parietal lobe epilepsy (n = 1). At least one seizure during sleep per year was reported for 81.1% (n= 30/37) of adolescents with epilepsy, and two or more seizures during sleep per year for 18.9% (n= 7/37). Of adolescents with epilepsy, 70.3% received monotherapy (n= 26/37) and 29.7% received polytherapy (n= 11/37) for seizure control.

Sleep problems

CSHQ subscales and total scores in adolescents with epilepsy and healthy controls are presented in Table II. The mean total sleep disturbance scores were 49.89 ± 7.15 in adolescents with epilepsy and 45.3 ± 6.07 in healthy controls. The mean daytime sleepiness scores were 13.9 ± 3.23 in adolescents with epilepsy and 10.9 ± 3.65 in healthy controls. There were significant differences in total sleep disturbance and daytime sleepiness scores of the CSHQ score (p= 0.003 and p= 0.001, respectively) between the groups.

When we examined the effect of antiseizure medications on sleep habits only in the study group, we found that there was no significant difference in the total CSHQ and daytime sleepiness scores between the mono- (n = 26) and polytherapy (n = 11) groups (56.9 ± 5.93 vs. 58.5 ± 9.3, p=0.59; and 15 ± 3.1 vs. 15.5 ± 3.4, p = 0.682, respectively) (Fig. 1a). The monotherapy group consisted of 26 patients using levetiracetam (16/26), valproic acid (9/26) and carbamazepine (1/26). Total CSHQ scores among the levetiracetam, valproic acid and carbamazepine groups were not significantly different (p=0.658). The polytherapy group consisted of 11 patients, 8 using the valproic acid plus levetiracetam combination, and 3 using the levetiracetam plus carbamazepine combination. There was no significant difference in the total CSHQ scores among patients using the valproic acid plus levetiracetam combination (56.5±6.07), or the levetiracetam plus carbamazepine combination (56.3±7.09) (p=0.970)

There was no significant difference in the total DSM-5 Sleep Disturbance Scale - Child Report scores between adolescents with epilepsy (18.5 ± 7.32) and healthy controls (16.7 ± 5.8) (p = 0.226).

The caregivers of adolescents with epilepsy had higher DSM -5 Sleep Disturbance Scale - Adult Report scores than the control group (49.86±10.76, 46.92 ± 8.08, respectively, p= 0.167). There was no significant difference between the groups. When we analyzed caregivers' sleep quality, 35.1% of the study group (n = 13/37) and 14% of the control group (n= 6/43)

Table II. The Children's Sleep Habits Questionnaire (CSHQ) subscales and total scores in adolescents with epilepsy and healthy controls.

	Adolescents with epilepsy (n=37)	Healthy controls (n=42)	p value
	Mean ± SD	Mean ± SD	
CSHQ total score	49.89 ± 7.15	45.3 ± 6.07	**.003**[1]
Bedtime resistance	8.5 ± 2.29	7.74 ± 1.69	.196[2]
Sleep onset delay	1.48 ± 0.69	1.46 ± 0.66	.919[2]
Sleep duration	6.21 ± 0.85	6.11 ± 1	.906[2]
Sleep anxiety	5.48 ± 1.92	4.88 ± 1.29	.185[2]
Night waking	4.05 ± 0.97	3.81 ± 1.05	.154[2]
Parasomnia	9.19 ± 1.8	8.97 ± 2.14	.382[2]
Sleep disordered breathing	3.78 ± 1.22	3.69 ± 1.18	.842[2]
Daytime sleepiness	13.9 ± 3.23	10.9 ± 3.65	**.001**[2]

CSHQ: Children's Sleep Habits Questionnaire, SD: standard deviation.
[1]Student's t-test, [2]Mann-Whitney U test.
Bold represents the significant p-values: p < 0.05

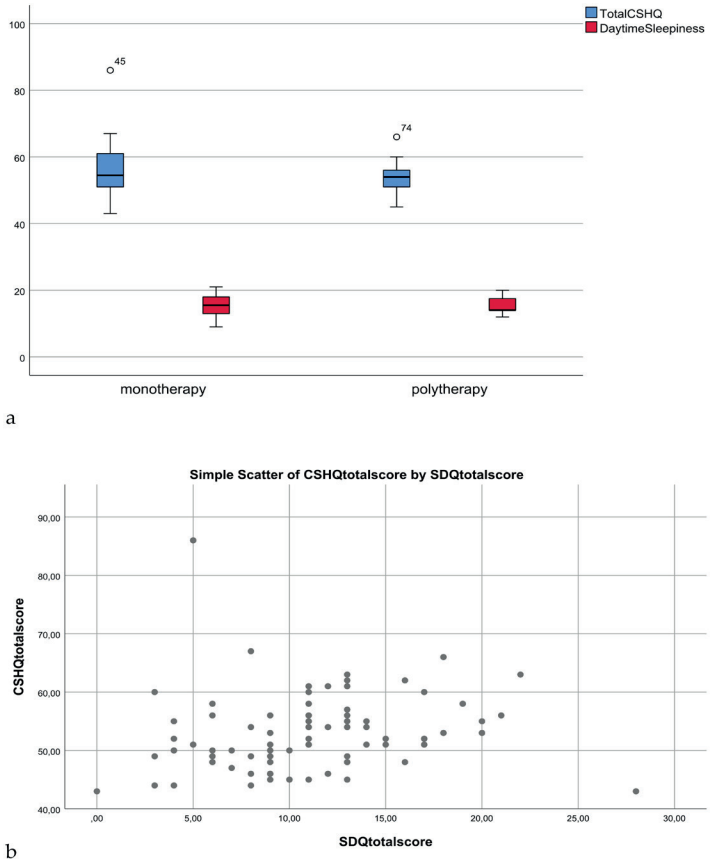


Fig. 1. a. The Children's Sleep Habits Questionnaire (CSHQ) total scores and daytime sleepiness scores in adolescents with epilepsy epilepsy on monotherapy and polytherapy. **b.** The relationship between the CSHQ and Strengths and Difficulties Questionnaire (SDQ) total scores (R-square= 0.085; P=.309).

were poor sleepers. The poor sleepers were more frequently the caregivers of epileptic adolescents (p = 0.036). Among these caregivers, 15.4% of their children were using polytherapy (2/13), while 84.6% of their children were using monotherapy (11/13) (p=0.160). DSM-5 Child Report showed nonsignificantly higher total scores of adolescents with epilepsy (18.5±7.3) compared to healthy controls (16.7±5.8) (p=0.120). Moreover, the DSM-5-Adult Report showed nonsignificant higher caregivers' total scores for adolescents with epilepsy (20.7±8.7) compared to caregivers of healthy controls (18.04±6.2) (p=0.226).

Behavioral difficulties

Table III summarizes the SDQ total and subscale scores based on self- and parent-report for the study participants. When we analyzed the adolescents' self-report of SDQ, behavioral scores showed no significant difference between the adolescents with epilepsy compared to the healthy controls. On the parents' reports of SDQ, we found significant differences in behavioral scores like total behavior, conduct problem, and hyperactivity/inattention scores between the groups ($p < 0.05$, $p < 0.01$, and $p < 0.05$).

Table III. The Strengths and Difficulties Questionnaire (SDQ) total and subscales scores in the participants of the study, based on self and parent-reports.

	Self-report			Parent-report		
	Adolescents with epilepsy Mean± SD	Healthy controls Mean±SD	p-Value	Adolescents with epilepsy Mean± SD	Healthy controls Mean±SD	p-Value
Total score	12.1 ± 5.4	11.6 ± 5.5	.668[1]	12.46 ± 4.9	9.9 ± 4.99	**.026[1]**
Emotional symptoms	3 ± 2.1	3.1 ± 2.3	.938[1]	3.05 ± 1.88	3.4 ± 2.36	.988[1]
Conduct problems	2 ± 1.5	1.5 ± 1.5	.159[1]	1.97 ± 1.42	1.18 ± 1.43	**.002[2]**
Hyperactivity / inattention	3.9 ± 1.9	4 ± 2	.683[1]	4.29 ± 2.36	3.07 ± 2.42	**.025[1]**
Peer relationship problems	3.2 ± 1.9	2.9 ± 1.8	.535[1]	3.13 ± 1.61	2.63 ± 1.23	.171[2]
Prosocial behavior	7.6 ± 2	8.4 ± 1.9	.064[1]	8.21 ± 1.9	9.3 ± 3.6	.124[2]

SD: standard deviation.
[1]Student's t-test, [2]Mann-Whitney U test.
Bold represents the significant p-values: p < 0.05.

Table IV. The correlation between the CSHQ and the SDQ total and subscale scores in adolescents with epilepsy (n=37).

	SDQ total difficulties score		Emotional problems score		Conduct problems score		Hyperactivity score		Peer problems score		Prosocial score	
	p	r	p	r	p	r	p	r	p	r	p	r
Total score[1]	.55	-.10	.77	-.04	.08	-.28	.78	.04	.70	-.06	.24	.19
Bedtime resistance[1]	.63	-.08	.67	-.07	.13	-.25	.82	.04	.97	.005	**.032**	**.35**
Sleep onset delay[1]	**.007**	**-.44**	**.03**	**-.47**	.11	-.26	.04	-.33	.70	-.06	.10	.27
Sleep duration[2]	.36	-.15	.30	-.17	**.046**	**-.33**	.75	-.05	.52	.10	**.004**	**.46**
Sleep anxiety[1]	.90	.02	.78	.04	.12	-.25	.25	.10	.65	.07	.17	.22
Night wakings[1]	**.033**	**.35**	.17	.22	.28	.18	**.02**	**.38**	.62	.08	.30	-.17
Parasomnia[1]	.25	.19	.19	.21	.98	-.00	**.036**	**.35**	.31	-.17	.40	.14
Sleep disordered breathing[2]	.61	.08	.30	.17	.21	-.21	.45	.12	.73	.05	.66	-.07
Daytime sleepiness[1]	.77	-.04	.84	-.34	.75	-.05	.62	.08	.28	-.18	.32	-.16

CSHQ: Children's Sleep Habits Questionnaire; r: Correlation coefficient (Pearson or Spearman as indicated below);
SDQ: Strengths and Difficulties Questionnaire.
[1]Pearson correlation, [2]Spearman's rank correlation.
Bold represents the significant p-values.

Associations between sleep problems & behavioral difficulties

Table IV displays the strengths and difficulties questionnaire (SDQ) total and subscale scores based on self- and parent-report for the study participants. There was a weak significant positive correlation between bedtime resistance and social score (r = 0.35, p = 0.032) in adolescents with epilepsy. Also a weak significant positive correlation was found between parasomnia and hyperactivity/inattention (r = 0.35, p = 0.036). The sleep onset delay showed a moderate significant negative correlation with both total behavioral difficulties (r = - 0.44, p = 0.007), and emotional problems (r = -0.47, p = 0.03). The sleep duration showed a weak significant negative correlation to conduct problems (r = - 0.33, p = 0.046). However it showed a moderate positive significant correlation with

social score (r = 0.46, p = 0.004) in adolescents with epilepsy. Also the night waking had a weak positive significant correlation between both total behavioral difficulties (r = 0.35, p < 0.05) and hyperactivity/inattention score (r = 0.38, p < 0.05) in adolescents with epilepsy.

The results of linear regression analysis, in order to evaluate the influence of sleep problems on behavioral difficulties in adolescents with epilepsy, were not statistically significant. Fig. 1b shows the correlation between the CSHQ and SDQ total scores (R-square = 0.085; P = 0.309).

Discussion

In the current study, we found that sleep disturbances were more frequent in adolescents with epilepsy compared to healthy controls. The homogeneity of the social-economic status of our cohort, which was configured by excluding any other debilitating chronic diseases, was supported by findings on the lack of statistical differences in BMI and the education level of caregivers between the groups.

Daytime sleepiness, which is a subscale of sleep disturbances, is a common problem among adolescents with epilepsy. The maladaptive behaviours such as conduct problems and hyperactivity were seen more often in adolescents with epilepsy. Furthermore, night waking and parasomnias were also positively correlated with maladaptive behaviors and induced hyperactivity inthis group. Also insufficient sleep duration was associated with conduct problems and social interaction problems in adolescents with epilepsy. Additionally, the caregivers of adolescents with epilepsy suffered more from sleep disturbances when compared with healthy controls. The sleep disturbances evaluated in this study were higher in adolescents with epilepsy and their caregivers compared with those of healthy controls and their caregivers.

There has been a general concern that children with epilepsy are more vulnerable to problematic sleep than their healthy counterparts.[2,4,8,26] We found a few studies in the literature that focused on the different types of sleep disturbances among adolescents with epilepsy. Primarily, the shorter 'sleep duration' is associated with poorer academic performance and it is an important contributor that affects functionality and quality of life in pediatric epilepsy.[3,27-29] Regarding the importance of sleep duration, Holley et al.[27] had established that sleep duration showed no difference between the idiopathic epilepsy group (9.19 h) and healthy controls (8.87 h). Our findings supported prior data that, adeloscents with epilepsy and healthy adolescents had similar sleep duration (8.83 h, SD 1.04; 8.81 h, SD 1.05, respectively). Parasomnia, night waking, and daytime sleepiness were other sleep disturbances detected in children with epilepsy.[3] Hansen et al.[8] found increased daytime sleepiness, parasomnia, and bedtime resistance in children and adolescents with epilepsy. In accordance with Hansen et al.[8], we revealed higher scores in bedtime resistance, sleep anxiety, daytime sleepiness, and total sleep disturbance score among adolescents with epilepsy. However, only daytime sleepiness and total sleep disturbance scores were statistically significant.

Sleep problems in children with epilepsy were associated with anti-seizure medication use in numerous studies.[3,4,10,30] In our study, adolescents with epilepsy who used polytherapy had higher problematic sleep scores than patients who used monotherapy. However, there were no significant differences between the groups. Our findings showed that the association between the monotherapy-polytherapy groups and problematic sleep in adolescents and their caregivers were statistically nonsignificant. The relatively small sample size of our cohort is an important determinant of these findings. Moreover, variations in side-effects of antiseizure medications may be pharmacodynamic due to hormonally induced changes in adolescents, and inter-individual differences in the response of receptors even in equal concentrations of these drugs could be unpredictable in the

developing brain. The estimate from our study supported the idea that antiseizure medications had possible unfavorable impacts on the sleep habits of adolescents with epilepsy.

Children and adolescents with epilepsy had more frequent behavioral problems, such as hyperactivity, opposition problems, anxiety and depression, than healthy ones.[4,11,15] Bektaş et al.[31] proposed that the timing of seizures in sleep relates to behavioral problems such as emotional symptoms, conduct problems, hyperactivity, concentration, and peer problems in patients with benign epilepsy with centrotemporal spikes. These studies provided important insights into the variance of behavioral problems in children with epilepsy. In our study, adolescents with epilepsy displayed statistically significant higher scores for conduct problems, hyperactivity, and overall behavioral problems than healthy controls. Moreover, we reported a non-significant difference in 'peer problems' scores between the groups. The current study also revealed that adolescents with epilepsy in Türkiye exhibit fewer 'emotional problems' than in the previously mentioned studies. This discrepancy could be attributed to parents of adolescents with epilepsy who may be less willing to report their children's 'emotional problems' due to psychiatric stigma in addition to epilepsy, or they may be less sensitive to identify emotional disturbances in them. The caregivers of adolescents with epilepsy and their teachers rated higher scores in emotional symptoms, conduct problems, hyperactivity, and peer problems than the adolescents themselves.[32] We found that the self-report on SDQ scores revealed some nonsignificant differences for adolescents with epilepsy with their conduct, peer, and total behavioral difficulties subscales than healthy controls. Interestingly, the subscales with significantly higher scores revealed by the reports of parents of adolescents with epilepsy on SDQ scores were conduct, hyperactivity, and total behavioral difficulties. This result may be explained by the fact that adolescents rated behavioral problems in atypical ways or had difficulty assuming responsibility reporting these difficulties on themselves. However, we concluded that using multiple informants' reports to assess psychiatric problems in adolescents increased the validity.

