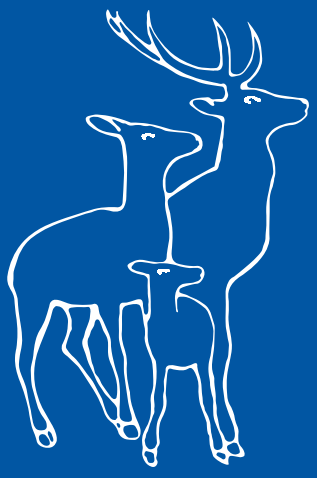
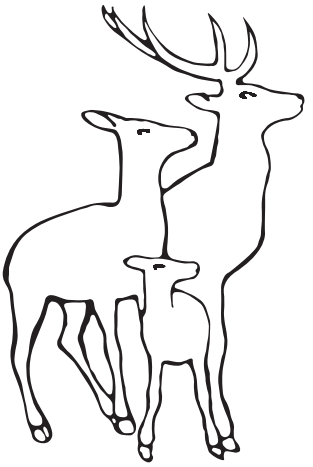


ISSN 0041-4301
Online ISSN 2791-6421
www.turkishjournalpediatrics.org

# THE TURKISH JOURNAL OF PEDIATRICS



**volume 64**
**number 2**
**March-April 2022**



# THE TURKISH JOURNAL OF PEDIATRICS

www.turkishjournalpediatrics.org

**Volume 64 ▪ Number 2**
**March-April 2022**

**ISSN: 0041-4301**
**Online ISSN: 2791-6421**

# THE TURKISH JOURNAL OF PEDIATRICS

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

Cilt: 64 Sayı: 2, Mart-Nisan 2022

**KURUCU**
İhsan DOĞRAMACI

**EDİTÖR**
Turgay COŞKUN

**YARDIMCI EDİTÖR**
Murat YURDAKÖK

**EDİTÖR YARDIMCILARI**
Ali DÜZOVA
Sinem AKGÜL

**YÜRÜTME KURULU**
Turgay COŞKUN
Murat YURDAKÖK
Ali DÜZOVA
Sinem AKGÜL

**YAYIN SAHİBİ**
**Yayınlayan Kurumlar Adına**
Elif Nursel ÖZMERT

**SORUMLU YAZI İŞLERİ MÜDÜRÜ**
Enver HASANOĞLU

**DÖKÜMANTASYON**
Ali DÜZOVA

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

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

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

**YAYININ TÜRÜ**
Yerel süreli yayın

**YAYIN ŞEKLİ**
İki aylık İngilizce

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

**BASIM TARİHİ:** XX.XX.2022

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

Vol: 64 Number: 2, March-April 2022

**FOUNDER**
İhsan DOĞRAMACI

**CHIEF-EDITOR**
Turgay COŞKUN

**DEPUTY EDITOR**
Murat YURDAKÖK

**ASSOCIATE EDITORS**
Ali DÜZOVA
Sinem AKGÜL

**MANAGING EDITORS**
Turgay COŞKUN
Murat YURDAKÖK
Ali DÜZOVA
Sinem AKGÜL

**PRODUCTION MANAGER**
**Owner on behalf of the Publishers**
Elif Nursel ÖZMERT

**ADMINISTRATOR**
Enver HASANOĞLU

**DOCUMENTATION and PUBLICATIONS**
Ali DÜZOVA

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

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

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

**PUBLICATION TYPE**
International peer-reviewed journal

**PUBLICATION FREQUENCY and LANGUAGE**
Bi-monthly ▪ English

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

**PRINT DATE:** XX.XX.2022

# THE TURKISH JOURNAL OF PEDIATRICS

**FOUNDER**
İhsan DOĞRAMACI

**CHIEF-EDITOR**
Turgay COŞKUN

**DEPUTY EDITOR**
Murat YURDAKÖK

**ASSOCIATE EDITORS**
Ali DÜZOVA
Sinem AKGÜL

**MANAGING EDITORS**
Turgay COŞKUN
Murat YURDAKÖK
Ali DÜZOVA
Sinem AKGÜL

**LANGUAGE EDITOR**
Deren RAMAN

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

**BIOSTATISTICS EDITORS**
Jale KARAKAYA
Sevilay KARAHAN

**EDITORIAL SECRETARY**
Derya TOLUNAY

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

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

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

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

# CONTENTS

VOLUME: 64 NUMBER: 2 MARCH-APRIL 2022

## REVIEW ARTICLE



## ORIGINAL ARTICLES

# CONTENTS

VOLUME: 64 NUMBER: 2 MARCH-APRIL 2022

# CONTENTS

VOLUME: 64 NUMBER: 2 MARCH-APRIL 2022

The Turkish Journal of Pediatrics 2022; 64: 187-209
https://doi.org/10.24953/turkjped.2020.2060

**Review**

# Maternal phthalate exposure during pregnancy and male reproductive disorders: a systematic review and meta-analysis

Chengjun Yu[1,2,3], Jiandong Lu[1,2,3], Jie Zhao[1,2,3], Tianxin Zhao[1,2,3,4], Chunlan Long[3,4,5], Tao Lin[1,2,3], Shengde Wu[1,2,3,5,6], Sheng Wen[1,2,3,5,6], Guanghui Wei[1,2,3,5,6]

*[1]Department of Urology, Children's Hospital of Chongqing Medical University, Chongqing, China; [2]Chongqing Key Laboratory of Children Urogenital Development and Tissue Engineering, Chongqing, China; [3]National Clinical Research Center for Child Health and Disorders, Chongqing, China; [4]Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing, China; [5]China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing, China; [6]Chongqing Key Laboratory of Pediatrics Chongqing, Chongqing, China.*

## ABSTRACT

**Background.** Phthalates are ubiquitous in the environment and they can penetrate the human body via multiple routes. However, the impact of phthalates on human male reproductive disorders remains unclear.

**Methods.** A critical review of published studies was conducted to clarify the association of phthalates and male reproductive disorders and to highlight future research needs. PubMed, Cochrane Library, and Web of Science Database were systematically searched for relevant articles written in English, independent of region and time period. If more than one paper overlapped in study design or participants included, the most recent manuscript was included in our review. Due to limited homogeneous statistical data, observed trends were summarized to draw approximate conclusions.

**Results.** Nineteen manuscripts were included in our final analysis. Exposure to di-(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and/or benzyl butyl phthalate (BBP) is associated with a shorter anogenital distance (AGD). Meanwhile, exposure to DEHP and/or di-isodecyl phthalate (DIDP) is associated with higher risks for cryptorchidism and hypospadias.

**Conclusions.** Generic exposure to phthalates has an adverse effect on human reproductive development, especially exposure to DEHP, DBP, DEP, BBP, and DIDP. A critical time for exposure sensitivity is during early pregnancy. Due to the lack of significant statistical power in this study, the conclusions drawn should be cautiously interpreted and they remain to be validated. Thus, additional well-designed studies, as well as propaganda and education regarding phthalate exposure and safer substitutes for these compounds, are greatly needed.

**Key words:** anogenital distance, cryptorchidism, hypospadias, male reproductive disorders, phthalates.

Phthalates are a class of manufactured chemicals that are commonly used to enhance the flexibility of plastics. By far, the major use of phthalates is as plasticizers in the production of polyvinyl chloride (PVC) products. PVC is the second most commonly used plastic in the world based on its use in pipes, construction materials, electronic wiring, and thousands of other applications. Phthalates are also used as additives in various consumer products, including food packaging, toys, shoes, cosmetics, and skin care products. Furthermore, phthalates are included in some medications and pesticides.[1]

✉ Shengde Wu and Sheng Wen
shengdewu@hospital.cqmu.edu.cn

Received 31st July 2020, revised 12th June 2021, accepted 6th July 2021.

Phthalate-containing products are quite common in daily life, yet they are not covalently bound to a product's matrix. Therefore, it is possible for phthalates to be released into the environment. As a result, phthalates have routinely been found to contaminate indoor air, dust, food, and water.[2-4] The major route of phthalate exposure is via ingestion of phthalate-contaminated food. However, phthalates can also permeate our body via dermal and inhalation pathways.[5]

Phthalates are endocrine disrupting chemicals (EDCs) which have been associated with male reproductive disorders (MRDs) in animal models over the past 20 years. For example, phthalates have been shown to act as anti-androgens and to downregulate the production of testosterone.[6,7] In rats, this is described as, "Phthalate Syndrome". Following *in* utero exposure to anti-androgen phthalates, a decrease in testosterone levels leads to an increased risk of conditions such as cryptorchidism and hypospadias.[7,8] Rodent studies have also identified that phthalates, especially di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP), can negatively affect androgen signaling when administered during a critical period of development in the genitourinary tract.[9,10] Furthermore, antenatal exposure to phthalates [notably DEHP, DBP, and butyl benzyl phthalate (BBzP)] induces a shorter anogenital distance (AGD) in rodents.[10,11]

To date, only a few studies have explored the relationship between prenatal phthalate exposure and the development of MRDs in humans. As a result, knowledge regarding this possible correlation remains incomplete. A key finding was made by Jensen et al.[12] who detected phthalate metabolites in amniotic fluid, thereby indicating the capacity for these compounds to cross the placental barrier and expose fetuses to their effects. Newborns are also vulnerable to endocrine disruption by chemicals, especially during the period of masculinization programming. The latter process of male sexual differentiation is driven by gonadal hormones. Here, we conducted a systemic review and meta-analysis to summarize the information currently available, to clarify associations between phthalate exposure and higher risks of cryptorchidism and hypospadias, as well as a shorter AGD, and to highlight future directions for studies of phthalates and their health risks.

## Methods

### *Definition of MRDs and phthalate exposure*

#### *MRDs examined*

Cryptorchidism refers to the testes being located somewhere along the path from the waist retroperitoneum down to the scrotum, yet not in accordance with the descent that normally occurs. Hypospadias is defined as displacement of the urethral meatus from the tip of glans penis to the ventral side of the phallus, scrotum, or perineum.[13] These conditions represent the two most common types of congenital defects that lead to abnormalities in male genitalia.[14,15] AGD is the distance from the center of the anus to an external genital landmark. In males, the external genital landmarks can include: (1) the base of the scrotum where the skin changes from rugated to smooth (AGDas); (2) the posterior or caudal insertion of the penis (AGDapp), and (3) the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone (AGDap).[16-18] Details regarding measurements of AGD are provided in Table I. AGD has been shown to serve as a readout of androgen concentrations during prenatal development in mammals.[19] Moreover, a shorter AGD in males is typically associated with higher risks for cryptorchidism and hypospadias. Since the AGD is easy to measure, it can have significant utility in clinical evaluations and epidemiological research studies. In this study, we identified risk of cryptorchidism, risk of hypospadias, and AGD as outcome parameters to assess the effect of prenatal phthalate exposure on MRDs.

#### *Exposure to phthalates*

Prenatal exposure to phthalates was defined as epidemiological exposure to generic phthalates

**Table I.** Anogenital distance (AGD) and exposure to phthalates (defined as urinary phthalate metabolites concentrations).

| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Definition and assessment of anogenital distance (AGD) (time of assessment) | Type of biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
|---|---|---|---|---|---|---|---|
| Adibi 2015 | Partly eligible subjects from The Infant Development and Environment Study (TIDES), prospective birth cohort | Maternal urine PMC detected by isotope-dilution tandem detection by isotope-dilution tandem mass spectrometry (first-trimester) | AGDas, AGDap in males and AGDaf, AGDac in females. Genital measurements were made by a trained study staff within a few days of birth. | Spot urine samples | Linear regression/ multivariate linear regression were used to estimate the relationship between phthalates and AGD z-score in male and female neonates with and without the hypothetical effect of hCG | MnBP, MBzP, MEHP, MEP, MiBP, MCPP, MCNP, MCOP | MEHP had a significant association with decreased AGDas in males before and after concerning the theoretical effect of hCG; higher MnBP induces shorter AGDas in males while higher MBzP induces longer AGDaf with significant difference when concerning the theoretical effect of hCG; |
| Bornehag 2014 | 196 mother-boy pairs were selected from Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA), a prospective birth cohort study | Urine samples, detected by isotope-dilution tandem mass spectrometry (first trimester, week 9-11 of pregnancy) | AGDas and AGDap, AGD was measured by two nurses (one pediatric staff nurse and one midwife) from the County Council of Värmland who were trained by research staff (20.8 ± 1.6 months, Mean ± SD) | First-trimester morning urine samples | Multiple linear regression modes were used to assess the relationship of log-transformed PMC and AGD after adjusted for adjustment for covariates (age, body size and creatinine concentrations); AGD was stratified and phthalate concentrations were compared by adjusted AGD quartile (short and long) | MBP, MEP, MBzP, oh-MMeOP, oxo-MMeOP, cx-MMeOP, MEHP, oh-MEHP, oxo-MEHP, cx-MEHP | Most of the phthalate metabolites were negatively associated with AGD before and after adjustment for covariates; all the 10 mentioned urine PMCs were much higher in short AGD group compared to long AGD group |

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

**Table I.** Continued.

| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Definition and assessment of anogenital distance (AGD) (time of assessment) | Type of biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
|---|---|---|---|---|---|---|---|
| Bustamante-Montes 2013 | 73 mothers–sons pairs, a hospital-based cohort study | Urine samples, detected by gas chromatography-mass spectroscopy (GC–MS), a modification of HPLC-MS /MS (third trimester) | AGDas, AGDap and AGDapp (the distance from the center of the anus to the posterior base of the penis), measured by 2 trained nurses (between 24 and 48 h after birth) | Third-trimester spot urine samples | Linear regression models were used to evaluate the association between prenatal MEHP and total phthalate exposure levels (μg/l), and AGDas, AGDap, AGDapp adjusted for creatinine and supine length at birth[a] | MEHP, MBP, MEP, MBzP | All three AGD measures showed inverse association trends with MEHP and "total phthalates"; shorter AGDap associated with "total phthalates" level with significance [β = −0.19mm/1μg/L (p = 0.04]; shorter AGDapp associated with MEHP [β = −0.07mm/1μg/L (p = 0.06] and "total phthalates" [β = −0.17mm/1μg/L (p = 0.09] with marginal significance |
| Huang 2009 | 65 mother–newborn pairs (33 boys, 32 girls), all participants and their fetuses were diagnosed as healthy after the chromosomes in the amniotic fluid had been evaluated | Five phthalate monoesters in amniotic fluid and urine samples from pregnant women were measured using liquid chromatography/tandem mass spectrometry (LC/MS-MS) | AGDaf in girls and AGDas in boys, measured twice by the same pediatrician and well-trained assistant (at birth) | Amniotic fluid and spot urine samples | Wilcoxon rank-sum test was used to evaluate differences between phthalates concentrations and fetal anthropometric measurements in high and low exposure group divided by Median levels; Spearman correlation coefficients was calculated to evaluate phthalates concentration and AGD | MBP, MEHP, MEP, MBzP, MMP | Association was detected between shorter AGDaf in girls and higher MBP in amniotic fluid with significance (p=0.024); and Spearman correlation coefficients showed marginally significant correlations between MBP in amniotic fluid and AGDaf in female newborns (p<0.06). Urine MBP was detected to be positively correlated with amniotic fluid MBP ($R^2$=0.156, p<0.05), so we may deduce: AGDaf in girls inversely associated with urinary MBP. In boys: no difference. |

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

**Table I.** Continued.

| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Definition and assessment of anogenital distance (AGD) (time of assessment) | Type of biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
|---|---|---|---|---|---|---|---|
| Jensen 2016 | 245 mother-boy pairs, eligibly selected from the Odense Child Cohort (Denmark) | Fasting spot urine samples, detected by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (median 28.7 weeks, range 26.4–30.4 weeks of gestation; second and third trimester) | AGDas, AGDap, measured three times by the same examiner, and calculated the arithmetic mean (3 months after the expected date of birth) | Fasting spot urine samples | An adjusted linear regression model was selected to evaluate the association between short AGD (AGDas) and long AGD (AGDap) in boys and quartiles of osmolality-adjusted concentrations of fasting urine phthalates metabolites | MEP, MiBP, MnBP, MBzP, ΣMBP(i + n) [b], ΣDiNPm[b], ΣDEHPm[b] | No significant dose-dependent association between any phthalate metabolites and AGDas & AGDap was found either in unadjusted or in adjusted analyses; but almost all estimates were negative, when comparing exposures above the first quartile in boys and exposures below the 25th percentile |
| Martino-Andrade 2016 | 168 mother-son pairs from TIDES | PMCs in urine samples, detected by the method of HPLC–MS⁄MS (first trimester, second trimester and third trimester) | AGDas and AGDap in boys, measured by two examiners for three times | Urine samples collected from first, second and third trimester | Multivariable linear regression models were used to determine the correlation between trimester-specific log10 SpG-adjusted concentrations of phthalate metabolites and AGDas &AGDap | MEHP, MEOHP, MEHHP, MECPP, ΣDEHP | Negative associations between log10 SpG-adjusted DEHP metabolite concentrations and both AGD measurements in T1 (data not show) |
| Sathyanarayana 2017 | 591 mother–newborn pairs selected from TIDES | Urine samples, detected by high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLCESI-MS/MS) (in early pregnancy) | AGDas and AGDap in boys, AGDaf and AGDac in girls, measured by examiners after 2-day training (shortly after birth) | Blood and urine samples | One multiple linear regression was used to explore the associations between log transformed prenatal urinary PMCs and log transformed maternal serum hormone concentrations; another multiple linear regression was used to explore the associations between log transformed prenatal hormone concentrations and AGDas, AGDap, and AGDac, and AGDaf, adjusted for several covariates[c] | MBP, MBzP, MEP, MiBP, MCNP, MEHP, MCOP, MEHHP, MEOHP, MECPP, sum DEHP | Only MCNP and MECPP correlated with lower free testosterone (FT) with statistical significance, but all DEHP metabolites were inversely related to FT. Higher maternal FT in early pregnancy was associated with a 25% lower prevalence of having a genital abnormality at birth in males, odds ratio, 0.10 (95% CI 0.01, 0.94), P<0.05. So we may deduce: higher DEHP metabolites (at least MECPP) and MCNP concentrations associated with increased prevalence of male reproductive disorders, and may induce shorter AGD |

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

**Table I.** Continued.

| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Definition and assessment of anogenital distance (AGD) (time of assessment) | Type of biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
|---|---|---|---|---|---|---|---|
| Suzuki 2011 | Selected 111 Japanese pregnant women and their male newborns | Spot urine samples, determined by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS⁄MS) (9th to 40th week of gestation) | AGD1: from the anus to the anterior genitalia and AGD2 to the posterior genitalia, examined by medical staff 1–3 days after birth using a plastic set of digital calipers. | Spot urine | A multiple regression model was used to assess the log-transformed PMC and AGI, AGD was corrected to birth weight and defined as the anogenital index (AGI, expressed in mm⁄kg) | DEHP, MMP, MEP, MnBP, MBzP, MEHHP, MEOHP | Shorter AGI1 for DEHP with significant difference (r=-0.189, p=0.047); ΣDEHP is associated with shorter AGI1 but did not reach significance (r=-0.140, p=0.144) and inverse relationships were also seen between AGI2 and ΣDEHP |
| Swan 2008 | 140 mothers–sons pairs from Study for Future Families (SFF), a multi-center pregnancy cohort study | Urine samples, detected by isotope-dilution tandem mass spectrometry (mid-pregnancy) | AGDap in males and AGDac in females. Examined by trained physicians (first batch at 12.8 months after delivery, the second one later) | Spot urine | Divided in “shorter AGD” (lowest 25%) “intermediate AGD” and “longer AGD” (upper 25%), and compared summary statistics (mean, median and geometric mean) for metabolite concentrations | MEHP, MEHHP, MEOHP, MEP, MiBP, MMP, MBzP, MCPP, MBP (data not show) | Phthalate metabolite concentrations in “shorter” compared to “longer” AGD: serveral times higher (MEHP, MEHHP, MEOHP); higher (MEP, MiBP, MMP, MBP); lower (MCPP, MBzP) |
| Swan 2015 | 172 women (85 mother-boy pairs) included in this study were originally recruited into the first phase of the Study for Future Families (SFFI), multicenter pregnancy cohort study | Detected by the method of HPLC–MS⁄MS, | AGDas and AGDap in boys. Anthropometric measurements were conducted by medical staff under the supervision of pediatric physicians who were trained in its administration (2-36 months of age) | Blood and urine samples | In several kinds of specific PMCs, AGIs in high level group PMC compared to those in low level group PMC, ORs and 95%CIs was used to describe the difference | MBP, MEP, MBzP, MiBP | The corresponding ORs for high compared with low concentration of MBP, MEP, MBzP, and MiBP were 10.2, 4.7, 3.8, and 9.1, respectively (all p-values < 0.05) |

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ethnicity and gestational age.

**Table I.** Continued.

| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Definition and assessment of anogenital distance (AGD) (time of assessment) | Type of biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
|---|---|---|---|---|---|---|---|
| | | | | | In several kinds of specific PMCs, the visual inspection of mean (median) PMC levels between long and short AGI category | MBP, MEP, MBzP, MiBP | |
| | | | | | Divided the PMC into three categories by summary phthalate score, and compare the ORs in each concentration group | - | |
| Wenzel 2018 | 187 African American and 193 white mothers-newborns pairs | 8 phthalate metabolites in urine measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), (second trimester) | AGDas, AGDap in males and AGDaf, AGDac in females, measured by one of eight trained observers in triplicate and averaged (within 48 hour of birth) | Urine specimen | Covariate-adjusted multivariable linear regression models were used to identify the correlation between PMCs and AGDas and AGDap | MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MEP, MMP, ΣDEHP, ΣDBP | Inverse associations between all measured urinary phthalates and AGDap was identified, albeit only MEHP reached significance; while statistically significant positive associations was detected for AGDas with MBP, MiBP, MBzP, and ΣDBP |

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

(details provided in eligibility criteria) or urinary phthalate metabolite concentrations (PMCs). The main phthalates examined and their urinary metabolites are listed in Table II.

### *Study registration*

This study was registered with PROSPERO describing the aims and methods in advance. The amendments specified the four target comparisons that were made between phthalate exposure and MRDs, and they discarded the initial goal of exploring AGD changes in male infants according to maternal epidemiological exposure to generic phthalates versus no exposure to phthalates. This study was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[20]

### *Information sources and search strategy terms*

Systematic literature searches of various databases were conducted. These databases included: PubMed, Cochrane Library, Web of Science Database, Russian Science Citation Index, and the SciELO Citation Index. Initially, a search was conducted to identify articles that considered antenatal phthalate exposure and MRDs/male genital abnormalities, or any outcome of cryptorchidism, hypospadias, or AGD in humans.

Using the Boolean approach, the above-mentioned databases were searched for the following key terms in titles, keywords, and Abstracts: ("male reproductive disorders" / "male genital abnormalities" OR "cryptorchidism" OR "cryptorchism" OR "undescended test*" OR "hypospadias" OR "anogenital distance" OR "AGD") AND ("phthalat*" and its specific types). In addition, two experts in the field advised us regarding the newest relevant studies and recommended the following articles published by Swan et al.[17], Sathyanarayana et al.[21], Martino-Andrade et al.[22], and Sathyanarayana et al.[23] A manual check of the reference lists of the relevant studies and reviews that were retrieved was also conducted. As a result, three additional manuscripts were selected according to the eligibility criteria of this study.

### *Eligibility and exclusion criteria*

*Criteria for study eligibility*

(1) Epidemiological studies examining generic exposure to total phthalates (with phthalates regarded as compounds rather than specific exposure to phthalate-containing products such as pesticides, cosmetics, and personal skin care products) which also provided data for an exposure group and a non-exposure group for examinations of risk of cryptorchidism, risk of hypospadias, or measurement of AGD.

(2) Clinical studies focused on exploring the relationship between risk of cryptorchidism, risk of hypospadias, or AGD and urine PMCs.

(3) Papers written in English, without restriction regarding the region studied or period of time studied, and involving human beings.

Manuscripts that satisfied criteria (1) and (3), or criteria (2) and (3), were considered eligible papers.

*2.4.2 Criteria for study exclusion*

(1) Animal models, reviews, guidelines, and *in vitro* or *in vivo* experimental studies.

(2) Studies addressing cellular or molecular mechanisms and other outcomes related to EDCs and MRDs (e.g., effects of hormone levels in tissues and measures of semen quality).

(3) Prenatal exposure to phthalates defined as concentrations in amniotic fluid, colostrum, or another biomarker matrix.

(4) Reports with a study design or participants that were updated in a subsequent report.

(5) Ecological studies with exposure information at the population level rather than at the individual level.

**Table II.** List of main specific phthalates and major urinary metabolites with abbreviations.

| Phthalates name | Abbreviation | Majority usage | Urinary metabolites | Abbreviation |
|---|---|---|---|---|
| Di-2-ethylhexyl phthalate, Di-(2-ethylhexyl) phthalate, Diethylhexyl phthalate | DEHP | Polychlorinated vinyl (PVC), flexible plastics (toys, film packaging, medical devices, building materials, garden hoses, etc.) | Mono-2-ethylhexyl phthalate | MEHP |
| | | | Mono-(2-ethyl-5-hydroxyhexyl) phthalate | MEHHP, oh-MEHP |
| | | | Mono-(2-ethyl-5-oxohexyl) phthalate | MEOHP, oxo-MEHP |
| | | | Mono-(2-ethyl-5-carboxypentyl) phthalate | MECPP, cx-MEPP |
| Di-*n*-butyl phthalate, Dibutyl phthalate | DBP | Personal care products, medications | Mono-n-butyl phthalate, | MnBP[a] |
| | | | Mono-isobutyl phthalate | MiBP[a] |
| Di-isobutyl phthalate | DiBP | Personal care products, medications | Mono-isobutyl phthalate | MiBP[a] |
| Benzylbutyl phthalate | BzBP | PVC, adhesives, sealants, car care products | Monobenzyl phthalate, Mono-benzyl phthalate | MBzP |
| Benzyl butyl phthalate | BBP | | | |
| Butyl benzyl phthalate | BBzP | | | |
| Di-isononyl phthalate | DINP, DiNP | PVC, flexible plastics | Mono-(carboxyoctyl) phthalate | MCOP |
| | | | Mono-isononyl phthalate | MiNP |
| | | | Mono-(4-methyl-7-hydroxyloctyl) phthalate | oh-MMeOP |
| | | | Mono-(4-methyl-7-oxo octyl) phthalate | oxo-MMeOP |
| | | | Mono-(4-methyl-7-carboxyheptyl) phthalate | cx-MMeHP |
| Di-isodecyl phthalate | DIDP, DiDP | PVC, flexible plastics | Mono-(carboxynonyl) phthalate | MCNP |
| Di-*n*-octyl phthalate | DOP, DnOP | PVC, flexible plastics | Mono-(3-carboxypropyl) phthalate | MCPP[b] |
| | | | Mono-n-octyl phthalate | MOP |
| Diethyl phthalate | DEP | Personal care products, fragrance | Mono-ethyl phthalate | MEP |
| Dimethyl phthalate | DMP | | Mono-methyl phthalate | MMP |

[a] sum of MnBP and MiBP is MBP.
[b] MCPP is also a minor metabolite of DBP and a non-specific metabolite of several high molecular weight phthalates( mainly refers to DEHP, BzBP, DOP, DiDP, DiNP, etc.)
AGD: anogenital distance, EDCs: endocrine disrupting chemicals, MRDs: male reproductive disorders, PMCs: phthalate metabolite concentrations.