As mentioned in the literature review, persistent sleep problems in healthy children were associated with an increased 16-fold risk of having psychosocial symptoms such as aggression, social-attention problems, and anxious/depressive mood.[6] A few studies outline a relationship between sleep disturbances and behavioral problems in epileptic children.[15,27,30,31] Furthermore, sleep disorders are closely associated with behavioral problems in children with epilepsy, regardless of seizure control.[2,30] Wang et al.[33] analyzed sleep disturbances associated with behavioral problems in healthy Chinese and Japanese children. They proposed that sleep disturbances such as sleep-disordered breathing, daytime sleepiness are associated with behavioral problems in Chinese children, while sleep anxiety and night waking are associated with behavioral problems in Japanese children.[33] Tsai et al.[30] reported that sleep problems, particularly sleep anxiety, were associated with more behavioral problems in children with epilepsy. Our results of correlation between multiple CSHQ and SDQ domains in this study affirmed that sleep disturbances and behavioral problems are linked to each other in adolescents with epilepsy. Firstly, to identify, adolescents with epilepsy exhibited bedtime resistance linked to difficulties in social behaviors. Secondly, the sleep onset delay appeared to have the most extensive relationship to emotional and behavioral problems. Moreover, we demonstrated that in adolescents with epilepsy, the conduct problems were associated with sleep duration, whilst, the parasomnias and night waking were associated with hyperactivity and inattention. Another significant aspect of night waking related to internalizing problems (sadness, anxiety, loneliness) was suggested in the study by Tsai et al.[30] Similarly, we noted that night waking was related to behavioral problems besides

hyperactivity. Furthermore, hyperactivity was associated with parasomnia.

Stigma is defined as ''an undesired differentness". Stigmatization causes several barriers that complicate establishing a social identity and impairs social interactions.[34] People with epilepsy experience feelings of stigmatization, which are strongly correlated with higher depressive symptoms.[35] The fear of stigma is a barrier for adolescents to discuss their behavioral difficulties. Anxiety about stigmatization as "mentally ill" can block revealing the feelings of an adolescent who has been labeled with epilepsy.[36] We found some fragile associations between sleep difficulties and behavioral problems. Our findings could be interpreted as cautionary under the consequences of stigmatization anxiety which could block the expression of behavioral symptoms in adolescents. We argue that our results on the associations between sleep difficulties and behavioral problems, are like the exposed part of an iceberg, failing to capture contradictions hidden under the water. Future studies on the current topic are therefore recommended.

Caregivers of adolescents with epilepsy are vulnerable to sleep problems more frequently than healthy adolescents' parents. This may result from the caregiver's concerns of missing a nocturnal seizure of adolescents which significantly increases the risk of sleep problems.[3,28] Similarly, the data from our study demonstrated that the caregivers of adolescents with epilepsy suffered from actual sleep problems compared to healthy adolescents' caregivers. However, total sleep disturbance scores of adolescents with epilepsy and their caregivers was higher compared to healthy controls without statistical significance. It is unclear whether adolescents with epilepsy and their caregivers were underestimating their concerns about sleep disturbances because of their reluctance or perception of unwillingness to accept new likely health problems besides nocturnal seizures.

The findings from our study should be interpreted considering its limitations. Our study was a single-centre evaluation with a small sample (n = 80). The study design was incompetent to control the effect of antiseizure medications on sleep. The other impediment to the study was the inability to exclude the possible comorbidity of attention deficit hyperactivity disorder, which is one of the most common problems in children with epilepsy. Sleep duration was assessed subjectively instead of using objective methods. The prevalence of sleep problems may be exaggerated or underestimated, because of the study design. This effect was minimized using different assessment tools.

The strength of the study was the detailed analysis of sleep distractions and emotional and behavioral problems with a reliable and valid measure for adolescents. Adolescents' scores were considered with both self and parent reports, thereby probably increasing sensitivity. The potential advantages of understanding the relationship between sleep disturbances and psychological problems in adolescents with epilepsy should be investigated in future interventional prospective control-case studies. The precautions to improve the sleep quality of adolescents with epilepsy and their parents will help mitigate psychiatric comorbidities. Moreover, this would reduce the burden of disease for their families.

In conclusion, our findings demonstrated a significantly elevated prevalence of sleep disturbances and behavioral problems in adolescents with epilepsy. The present study provided that the management protocols for adolescents with epilepsy should include assessments of sleep and emotional and behavioral problems correspondingly. There were still unanswered questions about the relationship of antiseizure medications and sleep in adolescents with epilepsy. A greater focus on this consent could produce interesting findings that improve the quality of life. The question raised by this study is what advantages this perspective would have. If the

debate is to be moved forward, a meticulous elaboration of sleep problems and behavioral difficulties in adolescents with epilepsy needs to be developed. Large randomized controlled trials could provide more definitive evidence. This would be a fruitful area for further work.

Ethical approval

This study was approved by the institutional ethical committee of Ege University (24.6.20-E.148753). The groups' caregivers agreed to participate in the study by signing the relevant informed consent form.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: IDÇ, GA, HT; data collection: IDÇ, BŞ, SK; analysis and interpretation of results: SG, MS, SY; draft manuscript preparation: IDÇ, SK. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Russ SA, Larson K, Halfon N. A national profile of childhood epilepsy and seizure disorder. Pediatrics 2012; 129: 256-264. https://doi.org/10.1542/peds.2010-1371
2. Roshan S, Puri V, Chaudhry N, Gupta A, Rabi SK. Sleep abnormalities in juvenile myoclonic epilepsy-A sleep questionnaire and polysomnography based study. Seizure 2017; 50: 194-201. https://doi.org/10.1016/j.seizure.2017.06.021
3. Larson AM, Ryther RCC, Jennesson M, et al. Impact of pediatric epilepsy on sleep patterns and behaviors in children and parents. Epilepsia 2012; 53: 1162-1169. https://doi.org/10.1111/j.1528-1167.2012.03515.x
4. Gibbon FM, Maccormac E, Gringras P. Sleep and epilepsy: unfortunate bedfellows. Arch Dis Child 2019; 104: 189-192. https://doi.org/10.1136/archdischild-2017-313421
5. Ringli M, Huber R. Developmental aspects of sleep slow waves: linking sleep, brain maturation and behavior. Prog Brain Res 2011; 193: 63-82. https://doi.org/10.1016/B978-0-444-53839-0.00005-3
6. Simola P, Liukkonen K, Pitkäranta A, Pirinen T, Aronen ET. Psychosocial and somatic outcomes of sleep problems in children: a 4-year follow-up study. Child Care Health Dev 2014; 40: 60-67. https://doi.org/10.1111/j.1365-2214.2012.01412.x
7. Chiang H-L, Gau SS-F, Ni H-C, et al. Association between symptoms and subtypes of attention-deficit hyperactivity disorder and sleep problems/disorders. J Sleep Res 2010; 19: 535-545. https://doi.org/10.1111/j.1365-2869.2010.00832.x
8. Hansen BH, Alfstad KÅ, van Roy B, Henning O, Lossius MI. Sleep problems in children and adolescents with epilepsy: associations with psychiatric comorbidity. Epilepsy Behav 2016; 62: 14-19. https://doi.org/10.1016/j.yebeh.2016.06.015
9. van Golde EGA, Gutter T, de Weerd AW. Sleep disturbances in people with epilepsy; prevalence, impact and treatment. Sleep Med Rev 2011; 15: 357-368. https://doi.org/10.1016/j.smrv.2011.01.002
10. Schmitt B, Martin F, Critelli H, Molinari L, Jenni OG. Effects of valproic acid on sleep in children with epilepsy. Epilepsia 2009; 50: 1860-1867. https://doi.org/10.1111/j.1528-1167.2009.02105.x
11. Myers KA, Davey MJ, Ching M, et al. Randomized controlled trial of melatonin for sleep disturbance in dravet syndrome: the DREAMS study. J Clin Sleep Med 2018; 14: 1697-1704. https://doi.org/10.5664/jcsm.7376
12. Rodriguez AJ. Pediatric sleep and epilepsy. Curr Neurol Neurosci Rep 2007; 7: 342-347. https://doi.org/10.1007/s11910-007-0052-0
13. Stores G. Sleep disturbance in childhood epilepsy: clinical implications, assessment and treatment. Arch Dis Child 2013; 98: 548-551. https://doi.org/10.1136/archdischild-2013-303825
14. Arhan E, Uçar HK, Aydın K, Hirfanoğlu T, Serdaroglu A. How do children with drug-resistant epilepsy sleep? A clinical and video-PSG study. Epilepsy Behav 2021; 114: 107320. https://doi.org/10.1016/j.yebeh.2020.107320

15. Tanabe T, Kashiwagi M, Shimakawa S, et al. Behavioral assessment of Japanese children with epilepsy using SDQ (strengths and difficulties questionnaire). Brain Dev 2013; 35: 81-86. https://doi.org/10.1016/j.braindev.2012.03.008

16. Dehne KL, Riedner G. Adolescence-a dynamic concept. Reprod Health Matters 2001; 9: 11-15. https://doi.org/10.1016/s0968-8080(01)90003-5

17. Wirrell E, Tinuper P, Perucca E, Moshé SL. Introduction to the epilepsy syndrome papers. Epilepsia 2022; 63: 1330-1332. https://doi.org/10.1111/epi.17262

18. Hirsch E, French J, Scheffer IE, et al. ILAE definition of the idiopathic generalized epilepsy syndromes: position statement by the ILAE task force on nosology and definitions. Epilepsia 2022; 63: 1475-1499. https://doi.org/10.1111/epi.17236

19. Specchio N, Wirrell EC, Scheffer IE, et al. International league against epilepsy classification and definition of epilepsy syndromes with onset in childhood: position paper by the ILAE task force on nosology and definitions. Epilepsia 2022; 63: 1398-1442. https://doi.org/10.1111/epi.17241

20. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. Sleep 2000; 23: 1043-1051.

21. Perdahlı Fiş N, Arman A, Ay P, et al. The validity and the reliability of Turkish version of children's sleep habits questionnaire. Anadolu Psikiyatri Dergisi 2010; 11: 151-160. Available at: https://alpha-psychiatry.com/en/the-validity-and-the-reliability-of-turkish-version-of-children-s-sleep-habits-questionnaire-131906

22. Erkuran HÖ, Sapmaz ŞY, Herdem A, et al. DSM-5 level 2 sleep disorders scale validity and reliability of Turkish form (Form for children aged 11-17 years and parent form for children aged 6-17 years). Noro Psikiyatr Ars 2018; 55: 256-260. https://doi.org/10.5152/npa.2017.19324

23. Yüzeren S, Herdem A, Aydemir Ö, Grubu D. Reliability and validity of Turkish form of sleep disorder scale. Anadolu Psikiyatri Derg 2017; 18: 79-84. https://doi.org/10.5455/apd.241499

24. Goodman R. Psychometric properties of the strengths and difficulties questionnaire. J Am Acad Child Adolesc Psychiatry 2001; 40: 1337-1345. https://doi.org/10.1097/00004583-200111000-00015

25. Yalın Ş, Özbek A, Güvenir T, Baydur H. The advanced psychometric properties of Turkish strength and difficulties questionnaire (SDQ). Turk J Child Adolesc Ment Health 2013; 20: 23-32.

26. Zambrelli E, Turner K, Vignoli A, et al. Sleep disturbances in Italian children and adolescents with epilepsy: a questionnaire study. Epilepsy Behav 2020; 106: 107014. https://doi.org/10.1016/j.yebeh.2020.107014

27. Holley S, Whitney A, Kirkham FJ, et al. Executive function and sleep problems in childhood epilepsy. Epilepsy Behav 2014; 37: 20-25. https://doi.org/10.1016/j.yebeh.2014.05.022

28. Urrila AS, Artiges E, Massicotte J, et al. Sleep habits, academic performance, and the adolescent brain structure. Sci Rep 2017; 7: 41678. https://doi.org/10.1038/srep41678

29. Ronen GM, Janssen I. Patterns of daily activity among young people with epilepsy. Dev Med Child Neurol 2019; 61: 1386-1391. https://doi.org/10.1111/dmcn.14223

30. Tsai SY, Lee WT, Jeng SF, Lee CC, Weng WC. Sleep and behavior problems in children with epilepsy. J Pediatr Health Care 2019; 33: 138-145. https://doi.org/10.1016/j.pedhc.2018.07.004

31. Bektaş G, Tekin U, Yıldız EP, et al. Psychosocial and behavioral functioning and their relationship to seizure timing in children with benign epilepsy with centrotemporal spikes. Brain Dev 2017; 39: 515-520. https://doi.org/10.1016/j.braindev.2017.01.013

32. Hanssen-Bauer K, Heyerdahl S, Eriksson A-S. Mental health problems in children and adolescents referred to a national epilepsy center. Epilepsy Behav 2007; 10: 255-262. https://doi.org/10.1016/j.yebeh.2006.11.011

33. Wang G, Takahashi M, Wu R, et al. Association between sleep disturbances and emotional/behavioral problems in Chinese and Japanese preschoolers. Behav Sleep Med 2020; 18: 420-431. https://doi.org/10.1080/15402002.2019.1605995

34. Abdel Ghaffar NF, Asiri RN, Al-Eitan LN, et al. Improving public stigma, sociocultural beliefs, and social identity for people with epilepsy in the Aseer region of Saudi Arabia. Epilepsy Behav Rep 2021; 16: 100442. https://doi.org/10.1016/j.ebr.2021.100442

35. Tombini M, Assenza G, Quintiliani L, et al. Epilepsy-associated stigma from the perspective of people with epilepsy and the community in Italy. Epilepsy Behav 2019; 98: 66-72. https://doi.org/10.1016/j.yebeh.2019.06.026

36. Dolphin L, Hennessy E. Labelling effects and adolescent responses to peers with depression: an experimental investigation. BMC Psychiatry 2017; 17: 228. https://doi.org/10.1186/s12888-017-1389-9

The Turkish Journal of Pediatrics 2023; 65: 512-522
https://doi.org/10.24953/turkjped.2022.268

Original Article

Measure of Processes of Care (MPOC-56 and 20): Turkish adaptation, reliability, and validity study

Duygu Türker[1], Cemil Özal[2], Sevilay Karahan[3], Mintaze Kerem Günel[2]

[1]University of Health Sciences, Faculty of Gülhane Physiotherapy and Rehabilitation, Ankara; [2]Faculty of Physical Therapy and Rehabilitation, Hacettepe University, Ankara; [3]Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Türkiye.

ABSTRACT

Background. The purpose of this study was to investigate the validity and reliability of the Turkish versions of the Measure of Processes of Care, MPOC-56 and MPOC-20, in children with disability aged 5-17 years.

Methods. A total of 290 parents of children with disability due to various disorders were evaluated with the MPOC-56 and MPOC-20. Internal consistency was determined with Cronbach's alpha, and test-retest reliability with the intraclass correlation coefficient (ICC). Confirmatory factor analysis was performed to investigate the factor structure of the Turkish MPOC-56 and -20.

Results. Cronbach's alpha values for the MPOC-56 and MPOC-20 ranged between 0.84-0.97 and 0.87-0.92, respectively. Test-retest ICC values were 0.96-0.99 for MPOC-56 and 0.94-0.98 for MPOC-20. The correlations of the subscale scores of MPOC- 56 and MPOC-20 were shown to be at very good to excellent levels for reliability. Factor structure for MPOC-20 and MPOC-56 were found to be acceptable.

Conclusions. This study has shown that the Turkish versions of MPOC-56 and MPOC-20 are valid, reliable, and applicable for the evaluation of parents' experiences of processes of care for children with disability aged 5-17 years.

Key words: measure of processes of care, children with disability, family-centered service, validity, reliability.

Family-centered care (FCC) has been described as a partnership approach to health care decision-making and has been recognized by multiple medical societies and health care systems. Family-centered care is considered the standard of pediatric health care by many clinical practices, hospitals, and health care groups.[1,2] A family-centered approach accepts parents as experts on the needs of their children, encourages partnerships between parents and health service providers, and supports the role of parents in making decisions about the services for their children.[3] Such an approach is accepted globally as one of the best practices when providing health care services to children with disabilities or chronic conditions.[3-5]

The concept of the family-centred approach is also quite compliant with the International Classification of Functioning, Disability and Health (ICF).[6] The ICF states that adaptation of the family to rehabilitation is extremely important as the family is the common point of all components.[7] Rosenbaum clearly emphasized that no aspect of the 'environment' of a child is more central than the family when investigating the child's environment.[8] It is therefore essential for pediatric health care services to be conducted with a family-centred approach to ensure successful and high-quality rehabilitation.[1] There is evidence indicating significant benefits from a family-centred

✉ Duygu Türker
duygu.turker@sbu.edu.tr

Received 23rd March 2022, revised 2nd August 2022, accepted 13th February 2023.

This study has been presented under the title of 'Measure of processes of care (MPOC-56 and MPOC-20), Turkish adaptation, reliability and validity study', at the 33rd Annual Meeting of the European Academy of Childhood Disability (EACD), May-June 2021 (online).

approach during pediatric rehabilitation and health care services, both for the children and the families.[1,4] The benefits for children include developmental gains and skill development as well as better psychological adjustment, while the parents benefit from increased knowledge of the child's development, increased participation in home therapy programs, feeling more competent as a parent, and improved psychosocial well-being.[2,9-12]

Evaluating whether the families feel the services provided are family-centred and obtaining feedback on the relevant changes in their perceptions over time, seems to be a critical requirement to provide partnership to health care decision-making between the family and health care provider. This helps in functional goal setting and coordination of a child's transition between programs which are important concepts for rehabilitation and health care services.[13] Scales have been developed to assess Family Centered Services (FCS) and to evaluate the value of adopting family-centred principles in clinical practice.