### *Study selection and data extraction*

*Study selection*

Our initial database search yielded 592 potential articles. Four additional records were identified based on recommendations made by expert authorities, and three articles were identified based on a manual review of reference lists. After 61 duplicate articles were eliminated, 538 articles were screened based on their titles and abstracts. A total of 507 reports were identified as irrelevant papers and were excluded, leaving 31 records to undergo full text screening. After a comprehensive screening of these texts, 19 manuscripts were found to conform to the eligibility criteria of this systemic review and meta-analysis. An overview of this selection process and its details are presented in Figure 1.

*Data extraction*

Each of the 19 articles selected was carefully reviewed and the following data were extracted: reference information; population; method and time of exposure assessment; assessment of cryptorchidism and hypospadias, measurement of AGD, and time of assessment if available; type of biomarker matrix; pivotal design and comparisons methods; specific phthalates identified; and main discoveries. For the epidemiological studies, statistical data regarding the proportion of cryptorchidism and hypospadias in exposed and non-exposed groups were recorded. These data were maintained in three electronic spreadsheets (Tables I, III–IV) for comparison of incidence of cryptorchidism, hypospadias, and AGD in relation to phthalate exposure.

### *Statistical analysis*

It was a challenge to assess the pooled risk estimate of epidemiological exposure to generic phthalates and MRDs (mainly cryptorchidism and hypospadias). Heterogeneity of the manuscripts selected was tested by using both the Chi-squared test ($P \geq 0.1$ indicated low heterogeneity) and $I^2$ index statistics. When $I^2$ was < 50%, the Mantel-Haenszel fixed effects model was applied; otherwise, the Mantel-Haenszel random effects model was applied.[24]

In the subsequent analyses of urinary PMCs and MRDs, we analyzed possible associations between: (1) generic phthalate exposure and risk of cryptorchidism, hypospadias, and AGD; (2) specific phthalate (DEHP, DBP, benzyl butyl phthalate (BBP), etc.) exposure and incidence of generic MRDs; and (3) specific phthalate exposure and incidence of cryptorchidism, incidence of hypospadias, or AGD. Log-transformed regression of phthalate metabolite coefficients and p-values of adjusted models for AGD/anogenital index in five manuscripts are summarized in Table V.

## Results

### *Literature search results*

Figure 1 provides an overview of the screening and selection procedure used. Due to the limited number of published studies regarding human exposure to phthalates and MRDs, only 19 manuscripts met the inclusion criteria for this study. The papers that were excluded after a screening of their full text, and the corresponding reasons, are listed in Supplemental Table I. Among the 19 selected papers, 15 focused on exploring possible correlations between urinary PMCs and risk of cryptorchidism, risk of hypospadias, or measurement of AGD. The remaining four papers presented generic epidemiological studies (Wagner-Mahler et al.[25], Morales-Surez-Varela et al.[26], Nassar et al.[27], and Vrijheid et al.[28]). To date, there are no published studies which have focused on examining the risk of MRDs between mothers with undetectable urinary PMCs and those with detectable urinary PMCs.

### *Cryptorchidism and phthalate exposure*

Two epidemiological studies compared the risk of cryptorchidism between populations exposed to generic phthalates and populations that were not exposed to phthalates. Due to the high heterogeneity between these studies, a random-

**Table III.** Cryptorchidism and exposure to phthalates.

| Epidemiological generic exposure to phthalates | | | | | | | |
|---|---|---|---|---|---|---|---|
| Reference | Study type, location | Period | Population | Phthalates exposure | | No phthalates exposure | |
| | | | | Cryptorchidism | Normal | Cryptorchidism | Normal |
| Morales-Surez-Varela 2011 | Danish National Birth Cohort (DNBC), Denmark | March, 1997 to November, 2002 | A large number of pregnant women enrolled from DNBC | 30 | 1441 | 899 | 43900 |
| Wagner-Mahler 2011 | prospective study, France | April, 2002 to April, 2005 | Pregnant women from two main maternity wards in Nice area. | 4 | 1 | 91 | 187 |

| Phthalates exposure defined by measured urine phthalate metabolites concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|
| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Assessment of cryptorchidism (time of assessment) | Type of phthalate metabolite biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
| Chevrier 2012 | 50 cryptorchidism and selected 149 controls nested in the EDEN and PELAGIE mother-child cohorts | Questionnaires and job-exposure matrix (JEM), the measurement methods of urinary PMCs not show. (between 6 and 30 weeks) | Examined by pediatricians or midwives during the first days after birth | Urine | The odds-ratios was calculated for undescended testis according to tertiles of urinary concentrations of phthalate metabolites to make a comparison | MEP, MBP, MiBP, MBzP,MCPP, MEHP, MEOHP, MEHHP, MECPP, MCOP, MCNP | No significant association was found between metabolites urinary levels and cryptorchidism. Only a decreased trend was found in MEP: 3rd tertile vs. 1st tertile [0.38 (0.10-1.10), p=0.06] |

**Table III.** Continued.

| Phthalates exposure defined by measured urine phthalate metabolites concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|
| Reference | Study type, location | Period | Population | Phthalates exposure | | No phthalates exposure | |
| | | | | Cryptorchidism | Normal | Cryptorchidism | Normal |
| Sathyanarayana 2017 | 591 mother–newborn pairs selected from TIDES | Urine PMCs detected by HPLCESI-MS/MS (in early pregnancy) | NA | Blood and urine samples | Multiple linear regressions were used, details showed in Table 4 | MBP, MBzP, MEP, MiBP, MCNP, MEHP, MCOP, MEHHP, MEOHP, MECPP, sum DEHP | Higher DEHP metabolites (at least MECPP) and MCNP concentrations associated with increased prevalence of male reproductive disorders, including cryptorchidism |
| Swan 2008 | 140 boys and 153 girls eligible pregnancy women and children selected from Study for Future Families (SFF), multi-center pregnancy cohort study | Urine samples, detected by isotope-dilution tandem mass spectrometry (mid-pregnancy) | Examined by trained physicians (first batch at 12.8 months after delivery, the second one later) | Urine | A logistic regression model controlled for age and weight percentile to see the relationship between several kinds of phthalates metabolites and cryptorchidism | Mainly DEHP metabolites: MEHP, MEHHP, MEOHP, ΣDEHP | Probably higher phthalate metabolite associated with higher risk of cryptorchidism with marginally significant difference in: MEHP ($\beta$ = 1.3, P<0.05) MEHHP ($\beta$ = 1.4, p=0.05) MEOHP ($\beta$ = 1.4, p=0.06) ΣDEHP ($\beta$ = 1.4, p=0.05) |

**Table IV.** Hypospadias and exposure to phthalates.

| Epidemiological generic exposure to phthalates | | | | | | | |
|---|---|---|---|---|---|---|---|
| Reference | Study type, location | Period | Population | Phthalates exposure | | No phthalates exposure | |
| | | | | Hypospadias | Control | Hypospadias | Control |
| Morales-Surez-Varela 2011 | Danish National Birth Cohort (DNBC), Denmark | March, 1997 to November, 2002 | A large number of pregnant women enrolled from DNBC | 17 | 1441 | 227 | 43900 |
| Nassar 2009 | Registry-based case-control study, Australia | 1980 to 2000 | Hypospadias children were identified from the Western Australian Birth Defects Registry (WABDR), | 48 | 80 | 1027 | 2209 |
| Vrijheid 2003a | Data based on National Congenital Anomaly System (NCAS), International Agency | 1980 to 1996 | Hypospadias and controls (congenital anomaly) recorded from the National Congenital Anomaly System (NCAS) | 341 | 3330 | 3130 | 32632 |

[a] Though control group was consist of other congenital abnormalities, we could approximately thought they were independent from each other in large-scale researches, but this must be interpreted with caution.
[b] Low-MWP comprises MEP, MBP and MiBP.

**Table IV.** Continued.

| Phthalates exposure defined by measured urine phthalate metabolites concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|
| Reference | Papulation, study type | Methods used for exposure assessment (time of assessment) | Assessment of hypospadias (time of assessment) | Type of biomarker matrix | Pivotal design methods and comparisons | Description of phthalates metabolite types and levels of concentrations | Main results and discoveries |
| Chevrier 2012 | Selected 19 hypospadias and 57 controls nested in the EDEN and PELAGIE mother–child cohorts | Questionnaires and job-exposure matrix (JEM), the measurement methods of urinary PMCs not show. (between 6 and 30 weeks) | Examined by pediatricians or midwives during the first days after birth | Urine | The odds-ratios was calculated for hypospadias according to tertiles of urinary concentrations of phthalate metabolites to make a comparison | MEP, MBP, MiBP, MBzP,MCPP, MEHP, MEOHP, MEHHP, MECPP, MCOP, MCNP | No significant association was found between metabolites urinary concentration levels and hypospadias. Only a decreased trend was found in MEOHP: 3rd tertile vs. 1st tertile [0.07(0.00-1.20), p=0.09] and Low-MWP[b] [0.20 (0.02-2.50), P=0.06] |
| Choi 2012 | Volunteer urine and plasma samples of 80 hypospadias patients and 80 controls were collected from a medical college located in Seoul (details not show) | Target compounds were detected by A GC/MS instrument consisting of gas chromatograph and mass selective detector from Agilent Technologies | NA | Urine and plasma samples | Calculated the average, maximum, and minimum concentrations, standard deviations and P-values for target compounds in the urine samples of hypospadias group and control group to make a conclusion | DBP, MBP, DEHP, MEHP | DEHP levels (P = 0.006) in the urine showed statistically significant relationships with hypospadias |
| Sathyanarayana 2017 | 591 mother–newborn pairs selected from TIDES | Urine PMCs detected by HPLCESI-MS/MS (in early pregnancy) | NA | Blood and urine samples | Multiple linear regressions were used, details showed in Table 4 | MBP, MBzP, MEP, MiBP, MCNP, MEHP, MCOP, MEHHP, MEOHP, MECPP, sum DEHP | Higher DEHP metabolites (at least MECPP) and MCNP concentrations associated with increased prevalence of male reproductive disorders, including hypospadias |

[a] Though control group was consist of other congenital abnormalities, we could approximately thought they were independent from each other in large-scale researches, but this must be interpreted with caution.
[b] Low-MWP comprises MEP, MBP and MiBP.

**Table V.** Log-transformed regression phthalate metabolite coefficients and p-value of adjusted models for anogenital distance (AGD) or anogenital index (AGI).

| Reference | Design strategy and method of comparison | p< 0.05[a] | | | p> 0.05 | | |
|---|---|---|---|---|---|---|---|
| | | Urine phthalate metabolite | Regression phthalate metabolite coefficients of adjusted models for AGD/ AGI | P-value | Urine phthalate metabolite | Regression phthalate metabolite coefficients of adjusted models for AGD/AGI | P-value |
| Log-transformed regression phthalate metabolite coefficients of adjusted models for AGD | | | | | | | |
| Barrett 2015 | An interactive models was used to assess the association between PMCs and AGDas measurements to vary by maternal overall stress (lower stress and higher stress group, data not show) | molar sum ΣDEHP[b] | -1.26 | 0.020 | MEHP | -1.14 | 0.300 |
| | | MEOHP | -1.44 | 0.010 | MnBP | -0.95 | 0.310 |
| | | MEHHP | -1.47 | 0.010 | MEP | -0.34 | 0.300 |
| | | MECPP | -0.97 | 0.040 | MiBP | -0.52 | 0.360 |
| | | | | | MCPP | 0.18 | 0.350 |
| | | | | | MBzP | 0.15 | 0.600 |
| Bornehag 2014 | Multiple linear regression mode was used to assess the relationship of log-transformed PMC and AGDas after adjusted for adjustment for covariates (age, body saze and creatinine concentrations) | oh-MMeOP | -1.61 | 0.029 | cx-MMeOP | -1.51 | 0.091 |
| | | oxo-MMeOP | -1.82 | 0.031 | MEHP | -1.28 | 0.304 |
| | | molar sum ΣDiNP (nmol/L) | -1.69 | 0.047 | oh-MEHP | -1.24 | 0.374 |
| | | | | | oxo-MEHP | -0.77 | 0.576 |
| | | | | | cx-MEHP | -0.89 | 0.534 |
| | | | | | MBP | -1.41 | 0.351 |
| | | | | | MEP | 0.63 | 0.518 |
| | | | | | MBzP | -1.66 | 0.088 |

AGD: anogenital distance (mm); AGI: anogenital index, anogenital distance divided by weight (mm/ Kg);
PMCs: phthalate metabolites concentrations;
[a] In this paper, we think p< 0.05 denotes "reach statistical significance".
[b] ΣDEHP: molar sum of four DEHP metabolites, ΣDEHP=[MEHP*(1/278)]+[MEHHP*(1/294)]+[MEOHP*(1/292)]+[MECPP*(1/308)].

**Table V.** Continued.

| Reference | Design strategy and method of comparison | p< 0.05[a] | | | p> 0.05 | | |
|---|---|---|---|---|---|---|---|
| | | Urine phthalate metabolite | Regression phthalate metabolite coefficients of adjusted models for AGD/ AGI | P-value | Urine phthalate metabolite | Regression phthalate metabolite coefficients of adjusted models for AGD/AGI | P-value |
| Swan 2008 | A mixed model was used to control for age and weight percentile, and assess the link of logarithmically PMCs and AGD | MEHP | -3.50 | 0.017 | MiBP | -2.96 | 0.097 |
| | | MEHHP | -4.98 | 0.002 | MBzP | -0.31 | 0.826 |
| | | MEOHP | -5.13 | 0.001 | MCPP | -0.97 | 0.591 |
| | | MEP | -2.93 | 0.005 | | | |
| | | MBP | -3.26 | 0.049 | | | |
| | | MMP | -4.00 | 0.053 | | | |
| Log-transformed regression phthalate metabolite coefficients of adjusted models for AGI | | | | | | | |
| Suzuki 2011 | A multiple regression model was used to assess the log-transformed PMC and AGI, AGD was corrected to birth weight and defined as the anogenital index (AGI, expressed in mm/kg) | MEHP | -0.25 | 0.011 | | | |
| Swan 2015 | Regression analyses of AGI on log10 PMCs controlled for age and age squared | MBP | -0.59 | 0.031 | MBzP | -0.39 | 0.097 |
| | | MEP | -0.40 | 0.017 | MCPP | -0.26 | 0.461 |
| | | MiBP | -0.77 | 0.007 | MMP | -0.28 | 0.383 |
| | | | | | MEHP | -0.05 | 0.833 |
| | | | | | MEHHP | -0.40 | 0.145 |
| | | | | | MEOHP | -0.41 | 0.114 |

AGD: anogenital distance (mm); AGI: anogenital index, anogenital distance divided by weight (mm/ Kg);
PMCs: phthalate metabolites concentrations;
[a] In this paper, we think p< 0.05 denotes "reach statistical significance".
[b] ΣDEHP: molar sum of four DEHP metabolites, ΣDEHP=[MEHP*(1/278)]+[MEHHP*(1/294)]+[MEOHP*(1/292)]+[MECPP*(1/308)].

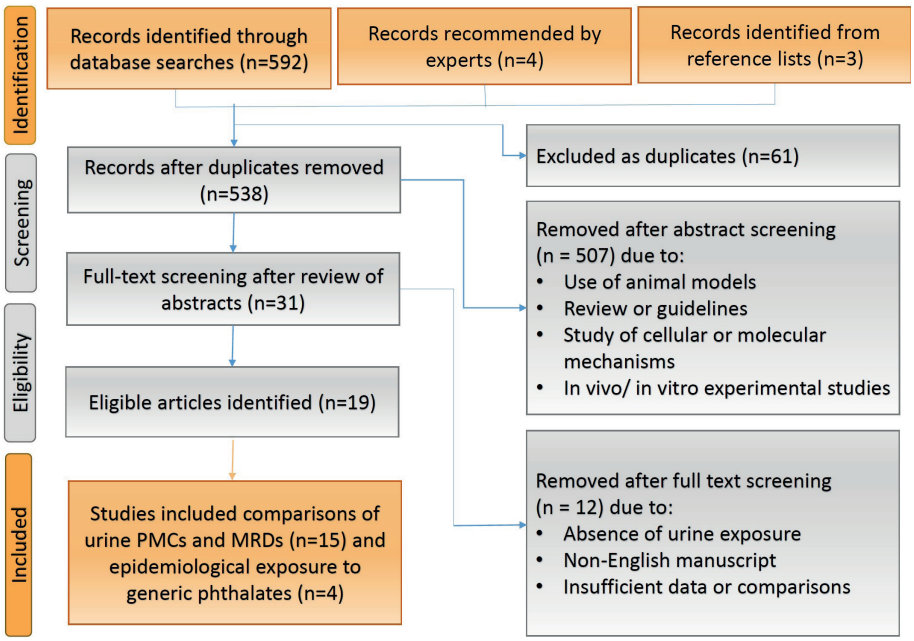



**Fig. 1.** Flow diagram of the systematic searches performed for various databases to identify potentially eligible publications for this study.

effect model was applied. Unfortunately, the results were not significant and a conclusion could not be made (pooled crude odds ratio (OR): 2.16; 95% confidence interval (CI): 0.30–15.45; $P = 0.44$; $I^2 = 70\%$) (Fig. 2).

Very few studies have investigated a possible association between urine PMCs and risk of cryptorchidism. However, it has been observed that the male offspring of mothers with higher PMCs of DEHP and di-isodecyl phthalate (DIDP) have a higher risk of cryptorchidism.[23,29] The design methods and findings for the phthalate-based and urine PMC-based studies are summarized in Table III.

### *Hypospadias and phthalate exposure*

Risk of hypospadias was also examined for populations with and without exposure to phthalates in three studies.[26,28,30] Similar to the studies of cryptorchidism described above, the studies selected exhibited high heterogeneity and a random-effect model was applied. A trend towards an increased risk of having a boy affected by hypospadias was observed for mothers who were exposed to phthalates versus those who were not, although this result did not achieve statistical significance (pooled crude OR: 1.38; 95% CI: 0.93–2.04; $P = 0.11$; $I^2 = 78\%$).

A few studies have clarified a possible correlation between urine PMCs and hypospadias.[24,31] For example, the greater the exposure of pregnant mothers to DEHP and DIDP, the greater the likelihood that they will have a boy affected by hypospadias. Thus, a similar trend as that observed for cryptorchidism is also relevant for hypospadias. The design methods and findings for the phthalate-based and urine PMC-based studies are summarized in Table IV.

### *AGD and phthalate exposure*

Eleven studies have explored a possible correlation between urine PMCs and AGD. The first study was published by Swan et al.[32] in 2005. Detailed information regarding the design methods, data comparisons, and results of all 11 studies are presented in Table IV. It was not possible to synthesize the data of these different studies to conduct a meta-analysis, since the pivotal designs of each article varied and the statistical data greatly

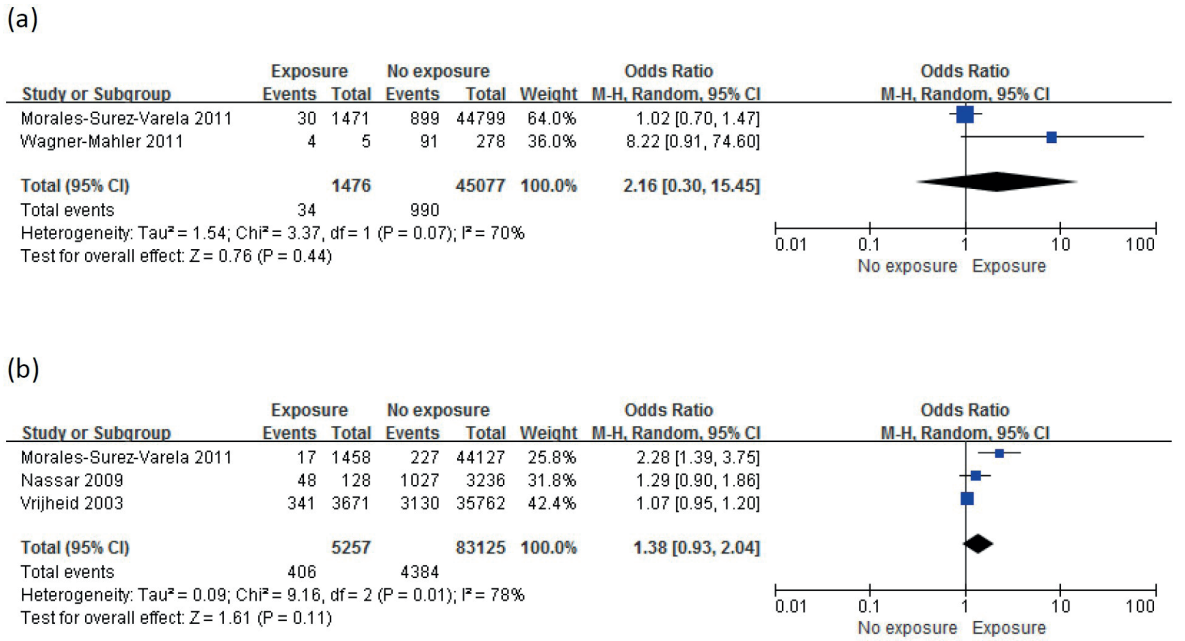



**Fig. 2.** Forest plot of epidemiological exposure to generic phthalates and risks of cryptorchidism (a) and hypospadias (b).

differed as well. However, we did identify an overall trend in which exposure to higher levels of phthalates was associated with a shorter AGD in humans. Moreover, a prominent association between exposure to DEHP, DBP, diethyl phthalate (DEP), or BBzP/BBP with a shorter AGD was observed. When the adjusted multiple regression model coefficients of log-transformed PMCs and AGD from 5 of the 11 articles were examined (Table V), almost all of the PMCs detected were found to be inversely associated with AGD, consistent with the overall trend observed for all 11 studies.

A graphical diagram, Figure 3, depicted these outcomes for convenience of detection of information at first sight.

## Discussion

In many animal models, exposure to phthalates leads to an adverse effect on reproductive development. This phenomenon derives from the ability of phthalates to inhibit the synthesis of testosterone and disrupt androgen signaling. As a result, higher risks of cryptorchidism and hypospadias, as well as a shorter AGD, have been observed in male laboratory animals. It has been speculated that human exposure to phthalates may result in similar effects. However, it is difficult to investigate this possible correlation because specific exposure in humans is rare. Swan et al.[32] published the first human study of phthalates exposure and MRDs in 2005. Currently, there are approximately 20 manuscripts which have focused on investigations of the effects of exposure to specific phthalates on MRDs.

To clarify the adverse effects of specific compounds on human developmental health, epidemiological studies are often considered the "gold standard". However, epidemiological studies are expensive and often involve a minimum of five years from conception to results.[29] As a result, we found only two epidemiological studies which have investigated phthalate exposure and risk of cryptorchidism, and four epidemiological studies which investigated phthalate exposure and hypospadias. (Note, Ormond et al. was excluded due to insufficient data even after requests for additional data were submitted

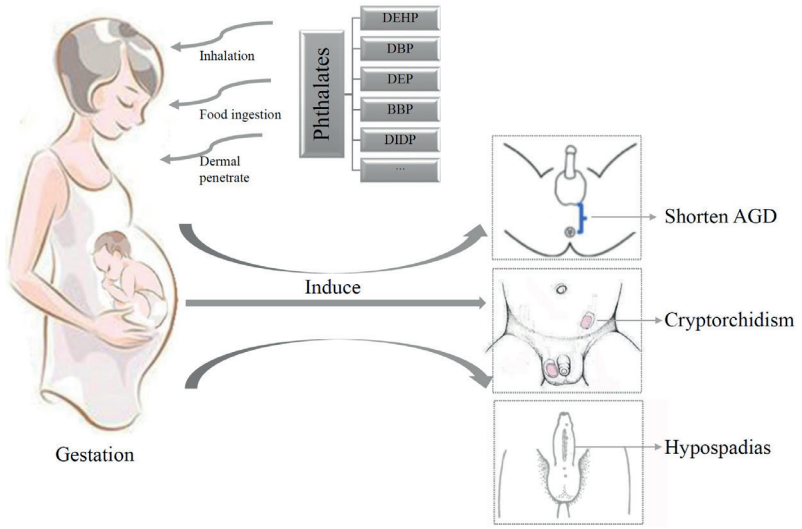



**Fig. 3.** Graphical diagram of effects of maternal phthalates exposure on male reproductive disorders in offsprings.

to the authors). Furthermore, we found that large-scale epidemiological studies of generic phthalate exposure were not published until after 2011. Due to this limited availability of epidemiological data, the results of our meta-analysis were rendered non-significant due to a lack of power. Moreover, our study design, which included manuscripts only written in English, may have increased the publication bias in the present study, thereby representing a limitation of this mini systematic review.

Detection and measurements of phthalate concentrations *in vitro* are complex and costly. In contrast, the excretion of high concentrations of phthalate metabolites in urine make it an optimal biomarker matrix.[33] However, a majority of phthalate metabolites may not exhibit a significant positive correlation between urine and amniotic fluid or with other biomarkers.[34] Hence, we emphasize that only urinary PMCs are regarded as indicating phthalate exposure in this paper.

Since the relationship between metabolites levels and AGD cannot be directly evaluated, the indirect methods most often used include: (1) a comparison of metabolite levels between "shorter" and "longer" AGD groups; (2) an examination of ORs of having a "shorter AGD" in individuals exposed to general or specific PMCs in different groups of higher and lower PMCs could reach statistical significance; and (3) the use of regression models to detect coefficients between log-transformed PMCs and AGD. However, the use of these various indirect methods in different studies has made it difficult to synthesize the data obtained to enhance statistical efficacy. Currently, advances in technology have allowed different phthalate metabolites to be detected in urine, and this has been applied in various studies. In this review, the majority of specific phthalates which were detected exhibited an inverse association with AGD in humans, particularly DEHP, DBP, DEP, and BBzP/BBP.[5,17,22,23,32,34-39] Furthermore, only a few studies focused on clarifying urine concentrations of phthalate metabolites and risks of cryptorchidism or hypospadias have been conducted. Thus, limited evidence is available regarding exposure to higher levels of DEHP and DIDP and increased risks of cryptorchidism and hypospadias. Furthermore, among these complex compounds, only a few have exhibited a protective effect in male genital disorders. The short-lived nature of these compounds, as well as analyses of single spot urine samples, may not effectively reflect the average exposure level to phthalates, and this

limitation existed in almost all of the included studies. In addition, all of the conclusions drawn in these studies derived from analyses of descriptive comparisons, and thus, they should be interpreted with caution.

Robust evidence has demonstrated that DEHP increases the risks of cryptorchidism and hypospadias, and shortens the AGD.[40] Di-isononyl phthalate (DINP/DiNP) has been introduced to replace DEHP, and consequently, DINP exposure has been rapidly increasing in populations worldwide. While animal data suggest that DINP may have an anti-androgen property that is similar to that of DEHP.[7,41] Bornehag et al.[5] demonstrated that urinary concentrations of DINP metabolites are also associated with a shorter AGD. Consequently, DINP exposure should be reexamined and safe replacements for harmful phthalates remains a critical need.

The effects mediated by phthalates depend on dosage, duration of action, and the stage of development for exposed individuals.[33] The primary programming period for human genital development is within the first 5–18 weeks of gestation.[23,42] During this time, phthalate metabolites can cross the placental barrier, thus, making the fetus in early pregnancy one of the most vulnerable groups to the effects of phthalate exposure. In a study conducted by Martino-Andrade et al.[22], exposure to DEHP metabolites only in the first trimester were found to be inversely associated with AGD. These findings are consistent with critical window data obtained in rodent studies[43], and they also support the biological plausibility of similar associations occurring in both humans and rodents. Therefore, avoiding exposure to phthalates during early pregnancy is the most efficient strategy for reducing the adverse effects of these compounds on male genital development in both humans and animals.