The Measure of Processes of Care (MPOC) is of one of the first and most widely used instruments to assess parents' self-reported experiences of family-centred behaviors.[14] The MPOC has been developed to evaluate family-centered care both in rehabilitation centers, inpatient or ambulant care[1] in pediatric settings including children's treatment or rehabilitation centers, children's hospitals, large urban hospitals, university hospitals, and community development programmes.[12,15] Additionally, the MPOC has been used with different disabilities or chronic health conditions such as cerebral palsy, neonatal intensive care patients, epilepsy, pediatric oncology, hospitalized for head injury, pediatric bowel problems, speech and language pathologies, hearing loss, and autism spectrum disorders.[4,16-23]

The MPOC questionnaire has two versions in order to evaluate the opinions of parents with children with disability regarding the institution where their children receive services and how the services they receive affect their psychosocial status. MPOC-56 contains 56 questions evaluating the parents' perceptions of the services and institutions from which the child received service and the employees such as medical doctors, therapists, psychologists, social workers and teachers of these institutions. MPOC-20 is the shortened version of MPOC-56 and consists of 20 selected questions.[9,14,24] The MPOC-56 and MPOC-20 has been translated into many languages and its cross-cultural validity has been demonstrated in various cultures and different countries around the world.[12,25-27]

Integration of family-centered practices into the health care system can be challenging especially in non-Western countries and FCS has been less studied in non-Western countries.[28] This current study is important as it assessed the reliability and validity of the scale, the most commonly used one for these purposes worldwide, by adapting it to Turkish for clinical use in Türkiye as well as Turkish speaking countries and communities, where a family-centered approach is used in the rehabilitation of children with disabilities. Therefore, the aim of this study was (1) to translate the MPOC-56 and -20 into Turkish and (2) assess its construct validity, test-retest reliability and internal consistency reliability in children with various disabilities.

Material and Methods

Study design

This study was designed as a psychometric evaluation study and consisted of two parts: The first part consisted of the translation of MPOC-20 and MPOC-56 into Turkish and the second part was to determine the reliability and validity of the Turkish version. The ethics committee approval necessary for the study was obtained from the University of Health Sciences Gülhane Non-Interventional Studies Ethics Committee (no: 2018/3-18/58). The clinical trial registration number is NCT03508583.

Translation process

In order to adapt MPOC-56 and MPOC-20 to Turkish, written permission was obtained from CanChild Centre that holds the publication rights of the original scale. Based on previous studies and guidelines[29], advanced and repeated translation methods were used to ensure intercultural adaptation. Two independent physiotherapists with a minimum of ten years experience in pediatric rehabilitation translated the MPOC-56 and MPOC-20 into Turkish (Stage 1). Once the translated versions were harmonized as first version (Stage 2), they were back translated into English by a professional translator (Stage 3). After the final versions of scales were approved by the original developers, the pre-final Turkish versions of MPOC-56 and MPOC-20 were assessed by an expert panel including researchers experienced in the field of children with disabilities and clinical professionals (Stage 4). This panel evaluated the translations in terms of suitability and comprehensibility. They decided that no cultural adaptation was required and approved the translations. Finally (Stage 5), a pilot administration of the Turkish version of MPOC-56 and MPOC-20 was conducted with 20 families of children with disability in order to make sure that all elements were clear and appropriate for Turkish parents; the feedback indicated that the questions were clear and understandable. Thus, the Turkish version of the questionnaire was created.

Participants

The study was conducted with 334 parents of children with disability who were receiving rehabilitation services in Türkiye. The sample size was calculated as general guidelines requiring 5–10 subjects per item in order to

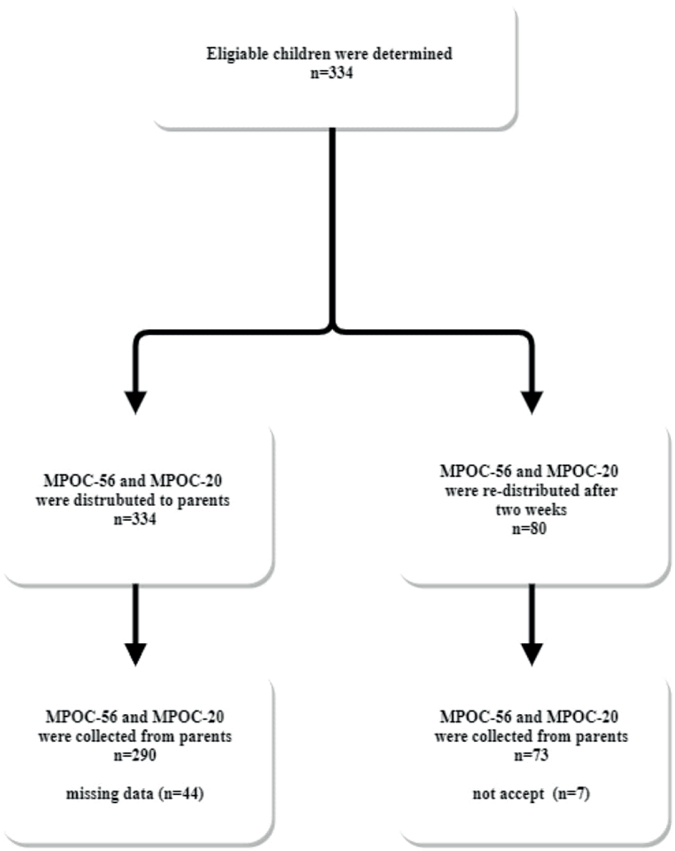


Fig. 1. Flow chart of participants

conduct the planned analyses.[30,31] The median time interval for test-retest reliability was two weeks, and the ratio of sample size for test-retest reliability to the number of items in each measure ranged between 1-4.[32]

Since MPOC has 56 items, a sample of between 280 and 560 subjects was appropriate and finally 290 subjects participated. Inclusion criteria were identified as: 1) accepting to participate to the study, 2) the parents being literate, 3) native speaker of Turkish, 4) having children with a disability aged 5-17 years old, and 5) the child with a disability was receiving rehabilitation services in Türkiye. Parents meeting the study inclusion criteria were asked to complete the Turkish version of both the MPOC-56 and MPOC-20. We excluded 44 parents because their forms were incomplete. For randomization every fourth parent was invited to participate in the test-retest procedure in order to evaluate the test-retest reliability of the Turkish MPOC-56 and MPOC-20. The test-retest procedure concluded with 73 parents who accepted to complete the scales two weeks later (Fig. 1). The socio-demographic characteristics (age, educational level, diagnosis) of the parents and the children were recorded before the scales were completed. Written approval was obtained from all parents based on the principles of the Helsinki Declaration.

The scales

MPOC is a scale published in two versions to evaluate the opinions of parents with children with disability regarding the institution where their children receives services and how the services they receive affect their psychosocial status. The scales examine through factor analysis the main characteristics of family-centered services in five sub-scales: (i) enabling and partnership; (ii) providing general information; (iii) providing specific information about the child; (iv) coordinated and comprehensive care for the child and the family; and (v) respectful and supportive care. The scales are easy to administer and low in cost.[3,9,24] All questions refer to behaviors occurring during the past year. Each item in the questionnaire starts "In the past year, to what extent do the people who work with your child…" followed by a description of a specific attitude or behavior of the health care professional in the organization or center.

The MPOC-56 consists of 56 questions evaluating the institutions where the child receives rehabilitation service and the employees of these institutions. The MPOC-56 includes 16 items addressing *Enabling and Partnership*, nine items on *Providing General Information*, five items on *Providing Specific Information about the Child*, 17 items on *Coordinated and Comprehensive Care*, and nine items on *Respectful and Supportive Care*. The MPOC-20 is a shorter version that was developed eight years later using conceptual and empirical approaches to improve the utility and the discriminative ability of the MPOC-56 to compare different service delivery models. MPOC-20 includes three items addressing *Enabling and Partnership*, five items on *Providing General Information*, three items on *Providing Specific Information about the Child*, four items on *Coordinated and Comprehensive Care*, and five items on *Respectful and Supportive Care*.[3]

The MPOC-20 and 56 scorings are based on the same system. The scoring consists of a Likert-type scale between 1 (Not at all) and 7 (To a very great extent). A score of 0 is also included to indicate 'non-applicable' items. An MPOC sub-scales score is calculated as mean of the ratings for the items in each sub-scale. As items are not weighted, a scale score can range from 1.00 to 7.00. A higher score reflects higher levels of satisfaction with the institution or people who provide rehabilitation services.[3,9,24]

Statistical analysis

The reliability (internal consistency, test-retest reliability) and validity (structural) of MPOC-56 and MPOC-20 were evaluated.

The Intraclass Correlation Coefficient (ICC) value was used to evaluate test-retest reliability. ICC varies between 0.00 and 1.00 and values of 0.60-0.80 indicate good reliability while values over 0.80 indicate excellent reliability. Internal consistency is related to whether the measurement of a result is homogeneous. We evaluated internal consistency using the "Cronbach's alpha" value, and an alpha value over 0.80 indicates high internal consistency.[33]

Construct validity was measured with Spearman's correlation coefficient. Validity coefficients were graded as follows: r≥ 0.81-1.0 excellent, 0.61-0.80 very good, 0.41-0.60 good, 0.21- 0.40 moderate, 0-0.21 poor.[31]

Confirmatory factor analysis was applied to confirm the factor structure. We expected a best-fit model with the following indices: a Satorra–Bentler scaled chi-square (S-Bχ2)/ degrees of freedom ratio (CMIN/DF) ≤3.0; a Trucker Lewis index (TLI) ≥0.90; a normed fit index (NFI) ≥0.90; a goodness-of-fit index (GFI) ≥0.90; an incremental fit index (IFI) ≥0.90 an adjusted goodness of fit index (AGFI) ≥0.90, and a low root mean square error of approximation (RMSEA) ≤0.08.[34]

Results

We included 290 parents of children with a disability due to various disorders aged 5-17 years old in the study. The children included 214 diagnosed with cerebral palsy, 23 with developmental delay, 22 with metabolic/ genetic disease, 11 with autism, eight with neuromuscular disease, six with Down syndrome, and six with myelomeningocele. The parents consisted of 224 mothers and 54 fathers, in addition to 12 other family members providing care. Table I presents the descriptive characteristics of the children and parents including the age, gender, diagnosis, and the rehabilitation centers providing the services.

Table I. Descriptive characteristics of participants.

	N (%)
Age (years)	9.40 ± 3.17
Sex	N (%)
Male	169 (58.3)
Female	121 (41.7)
Main Diagnoses	N (%)
Cerebral Palsy	214 (73.8)
Developmental Delay	23 (7.9)
Metabolic / Genetic Disease	22 (7.6)
Autism	11 (3.8)
Neuromuscular Disease	8 (2.8)
Down Syndrome	6 (2.1)
Myelomeningocele	6 (2.1)
Type of Centre	N (%)
Special Education and Rehabilitation Centre	121 (41.7)
Public Hospital	104 (35.9)
Private Medical Centre	65 (22.4)

Reliability

Internal consistency

When the descriptive statistics of the five subscales of MPOC-56 and MPOC-20 were investigated, the *enabling and partnership* subscale obtained the highest score in both scales whereas the *providing specific information about the child* subscale obtained lowest scores. Cronbach's Alpha coefficient for the internal consistency of the five subscales of MPOC-20 and MPOC-56 varied between 0.84 and 0.97, indicating excellent internal consistency for each subscale (Table II).

Test-retest reliability

Seventy-three parents agreed to complete the scales two weeks later for test-retest reliability. The test-retest reliability was found to be excellent for both MPOC-56 and MPOC-20. The ICC values were 0.96-0.99 for MPOC-56 and 0.94-0.98 for MPOC-20 (Table III).

Table II. Descriptive statistics and internal consistency measurements of Cronbach's alpha for MPOC-56 and MPOC-20.

Scale	Version	NI	NS	M (SD)	Range	α
Enabling and Partnership	MPOC-56	16	290	5.25 (SD=1.03)	2.25-7.00	0.972
	MPOC-20	3	290	5.45 (SD=1.05)	2.00-7.00	0.883
Providing General Information	MPOC-56	9	290	4.46 (SD=0.93)	2.00-7.00	0.912
	MPOC-20	5	290	4.99 (SD= 0.93)	2.20-7.00	0.882
Providing Specific Information About the Child	MPOC-56	5	290	3.83 (SD= 1.15)	1.40-7.00	0.848
	MPOC-20	3	290	3.71 (SD= 1.32)	1.33-7.00	0.878
Coordinated and Comprehensive Care	MPOC-56	17	290	4.90 (SD= 1.12)	1.94-700	0.972
	MPOC-20	4	290	5.05 (SD= 1.08)	1.75-7.00	0.881
Respectful and Supportive Care	MPOC-56	9	290	4.90 (SD=1.08)	2.00-7.00	0.942
	MPOC-20	5	290	4.51 (SD=1.20)	1.60-700	0.920

α: Cronbach's alpha, M: mean scale score, MPOC: measure of process of care, NI: number of items, NS: number of subjects, SD: standard deviation.

Table III. Results of test-retest analysis for MPOC (n=73).

Scale	Version	ICC	95% CI	First rating	Second rating
Enabling and Partnership	MPOC-56	0.982	-0.03-0.03	5.73 (SD=0.87)	5.73 (SD=0.84)
	MPOC-20	0.949	-0.07-0.06	5.79 (SD=0.97)	5.79 (SD=0.92)
Providing General Information	MPOC-56	0.982	0.00-0.08	4.74 (SD=0.92)	4.69 (SD=0.93)
	MPOC-20	0.975	-0.03-0,06	5.28 (SD=0.93)	5.27 (SD=0.95)
Providing Specific Information About the Child	MPOC-56	0.962	-0.00-0.13	4.41 (SD=1.09)	4.35 (SD=1.05)
	MPOC-20	0.951	-0.00-0.17	4.47 (SD=1.25)	4.38 (SD=1.24)
Coordinated and Comprehensive Care	MPOC-56	0.992	-0.07- -0.01	5.34 (SD=1.05)	5.39 (SD=1.02)
	MPOC-20	0.982	-0.11- -0.01	5.43 (SD=1.02)	5.49 (SD=0.99)
Respectful and Supportive Care	MPOC-56	0.979	-0.02-0.06	5.62 (SD=0.91)	5.60 (SD=0.88)
	MPOC-20	0.975	-0.02-0.07	5.34 (SD=1.03)	5.31 (SD=1.03)

CI: confidence interval, ICC: intraclass correlation coefficient, MPOC: measure of Process of Care.

Validity

Construct validity

Spearman's Correlation coefficient between each evaluated subscale of MPOC-56 varied between 0.72 and 0.93. The strongest correlation between MPOC-56 subscales was between *Enabling and Partnership* and *Coordinated and Comprehensive Care for the Child and the Family* (r= 0.93) while the weakest was between *Enabling and Partnership* and *Providing General Information* (r=0.72). The correlation coefficient between the subscales of MPOC-20 varied 0.60 and 0.86; with the strongest between *Coordinated and Comprehensive Care for the Child and the Family* and *Respectful and Supportive Care* (r=0.86); and the weakest between *Providing General Information* and *Providing Specific Information about the Child* (r=0.60) (Table IV).

As a result of confirmatory factor analysis, index values were calculated for MPOC-56 as CMIN/DF: 2.83, RMSEA: 0.08, GFI: 0.59, CFI: 0.87, AGFI: 0.55, NFI: 0.80 and TLI: 0.85. For MPOC-20, these values were found as CMIN/DF: 2.98, RMSEA: 0.08, GFI: 0.84, CFI: 0.95, AGFI: 0.79, NFI: 0.91 and TLI: 0.93. According to these results factor structure of the scales were found to be acceptable (Table V).[33]

Construct validity of the MPOC- 56 was found to be acceptable in the subdomains of *Enabling and Partnership* (RMSEA: 0,09 / GFI: 0,85),

Table IV. Spearman's rank correlation coefficients between MPOC subscales.