Phthalates are not covalently bound to a product matrix and they are currently used in a wide array of consumer products. In addition, their presence in the environment has made exposure to these compounds ubiquitous in daily life over the past 30 years. Consequently, phthalates have opportunities to penetrate the human body via multiple routes. Thus, nearly all human beings are exposed to phthalates, albeit at different levels. Consequently, there is not a need for more studies to evaluate the differences in risk of MRDs between mothers with and without exposure to phthalates. Rather, it would provide greater insight if well-designed studies were conducted to learn the effect of different concentration intervals of phthalate metabolites on MRDs.

The limited number of studies that have been published suggest that generic exposure to phthalates induces an adverse effect on human genital development, especially exposure to DEHP, DBP, DEP, BBP, and DIDP. Among these phthalates, DBP and DEP are most often found in personal skin care products, cosmetics, and fragrance, and this increases the exposure of pregnant women to these phthalates. As a result, public awareness of approaches to reduce phthalate exposure is important and necessary, especially during the early stages of pregnancy. Furthermore, safer substitutes that provide similar properties for plastics are greatly needed. These advances, in combination with additional well-designed and multi-center human studies, could provide valuable insight into the mechanisms mediated by phthalates and possible opportunities to prevent their adverse effects.

Multiple studies support the association between exposure to DEHP, DBP, DEP, and/or BBP and a shorter AGD. In addition, DEHP and DIDP are associated with higher risks of cryptorchidism and hypospadias. Thus, generic exposure to phthalates has an adverse effect on MRDs in both animals and humans, especially exposure to DEHP, DBP, DEP, BBP, and DIDP. Moreover, a critical time window for exposure to phthalates is during the first trimester of pregnancy, which supports the biological plausibility that similar effects are induced in both rodents and humans. However, due to the lack of significant statistical power in our

present meta-analysis, these conclusions should be interpreted with caution, and they remain to be confirmed in future well-designed studies. In the meantime, education of the public regarding phthalate exposure and the development of safer substitutes for these compounds are greatly needed.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: CY, SW, SW; data collection: CY, JL, JZ; analysis and interpretation of results: CY, JL, TZ, CL, TL; draft manuscript preparation: CY, SW, GW. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

Shengde Wu was rewarded by National Natural Science Foundation of China (NO.81873828), Chongqing Municipal Health Commission (High-level Medical Reserved Personnel Training Project of Chongqing), Innovation Program for Chongqing's Overseas Returnees (cx2019030).

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Bornehag C, Sundell J, Weschler C, et al. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. Environ Health Perspect 2004; 112: 1393-1397. https://doi.org/10.1289/ehp.7187
2. Abb M, Heinrich T, Sorkau E, Lorenz W. Phthalates in house dust. Environ Int 2009; 35: 965-970. https://doi.org/10.1016/j.envint.2009.04.007
3. Bergh C, Torgrip R, Emenius G, Ostman C. Organophosphate and phthalate esters in air and settled dust - a multi-location indoor study. Indoor Air 2011; 21: 67-76. https://doi.org/10.1111/j.1600-0668.2010.00684.x
4. Shi W, Hu X, Zhang F, et al. Occurrence of thyroid hormone activities in drinking water from eastern China: contributions of phthalate esters. Environ Sci Technol 2012; 46: 1811-1818. https://doi.org/10.1021/es202625r
5. Bornehag CG, Carlstedt F, Jonsson BAG, et al. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ Health Perspect 2015; 123: 101-107. https://doi.org/10.1289/ehp.1408163
6. Fisher J. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction 2004; 127: 305-315. https://doi.org/10.1530/rep.1.00025
7. Gray L, Ostby J, Furr J, Price M, Veeramachaneni D, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 2000; 58: 350-365. https://doi.org/10.1093/toxsci/58.2.350
8. Wilson V, Howdeshell K, Lambright C, Furr J, Earl Gray L. Differential expression of the phthalate syndrome in male Sprague-Dawley and Wistar rats after in utero DEHP exposure. Toxicol Lett 2007; 170: 177-184. https://doi.org/10.1016/j.toxlet.2007.03.004
9. Macleod D, Sharpe R, Welsh M, et al. Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl 2010; 33: 279-287. https://doi.org/10.1111/j.1365-2605.2009.01005.x
10. van den Driesche S, Scott H, MacLeod D, Fisken M, Walker M, Sharpe R. Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. Int J Androl 2011; 34: e578-586. https://doi.org/10.1111/j.1365-2605.2011.01175.x
11. Foster P. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl 2006; 29: 140-147; discussion 181-145. https://doi.org/10.1111/j.1365-2605.2005.00563.x
12. Jensen M, Nørgaard-Pedersen B, Toft G, et al. Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: temporal trends and timing of amniocentesis in pregnancy. Environ. Health Perspect 2012; 120: 897-903. https://doi.org/10.1289/ehp.1104522
13. Pierik F, Burdorf A, Nijman J, de Muinck Keizer-Schrama S, Juttmann R, Weber R. A high hypospadias rate in The Netherlands. Hum Reprod 2002; 17: 1112-1115. https://doi.org/10.1093/humrep/17.4.1112

14. Ormond G, Nieuwenhuijsen MJ, Nelson P, et al. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: case-control study. Environ Health Perspect 2009; 117: 303-307. https://doi.org/10.1289/ehp.11933

15. Braga L, Lorenzo A, Romao R. Canadian Urological Association-Pediatric Urologists of Canada (CUA-PUC) guideline for the diagnosis, management, and followup of cryptorchidism. Can Urol Assoc J 2017; 11: E251-E260. https://doi.org/10.5489/cuaj.4585

16. Barrett ES, Parlett LE, Redmon JB, Swan SH. Evidence for sexually dimorphic associations between maternal characteristics and anogenital distance, a marker of reproductive development. Am J Epidemiol 2014; 179: 57-66. https://doi.org/10.1093/aje/kwt220

17. Swan SH, Sathyanarayana S, Barrett ES, et al. First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod 2015; 30: 963-972. https://doi.org/10.1093/humrep/deu363

18. Sathyanarayana S, Grady R, Redmon JB, et al. Anogenital distance and penile width measurements in The Infant Development and the Environment Study (TIDES): methods and predictors. J Pediatr Urol 2015; 11: 76.e1-76.e766. https://doi.org/10.1016/j.jpurol.2014.11.018

19. Mendiola J, Onate-Celdran J, Samper-Mateo P, et al. Comparability and reproducibility of adult male anogenital distance measurements for two different methods. Andrology 2016; 4: 626-631. https://doi.org/10.1111/andr.12202

20. Moher D, Liberati A, Tetzlaff J, Altman D. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009; 339: b2535. https://doi.org/10.1136/bmj.b2535

21. Sathyanarayana S, Grady R, Barrett ES, et al. First trimester phthalate exposure and male newborn genital anomalies. Environ Res 2016; 151: 777-782. https://doi.org/10.1016/j.envres.2016.07.043

22. Martino-Andrade AJ, Liu F, Sathyanarayana S, et al. Timing of prenatal phthalate exposure in relation to genital endpoints in male newborns. Andrology 2016; 4: 585-593. https://doi.org/10.1111/andr.12180

23. Sathyanarayana S, Butts S, Wang C, et al. Early Prenatal Phthalate Exposure, Sex Steroid Hormones, and Birth Outcomes. J Clin Endocrinol Metab 2017; 102: 1870-1878. https://doi.org/10.1210/jc.2016-3837

24. Higgins J, Thompson S, Deeks J, Altman D. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560. https://doi.org/10.1136/bmj.327.7414.557

25. Wagner-Mahler K, Kurzenne JY, Delattre I, et al. Prospective study on the prevalence and associated risk factors of cryptorchidism in 6246 newborn boys from Nice area, France. Int J Androl 2011; 34: e499-e510. https://doi.org/10.1111/j.1365-2605.2011.01211.x

26. Morales-Suárez-Varela MM, Toft GV, Jensen MS, et al. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: a study in the Danish National Birth Cohort study. Environ Health 2011; 10: 3. https://doi.org/10.1186/1476-069X-10-3

27. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to endocrine disrupting chemicals and risk of hypospadias. Am J Epidemiol 2009;169(Suppl):S45. https://doi.org/10.1093/aje/kwp124

28. Vrijheid M, Armstrong B, Dolk H, van Tongeren M, Botting B. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med 2003; 60: 543-550. https://doi.org/10.1136/oem.60.8.543

29. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res 2008; 108: 177-184. https://doi.org/10.1016/j.envres.2008.08.007

30. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup Environ Med 2010; 67: 585-589. https://doi.org/10.1136/oem.2009.048272

31. Choi H, Kim J, Im Y, Lee S, Kim Y. The association between some endocrine disruptors and hypospadias in biological samples. J Environ Sci Health A Tox Hazard Subst Environ Eng 2012; 47: 2173-2179. https://doi.org/10.1080/10934529.2012.680387

32. Swan SH, Main KM, Liu F, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 2005; 113: 1056-1061. https://doi.org/10.1289/ehp.8100

33. Bajkin I, Bjelica A, Icin T, Dobrić V, Zavisić BK, Stojanoska MM. Effects of phthalic acid esters on fetal health. Med Pregl 2014; 67: 172-175. https://doi.org/10.2298/MPNS1406172B

34. Huang PC, Kuo PL, Chou YY, Lin SJ, Lee CC. Association between prenatal exposure to phthalates and the health of newborns. Environ Int 2009; 35: 14-20. https://doi.org/10.1016/j.envint.2008.05.012

35. Adibi JJ, Lee MK, Naimi AI, et al. Human chorionic gonadotropin partially mediates phthalate association with male and female anogenital distance. J Clin Endocrinol Metab 2015; 100: E1216-E1224. https://doi.org/10.1210/jc.2015-2370

36. Bustamante-Montes LP, Hernández-Valero MA, Flores-Pimentel D, et al. Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns. J Dev Orig Health Dis 2013; 4: 300-306. https://doi.org/10.1017/S2040174413000172

37. Jensen TK, Frederiksen H, Kyhl HB, et al. Prenatal exposure to phthalates and anogenital distance in male infants from a low-exposed Danish cohort (2010-2012). Environ Health Perspect 2016; 124: 1107-1113. https://doi.org/10.1289/ehp.1509870

38. Suzuki Y, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H. Foetal exposure to phthalate esters and anogenital distance in male newborns. Int J Androl 2012; 35: 236-244. https://doi.org/10.1111/j.1365-2605.2011.01190.x

39. Wenzel AG, Bloom MS, Butts CD, et al. Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls. Environ Int 2018; 110: 61-70. https://doi.org/10.1016/j.envint.2017.10.007

40. Dorman DC, Chiu W, Hales BF, et al. Systematic reviews and meta-analyses of human and animal evidence of prenatal diethylhexyl phthalate exposure and changes in male anogenital distance. J Toxicol Environ Health B Crit Rev 2018; 21: 207-226. https://doi.org/10.1080/10937404.2018.1505354

41. Boberg J, Christiansen S, Axelstad M, et al. Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol 2011; 31: 200-209. https://doi.org/10.1016/j.reprotox.2010.11.001

42. Rey R, Picard JY. Embryology and endocrinology of genital development. Baillieres Clin Endocrinol Metab 1998; 12: 17-33. https://doi.org/10.1016/s0950-351x(98)80427-8

43. Welsh M, Saunders PT, Fisken M, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest 2008; 118: 1479-1490. https://doi.org/10.1172/JCI34241

The Turkish Journal of Pediatrics 2022; 64: 210-220
https://doi.org/10.24953/turkjped.2021.4

**Original Article**

# The effectiveness of the ketogenic diet in drug-resistant childhood epilepsy

Ünsal Yılmaz[1], Selvinaz Edizer[1], Zeynep Akışin[2], Melis Köse[3], Yiğithan Güzin[1], Gürkan Gürbüz[4], Bahar Toklu Baysal[5], Serdar Sarıtaş[1], Serdar Pekuz[1], Hatice Hilal Kırkgöz[1], Merve Yavuz[1], Aycan Ünalp[1]

*Departments of [1]Pediatric Neurology and [2]Nutrition and Dietetics, University of Health Sciences Turkey, Dr. Behçet Uz Children's Education and Research Hospital, İzmir; [3]Department of Pediatric Metabolism and Nutrition, İzmir Katip Çelebi University, Izmir; [4]Department of Pediatric Neurology, Tekirdağ Namık Kemal University Medical Faculty Tekirdağ; [5]Department of Pediatric Neurology, T. C. Ministry of Health Uşak Education and Research Hospital, Uşak, Turkey.*

## ABSTRACT

**Background.** We aimed to investigate the effectiveness of ketogenic diet (KD) in children with various types of refractory epilepsy.

**Methods.** A total of 91 children (49 females) aged 3 to 193 months (median, 52 months) with drug resistant epilepsy who received KD treatment for at least 12 months were enrolled in the study. Seizure frequency, adherence to diet, reason for discontinuation of KD, and adverse effects were recorded. Response was defined as ≥50% improvement in seizure frequency compared to baseline. We also searched for influences of different variables on the outcome.

**Results.** Intent-to-treat analysis revealed an improvement in seizure frequency for ≥50% in 73.6%, 80.2%, 75.8%, 73.6%, and 70.3% of patients at month-1, -3, -6, -9, and month-12, respectively. Overall, 32 (35.2%) patients remained seizure-free at month-12. There was no significant differences between responders and non-responders in terms of age at onset of epilepsy, age at onset of KD, gender, or etiology. Mild hyperlipidemia was associated with a higher response rate. At the last follow-up (median: 20 months), 38 (41.8%) patients were still maintained on KD. While 15.4% of patients completed the diet with a success in seizure control, remainder discontinued KD due to lack of efficacy (23.1%), non-adharence to diet (11%), intercurrent infection (4.4%), adverse effects (3.3%), and death (1.1%).

**Conclusion.** Ketogenic diet treatment appears to be effective in about two-thirds of children with various types of drug-resistant epilepsy, including one-third remaining seizure free. Mild hyperlipidemia seems to be associated with a higher response rate. Discontinuation of KD is mostly due to lack of efficacy or non-adherence, and rarely side effects.

**Key words:** ketogenic diet, drug-resistant epilepsy, effectiveness, tolerability.

Epilepsy is the most common chronic neurologic disorder that requires continued medication for long-term management in children. However approximately 20-30% of all children with epilepsy do not respond sufficiently to antiepileptic drugs (AED), and are considered medically intractable, despite recent development of a number of anticonvulsant agents.[1] The ketogenic diet (KD), defined as a high-fat, adequate-protein, and low-carbohydrate diet, is one of the most effective alternative treatment options for drug resistant epilepsy.[2] It is not a benign therapy, however, being associated with a number of adverse effects.[3] Moreover, compliance with

✉ Ünsal Yılmaz
drunsalyilmaz@yahoo.com

Received 16th April 2021, revised 18th July 2021, accepted 16th August 2021.

This study was presented at the 2nd International Dr. Behçet Uz Children's Congress, March 4-7 2020, (P-72), İzmir, Turkey.

diet for both patients and their caregivers appears to be one of the main concerns in KD implementation. Differences in food culture, locally available food, eating habits and children's preferences may affect dietary compliance, and therefore the effectiveness of the diet in various parts of the world. For instance, since Asian diet is predominantly cereal-based and its carbohydrate content is much higher compared to Western diets, it is not surprising that compliance with the diet and, accordingly, the response to treatment in children from East Asian countries is lower than those from Western countries where ordinary diet is based mainly on high-lipid, low carbohydrate foods such as butter and cream.[4,5] In this study, we aimed to investigate effectiveness of KD in different types of drug resistant childhood epilepsy in Turkey where the fat content of ordinary diet consists mainly of olive oil.

## Material and Methods

### *Patients and settings*

Hospital charts of children treated with KD at the University of Health Sciences Turkey, Dr. Behçet Uz Children's Education and ResearchHospital between the years 2013 and 2019 were retrospectively reviewed. Patients who had drug resistant epilepsy (defined as failure to ≥2 appropriate antiepileptic drugs), who had more than four seizures per month, and patients who continued KD treatment for at least 12 months were identified. Patients who discontinued treatment before 12 months due to ineffectiveness, side effects or nonadherence to KD were also included into the analysis. Ethical permission for the study was obtained from the institutional ethical committee (2020/08-05).

### *Dietary protocol*

A standardized KD with a non-fasting gradual initiation protocol during a 5-day hospital stay was started on a 3:1-ratio. During the inpatient treatment, parents were educated about diet and ways to prepare KD at home. Ketogenic diet was started with full calories. A 1:1 ratio on the 1st day, 2:1 ratio on the 2nd day, and 3:1 ratio thereafter was administered. Continuing adjustments to dietary ratio between 2:1 to 4:1, energy, and protein intake were made while children remained on the diet in order to optimize ketone levels and seizure control, minimize side effects, and maintain appropriate growth. We tried to maintain ketone levels at ≥3+ (50–150 mg/dl) in routine urinalysis or 4–6 mM for serum β-hydroxybutyrate. No water restriction was applied. As there is evidence showing similar efficacy at ratios of 2:1 to 4:1,[6,7] we included all children receiving KD at ratios between 2:1 and 4:1. The recipes were individually prepared with locally available foods, taking into account the preferences of families and the children, and cultural differences. The principal unsaturated fat source of the diet was olive oil (about 80% of all fats in the diet), which is widely used, readily available, and relatively inexpensive in Turkey. Saturated fats including cream, butter, and fatty beef made up about remaining 20% of the fat in the diet, and no other types of fat were used. We tried to manage patients who developed hyperlipidemia using simple dietary modifications such as replacing saturated fats with extra-virgin olive oil, carnitine and/or omega-3 fatty acid supplementation, or reducing the ketogenic ratio gradually down to the level of 2:1, trying not to alter ketosis and seizure control. We had to reduce the KD ratio to 2:1 in only two patients. Omega-3 supplements (docosahexaenoic acid / eicosapentaenoic acid) were given to five patients whose high triglyceride levels persisted despite dietary modifications. Carnitine supplements were given to six patients with low carnitine concentrations, because carnitine supplementation has been reported to reduce triglyceride levels in adults.[8] In order to reach optimum ketone levels, medium-chain triglyceride (MCT) oil was added to the diet in seven children. The initial daily calorie requirement was calculated individually for each patient as the average between their pre-diet intake and their energy requirements for

ideal body weight for children younger than two years of age and ideal body mass index for children aged two years or older (approximately 60 – 80 kcal/kg/day), taking into account their levels of physical activity, and trying to bring patients to their ideal weight. Calories were generally adjusted in increments or decrements of 100 kcal at intervals of at least 2 weeks before any further changes were made in order to optimize ketone levels and seizure control. Protein content was generally calculated according to World Health Organization (WHO) minimum requirements for age (about 1 – 1.5 g/kg/day).[9] All foods and beverages weighed precisely on a gram scale, and the recipes were largely planned with a computer program called "ketodietcalculator" freely released by Charlie Foundation.[10] The amount of fat, carbohydrate, protein, and calories contained in all locally available food and beverages were entered into the programme by our dietitian (Z.A.). A formula with 4:1 ratio produced for KD therapy were added to the diet for formula fed babies. Urine or blood ketone and glucose levels were checked and recorded by parents using ketostix and dextrostix strips twice daily during the first week and as needed clinically thereafter. Medications were changed to carbohydrate free preparations wherever available. Multivitamins were given to all patients, as the KD is not nutritionally complete.

### *Data collection and variables*

Detailed demographic and clinical information including age at onset of seizures, age at initiation of KD, gender, developmental status, etiology, type of seizure and epilepsy syndrome, improvement in alertness, seizure frequency, and treatment history were noted. Seizure types, etiology and epilepsy syndromes were classified, where possible, according to the criteria proposed by the International League Against Epilepsy (ILAE).[11,12] Before the diet was started, each patient underwent detailed metabolic screening in order to rule out disorders contraindicated for KD treatment such as primary carnitine deficiency, fatty acid oxidation disorders, or pyruvate carboxylase deficiency. A thorough laboratory investigation was carried out, as well, including complete blood count, biochemical profile (liver and kidney function tests, fasting lipid profile), and abdominal ultrasonography. Brain magnetic resonance imaging and electroencephalogram (EEG) were performed in all cases.

Patients were assessed through clinic visits at 1, 3, 6, 9, and 12 months, and every 6 months thereafter. At each visit, seizure frequency, adverse events, compliance with the diet, adherence, and the reason for KD discontinuation were recorded. The main measure of efficacy was the decrease in seizure frequency as assessed by parental report and seizure diaries. The average seizure number in one month prior to each visit was compared to the baseline average seizure number in the month before the start of the diet, and expressed as a percentage of reduction. Improvement in seizure frequency was classified into four categories: seizure-free, ≥50% improvement, <50% improvement, or no improvement/ increase in seizure frequency compared to baseline. Response was defined as ≥50% seizure reduction. An intent-to-treat analysis was employed, and patients who discontinued KD for any reason (ineffectiveness, incompatibility, or adverse effects) were counted as non-responders from the point of discontinuation of KD. Assessment of improvement in alertness was based on parental reports and clinical examination; no formal developmental tests were performed. Tolerability and adverse events were assessed with physical examination, laboratory testings, and adverse events spontaneously reported by the parents or the children. Hyperlipidemia was defined as serum total cholesterol levels ≥ 200 mg/dl and/or serum triglyceride levels ≥ 130 mg/dl, and graded as mild, moderate, or severe hyperlipidemia (serum total cholesterol level between 200 - 399 mg/dl; 400 - 599 mg/dl; ≥ 600 mg/dl, and/or serum triglyceride levels between 130 -259 mg/dl; 260 -389 mg/dl; ≥ 390 mg/dl, respectively). Adherence was assessed by direct questioning and by measurement

of serum/urine ketone concentrations. The decision to continue antiepileptic medications was made by the treating physician based on seizure frequency, epilepsy type, and EEG results at each visit.

### *Statistics*

The data were analyzed using SPSS for Windows software package, version 20.0 (SPSS, Chicago, IL). Due to non-parametric distribution of data, Mann—Whitney U and Friedmann tests were used for continuous variables, and Pearson Chi Square, Fisher exact or Cochrane Q tests for categorical variables. A p-value less than 0.05 was regarded as statistically significant.

## Results

A total of 91 children (49 females) aged from 3 to 193 months (median, 52 months) at the onset of KD treatment with drug resistance epilepsy due to various types of etiology, who received a KD for at least 12 months were enrolled in the study. Etiologic distribution of study sample is shown in Table I.

Of the 91 patients, 16 (17.6%) stopped KD before 12 months due to lack of efficacy (7 patients), non-adherance to the diet (5 patients), adverse effects (2 patients) and severe intercurrent infection (2 patients), and these patients were counted as non-responders in the analysis. Intent-to-treat analysis revealed an improvement in seizure frequency for ≥50% in 73.6%, 80.2%, 75.8%, 73.6%, and 70.3% of patients at month-1, -3, -6, -9, and month-12, respectively. Overall, 32 (35.2%) patients remained seizure-free at month-12. The median number of AEDs used during this 12-month period decreased significantly (Table II).

The comparative demographic and clinical characteristics of the study sample between responder and non-responder groups are presented in Table III. There was no significant difference between these groups in terms of age at onset of epilepsy, age at onset of KD, gender, and etiology. Mild hyperlipidemia was significantly higher in the responder group compared to non-responders. Number of children with individual syndrome groups was too small for intergroup statistical analysis. However KD appeared to be particularly effective in patients with glucose transporter 1 (GLUT-1) deficiency, pyruvate dehydrogenase deficiency, and Dravet syndrome. At the last follow-up (median: 20 months), 38 (41.8%)

**Table I.** Etiologic distribution of the patients treated with ketogenic diet.

| Etiology | n (%) |
|---|---|
| Total | 91 (100) |
| Unknown | 36 (39.6) |
| Structural causes | 21 (23.1) |
| Hypoxic ischemic encephalopathy | 7 (7.7) |
| Cortical malformation | 4 (4.4) |
| Encephalitis with unknown etiology | 3 (3.3) |
| Herpes encephalitis | 1 (1.1) |
| Stroke | 2 (2.2) |
| Congenital cytomegalovirus infection | 1 (1.1) |
| Hypothalamic hamartoma | 1 (1.1) |
| Traumatic brain injury | 1 (1.1) |
| Neonatal cerebral hemorrhage | 1 (1.1) |
| Epileptic encephalopathies | 23 (25.3) |
| West syndrome | 6 (6.6) |
| Lennox-Gastaut syndrome | 6 (6.6) |
| Dravet syndrome | 3 (3.3) |
| Rett syndrome | 2 (2.2) |
| SCN1B Encephalopathy | 1 (1.1) |
| CDKL5 encephalopathy | 1 (1.1) |
| KCNB1 related encephalopathy | 1 (1.1) |
| STXBP1 related encephalopathy | 1 (1.1) |
| EMC1 gene mutation related disorder | 1 (1.1) |
| DYRK1A related disorder | 1 (1.1) |
| Metabolic causes | 11 (12.1) |
| Glucose transporter-1 deficiency | 3 (3.3) |
| Pyruvate dehydrogenase deficiency | 2 (2.2) |
| Nonketotic hyperglysinemia | 1 (1.1) |
| Tay Sachs Disease | 1 (1.1) |
| Cobalamin synthesis defect | 1 (1.1) |
| Mitochondrial DNA depletion syndrome type 14 | 1 (1.1) |
| Congenital glycosylation defect 1D | 1 (1.1) |
| Hypomyelinating leukodystrophy | 1 (1.1) |

**Table II.** Seizure outcomes and number of antiepileptic drugs currently used at 1, 3, 6 and 12 months after initiation of the ketogenic diet.

| | Baseline N:91 | Month 1 N:91 | Month 3 N:91 | Month 6 N:91 | Month 9 N:91 | Month 12 N:91 | p |
|---|---|---|---|---|---|---|---|
| Responders, %, (n) | | 73.6 (67) | 80.2 (73) | 75.8 (69) | 73.6 (67) | 70.3 (64) | 0.086 |
| Seizure free | | 18.7 (17) | 25.3 (23) | 27.5 (25) | 30.8 (28) | 35.2 (32) | |
| Reduction ≥50% | | 54.4 (50) | 54.9 (50) | 48.4 (44) | 42.9 (39) | 35.2 (32) | |
| Non-responders, %, (n) | | 26.4 (24) | 19.8 (18) | 24.2 (22) | 26.4 (24) | 29.7 (27) | |
| Reduction <50% | | 11.0 (10) | 8.8 (8) | 6.6 (6) | 8.8 (8) | 8.8 (8) | |
| No change | | 15.4 (14) | 11.0 (10) | 17.6 (16) | 17.6 (16) | 20.9 (19) | |
| Number of AEDs currently used, n±S.D, (median) | 2.96±1.01 (3) | 2.71±0.98 (3) | 2.54±1.03 (3) | 2.43±1.11 (3) | 2.37±1.15 (2) | 2.34±1.1 (2) | <0.0001 |

AEDs: antiepileptic drugs, SD: standard deviation.