Scale	Version	Providing General Information	Providing Specific Information About the Child	Coordinated and Comprehensive Care	Respectful and Supportive Care
Enabling and Partnership	MPOC-56	0.729	0.815	0.939	0.920
	MPOC-20	0.713	0.682	0.857	0.771
Providing General Information	MPOC-56		0.768	0.743	0.733
	MPOC-20		0.600	0.735	0.699
Providing Specific Information About the Child	MPOC-56			0.838	0.844
	MPOC-20			0.719	0.805
Coordinated and Comprehensive Care	MPOC-56				0.934
	MPOC-20				0.863

MPOC: measure of process of care.

Table V. Results of confirmatory factor analysis.

			Model Fit			
Domain		Items included in domain	CMIN/DF	RMSEA	GFI	CFI
Total Score	MPOC 56	1-56	2.83	0.08	0.59	0.87
	MPOC-20	1-20	2.98	0.08	0.84	0.95
Enabling and Partnership	MPOC-56	2,3,8,12,15,16,17,19,22,23,25, 28,30,35,36,43	3.72	0.09	0.85	0.95
	MPOC-20	4,7,8	0	0	1	1
Providing General Information	MPOC-56	46,48,49,50,51,53,54,55,56	5.45	0.12	0.90	0.94
	MPOC-20	16,17,18,19,20	1.58	0.04	0.99	0.99
Providing Specific Information About the Child	MPOC-56	24,26,27,39,52	5.11	0.12	0.97	0.99
	MPOC-20	2,14,15	0	0	1	1
Coordinated and Comprehensive Care	MPOC-56	1,4,5,6,7,10,11,13,14,20,21,32, 34,37,40,44,45	3.51	0.09	0.86	0.95
	MPOC-20	5,6,10,12	1.90	0.05	0.99	0.99
Respectful and Supportive Care	MPOC-56	9,18,29,31,33,38,41,42,47	5.84	0.13	0.90	0.95
	MPOC-20	1,3,9,11,13	4.43	0.11	0.97	0.98

CFI: comparative fit index, CMIN/DF: satorra–bentler scaled chi-square/degrees of freedom ratio, GFI: goodness-of-fit index, MPOC: measure of process of care, RMSEA: low root mean square error of approximation.

Providing General Information (RMSEA: 0.12 / GFI: 0.90), *Providing Specific Information About the Child* (RMSEA: 0.12 / GFI:0.97), *Coordinated and Comprehensive Care* (RMSEA: 0.09 / GFI:0.86), and *Respectful and Supportive Care* (RMSEA: 0.13 / GFI: 0.90). Construct validity of the MPOC- 20 was found to be acceptable in the subdomains of *Enabling and Partnership* (RMSEA: 0.00 / GFI: 1.00), *Providing General Information* (RMSEA: 0.04 / GFI: 0.99), *Providing Specific Information About the Child* (RMSEA: 0.00 / GFI: 1.00), *Coordinated and Comprehensive Care* (RMSEA: 0.05 / GFI: 0.99), and *Respectful and Supportive Care* (RMSEA: 0.11 / GFI: 0.97). These results show that both MPOC-56 and MPOC-20 fitted well according to the confirmatory factor analysis, on the other hand MPOC-20 was better fitted than MPOC-56 (Table V).

Discussion

The aim of this study was to investigate the validity and reliability of the Turkish versions of MPOC-56 and MPOC-20, in children with disability aged 5-17 years. In this study, we investigated the validity and reliability of the

MPOC 56- and -20 in children with disability and both scales showed an acceptable construct validity and high test–retest reliability and acceptable factor distribution.

The findings of this study show that the Turkish versions of MPOC-56 and MPOC-20 are strong in terms of psychometric characteristics and are reliable and valid similar to the original Canadian version of the scale.[9]

Both the descriptive statistics of the Turkish MPOC-56 and MPOC-20 coincided with the original scale, they were also similar to the versions from Sweden, Japan, Korea and Jordan.[27,35-37] Although the mean scores of the descriptive statistics are in line with the original Canada study, there were some differences in the patterns of the high and low scale scores.[9] In the original Canadian study, the highest mean among subscales both in MPOC-56 and MPOC-20 was *Respectful and Supportive Care*, and the lowest mean among subscales was *Providing General Information*; whereas in current study the highest mean among subscales was *Enabling and Partnership*, and the lowest mean among subscales was *Providing General Information* both in MPOC-56 and MPOC-20. Therefore, unlike other cultural adaptations, reliability and validity studies[27,35-37], it can be considered that service providers in Turkish population contribute more in terms of enabling and partnership, rather than providing specific information to parents of children with disabilities during their care processes.

The mean values of the subscales were between 3.83-5.25 for MPOC-56 and 3.71-5.45 for MPOC-20. These values are similar with studies of Dutch, Korea and Iceland.[26,36,38] The mean score range was 1.40-7.00 for MPOC-56 and 1.33-7.00 for MPOC-20. These findings show that the entire range of item levels were used by the parents in their responses.

Cronbach's alpha was used in the current study to evaluate the internal consistency of the questionnaire items. The Cronbach alpha coefficient was calculated for each subscale as a measure of internal consistency. Reliability was found to be comparable with other versions. The present study shows that the Turkish versions of the MPOC-56 and MPOC-20 have high internal consistency for all subscales as in the Sloven, Norwegian, Dutch, Malaysian and Icelandic versions that had subscale internal consistencies ranging between 0.70 and 0.97.[25,26,38-40]

According to the test-retest reliability results in this study, the ICC of the total score was 0.9, which indicates high reliability of this questionnaire. ICCs were also reported in the Canadian, Japanese and Korean versions for test-retest reliability.[9,35,36] For MPOC-56 ICCs ranged from 0.78-0.88 in the Canadian version, 0.83 to 0.96 in the Korean version, and from 0.80-0.89 in the Japanese version. In the Korean version ICCs ranged between 0.86-0.97, and between 0.76-0.87 in the Japanese version for MPOC-20.[9,35,36] In the present study, however, ICCs ranged between 0.97 to 0.99 for MPOC-56 and 0.94-0.98 for MPOC-20. Compared to other versions, the Turkish versions of the MPOC-56 and MPOC-20 had better test-retest reliability according to ICC findings.

The correlation coefficients of subscales within each scale and between the five subscales were found to be very good to excellent in the Turkish version of the MPOC. These findings therefore show that the items are highly related to their own scales and the five subscales are related to each other. For this reason, the Turkish version of the MPOC-56 and MPOC-20 have high construct validity like the Japanese and Korean versions.[35,36]

The most frequently used method to determine the validity of a scale used in a different culture is by using confirmatory factor analysis.[41] As a result of the confirmatory factor analysis conducted in this study, the factors in the Turkish version of MPOC-56 were different from the original Canadian factors. The reasons for analysing the factor distribution in the Turkish and Canadian versions were different. In the Canadian study the factor analysis was performed to determine the final

version of the scale and as a result 92 items were reduced to 56 items and the MPOC-56 was created, whereas the factor analysis in the Turkish version was carried out to confirm the factor structure. Similarly, in factor analysis for the Dutch and Japanese versions of MPOC, there are both differences and similarities in the distribution of factor structures.[26,35] In this study, all other analyses strongly validated the original Canadian factor structure. Therefore, in this case it was decided that it was unnecessary to adapt another factor structure. The factor structure of the MPOC-20 is largely similar to the Canadian version.

In Türkiye, children may get rehabilitation and care services from three main centres such as public hospitals, private medical centers or special education and rehabilitation centers. Since this current study includes these centers, it covers the rehabilitation landscape of Türkiye. It also indicates that these results can be generalized. The presence of subjects within a wide age range and with various diagnoses is also important in terms of reflecting the general population. The main limitation of the study was, the small numbers of children with different kinds of disabilities or chronic health conditions beside children with cerebral palsy, and it is therefore recommended to investigate findings with larger samples of different kinds of disabilities. One other limitation was the lack of concurrent validity. Although we showed construct validity by confirmatory factor analysis, due to the lack of a valid and reliable gold standard measure to evaluate family-centered care and services in Turkish, no other additional tests parallel to the MPOC-56 and MPOC-20 were used.

In order to plan and deliver health services effectively it is essential to understand the processes of care from children and their parents' perspectives. Researchers and policymakers look for reliable measures that will make it possible to capture the extent to which children with disability as well as their families' wishes, and needs are addressed.

The present study shows that the Turkish version of MPOC is an important instrument that can increase the evaluation of quality of care provided by service providers. It can be used as an evaluation tool to shed light on the understanding and assessment of rehabilitation services received from service providers for politicians and researchers in our country.

Our findings indicate that the Turkish versions of MPOC-56 and MPOC-20 are reliable and valid. Since the values in the confirmatory factor analysis of MPOC-20 better fitted than MPOC-56, we believe that MPOC-20 is a more suitable scale for clinical studies and research on t Turkish population. The Turkish version of MPOC-56 and MPOC-20 is an important instrument that can increase the evaluation of quality of care provided by service providers and health care organizations and enables the integration of families into care processes.

Acknowledgements

We thank Professor Peter Rosenbaum from Canada Research Chair in Childhood Disability Co-Founder, CanChild Centre for Childhood Disability Research for his great support in all the steps of the study process.

Ethical approval

The ethics committee approval necessary for the study was obtained from the University of Health Sciences Gülhane Non-Interventional Studies Ethics Committee (no: 2018/3-18/58).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DT, CÖ, MKG Author; data collection: DT, CÖ. Author; analysis and interpretation of results: SK; draft manuscript preparation: DT, CÖ, MKG, SK Author. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Kuo DZ, Houtrow AJ, Arango P, Kuhlthau KA, Simmons JM, Neff JM. Family-centered care: current applications and future directions in pediatric health care. Matern Child Health J 2012; 16: 297-305. https://doi.org/10.1007/s10995-011-0751-7
2. Rosenbaum P, King S, Law M, King G, Evans J. Family-centered service: a conceptual framework and research review. J Physical Occupational Therapy in Pediatrics 1998; 18: 1-20. https://doi.org/10.1300/j006v18n01_01
3. King S, King G, Rosenbaum P. Evaluating health service delivery to children with chronic conditions and their families: development of a refined Measure of Processes of Care (MPOC-20). Children's Health Care 2004; 33: 35-57. https://doi.org/10.1207/s15326888chc3301_3
4. King S, Teplicky R, King G, Rosenbaum P. Family-centered service for children with cerebral palsy and their families: a review of the literature. Semin Pediatr Neurol 2004; 11: 78-86. https://doi.org/10.1016/j.spen.2004.01.009
5. Bamm EL, Rosenbaum P, Stratford P. Validation of the measure of processes of care for adults: a measure of client-centred care. Int J Qual Health Care 2010; 22: 302-309. https://doi.org/10.1093/intqhc/mzq031
6. World Health Organization (WHO). International Classification of Functioning, Disability and Health (ICF). WHO; 2001.
7. Trabacca A, Vespino T, Di Liddo A, Russo L. Multidisciplinary rehabilitation for patients with cerebral palsy: improving long-term care. J Multidiscip Healthc 2016; 9: 455-462. https://doi.org/10.2147/JMDH.S88782
8. Rosenbaum P. The environment and childhood disability: opportunities to expand our horizons. Dev Med Child Neurol 2007; 49: 643. https://doi.org/10.1111/j.1469-8749.2007.00643.x
9. King SM, Rosenbaum PL, King GA. Parents' perceptions of caregiving: development and validation of a measure of processes. Dev Med Child Neurol 1996; 38: 757-772. https://doi.org/10.1111/j.1469-8749.1996.tb15110.x
10. King G. Family-centered caregiving and well-being of parents of children with disabilities: linking process with outcome. Journal of Pediatric Psychology 1999; 24: 41-53. https://doi.org/10.1093/jpepsy/24.1.41
11. King G, Law M, King S, Rosenbaum P. Parents' and service providers' perceptions of the family-centredness of children's rehabilitation services. Physical & Occupational Therapy in Pediatrics 1998; 18: 21-40. https://doi.org/10.1080/j006v18n01_02
12. Cunningham BJ, Rosenbaum PL. Measure of processes of care: a review of 20 years of research. Dev Med Child Neurol 2014; 56: 445-452. https://doi.org/10.1111/dmcn.12347
13. Darrah J, Wiart L, Magill-Evans J, Ray L, Andersen J. Are family-centred principles, functional goal setting and transition planning evident in therapy services for children with cerebral palsy? Child Care Health Dev 2012; 38: 41-47. https://doi.org/10.1111/j.1365-2214.2010.01160.x
14. King S, Rosenbaum P, King G. The Measure of processes of care: a means to assess family-centered behaviours of health care providers. Hamilton, Ontario: McMaster University; 1995.
15. Dyke P, Buttigieg P, Blackmore AM, Ghose A. Use of the measure of process of care for families (MPOC-56) and service providers (MPOC-SP) to evaluate family-centred services in a paediatric disability setting. Child Care Health Dev 2006; 32: 167-176. https://doi.org/10.1111/j.1365-2214.2006.00604.x
16. Himuro N, Miyagishima S, Kozuka N, Tsutsumi H, Mori M. Measurement of family-centered care in the neonatal intensive care unit and professional background. J Perinatol 2015; 35: 284-289. https://doi.org/10.1038/jp.2014.204
17. Joachim KC, Wilk P, Ryan BL, Speechley KN. Family-centered care in children with epilepsy: evaluating the Measure of Processes of Care (MPOC-20). Epilepsia 2016; 57: 1660-1668. https://doi.org/10.1111/epi.13494
18. Klassen AF, Dix D, Cano SJ, Papsdorf M, Sung L, Klaassen RJ. Evaluating family-centred service in paediatric oncology with the measure of processes of care (MPOC-20). Child Care Health Dev 2009; 35: 16-22. https://doi.org/10.1111/j.1365-2214.2008.00887.x
19. Swaine BR, Pless IB, Friedman DS, Montes JL. Using the measure of processes of care with parents of children hospitalized for head injury. Am J Phys Med Rehabil 1999; 78: 323-329. https://doi.org/10.1097/00002060-199907000-00006

20. Poenaru D, Roblin N, Bird M, et al. The pediatric bowel management clinic: initial results of a multidisciplinary approach to functional constipation in children. J Pediatr Surg 1997; 32: 843-848. https://doi.org/10.1016/s0022-3468(97)90633-3

21. McKean K, Phillips B, Thompson A. A family-centred model of care in paediatric speech-language pathology. Int J Speech Lang Pathol 2012; 14: 235-246. https://doi.org/10.3109/17549507.2011.604792

22. Stewart V, Slattery M, McKee J. Deaf and hard of hearing early intervention: Perceptions of family-centered practice. Journal of Early Intervention 2021; 43: 221-234. https://doi.org/10.1177/1053815120962547

23. Russell S, McCloskey CR. Parent perceptions of care received by children with an autism spectrum disorder. J Pediatr Nurs 2016; 31: 21-31. https://doi.org/10.1016/j.pedn.2015.11.002

24. Woodside JM, Rosenbaum PL, King SM, King GA. Family-centered service: developing and validating a self-assessment tool for pediatric service providers. Children's Health Care 2001; 30: 237-252. https://doi.org/10.1207/s15326888chc3003_5

25. Hagen A-K, Bjorbækmo WS. Parents evaluation of the processes of care in child rehabilitation: a reliability study of the Norwegian translation of MPOC-20. Child Care Health Dev 2012; 38: 48-53. https://doi.org/10.1111/j.1365-2214.2010.01192.x

26. van Schie PEM, Siebes RC, Ketelaar M, Vermeer A. The measure of processes of care (MPOC): validation of the Dutch translation. Child Care Health Dev 2004; 30: 529-539. https://doi.org/10.1111/j.1365-2214.2004.00451.x

27. Bjerre IM, Larsson M, Franzon A-M, Nilsson MS, Strömberg G, Westbom LM. Measure of Processes of Care (MPOC) applied to measure parent's perception of the habilitation process in Sweden. Child Care Health Dev 2004; 30: 123-130. https://doi.org/10.1111/j.1365-2214.2003.00403.x

28. Zengin Akkus P, Ilter Bahadur E, Coskun A, Koken G, Karahan S, Ozmert EN. Family-centred service: perspectives of paediatric residents from a non-Western country. Child Care Health Dev 2020; 46: 275-282. https://doi.org/10.1111/cch.12753

29. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine (Phila Pa 1976) 2000; 25: 3186-3191. https://doi.org/10.1097/00007632-200012150-00014

30. Nunnally JC, Wilson WH. Method and theory for developing measures in evaluation research. In: Struening EL, Guttentag M, editors. Handbook of Evaluation Research. Beverly Hills, Calif: Sage; 1975: 227-289.