**Table III.** Comparison of variables between Responders and Non-responders at month-12.

| Characteristics | Total | Responders | Non-responders | p |
|---|---|---|---|---|
| Patients enrolled, n (%) | 91 | 64 (70.3) | 27 (29.7) | |
| Gender | | | | 0.479 |
| Female, n (%) | 49 (53.8) | 36 (56.3) | 13 (48.1) | |
| Male, n (%) | 42 (46.2) | 28 (43.7) | 14 (51.9) | |
| Age at onset of epilepsy, yr; mean ± SD | 12.0 ± 20.4 | 12.3 ± 21.4 | 11.3 ± 18.1 | 0.885 |
| Duration of epilepsy at onset of KD, mon; mean ± SD | 51.9 ± 42.4 | 52.9 ± 40.3 | 49.3 ± 47.8 | 0.414 |
| Age at onset of KD, yr; mean ± SD | 64.5 ± 49.6 | 65.7 ± 48.8 | 61.7 ± 52.1 | 0.578 |
| Patients under the age of 2 years, n (%) | 25 (27.5) | 17 (26.6) | 8 (29.6) | 0.765 |
| Patients under the age of 6 years, n (%) | 56 (61.5) | 40 (62.5) | 16 (59.5) | 0.772 |
| Patients under the age of 12 years, n (%) | 79 (86.8) | 55 (85.9) | 24 (88.9) | 0.498 |
| Duration of KD at last follow up, mon; mean ± SD | 25.8 ± 17.0 | 29.7 ± 15.6 | 16.6 ± 16.9 | <0.001 |
| Improvement in alertness | 76 (84.4) | 59 (93.7) | 17 (63.0) | 0.001 |
| Continuation of the ketogenic diet at month 12 | 75 (82.4) | 63 (98.4) | 12 (44.4) | 0.001 |
| Constipation during KD treatment, n (%) | 22 (24.2) | 15 (23.4) | 7 (25.9) | 0.800 |
| Constipation at baseline, n (%) | 17 (18.7) | 11 (17.2) | 6 (22.2) | 0.574 |
| Renal stone, n (%) | 11 (12.1) | 8 (12.5) | 3 (11.1) | 0.853 |
| Hyperlipidemia | 69 (75.8) | 52 (81.3) | 17 (63.0) | 0.063 |
| • Mild | 47 (51.6) | 39 (60.9) | 8 (29.6) | 0.006 |
| • Moderate | 11 (12.1) | 6 (9.4) | 5 (18.5) | 0.292 |
| • Severe | 11 (12.1) | 7 (10.9) | 4 (14.8) | 0.726 |
| Ambulant, n (%) | 57 (62.6) | 40 (62.5) | 17 (63.0) | 0.967 |
| Number of AEDs at baseline, mean ± SD | 2.96±1.01 | 2.91±1.05 | 3.07±0.92 | 0.473 |
| Number of AEDs at month 12, mean ± SD | 2.34±1.16 | 2.09±1.18 | 2.96±0.84 | 0.002 |

**Table III.** Continued.

| Characteristics | Total | Responders | Non-responders | p |
|---|---|---|---|---|
| Etiology, n (%) | | | | |
| • Unknown | 36 (39.6) | 28 (77.8) | 8 (22.2) | 0.208 |
| • Structural causes | 21 (23.1) | 13 (72.9) | 8 (27.1) | 0.335 |
| ° Hypoxic ischemic encephalopathy | 7 (7.7) | 4 (57.1) | 3 (42.9) | 0.419 |
| ° Cortical malformation | 4 (4.4) | 1 (25) | 3 (75) | 0.077 |
| • Epileptic encephalopathies | 23 (25.3) | 14 (73.5) | 9 (26.5) | 0.251 |
| ° West syndrome | 6 (6.6) | 2 (33.3) | 4 (66.7) | 0.061 |
| ° Lennox-Gastaut syndrome | 6 (6.6) | 3 (50) | 3 (50) | 0.357 |
| ° Dravet syndrome | 3 (3.3) | 3 (100) | 0 | |
| ° Rett syndrome | 2 (2.2) | 1 (50) | 1 (50) | |
| • Metabolic causes | 11 (12.1) | 9 (68.8) | 2 (31.2) | 0.496 |
| ° GLUT-1 deficiency | 3 (3.3) | 3 (100) | 0 | |
| ° Pyruvate dehydrogenase deficiency | 2 (2.2) | 2 (100) | 0 | |

AEDs: antiepileptic drugs, GLUT-1: glucose transporter 1, KD: ketogenic diet, mo: months, SD: standard deviation, yr: years.

**Table IV.** Continuation of Ketogenic Diet at month-12 and at the last follow up.

| Characteristics | n (%) |
|---|---|
| Patients enrolled | 91 |
| Continuation of KD at month 12 | 75 (82.4) |
| Reason for discontinuation of KD at month 12 | |
| Lack of efficacy | 7 (7.7) |
| Completion of treatment | |
| Nonadharence to KD | 5 (5.5) |
| Adverse effects | 1 (1.1) |
| Nephropathy | 1 (1.1) |
| Intercurrent infection | 3 (3.3) |
| Continuation of KD at last follow up (median 20 months) | 38 (41.8) |
| Reason for discontinuation of KD at last follow up (median 20 months) | |
| Lack of efficacy | 21 (23.1) |
| Completion of treatment | 14 (15.4) |
| Nonadharence to diet | 10 (11.0) |
| Adverse effects | 3 (3.3) |
| Nephropathy | 2 (2.2) |
| Elevation of liver enzymes | 1 (1.1) |
| Intercurrent infection | 4 (4.4) |
| Death | 1 (1.1) |

KD: Ketogenic diet

patients were still on the KD. While 14 (15.4%) patients completed the diet due to success in seizure control, remainder stopped KD due to lack of efficacy (23.1%), nonadharence to diet (11%), adverse effects (3.3%), intercurrent infection (4.4%), and death (1.1%) (Table IV).

## Discussion

Due to the difficulties in developing a blinded placebo-controlled prospective trial of the KD, so far, no placebo-controled study has been conducted for the efficacy of KD in children with

drug resistant epilepsy. Few randomized non-placebo-controlled trials provided an overall low to very low-quality evidence for the efficacy of KD due to the limited number of studies and small sample sizes.[2] Furthermore, except for one study that provided data on 12-month outcomes.[13] all other randomized controlled trials reported outcome measures for short periods ranging from 3 to 6 months.[2] On the other hand, many more observational studies have provided evidence of the effectiveness of KD for longer periods.[14] However most of these studies are heterogenous in terms of patient population, age, and duration of follow-up.[14] Therefore, more studies are still needed to address the effectiveness and safety of KD in daily clinical practice. The present study, although observational, provides outcome measures at different time points including short and long term data, and showed that about 70% of the children with various types of drug-resistant epilepsy, including one third of all patients remained seizure free, achieved ≥50% improvement in seizure frequency. Our rates are higher than those of most of studies reporting 50% or greater reduction in seizure frequency from 18 to 53%[4,5,13,15,16], and are comparable to others with reported response rates between 63 and 72%.[17,18] Various factors, such as differences in eating habits, culinary culture and traditional dishes, heterogeneity of study samples in terms of age at onset of KD or etiology may contribute to the wide range of effectiveness rates. Our high response rate, likely due in part to high retention rate, may be partly explained by the high olive oil content in the customary diet, which seems slightly more similar to KD than, for instance, cereal based high-carb Asian diet. Low retention rates from Asian countries reported between 24 and 46% at 12 months support this hypothesis.[4,16] Our retention rate at month 12 (82%) was even higher than those of many Western countries (55%)[5,13], suggesting olive oil based KD might be more palatable regarding KD. Similar to previous reports, lack of effectiveness was the most common reason for dietary withdrawal in our series.[3-5,16] However, unlike previous studies that reported discontinuation rates due to non-compliance between 14-23%[4,16], in the present study, only 6% of children stopped the diet due to non-adherence, which underlines the importance of individualized diet preparation taking into account various factors such as the families and the child's preferences, cultural differences, close follow up and rapid changes in recipes in case of refusal to eat.

Few data, limited to case reports or small case series, are available on the effectiveness of KD by etiology.[16,19-21] In our series there was no difference between main etiologic groups in terms of effectiveness. Although the effectiveness of the KD treatment for individuals with a specific etiology could not be determined due to the small numbers of patients in each specific group in our series, KD appeared to be particularly effective in patients with GLUT-1 deficiency, pyruvate dehydrogenase deficiency, hypoxic ischemic encephalopathy, and Dravet and Rett syndrome, in line with previous reports.[4,6,15,19,20] It is also noteworthy that KD treatment was found to be safe and effective in some single cases for which the effectiveness and tolerability of KD have not been previously reported including SCN1B encephalopathy, hypotalamic hamartoma, hypomyelinating leukodystrophy, cobalamin synthesis defect, EMC1 encephalopathy, KCN1B encephalopathy, DYRK1A related disorder, STXBP1 related encephalopathy, congenital glycosylation defect 1D, and nonketotic hyperglysinemia. However an infant with Tay-Sachs syndrome did not respond to KD treatment, and another infant with a diagnosis of mitochondrial DNA depletion syndrome type 14 developed severe pneumonia and we stopped KD during the initiation period.

Age at onset of KD did not have any effect on effectiveness in our series similar to some studies[15,16,21,22], but contrary to the others that demonstrated higher effectiveness in younger ages.[4,5,23,24] One of the suggested explanations for the question of why KD treatment in older children is less effective, is the longer duration of epilepsy.[25,26] This has been supported by

the observation that KD was more effective in patients with shorter duration of epilepy before epilepsy becomes intractable.[22] However this explanation was not supported by our study where the duration of epilepsy had no effect on effectiveness. Thus, the lower efficacy of KD in older children may be due to decreased compliance to the diet rather than the longer duration of epilepsy or age of the patients, as suggested before.[24]

Improvement in cognition and alertness has been reported as an additional therapeutic effect in children receiving KD.[24,27] This was also remarkable in our series, in which parents of more than 90% of responders, and also about two third of patients who did not achieve improvements in seizure frequency reported improvement in alertness which was the most important reason for continuation of diet for non-responders.

Our rates of adverse effects were comparable to those of previous reports.[3,6,13,28] Among more than 40 known categories, the most commonly reported side effects of KD therapy include gastrointestinal disturbances, hyperlipidemia, and kidney stones.[3,6,15] Consistent with previous reports, constipation was the most frequently observed advers effect in our series affecting about a quarter of the children at any time during the KD therapy.[3,6,13,15,20] However, 19% of our patients already had constipation prior to the onset of KD, and in line with published studies, no patient stopped the diet because of constipation.[3,15] Thus, it appears that patients with drug resistant epilepsy are prone to having constipation but this problem can be managed by increasing dietary water and fibers, enema, or polyethylene glycol.

Hyperlipidemia, another frequently reported adverse effect in children receiving KD, was also observed in approximately three quarters of the patients in our series.[3,15,29] However, as expected, most of them had mild hyperlipidemia and none needed antilipidemic medication. Since children with mild hyperlipidemia were found to be more likely to respond to KD therapy in our series, it would be more appropriate to call it a desired effect rather than an adverse effect. Severe hyperlipidemia were observed in 12% of our patients and similar to a previous report, it was successfully managed by reducing KD ratio and/or the amount of saturated fat in the diet.[30]

Ketogenic diet has been associated with a reported incidence of kidney stones between 2.2 and 6.7%, which rarely leads to cessation of KD therapy.[4,5,30,31] Although the incidence of kidney stones, which was 12% in our study, was slightly higher than those in previous studies, no child had to terminate the diet because of stones.[5,31] Following potassium citrate supplementation and increasing fluid intake, the size of the stones decreased in two patients, remained unchanged in most, and one patient required lithotripsy. In addition, a patient who had kidney stones before starting diet successfully received KD without any renal complications. Surprisingly two patients stopped the diet due to grade II nephropathy detected on ultrasonography, which was very rarely reported[32], and none of these patients had calculi on ultrasonography. Nephropathy might have developed due to chronic acidosis and dehydration since these two children admitted emergency room with poor food and fluid intake, severe acidosis, persistent vomiting, and dehydration. Therefore hydration with larger amounts of water should be encouraged to prevent the formation of renal calculi, dehydration, acidosis and nephropathy.

In our series, the most common reason for discontinuation of KD therapy was a lack of efficacy, in line with previous studies reporting that up to half of patients stopped the diet due to limited efficacy.[3,5,6,16] Conversely, patients likely remain on diet for as long as they respond to treatment.[5] Indeed, nearly all patients in our series who showed more than 50% improvement in seizure frequency continued on the diet for more than 12 months; this is comparable to the 80% probability rate of remaining on the diet at 12 months reported previously.[5] Similar to previous reports, being

too restrictive for patients or parents, and refusal to eat was the second most common reason for diet cessation.[3,5,7,16] In agreement with previous reports, side effects and intercurrent ilnesses were not common causes of KD discontinuation in our study.[3,5,30] Except for two patients with acidosis, dehydration and nephropathy and one patient with elevated liver enzymes, most of the complications in our series were transient and improved with conservative management, and as reported previously, did not require cessation of the KD.[30] Consistent with previous studies, the cause of death of one patient in our series was attributed not to KD but to severe global developmental delay, malnutrition, and severe infection.[3,5]

### *Limitations*

The main limitations of this study are its retrospective nature and the heterogeneity of the study sample. There is also a risk of subjective error due to the use of parental or caregiver seizure records and determination of certain parameters, including improvement in alertness, based on verbal expressions of the parents. Our inability to evaluate the effectiveness of KD treatment for individual syndromes due to the small number of patients in each group was another significant limitation. In this study, the effectiveness of olive oil-based (approximately 80% of dietary fats) KD in daily clinical practice was investigated. To reflect the results of our daily clinical practice, we included all children who received KD between 2:1 and 4:1 ratios; children whose diets differed slightly in olive oil content, and also children who had dietary modifications such as replacing saturated fats with unsaturated fats or medium-length chain triglycerides, carnitine and/or omega-3 fatty acid supplementation, or changing olive oil content or KD ratio during the study period, to optimize ketone levels, minimize adverse effects or normalize serum lipid concentrations. Thus, this is not a study of the effectiveness of well-controlled olive-oil-based KD, but a study that reflects the results of daily clinical practice of olive-oil-based KD. Future comparative studies are needed for the effectiveness of well-controlled olive oil-based KD.