31. Norman GR, Streiner DL. PDQ Statistics. PMPH-USA; 2003.

32. Park MS, Kang KJ, Jang SJ, Lee JY, Chang SJ. Evaluating test-retest reliability in patient-reported outcome measures for older people: a systematic review. Int J Nurs Stud 2018; 79: 58-69. https://doi.org/10.1016/j.ijnurstu.2017.11.003

33. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012; 22: 276-282

34. Brown TA. Confirmatory factor analysis for applied research. Guilford Publications; 2015.

35. Himuro N, Kozuka N, Mori M. Measurement of family-centred care: translation, adaptation and validation of the Measure of Processes of Care (MPOC-56 and -20) for use in Japan. Child Care Health Dev 2013; 39: 358-365. https://doi.org/10.1111/j.1365-2214.2012.01371.x

36. An M, Kim JG, Kim J. Measure of processes of care (MPOC): translation and validation for use in Korea. Child Care Health Dev 2018; 44: 545-551. https://doi.org/10.1111/cch.12577

37. Saleh M, Almasri NA. Use of the Measure of Processes of Care (MPOC-20) to evaluate health service delivery for children with cerebral palsy and their families in Jordan: validation of Arabic-translated version (AR-MPOC-20). Child Care Health Dev 2014; 40: 680-688. https://doi.org/10.1111/cch.12116

38. Arnadottir U, Egilson ST. Evaluation of therapy services with the Measure of Processes of Care (MPOC-20): the perspectives of Icelandic parents of children with physical disability. J Child Health Care 2012; 16: 62-74. https://doi.org/10.1177/1367493511423769

39. Fauzi NFM, Ming ACE, Rasdi HFM, Saat NZM. The Measure of Processes of Care 20-Item (MPOC-20): validity and reliability of the Malay Version. Malaysian Journal of Health Sciences 2015; 13: 61-67. https://doi.org/10.17576/JSKM-2015-1302-07

40. Groleger Sršen K, Vidmar G, Zupan A. Validity, internal consistency reliability and one-year stability of the Slovene translation of the Measure of Processes of Care (20-item version). Child Care Health Dev 2015; 41: 569-580. https://doi.org/10.1111/cch.12198

41. Streiner DL, Norman GR, Cairney J. Health measurement scales: a practical guide to their development and use. USA: Oxford University Press; 2015. https://doi.org/10.1093/med/9780199685219.001.0001

The Turkish Journal of Pediatrics 2023; 65: 523-530
https://doi.org/10.24953/turkjped.2022.598

Case Report

Rhombencephalitis and longitudinal extensive myelitis associated with dinutuximab use in high-risk neuroblastoma

Fırat Atak[1], Güzide Burça Aydın[2], İbrahim Öncel[3], Sibel Öz[3], Kader Karlı Oğuz[1]

[1]Department of Radiology, Hacettepe University School of Medicine Ankara; [2]Department of Pediatric Oncology, Hacettepe University School of Medicine Ankara; [3]Department of Pediatric Neurology, Hacettepe University School of Medicine Ankara, Türkiye.

ABSTRACT

Background. Dinutuximab is a monoclonal antibody that targets the GD2 antigen used in the treatment of high-risk neuroblastoma. Dinutuximab-associated rhombencephalitis and myelitis is a rare, steroid-responsive, serious, but reversible pathology. To date, three transverse myelitis cases and one rhombencephalitis case due to dinutuximab have already been reported. Moreover, a recently published article identified five inflammatory CNS demyelination cases (four myelitis and one rhombencephalitis). We present a 5-year-old patient with rhombencephalitis and myelitis following dinutuximab-beta treatment.

Case. A 5-year-old patient with a left-sided retroperitoneal mass infiltrating the left kidney and multiple lytic bone lesions was diagnosed with neuroblastoma with a percutaneous biopsy from the abdominal mass. Surgery was performed after a prominent treatment response was detected on the abdominal CT. Radiotherapy was applied to the abdomen. While she was still undergoing maintenance treatment with 13-cis retinoic acid, a metaiodobenzylguanidine (MIBG) scan detected new bone lesions, and brain MRG identified pachymeningeal involvement. A new chemotherapy regimen was started and decreased MIBG uptake was seen in all previous bone lesions. However, newly developed eighth rib metastasis was seen in the following MIBG scan. Autologous stem cell transplantation was done. Soon after, dinutuximab-beta, together with temozolomide and irinotecan, was initiated. Following the third cycle hypotension, somnolence, paraparesis, and unilateral fixed dilated pupil were developed. Afterward, hemiballismus-like irregular limb movements were observed. Work-up studies were unremarkable, except for hypodensity in the brain stem on the brain CT. MRI revealed T2 hyperintensity of the brainstem and spinal cord extending from the cervicomedullary junction to the T7 level. Moreover, incomplete contrast enhancement and facilitated diffusion were observed. Imaging findings suggested demyelination. Steroids and intravenous immune globulin (IVIG) treatment were initiated. Both imaging abnormalities and clinical symptoms resolved partially at one month and disappeared at six months.

Conclusions. Awareness of the radiological findings of dinutuximab toxicity will lead to prompt diagnosis and treatment.

Key words: dinutuximab, rhombencephalitis, myelitis, neuroblastoma.

Monoclonal antibody treatment is an emerging and effective modality for cancer treatment. It has become possible with the demonstration of relatively specific surface proteins in cancer cells and the development of monoclonal antibodies in mice with the developing hybridoma technology.[1,2] Cell destruction can be brought about by several mechanisms: direct action (inhibition of cell survival signaling through receptor binding, induction of apoptosis, delivery of a drug or cytotoxic agent by conjugated antibodies), immune-mediated cell killing mechanisms (antibody-dependent cellular toxicity, complement-dependent cytotoxicity, regulation of T cell function) and disruption of tumor vasculature or stroma.[1,2]

✉ Fırat Atak
firatmd@gmail.com

Received 9th June 2022, revised 25th September 2022, 8th March 2023, accepted 26th March 2023.

Gangliosides are glycosylated lipid molecules. Monoclonal antibodies targeting them have been used in the treatment of various tumors. GD2 antigen is an example of a ganglioside, which is found in small amounts on the surface of neurons, skin melanocytes, and peripheral sensory nerves.[3,4] Anti-GD2 monoclonal antibodies are currently used to treat neuroblastoma based on their high expression on the surface of neuroblastoma cells.

Dinutuximab, a monoclonal antibody that targets the GD2 antigen, has been routinely used in the maintenance treatment of pediatric patients with high-risk neuroblastoma who have partially responded to first-line therapy or with relapsed or refractory disease.[3,5] Pain, tachycardia, hypertension, hypotension, fever, and urticaria are the most common side effects of dinutuximab.[3,4]

So far, three cases of transverse myelitis and one rhombencephalitis case associated with dinutuximab have been published in the literature.[3,4] In addition, a recent study reported a case with brain stem lesions and four cases of transverse myelitis due to dinutuximab.[5] However, to the best of our knowledge, there are still no cases involving the spinal cord and brain stem simultaneously, as in our case. Herein, we present a case with some distinct clinical features, concurrent brainstem and spinal cord involvement, and one-year follow-up information.

Case Report

A 5-year-old patient was admitted to our hospital with a large intraabdominal tumor in the left retroperitoneal compartment three days after the ultrasound was conducted due to recurrent abdominal pain. Abdominal computed tomography (CT) revealed a left-sided retroperitoneal mass extending between the suprarenal space and iliac bifurcation (TR 8.7 cm, AP 6.5 cm), infiltrating the left kidney, encircling the renal artery and vein, and crossing to the contralateral side through the retroaortic area. Furthermore, metaiodobenzylguanidine (MIBG) scan identified multiple bone lesions at the time of diagnosis. Neuroblastoma with MYCN amplification was diagnosed from a percutaneous biopsy of the abdominal mass. The bone marrow aspiration biopsy was positive for infiltration. According to the Turkish Pediatric Oncology Group, the patient received a high-risk neuroblastoma chemotherapy regimen, including ifosfamide, doxorubicin, dacarbazine, vincristine, cisplatin, cyclophosphamide, and etoposide. After six months of chemotherapy, CT showed an excellent response to induction chemotherapy with a tumor volume decrease (TR 3.8 cm, AP 1.8 cm). But, the tumor still infiltrated the left kidney and involved the renal hilum. The patient underwent surgery, and the tumor, along with the left adrenal gland, kidney, and perirenal fat (four lymph nodes were metastatic on the pathology specimen), were grossly resected. Following radiotherapy for the abdomen, the MIBG scan was normal. Maintenance treatment with 13-cis retinoic acid was started.[6]

While she was still on maintenance treatment for 18 months, multiple MIBG-positive new medullary bone lesions involving the vertebral column, pelvis, sacrum, bilateral proximal lower extremities, and bilateral orbital walls were detected. Brain MRI revealed some calvarial lesions, some of which were accompanied with soft tissue lesions and pachymeningeal focal thickening, which was suspicious for neuroblastoma involvement. The patient was received six cycles of ifosfamide, carboplatin, and etoposide. Decreased uptake was seen in all previous bone lesions on the first MIBG scan, but two months after a new focal lesion on the left eighth rib was revealed. Afterward, she underwent autologous stem cell transplantation after six courses of busulfan and melphalan. Dinutuximab-beta (DB) with irinotecan and temozolomide (TEMIRI) chemotherapy was initiated due to a lack of regression in bone lesions.

Following the third cycle of DB and TEMIRI, he experienced hypotension, tachycardia,

somnolence, and paraparesis. Also, the patient had a unilaterally fixed dilated pupil. Soon after, left-sided hemiballismus-like high-amplitude irregular movements were detected. In addition, she became unresponsive to verbal stimuli within hours. The chronological flow of chemotherapy protocols, imaging studies, and essential dates from the diagnosis to the incident are shown in Figure 1. Work-up studies were unremarkable, except for an expansile diffuse hypodensity in the brain stem on the CT. A brain MRI revealed swelling and T2 hyperintensity in the brain stem. An incomplete ring enhancement was seen on T1-weighted images following intravenous Gadolinium-based contrast material injection, and the lesions showed facilitated diffusion as shown by increased ADC values. Linear and punctate susceptibility foci in the pons were seen on SWI (Fig. 2). Cervical spinal MRI showed longitudinal extensive T2 hyperintense lesion extending from the cervicomedullary junction to the T7 level, involving most of the spinal cord's cross-sectional area and showing eccentric, patchy contrast enhancement similar to that in the brain stem (Fig. 3). Due to the swelling of the brain stem, a lumbar puncture was not performed. After DB treatment was discontinued, the patient received 30 mg/kg/day pulse methylprednisolone for seven days and 0.4 g/kg/day intravenous immune globulin (IVIG) for five days. Left-sided hemiballismus-like symptoms quickly disappeared, her consciousness began to recover, and she gradually regained lower extremity motor strength. Then, she continued to take prednisolone at a dose of 1 mg/kg/day for 4 weeks. Following the regression of the brainstem and spinal cord lesions on follow-up MRI at one month, steroids were tapered and discontinued in four weeks (Fig. 4).

After her discharge from the hospital, she continued receiving TEMIRI chemotherapy, and her last dose (8th cycle) was in September 2021. At the 9th-month follow-up examination, her neurological examination was normal. The patient still has several sequela bone lesions on the pelvis and bilateral orbital walls. Her last brain MRI was normal except for the stable lesion in the orbital portion of the right frontal bone. She is still in remission. Informed consent was received from the family.

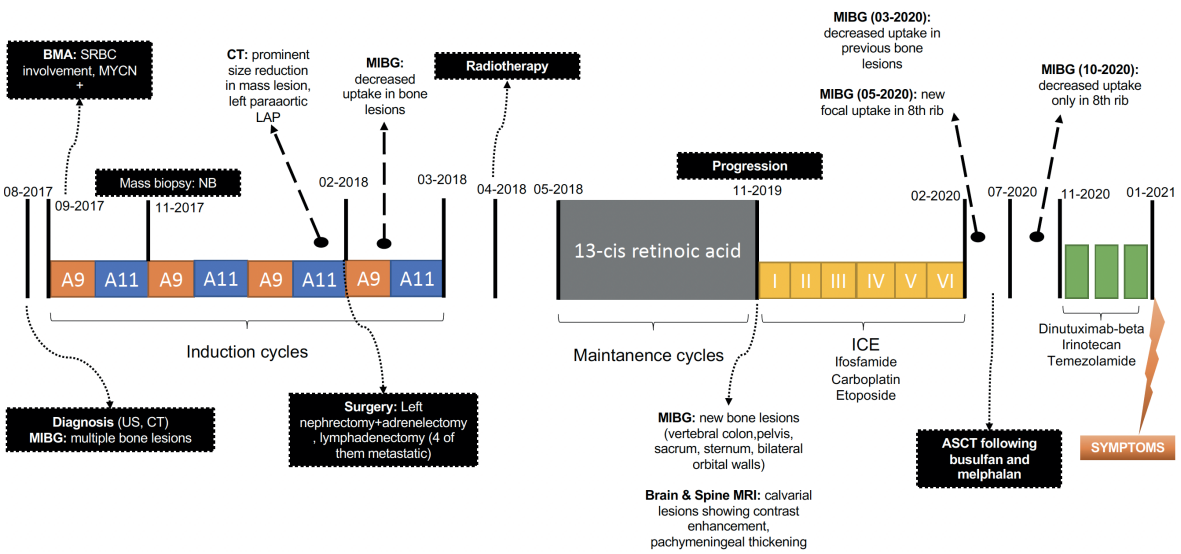


Fig. 1. Flow chart of treatment and important date marks (A9, A11: chemotherapy cycles; BMA: bone marrow aspiration; SRBC: small round blue cell; NB: neuroblastoma; MIBG: Metaiodobenzylguanidine; ASCT: autologous stem cell transplant).

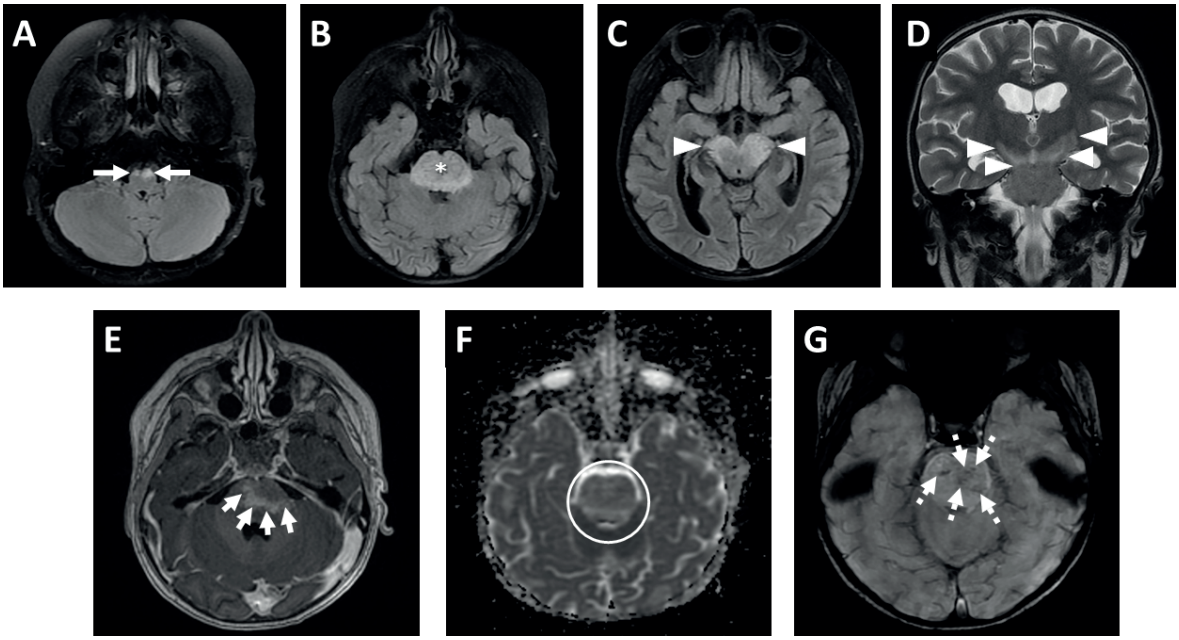


Fig. 2. Brain MRI at the time of diagnosis of a 5-year-old girl with recurrent high-risk neuroblastoma who was unresponsive to verbal stimuli had paraparesis and left unilateral fixed dilated pupil after the third dose of dinutuximab. Axial FLAIR (*A-C*) and coronal T2-weighted (*D*) images demonstrate hyperintense signal on medullary pyramids (*long arrows*), pons (*asterisk*), and cerebral peduncles extending to the left internal capsule posterior limb (*arrowheads*). The lesion also demonstrated incomplete ring enhancement (*short arrows*) on the T1-weighted postcontrast thin-section image (*E*) and increased diffusion (*circle*) on the ADC map (*F*). In addition, linear and dot-like susceptibility foci (*dashed arrow, G*) in the pons on SWI images should be noted.

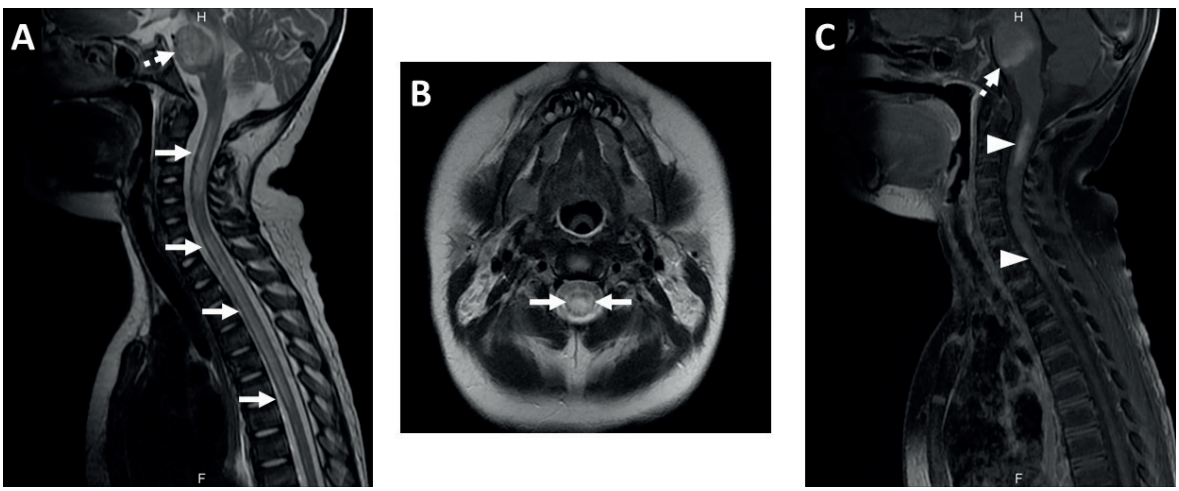


Fig. 3. Sagittal and axial T2-weighted images of spinal MRI performed at the time of diagnosis (*A-B*) demonstrate longitudinally and transversally extensive (*arrows, A*) increased T2 signal extending from cervicomedullary junction to T7, with patchy contrast enhancement (*arrowheads, C*) on sagittal fat-suppressed T1-weighted images. In addition, note large T2 hyperintensity and contrast enhancement (*dashed arrow, A-C*) within the pons.

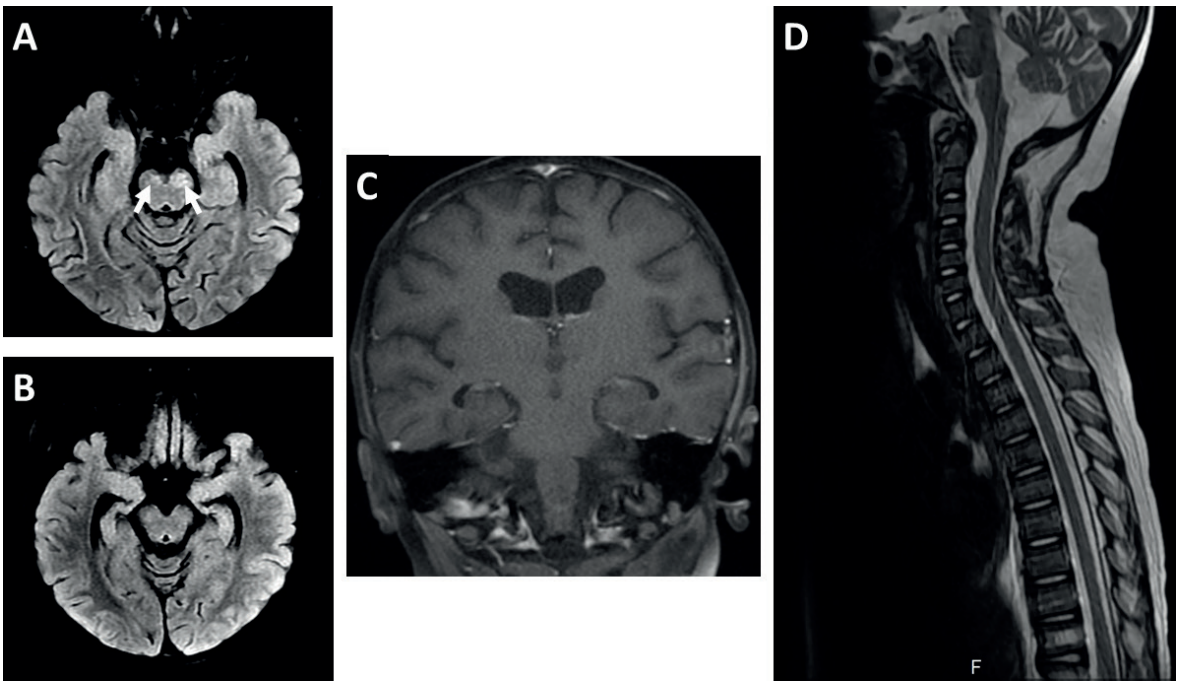


Fig. 4. The residual hyperintensity on bilateral cerebral peduncles on axial FLAIR images (*arrows, A*) after one-month, is completely resolved on six-month follow-up (*B*). Neither contrast enhancement on brain stem on coronal T1-weighted spin-echo image (*C*) nor residual spinal cord lesions on sagittal T2-weighted image (*D*) is seen on one-month follow-up brain and cervicothoracic spinal MRI.

Discussion

Two cases of rhombencephalitis and seven cases of transverse myelitis that developed following dinutuximab therapy have already been described in the literature.[3-5] Herein, we present the first case of concurrent rhombencephalitis and transverse myelitis with a detailed description of peculiar radiological features.

Some monoclonal antibodies might impair the balance of the immune system and promote an abnormal inflammatory reaction and demyelination. Until 2017, of 64 monoclonal antibodies identified, 54 reported with neurological adverse effects including peripheral neuropathy, neuromuscular diseases, polyradiculopathies, myelitis, central nervous system (CNS) demyelinating diseases (multiple sclerosis [MS], MS-like demyelinating multifocal lesions, pre-existing MS exacerbation, neuromyelitis optica spectrum disorder, optic neuritis), CNS non-demyelinating diseases (leptomeningitis, meningoencephalitis, vasculitis, cranial nerve involvement, autoimmune encephalitis).[7,8] Commonly described agents causing neurotoxicities in the literature were TNF-alfa blockers, immune checkpoint inhibitors, and kinase inhibitors.[8,9]

Various central and peripheral neurotoxicities have also been reported in association with anti-GD2 immunotherapy ranging from neuropathic pain, confusion, and sensorimotor neuropathy to coma, seizure, and psychosis.[5] In the Children's Oncology Group trial, in children who received dinutuximab combined with interleukin 2 (IL-2) and granulocyte monocyte - colony stimulating factor (GM-CSF), CNS toxicity manifested as encephalopathy, confusion, psychosis, and coma was reported in 4.4% of patients.[5,10] Furthermore, in the recently published study investigating DB-related central neurotoxicities in a total of 1102 patients at least grade 3 neurotoxicity was found in 4% of the patients and severe neurotoxicity in 2.2%.[5] Also, they concluded that the events

predominantly occurred in the patients receiving combined treatment with DB and IL-2. Reported CNS findings in ten available MRI studies were as follows: four transverse myelitis (one thoracolumbar involvement, two thoracic involvement, and one not reported), one cytotoxic brain stem lesions, two posterior reversible encephalopathy syndrome (PRES), one encephalomyelitis, two encephalitis, one sensory axonal neuropathy, one demyelinating neuropathy of the dorsal roots, one involvement of the vestibulocochlear nerve.[5] One study reported that another chimeric anti-GD2 antibody, m3F8, was associated with PRES in five patients presenting with visual symptoms, headache, and seizure.[11] In our patient, the clinical and radiological hallmarks of PRES were excluded.

Some debates continue regarding the utility of IL-2 and its role in neurotoxicity. It has been previously described that neurotoxicity is more common in those receiving combination therapy with IL-2.[5] Wieczorek et al.[5] reported that the majority of grade 3/4 neurotoxicities (79.5%) occurred in patients treated with DB plus IL-2. Five CNS demyelination cases manifested as myelitis and cytotoxic brain stem lesions due to dinutuximab and IL-2 combination therapy. Our patient was receiving dinutuximab combined with chemotherapy. A case of transverse myelitis developed due to the same treatment regimen was already published by Ding et al.[3]

Our patient had a rapid recovery after treatment with steroids and IVIG. At the 6-month follow-up, no residual clinical symptoms were observed, while some subtle changes were identified within the brain stem. Wieczorek et al.[5] stated that most patients (33/38, 86.8%) with neurotoxicity recovered from symptoms with steroid and IVIG treatment. However, complete resolution was achieved with plasmapheresis in two patients who did not respond to first-line therapy.[5]

Symptoms appeared following the third cycle in our patient. As all patients develop neurotoxicity after consecutive courses with dinutuximab, as reported by Ding et al.[3] and Zama et al.[4], the authors proposed that prior exposure to the drug might cause alloreactivity against neural tissues. However, in another study, the fact that severe neurotoxicity was observed during the first cycle in most patients raises doubt on this interpretation.[5]

Altered consciousness, paraparesis, and unilateral fixed dilated pupil were observed in our patient. In addition, left-sided irregular proximal limb movements were seen, and due to the similarity of hemiballismus, it was evaluated primarily in favor of an extrapyramidal symptom. The neurological symptoms observed in our patient are among the most common symptoms mentioned by Wieczorek et al.[5] Authors reported that the most common side effects were paraparesis or hypotonia, urinary retention, seizures, ataxia or gait disturbances, and cranial nerve palsies.[5] Altered consciousness (somnolence and coma) was reported in three patients, two of which were manifested as encephalitis in MRI.[5] Hyperkinesis, which may be similar to hemiballismus-like limb movements in our patient, was detected in one patient, but the authors gave no other characteristic details. MRI findings of that patient were reported to be compatible with encephalitis. Also, case #1 with fixed pupil and brain stem lesions in the predefined study was similar to our case.[5] In addition, sixth cranial nerve palsy was found to cause strabismus in a patient who manifested as brain stem lesions.[4] In light of these, it should be kept in mind that cases with brain stem involvement may present with cranial nerve palsy.

Brainstem lesions showed T2 hyperintensity, facilitated diffusion, and open-ring contrast enhancement without an accompanying extra-axial mass. These features suggested a tumefactive demyelinating lesion in the brain, which led to the radiological differential diagnosis of inflammatory and demyelinating diseases in this case. These include NMOSD, neuro-Behçet disease (NBD), and neurosarcoidosis (NS), all of

which occur very rarely in childhood. Of these, no area postrema, optic nerve, periaqueductal white matter lesions, or T2 bright spotty lesions in the spinal cord were observed in favor of NMO. Neither of our patients had any systemic findings to suggest other inflammatory diseases like systemic lupus erythematosus (SLE) and Sjögren syndrome (SS), which may present with extensive myelitis rarely in children.[12] Although the MRI pattern of brainstem involvement could suggest NBD, especially in a Turkish patient, diagnosis is challenging in the absence of other clinical components of the disease, mainly oral and genital aphthous ulcers. For NS, which is extremely rare in the pediatric age group, our patient had no systemic manifestations, as seen in approximately 90% of the patients.[13] Our case was on follow-up of high-risk neuroblastoma with no such confounding findings on his already available imaging studies of thoracoabdominal CT and MIBG scan. Although a possible diagnosis in a patient with malignancy and receiving chemotherapy, infectious involvement mainly due to listeria, enterovirus 71, and herpes viruses was precluded due to the absence of fever or systemic findings and lack of relatively specific imaging findings. Neuroblastoma involvement could be considered obvious; however, in the present case, the brainstem and spinal cord were affected intrinsically rather than through external compression or infiltration of an extra-axial mass.[14] Therefore, CNS involvement in neuroblastoma was ruled out.

To summarize, myelitis and rhombencephalitis following DB treatment is a steroid-responsive, serious but transient pathology that can develop mostly after the second dose regimen. Clinical and radiological findings seen in dinutuximab toxicity are, however, nonspecific. Therefore, differentiation can only be made by combining clinical, laboratory, and radiological findings. Considering the efficacy of the steroid treatment, radiologists should be familiar with imaging findings of dinutuximab-related neurotoxicities for prompt diagnosis and treatment.

Ethical approval

Informed consent was received from the family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KKO and GBA; data collection: FA; analysis and interpretation of results: FA, IO, SO, KKO; draft manuscript preparation: FA, KKO. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer 2012; 12: 278-287. https://doi.org/10.1038/nrc3236
2. Coulson A, Levy A, Gossell-Williams M. Monoclonal antibodies in cancer therapy: mechanisms, successes and limitations. West Indian Med J 2014; 63: 650-654. https://doi.org/10.7727/wimj.2013.241
3. Ding YY, Panzer J, Maris JM, Castañeda A, Gomez-Chiari M, Mora J. Transverse myelitis as an unexpected complication following treatment with dinutuximab in pediatric patients with high-risk neuroblastoma: a case series. Pediatr Blood Cancer 2018; 65: e26732. https://doi.org/10.1002/pbc.26732
4. Zama D, Morello W, Masetti R, et al. Inflammatory disease of the central nervous system induced by anti-GD2 monoclonal antibody in a patient with high risk neuroblastoma. Pediatr Blood Cancer 2014; 61: 1521-1522. https://doi.org/10.1002/pbc.24982
5. Wieczorek A, Manzitti C, Garaventa A, et al. Clinical phenotype and management of severe neurotoxicity observed in patients with neuroblastoma treated with dinutuximab beta in clinical trials. Cancers (Basel) 2022; 14: 1919. https://doi.org/10.3390/cancers14081919

6. Aksoylar S, Varan A, Vergin C, et al. Treatment of high-risk neuroblastoma: National protocol results of the Turkish Pediatric Oncology Group. J Cancer Res Ther 2017; 13: 284-290. https://doi.org/10.4103/0973-1482.183205

7. Gill C, Rouse S, Jacobson RD. Neurological complications of therapeutic monoclonal antibodies: trends from oncology to rheumatology. Curr Neurol Neurosci Rep 2017; 17: 75. https://doi.org/10.1007/s11910-017-0785-3

8. Pérez-De-Lis M, Retamozo S, Flores-Chávez A, et al. Autoimmune diseases induced by biological agents. A review of 12,731 cases (BIOGEAS Registry). Expert Opin Drug Saf 2017; 16: 1255-1271. https://doi.org/10.1080/14740338.2017.1372421

9. Rimkus CM, Schoeps VA, Boaventura M, et al. Drug-related demyelinating syndromes: understanding risk factors, pathophysiological mechanisms and magnetic resonance imaging findings. Mult Scler Relat Disord 2021; 55: 103146. https://doi.org/10.1016/j.msard.2021.103146

10. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med 2010; 363: 1324-1334. https://doi.org/10.1056/NEJMoa0911123

11. Kushner BH, Modak S, Basu EM, Roberts SS, Kramer K, Cheung N-KV. Posterior reversible encephalopathy syndrome in neuroblastoma patients receiving anti-GD2 3F8 monoclonal antibody. Cancer 2013; 119: 2789-2795. https://doi.org/10.1002/cncr.28137

12. Law LY, Riminton DS, Nguyen M, Barnett MH, Reddel SW, Hardy TA. The spectrum of immune-mediated and inflammatory lesions of the brainstem: Clues to diagnosis. Neurology 2019; 93: 390-405. https://doi.org/10.1212/WNL.0000000000008015

13. Ungprasert P, Ryu JH, Matteson EL. Clinical manifestations, diagnosis, and treatment of sarcoidosis. Mayo Clin Proc Innov Qual Outcomes 2019; 3: 358-375. https://doi.org/10.1016/j.mayocpiqo.2019.04.006

14. D'Ambrosio N, Lyo JK, Young RJ, Haque SS, Karimi S. Imaging of metastatic CNS neuroblastoma. AJR Am J Roentgenol 2010; 194: 1223-1229. https://doi.org/10.2214/AJR.09.3203

The Turkish Journal of Pediatrics 2023; 65: 531-535
https://doi.org/10.24953/turkjped.2022.513

Case Report

Expanding the phenotype of *DYNC1H1*-associated diseases with a rare variant resulting in spinal muscular atrophy with lower extremity predominance (SMA-LED) and upper motor neuron signs

Jessica Lee[1], Philip Millington[1], Kavinda Dayasiri[2], Sithara Ramdas[1], Sandeep Jayawant[1], Geetha Anand[1]

[1]Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom; [2]Faculty of Medicine, University of Kelaniya, Sri Lanka, and, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom.

ABSTRACT

Background. Spinal muscular atrophy with lower extremity predominance (SMA-LED) is an autosomal dominant disorder. Since SMA-LED affects lower motor neurons, the disease is characterized by weakness and atrophy of lower limb muscles. We present a familial case series of SMA-LED with upper motor neuron signs associated with a rare variant in *DYNC1H1*.

Case. The index case was referred to Pediatric Neurology at the age of two and half years, due to delayed mobility. The child was diagnosed with congenital vertical talus at birth, which was managed with serial bilateral casting and surgery. The delayed mobility was initially attributed to lower limb weakness secondary to prolonged periods of immobilization from casting of his lower limbs. He had a striking waddling gait and proximal muscle weakness on neurological assessment. He had lower motor neuron signs predominantly in his lower limbs that were in keeping with SMA-LED. Surprisingly, he also demonstrated a brisk crossed adductor response that was not in keeping with an isolated primary neuro-muscular disorder and suggested a mixed upper and lower motor neuron pathology. The inherited neuropathy gene panel revealed a heterozygous sequence change in the *DYNC1H1* gene which was present in all affected family members.

Conclusions. We present the first report of a familial case series of SMA-LED with upper motor neuron signs associated with an extremely rare variant in *DYNC1H1*: c.1808A>T (p.Glu603Val). As per the American College of Medical Genetics and Genomics (ACMG) guidelines for variant classification, we would recommend that this variant be reclassified as "Likely Pathogenic" due to matching 1 moderate (PM1–PM6) and ≥4 supporting (PP1–PP5) criteria in the reported case series.

Key words: spinal muscular atrophy with lower extremity predominance, upper motor neuron signs.

Spinal muscular atrophy with lower extremity predominance is a small group of autosomal dominant neurological disorders. SMA-LED affects lower motor neurons and is thus characterized by wasting and weakness of the lower limb muscles. Two genetic variants have been associated with SMA-LED: *BICD2* and *DYNC1H1*. The *DYNC1H1* gene encodes cytoplasmic dynein 1 heavy chain 1, a protein of the dynein family.[1] Variants in the *DYNC1H1* gene have previously been associated with SMA-LED, with Charcot-Marie-Tooth disease, and separately with hereditary spastic paraplegia (HSP).[1-3] *BICD2* encodes a golgin (a component of dynein-based transport). Pathogenic variants of *BICD2* have been reported with SMA-LED, with SMA-LED with upper motor neuron signs, and separately with the HSP phenotype.[4-6] We present a familial case series of SMA-LED with

✉ Kavinda Dayasiri
kavindadayasiri@gmail.com

Received 6th June 2022, revised 9th October 2022, accepted 18th April 2023.

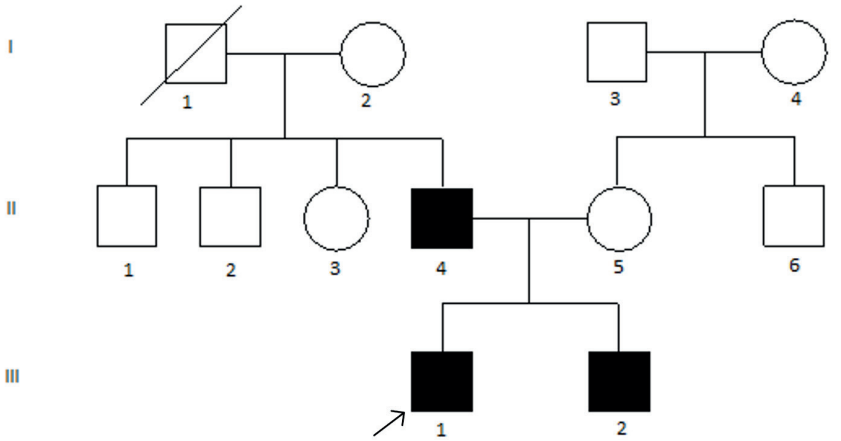


Fig. 1. Family Tree - Index case identified by arrow. Three affected family members as shown by filled squares.

upper motor neuron signs associated with a rare variant in *DYNC1H1*. We describe the clinical phenotypes of three family members across two generations - a father and two sons (Fig. 1).

Case Report

The index case is a five-year-old male child born at term after an uneventful pregnancy and a forceps delivery. He was noted to have congenital vertical talus, which was managed with serial bilateral casting and surgery. His walking was delayed but the rest of his developmental milestones were within normal range. This delay in ambulation was initially attributed to lower limb weakness secondary to prolonged periods of immobilization from casting of his lower limbs. However, at two and a half years he was still able to mobilize only when in bilateral ankle-foot orthoses and with the assistance of a walking frame, and so was referred to Pediatric Neurology by his Orthopedics team. He was unable to take more than a couple of steps unsupported, falling primarily due to proximal weakness. On examination, the most striking abnormality was a waddling gait. He walked with externally rotated lower limbs and his hip mobility demonstrated excessive external rotation and limited internal rotation bilaterally. He had almost lichenified prepatellar skin thickening, as a result of ambulating on his knees. The proximal and distal muscles of his lower limbs were markedly wasted. Tone was generally decreased in both lower limbs but with tightness noted bilaterally in his iliotibial bands, hamstrings, and Achilles tendons. Power in all muscle groups of his lower limbs was diminished. Deep tendon reflexes in his lower limbs were difficult to elicit given the ankle deformity and lichenification of the skin overlying the patella. However, a crossed adductor response was present. Tone, power, and deep tendon reflexes were normal in his upper limbs, with no evidence of muscle wasting. Sensation was normal in both his upper and lower limbs. Cranial nerve and cerebellar examinations were normal, as was cognition. He was attending a mainstream school without any special educational needs.

The father of the index case was not able to recollect his age of independent ambulation. He recalled requiring a wheelchair during his childhood. He reported gradual improvement in his strength during his youth and that he was eventually able to walk unsupported on flat surfaces. Nonetheless, he still requires the support of a banister when ascending stairs, while able to descend stairs unsupported. When examined he also exhibited bilateral lower limb proximal weakness with a waddling gait, proximal and distal lower limb muscle atrophy, and brisk crossed adductor responses.

Additionally, his youngest son, the index case's infant brother, demonstrated a similar phenotype, including the crossed adductor response (Fig. 2). Despite the proximal and distal lower limb wasting and weakness, the presence of a brisk crossed adductor response was not in keeping with an isolated primary neuromuscular disorder and suggested a mixed upper and lower motor neuron pathology.

Following the initial consultation, an inherited neuropathy gene panel was sent. Electromyography, nerve conduction tests and genetic tests looking at dynein heavy chain variants were performed. Imaging was requested, including magnetic resonance imaging (MRI) of his spine as well as anteroposterior and lateral X-ray views of his thoracolumbar spine and pelvis.

Thoracolumbar and pelvic X-rays did not show any evidence of spondyloepiphyseal dysplasia. The spine MRI was normal. Given the normal cognition in the father, lack of cognitive concerns in the index case, and clinical features pointing to a likely primary lower motor neuron pathology, brain MRI was felt not to be clinically indicated. Neurophysiological testing showed moderate to severe chronic denervation of the lower limb muscles with normal sensory responses. Creatine kinase was 210 IU/L (normal range: 30-200). The inherited neuropathy gene panel revealed a heterozygous sequence change in the *DYNC1H1* gene: c.1808A>T p.(Glu603Val), a single nucleotide missense variant, which was present in all affected family members.

Since diagnosis, the index case and his sibling have been followed up by the Pediatric Neuromuscular team. Further care has also been provided by Neuromuscular Physiotherapy, Community Pediatrics, and Orthopedics. Clinical progress is slow, with both brothers stable and ambulant with support.

Fig. 2. Findings of bilateral vertical talus, lower limb muscle atrophy, and thickened skin overlying patella shown in the index case (right), with similar features demonstrated in his father (left) and younger brother (center).

Discussion

We present a familial case series of SMA-LED with upper motor neuron signs associated with a rare variant in *DYNC1H1*. This was the first observation of the c.1808A>T (p.Glu603Val) variant at the reference laboratory and has not previously been listed in the Genome Aggregation Database (gnomAD), suggesting an extremely rare variant. Due to the segregation of the variant within the family, this was presumed to be a *de novo* variant in our patient's father. Searching the ClinVar database revealed one reported case with the same variant, in a patient with SMA-LED.[1] This case demonstrated similar findings to our index case, presenting with congenital talipes, lower limb muscle atrophy and weakness, and a requirement for ankle-foot orthoses for independent walking. A sibling without diagnostic genetic confirmation was noted to have similar features and an autosomal dominant mode of inheritance was postulated. However, in contrast to our index case, there were no associated upper motor neuron signs. Previous work has expanded the clinical phenotype of *DYNC1H1* to include upper motor neuron disease, describing a family with hereditary spastic paraplegia, complicated by intellectual disability, epilepsy, cataracts, and a thin corpus callosum.[2,3] Additionally, the clinical phenotype of SMA-LED with upper motor neuron signs has also been previously demonstrated with mutations in *BICD2*.[6]

According to ClinVar, the *DYNC1H1* gene c.1808A>T (p.Glu603Val) variant was hitherto classified as being a variant of uncertain significance. As per the American College of Medical Genetics and Genomics (ACMG) guidelines for variant classification, we would recommend that this variant be reclassified as "Likely Pathogenic" due to matching 1 moderate (PM1–PM6) and ≥4 supporting (PP1–PP5) criteria[7]; the variant has not been detected in population controls and is thus not listed in the gnomAD database (PM2). Other missense variants in the *DYNC1H1* gene are known to cause similar neurological disease[1] and we have shown this variant has been demonstrated to co-segregate with disease in members of one family (PP1, PP2). The family's phenotype and presentation are highly specific for a disease with a single genetic etiology (PP4) and *in silico* analysis performed in the laboratory predicts a deleterious effect of this variant on the gene product (PP3).

We have submitted this variant to the ClinVar database, accession number: SCV001793786.1 https://www.ncbi.nlm.nih.gov/clinvar/variation/637517/

Ethical approval

Written informed consent for patient information and images to be published was provided by parents of reported children.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SJ, GA; data collection: JL, PM, KD; analysis and interpretation of results: SR, SJ, GA; draft manuscript preparation: KD, JL, PM. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Scoto M, Rossor AM, Harms MB, et al. Novel mutations expand the clinical spectrum of DYNC1H1-associated spinal muscular atrophy. Neurology 2015; 84: 668-679. https://doi.org/10.1212/WNL.0000000000001269
2. Strickland AV, Schabhüttl M, Offenbacher H, et al. Mutation screen reveals novel variants and expands the phenotypes associated with DYNC1H1. J Neurol 2015; 262: 2124-2134. https://doi.org/10.1007/s00415-015-7727-2
3. Becker LL, Dafsari HS, Schallner J, et al. The clinical-phenotype continuum in DYNC1H1-related disorders-genomic profiling and proposal for a novel classification. J Hum Genet 2020; 65: 1003-1017. https://doi.org/10.1038/s10038-020-0803-1
4. Martinez-Carrera LA, Wirth B. Dominant spinal muscular atrophy is caused by mutations in BICD2, an important golgin protein. Front Neurosci 2015; 9: 401. https://doi.org/10.3389/fnins.2015.00401
5. Rossor AM, Oates EC, Salter HK, et al. Phenotypic and molecular insights into spinal muscular atrophy due to mutations in BICD2. Brain 2015; 138: 293-310. https://doi.org/10.1093/brain/awu356
6. Oates EC, Rossor AM, Hafezparast M, et al. Mutations in BICD2 cause dominant congenital spinal muscular atrophy and hereditary spastic paraplegia. Am J Hum Genet 2013; 92: 965-973. https://doi.org/10.1016/j.ajhg.2013.04.018
7. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-424. https://doi.org/10.1038/gim.2015.30

The Turkish Journal of Pediatrics 2023; 65: 536-541
https://doi.org/10.24953/turkjped.2020.1934

Case Report

DOCK8 deficiency with hypereosinophilia and the syndrome of inappropriate antidiuretic hormone secretion during herpes infection

Ayşe Mete Yeşil[1], Başak Kayaoğlu[3], Ersin Gül[3], Nazlı Gönç[4], Alev Özön[4], İlhan Tezcan[2], Mayda Gürsel[3], Deniz Çağdaş[2]

[1]Department of Pediatric, Hacettepe University Faculty of Medicine, Ankara; [2]Department of Pediatric Immunology, Hacettepe University Faculty of Medicine, Ankara; [3]Department of Biological Sciences, Middle East Technical University, Ankara; [4]Department of Pediatric Endocrinology, Hacettepe University Faculty of Medicine, Ankara, Türkiye.

ABSTRACT

Background. Hyperimmunoglobulin E syndrome (HIES) due to dedicator of cytokinesis8 (DOCK8) deficiency may present in infancy and childhood with different clinical features involving recurrent infections, allergic dysregulation, and autoimmunity.

Case. In this report, we describe a patient who first presented with severe hypereosinophilia and went on to develop the syndrome of inappropriate antidiuretic hormone secretion (SIADH) in the context of a severe herpes infection. Investigation revealed the presence of underlying DOCK8 deficiency presenting with atypical clinical features.

Conclusions. Distinct inflammatory features associated with infections may be seen in the course of primary immunodeficiency diseases, and early functional and molecular genetic tests will aid the proper management.

Key words: dedicator of cytokinesis (DOCK8) deficiency, syndrome of inappropriate antidiuretic hormone secretion (SIADH), interferon response.

Hyperimmunoglobulin E syndrome (HIES) is a form of primary immunodeficiency characterized by elevated serum IgE, rash, and recurrent skin and sinopulmonary bacterial infections.[1] Heterozygous mutations in the *STAT3* gene cause autosomal dominant (AD) HIES[2,3] and biallelic mutations in the dedicator of cytokinesis8 (*DOCK8*) gene cause autosomal recessive (AR) form of HIES, which was first described in 2009.[4,5]

Patients with AR-HIES, caused by the defects in the *DOCK8*, *PGM3*, *ERBIN*, and *ZNF341* genes, do not generally fulfill the criteria described for AD-HIES caused by *STAT3* gene defect.[5-7] DOCK8 deficiency presents with recurrent viral cutaneous infections, asthma, food/airborne allergies, and changes in immunoglobulin and T cell levels that are typical features for differentiation of DOCK8 deficiency from other AR-HIES. In a large case series of DOCK8 deficiency, eczema was detected in 99% of patients whereas, recurrent respiratory and persistent viral infections occurred in 91 and 80% of cases, respectively.[8] Allergies were observed in 71% of patients and were mostly caused by food allergens and abscesses (60%). Pneumonia, sepsis, and cerebral infections were common life-threatening infections, respectively experienced in 32%, 29%, and 22% of patients, in this study.[8]

Herein, we present a child with severe hypereosinophilia, cow milk allergy, recurrent herpetic skin lesions, and syndrome of inappropriate antidiuretic hormone secretion (SIADH) in the presence of DOCK8 deficiency.

✉ Ayşe Mete Yeşil
draysemeteyesil@gmail.com

Received 6th August 2020, revised 29th March 2021, accepted 29th November 2021.

Case Report

A 4-month-old girl, the first child of parents from the same village, had severe eosinophilia (30%) on admission to the emergency department with fever. There was no evidence of dysplasia in the bone marrow smear. Platelet-derived growth factor receptor alpha polypeptide (*PDGFRA1*) gene defect responsible for the myeloproliferative variant of hypereosinophilic syndrome was negative. Evaluation of eosinophilia revealed cow milk protein allergy in the patient, and the elimination of cow milk led to the regression of eosinophilia. At 9 months of age, she had an urticarial rash after ingesting cow milk, which regressed following oral antihistamines. She was hospitalized for severe oral lesions, possibly due to herpetic gingivostomatitis. A week later, widespread vesicular lesions on the face and hands and ear discharge developed, and she was admitted to our center. On physical examination, her weight was 8.5 kg (25-50 percentile), height was 70 cm (25th percentile), and head circumference was 44 cm (<3 percentile). She had a widespread herpes infection affecting the right hemifacial region, mouth, and hands. Complete blood count showed anemia, leukocytosis with mild neutropenia, and severe eosinophilia (Table I). Erythrocyte sedimentation rate was 76 mm/hr (normal range [NR]: 0-20); C-reactive protein (CRP) was 2.07 mg/dL (NR: 0-0.8). Sulbactam-ampicillin and acyclovir treatment were started for the herpes infection. Combined immunodeficiency was considered due to the severe and recurrent viral skin infection, food allergy, eosinophilia, and elevated serum IgE. Monthly intravenous immunoglobulin (IVIG) was started together with antibacterial and antifungal prophylaxis. Abdominal ultrasonography (USG) and echocardiography

Table I. Laboratory findings of the patient.

	First admission	Second month of the assessment	References
White blood cells, mm^3	15900	17500	6400-13000
Absolute lymphocyte count, mm^3	6100	7800	3400-9000
Absolute neutrophil count, mm^3	1400	2600	1500-8500
Absolute eosinophil count, mm^3	1500	5600	0-500
IgA (mg/dL)	13.6	19.9	17-69
IgG (mg/dL)	820	939	463-1006
IgM (mg/dL)	114	132	46-159
Total IgE (IU/mL)	14	511	(0.76-7.31)
Lymphocyte subsets, % (/mm^3)			
CD3	42% (2226)	28% (2128)	53-75% (3600-8900)
CD4	22% (1166)	16% (1216)	32-51% (1300-3400)
CD8	18% (954)	14% (1064)	14-30% (620-2000)
CD16/56	3% (159)	2% (152)	3-15% (180-920)
CD19	49% (2597)	66% (5016)	16-35% (2100-6200)
Lymphocyte proliferation test, SI (P/C)			
PHA	50/60		
Con A	39/48		
PMA/Ion	8/2		

Con A: concanavalin A, Ig: mmunoglobulin, P/C: patient/control, PHA: phytohemaglutinin, PMA/Ion: PMA/ionomycin, SI: stimulation index

were normal. There were few eosinophils in microscopy of the bronchoalveolar lavage (BAL) fluid of the patient, who had ground glass changes on thoracal computerized tomography (CT). CMV PCR was 496426 copies/mL in BAL fluid. Gancyclovir, and due to severe hypereosinophilia, methylprednisolone (2 mg/kg/day) was added to the therapy. Eosinophilia resolved, and the patient was discharged with steroids after 6 weeks of hospitalization. In the *DOCK8* gene analysis, a homozygous large deletion was detected, and the DOCK8 protein expression was absent (Fig. 1).

One week after her discharge, the patient developed blurred consciousness, and severe hyponatremia was detected without any signs of dehydration. Her vital signs were normal. No edema and hepatomegaly were present on physical examination. Laboratory tests showed hyponatraemia (114 mEq/L), hypochloremia (86 mEq/L), and normokalemia (5.17 mEq/L) with low plasma osmolality (245 mOsm/kg, NR: 275-295) and inappropriately high urinary osmolality (451 mOsm/kg). Urinary sodium (112 mEq/L, NR: 15-267 mEq/L), and β-2 microglobulin levels (192 ng/mL, NR: 0-300 ng/mL) were normal. Serum glucose and lipid profile were within normal limits, whereas serum uric acid was very low (0.7 mg/dL, NR: 1.8-5.0 mg/dL), and serum blood urea nitrogen (8.04 mg/dL, NR: 5-15) and creatinine levels (0.18 mg/dL, NR: 0.03-0.5) were normal. Blood gas analysis, thyroid function tests (free T4: 11.8 pmol/L, NR: 9.0-25.7; TSH: 0.53 mIU/mL, NR: 0.5-4.5 mIU/ml) were normal. Plasma renin and aldosterone concentrations were in the low-normal range showing a lack of dehydration (7.31 pg/mL, NR: 2.71-16.51; and 36 pg/mL, NR: 30.7-275 respectively). Regarding severe hyponatremia, plasma ADH level was inappropriately high (1.78 pmol/L), suggesting a lack of ADH secretion suppression. These confirmed the diagnosis of SIADH. Magnetic resonance imaging of the brain and hypophysis of the patient revealed normal brain and anterior pituitary gland with the absence of a bright spot of the neurohypophysis. Acyclovir was given for the recurrent herpetic lesions around the mouth, and fluid restriction was commenced for the SIADH management. Oral salt and loop-diuretics were applied, as fluid restriction was not possible. SIADH resolved in a four-month period.

The interferon response to stimulation with various nucleic acids (immune-stimulatory DNA (ISD), polydA:dT, polyI:C, D type CpG ODN) and cyclic dinucleotide cGAMP was tested in the patient's peripheral blood mononuclear cells during the hospitalization for herpes infection. The analysis revealed a markedly reduced type I interferon response compared to healthy controls (Fig. 2). The defective interferon (IFN) response was not only restricted to stimulation with the plasmacytoid dendritic cell targeting toll-like receptor 9 (TLR9) ligand D35 as previously

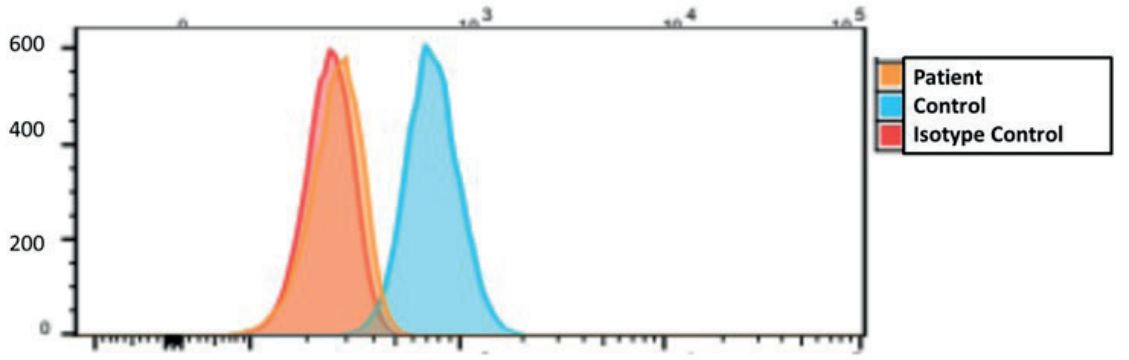


Fig. 1. DOCK8 expression was absent in the patient

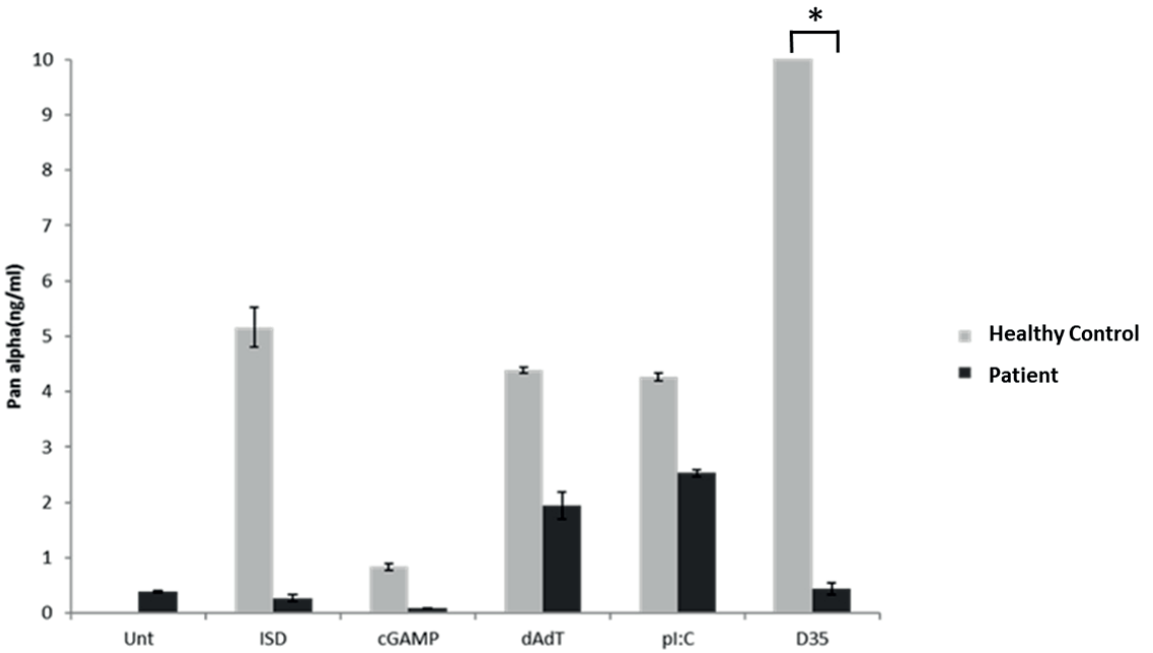


Fig. 2. Interferon response to several stimulating agents were low compared to healthy controls. Experiments were performed at least three times and each reaction was run in triplicate. cGAMP: cyclic dinucleotide guanosine monophosphate – adenine monophosphate, D35: D type CpG ODN, dAdT: polydA:dT, ISD: immune stimulatory DNA, Pan alpha: pan-specific interferon-α, pI:C: polyC, Unt: Untreated. *Statistically significant ($p<0.05$).

reported[9] but was also pronounced when cyclic GMP-AMP synthase (cGAS) and STING (Stimulator of Interferon Genes) ligands ISD and cGAMP were employed. The patient was discharged at 13 months of age and underwent stem cell transplantation in another center one year later.

Informed consent was obtained from the family for the publication of the case report.

Discussion

When eosinophilia accompanies recurrent viral infections and allergic symptoms (asthma, food/airborne allergies), the DOCK8 deficiency should be in the differential diagnosis. Since there is a high risk of mortality and malignancy, prompt diagnosis and evaluation for bone marrow transplantation as well as confirmation of the diagnosis using molecular genetic analysis are critically important for immediate and proper management.[10] Although our case had distinctive clinical features, a prompt diagnosis was achieved within a short time, and bone marrow transplantation was performed.

Another important finding in our patient was the low level of IgE when she presented firstly. We also observed that the IgE level may be low[8] in the early course of the disease and increases progressively in other patients with the *DOCK8* gene defect.

The skin is one of the major affected organs in patients with DOCK8 deficiency. Recurrent herpes infections, as in our patient, molluscum contagiosum infection, mucocutaneous candidiasis, and malignancies have been reported.[8] In a multicenter study, cutaneous viral infections have been reported in 68% of cases (n=40).[10] It is difficult to manage the viral cutaneous infections in DOCK8-deficient patients.[3] Recurrent herpetic infections in our patient caused poor oral intake of the patient and required intravenous antiviral therapy.

Hyponatremia due to SIADH is a presenting feature of several diseases, such as pulmonary infections, malignancies, or CNS infections.[11] A localized herpes infection in the patient was associated with the SIADH. Severe infections of Epstein –Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus (VZV) may also cause SIADH.[12,13] Although SIADH due to disseminated herpes infections due to CNS involvement has been well described[13], SIADH associated with localized herpes has been rarely reported. It has been first reported in two adult patients with localized herpes zoster ophthalmic infection. In this study, it is reported that SIADH was treated with fluid restriction and a high salt diet, and lasted for 3 to 4 months.[12] As SIADH may be associated with central nervous system pathologies[13], we suggest that these patients and our patient who has infections around the head region may have yet asymptomatic central nervous system involvement, which may have regressed due to antiviral therapies.

Inadequate IFN-α production and plasmocytoid dendritic cell deficiency have been reported in DOCK8 deficiency. A patient diagnosed with refractory warts in addition to DOCK8 deficiency was reported to have benefited from IFN- α 2b treatment.[9] During SIADH, which seems to occur due to asymptomatic central nervous system involvement, the patients may benefit from IFN-α 2b treatment.

The expression of IFN-γ was known to be decreased in DOCK8 deficiency while interleukin 4 and interleukin 5 are increased leading to T helper-2 response and hypereosinophilia respectively. In a DOCK8 defective patient, IFN-γ is measured as about half of the values in the healthy control.[14] We planned to test the IFN-α response in the patient during hospitalization for severe herpes infection before the test for DOCK8 protein expression and molecular genetic analysis. A benefit of functional studies is the definition of the new findings in a known disease. It is known that low IFN-α response is seen in DOCK8 deficiency. However, it is reported previously that the defective IFN-α response was due to stimulation with the plasmacytoid dendritic cell targeting TLR9 ligand D35.[9] The novel finding in our report is that the IFN-α response is also low when cGAS and STING ligands ISD and cGAMP were employed. The defective interferon alpha response is not only mediated with TLR9, but also mediated with cGAS and STING.

The presentation of the present patient with DOCK8 deficiency was diverse. The absence of IgE elevation at presentation and the SIADH in the course of the disease were important findings. Low interferon response and low DOCK8 protein expression helped the early diagnosis of the patient.

Uncontrolled herpes infection in the present patient likely triggered the SIADH. Distinct inflammatory features associated with infections may be seen in the course of primary immunodeficiencies, and early functional and molecular genetic tests will aid the proper management.

Acknowledgement

We appreciate Dr. Talal Chatila and Janet Chou for testing the DOCK8 protein expression and *DOCK8* gene analysis. We thank the patient and the family, and the healthcare workers.

Ethical approval

Informed consent was obtained from the family for the publication of the case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AMY, DC; data generation and collection: AMY, DC, MG, BK, EG; analysis and interpretation of results: AMY, DC, MG, İT, NG, AÖ, İT, MG, DÇ; draft manuscript preparation: AMY, DÇ. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

Molecular genetic tests of the patient and functional analysis were performed within the scope of two projects together with other international collaborative efforts. The projects were supported by the Scientific and Technological Research Council of Turkey (TUBITAK 1003), and Hacettepe University Coordination Unit for Scientific Research Projects (Project Numbers: 315S125 and 11/19-23).

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Freeman AF, Holland SM. The hyper-IgE syndromes. Immunol Allergy Clin North Am 2008; 28: 277-291. https://doi.org/10.1016/j.iac.2008.01.005
2. Freeman AF, Collura-Burke CJ, Patronas NJ, et al. Brain abnormalities in patients with hyperimmunoglobulin E syndrome. Pediatrics 2007; 119: e1121-e1125. https://doi.org/10.1542/peds.2006-2649
3. Ling JC, Freeman AF, Gharib AM, et al. Coronary artery aneurysms in patients with hyper IgE recurrent infection syndrome. Clin Immunol 2007; 122: 255-258. https://doi.org/10.1016/j.clim.2006.10.005
4. Zhang Q, Davis JC, Lamborn IT, et al. Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med 2009; 361: 2046-2055. https://doi.org/10.1056/NEJMoa0905506
5. Engelhardt KR, McGhee S, Winkler S, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol 2009; 124: 1289-1302. https://doi.org/10.1016/j.jaci.2009.10.038
6. Cavkaytar O, Cagdas Ayvaz D, Keskin O, et al. A case of DOCK8 deficient hyper-IgE syndrome presenting primarily with eczema, food allergy, and asthma. Pediatr Allergy Immunol Pulmonol 2013; 26: 48-51. https://doi.org/10.1089/ped.2012.0179
7. Shahin T, Aschenbrenner D, Cagdas D, et al. Selective loss of function variants in IL6ST cause Hyper-IgE syndrome with distinct impairments of T-cell phenotype and function. Haematologica 2019; 104: 609-621. https://doi.org/10.3324/haematol.2018.194233
8. Aydin SE, Kilic SS, Aytekin C, et al. DOCK8 deficiency: clinical and immunological phenotype and treatment options - a review of 136 patients. J Clin Immunol 2015; 35: 189-198. https://doi.org/10.1007/s10875-014-0126-0
9. Al-Zahrani D, Raddadi A, Massaad M, et al. Successful interferon-alpha 2b therapy for unremitting warts in a patient with DOCK8 deficiency. Clin Immunol 2014; 153: 104-108. https://doi.org/10.1016/j.clim.2014.04.005
10. Engelhardt KR, Gertz ME, Keles S, et al. The extended clinical phenotype of 64 patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol 2015; 136: 402-412. https://doi.org/10.1016/j.jaci.2014.12.1945
11. Cousland Z, Payne C, El-Mahmoudi B. Hyponatraemia and SIADH as a presenting feature of acquired immunodeficiency syndrome. In: Society for Endocrinology BES 2011. Birmingham, UK: BioScientifica, 2011.
12. Wang CC, Shiang JC, Chen JT, Lin SH. Syndrome of inappropriate secretion of antidiuretic hormone associated with localized herpes zoster ophthalmicus. J Gen Intern Med 2011; 26: 216-220. https://doi.org/10.1007/s11606-010-1517-4
13. Kuriki A, Ishihara K, Satoh H, Sugie M, Kato H, Kawamura M. Syndrome of inappropriate secretion of anti-diuretic hormone associated with limbic encephalitis due to herpes simplex virus infection--a case report. Rinsho Shinkeigaku 2008; 48: 184-190. https://doi.org/10.5692/clinicalneurol.48.184
14. Aryan Z, Nabavi M, Shabani M, et al. Hypomorphic DOCK8 deletion causes hypereosinophilic syndrome. Pediatr Blood Cancer 2020; 67: e28084. https://doi.org/10.1002/pbc.28084

CASE REPORTS

Printed by METEKSAN in Ankara, Turkey