In conclusion, individualized olive oil-based KD appears to be effective in about two-thirds of children with various types of drug-resistant epilepsy, including one-third who remain seizure free. Mild hyperlipidemia seems to be associated with a higher response rate. Although the small number of patients with specific etiologies did not allow us to provide evidence for effectiveness, KD appears to be particularly effective in patients with GLUT-1 deficiency, pyruvate dehydrogenase deficiency, and Dravet Syndrome. Discontinuation of KD is mostly due to lack of efficacy or nonadherence to diet, and rarely side effects.

**Acknowledgements**

This work was completed at the Department of Pediatric Neurology, Health Sciences University, İzmir Dr. Behçet Uz Children's Hospital. The authors thank all patients, their parents and all colleagues for their help in follow-up of the patients.

**Ethical approval**

This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Dr. Behçet Uz Children's Education and Research Hospital Clinical Research Ethics Committee on May 21, 2020 (Number: 2020/08-05).

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: SE, ÜY; data collection: ZA, YG, GG, BTB, SS, SP, HHK, MY; analysis and interpretation of results: SE, ÜY, MK, AÜ; draft manuscript preparation: SE, ÜY. 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. Kwan P, Arzimanoglou A, Berg AT, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 2010; 51: 1069-1077. https://doi.org/10.1111/j.1528-1167.2009.02397.x
2. Martin-McGill KJ, Jackson CF, Bresnahan R, Levy RG, Cooper PN. Ketogenic diets for drug-resistant epilepsy. Cochrane Database Syst Rev 2018; 11: CD001903. https://doi.org/10.1002/14651858.CD001903.pub4
3. Cai QY, Zhou ZJ, Luo R, et al. Safety and tolerability of the ketogenic diet used for the treatment of refractory childhood epilepsy: a systematic review of published prospective studies. World J Pediatr 2017; 13: 528-536. https://doi.org/10.1007/s12519-017-0053-2
4. Suo C, Liao J, Lu X, Fang K, Hu Y, Chen L, et al. Efficacy and safety of the ketogenic diet in Chinese children. Seizure 2013; 22: 174-178. https://doi.org/10.1016/j.seizure.2012.11.014
5. Freeman JM, Vining EP, Pillas DJ, Pyzik PL, Casey JC, Kelly LM. The efficacy of the ketogenic diet-1998: a prospective evaluation of intervention in 150 children. Pediatrics 1998; 102: 1358-1363. https://doi.org/10.1542/peds.102.6.1358
6. Raju KN, Gulati S, Kabra M, et al. Efficacy of 4:1 (classic) versus 2.5:1 ketogenic ratio diets in refractory epilepsy in young children: a randomized open labeled study. Epilepsy Res 2011; 96: 96-100. https://doi.org/10.1016/j.eplepsyres.2011.05.005
7. Lee HF, Chi CS, Liao JH. Use of cooking oils in a 2:1 ratio classical ketogenic diet for intractable pediatric epilepsy: Long-term effectiveness and tolerability. Epilepsy Res 2018; 147: 75-79. https://doi.org/10.1016/j.eplepsyres.2018.09.002
8. Asadi M, Rahimlou M, Shishehbor F, Mansoori A. The effect of l-carnitine supplementation on lipid profile and glycaemic control in adults with cardiovascular risk factors: A systematic review and meta-analysis of randomized controlled clinical trials. Clin Nutr 2020; 39: 110-122. https://doi.org/10.1016/j.clnu.2019.01.020
9. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. World Health Organ Tech Rep Ser 1985; 724: 1-206.
10. Ketodietcalculator. Available at: https://ketodietcalculator.org/ketoweb/KetoStart (Accessed on June 28, 2021).
11. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017; 58: 522-530. https://doi.org/10.1111/epi.13670
12. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017; 58: 512-521. https://doi.org/10.1111/epi.13709
13. Neal EG, Chaffe H, Schwartz RH, et al. A randomized trial of classical and medium-chain triglyceride ketogenic diets in the treatment of childhood epilepsy. Epilepsia 2009; 50: 1109-1117. https://doi.org/10.1111/j.1528-1167.2008.01870.x
14. Wells J, Swaminathan A, Paseka J, Hanson C. Efficacy and safety of a ketogenic diet in children and adolescents with refractory epilepsy-a review. Nutrients 2020; 12: E1809. https://doi.org/10.3390/nu12061809
15. Coppola G, Verrotti A, Ammendola E, et al. Ketogenic diet for the treatment of catastrophic epileptic encephalopathies in childhood. Eur J Paediatr Neurol 2010; 14: 229-234. https://doi.org/10.1016/j.ejpn.2009.06.006
16. Kang HC, Kim YJ, Kim DW, Kim HD. Efficacy and safety of the ketogenic diet for intractable childhood epilepsy: Korean multicentric experience. Epilepsia 2005; 46: 272-279. https://doi.org/10.1111/j.0013-9580.2005.48504.x
17. Bergqvist AG, Schall JI, Gallagher PR, Cnaan A, Stallings VA. Fasting versus gradual initiation of the ketogenic diet: a prospective, randomized clinical trial of efficacy. Epilepsia 2005; 46: 1810-1819. https://doi.org/10.1111/j.1528-1167.2005.00282.x

18. Seo JH, Lee YM, Lee JS, Kang HC, Kim HC. Efficacy and tolerability of the ketogenic diet according to lipid: nonlipid ratios - comparison of 3:1 with 4:1 diet. Epilepsia 2007; 48: 801-805. https://doi.org/10.1111/j.1528-1167.2007.01025.x

19. Thammongkol S, Vears DF, Bicknell-Royle J, et al. Efficacy of the ketogenic diet: which epilepsies respond?. Epilepsia 2012; 53: e55-e59. https://doi.org/10.1111/j.1528-1167.2011.03394.x

20. Riantarini I, Kim HD, Ko A, et al. Short- and long-term seizure-free outcomes of dietary treatment in infants according to etiology. Seizure 2019; 71: 100-104. https://doi.org/10.1016/j.seizure.2019.06.002

21. Kim SH, Shaw A, Blackford R, et al. The ketogenic diet in children 3 years of age or younger: a 10-year single-center experience. Sci Rep 2019; 9: 8736. https://doi.org/10.1038/s41598-019-45147-6

22. Dressler A, Stöcklin B, Reithofer E, et al. Long-term outcome and tolerability of the ketogenic diet in drug-resistant childhood epilepsy--the Austrian experience. Seizure 2010; 19: 404-408. https://doi.org/10.1016/j.seizure.2010.06.006

23. Dressler A, Trimmel-Schwahofer P, Reithofer E, et al. The ketogenic diet in infants--Advantages of early use. Epilepsy Res 2015; 116: 53-58. https://doi.org/10.1016/j.eplepsyres.2015.06.015

24. Maydell BV, Wyllie E, Akhtar N, et al. Efficacy of the ketogenic diet in focal versus generalized seizures. Pediatr Neurol 2001; 25: 208-212. https://doi.org/10.1016/S0887-8994(01)00310-1

25. Henderson CB, Filloux FM, Alder SC, Lyon JL, Caplin DA. Efficacy of the ketogenic diet as a treatment option for epilepsy: meta-analysis. J Child Neurol 2006; 21: 193-198. https://doi.org/10.2310/7010.2006.00044

26. Rubenstein JE, Kossoff EH, Pyzik PL, Vining EP, McGrogan JR, Freeman JM. Experience in the use of the ketogenic diet as early therapy. J Child Neurol 2005; 20: 31-34. https://doi.org/10.1177/08830738050200010501

27. Nordli DR Jr, Kuroda MM, Carroll J, et al. Experience with the ketogenic diet in infants. Pediatrics 2001; 108: 129-133. https://doi.org/10.1542/peds.108.1.129

28. Lambrechts DA, Wielders LH, Aldenkamp AP, Kessels FG, de Kinderen RJ, Majoie MJ. The ketogenic diet as a treatment option in adults with chronic refractory epilepsy: efficacy and tolerability in clinical practice. Epilepsy Behav 2012; 23: 310-314. https://doi.org/10.1016/j.yebeh.2012.01.002

29. Yılmaz Ü, Edizer S, Köse M, et al. The effect of ketogenic diet on serum lipid concentrations in children with medication resistant epilepsy. Seizure 2021; 91: 99-107. https://doi.org/10.1016/j.seizure.2021.06.008

30. Kang HC, Chung DE, Kim DW, Kim HD. Early- and late-onset complications of the ketogenic diet for intractable epilepsy. Epilepsia 2004; 45: 1116-1123. https://doi.org/10.1111/j.0013-9580.2004.10004.x

31. Sampath A, Kossoff EH, Furth SL, Pyzik PL, Vining EP. Kidney stones and the ketogenic diet: risk factors and prevention. J Child Neurol 2007; 22: 375-378. https://doi.org/10.1177/0883073807301926

32. Ballaban-Gil K, Callahan C, O'Dell C, Pappo M, Moshé S, Shinnar S. Complications of the ketogenic diet. Epilepsia 1998; 39: 744-748. https://doi.org/10.1111/j.1528-1157.1998.tb01160.x

The Turkish Journal of Pediatrics 2022; 64: 221-227
https://doi.org/10.24953/turkjped.2021.1943

**Original Article**

# Retinopathy of prematurity: applicability of international and national screening guidelines in the north of Iran

Yousef Alizadeh, Hassan Behboudi, Maryam Dourandeesh, Reza Soltani-Moghadam, Mitra Akbari, Abdolreza Medghalchi, Ebrahim Azaripour, Zahra Moravvej

*Eye Research Center, Department of Eye, Amiralmomenin Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.*

## ABSTRACT

**Background.** To determine the applicability of current international and national retinopathy of prematurity (ROP) screening guidelines and to identify a suitable community-based screening criterion.

**Methods.** A retrospective study on premature neonates (≤37 weeks gestation) referred to a tertiary eye hospital ROP clinic in the north of Iran was conducted over a 10-year period. Neonates were classified as no ROP, with ROP and type 1 ROP. Data consisting of birth weight (BW), gestational age (GA) and chief risk factors were evaluated. Various screening criteria and currently established screening guidelines were applied and compared for applicability using a receiver operating characteristic curve.

**Results.** A total of 716 neonates with a mean GA of 31.4 ± 2.8 weeks and BW of 1629 ± 502 grams were screened. The incidence of ROP was 22.9% and type 1 ROP requiring treatment was 0.28%. When applying the national Ministry of Health Guidelines, all neonates with type 1 ROP requiring treatment were identified; These criteria had a specificity of 7% for the diagnosis of type 1 ROP, and a large number of neonates (n=645) who are not at risk for type 1 ROP will be redundantly screened. Guidelines of the American Academy of Pediatrics and the UK would miss 4.5% of patients requiring ROP treatment. According to our data a threshold of GA≤32 weeks and/or BW ≤1600 grams demonstrated a sensitivity of 95.7% and specificity of 33.6% for the diagnosis of any ROP and a sensitivity of 100% and specificity of 26.8% for type 1 ROP requiring treatment.

**Conclusions.** The ideal ROP screening guideline is one that is very sensitive and identifies patients requiring treatment without delay. To minimize redundant screening while maintaining optimum ROP requiring treatment diagnosis, we proposed a new local evidence-based screening guideline.

**Key words:** retinopathy of prematurity, prematurity, screening guideline, neonate.

Retinopathy of prematurity (ROP), a vasoproliferative retinal disease in premature neonates, is a leading cause of avoidable childhood blindness and impaired vision.[1,2] The advancement of neonatal care in developing countries including Iran has led to an increase in the incidence of ROP.[3,4] The main preventive measure in these neonates is serial fundus examinations for timely diagnosis of vision-threatening ROP.

An adequate ROP screening program should be cost-effective, detect those in need of treatment and avoid redundant examinations which are stressful for infants and families. As various population-based and prenatal care factors influence ROP and its severity, the development of specific screening criteria tailored to the local population seem necessary.[5-7] The current ROP screening guideline of the American Academy of Pediatrics (AAP) has shown to be inadequate in developing countries.[5,6] Furthermore, the

✉ Maryam Dourandeesh
maryam.dourandeesh.dl@gmail.com

Received 30th June 2021, revised 15th September 2021, accepted 24th September 2021.

current Iranian screening guideline which recommends screening neonates of birth weights (BWs) ≤2000 grams and/or gestational age (GA) <34 weeks seems to place a large burden on the health-care system.

The purpose of this study was to access the applicability of international guidelines (including the AAP's and United Kingdom's) and our current national screening guideline in premature neonates in the north of Iran. This study was carried out at the main referral hospital for ROP in the north of Iran and is one of the few of its kind in Iran. We aimed to modify and determine a screening threshold that would be safer and more efficient to identify type 1 ROP requiring treatment in neonates.

## Material and Methods

Data were retrospectively collected from the records of all premature neonates (≤37 weeks gestation) examined in the ROP clinic from 2008 to 2018 at Amiralmomenin Hospital, a tertiary hospital in the Guilan province. The study was approved by our institutional ethics review board and was conducted in accordance with the tenets of the Declaration of Helsinki with the code of IR.GUMS.REC.1394.195. The patients were all referred from local neonatal intensive care units or by a pediatrician. The same protocol was applied for all neonates and patients were examined by retina specialists with expertise in ROP. After the instillation of mydriatic eye drops (0.5% tropicamide and 1% phenylephrine) indirect ophthalmoscopy was performed using a sterile eyelid speculum, depressor and 2.2 or 30 diopter lenses. The staging of ROP was recorded according to the International Classification of ROP.

Medical data regarding gestational age (GA), birth weight (BW) and additional risk factors for the development of ROP, such as twin birth, O2 therapy, mechanical ventilation, acute respiratory distress syndrome, apnea, intra-ventricular hemorrhage and transfusion were extracted. ROP staging was done according to the International Classification of Retinopathy of Prematurity (2005). Neonates requiring treatment were indicated based on the early treatment of ROP (ETROP) study and included (Type 1 ROP): eyes with any stage of ROP with plus disease in zone 1, stage 3 without/with plus disease in zone 1, and stage 2 or 3 with plus disease in zone 2. Treatment included indirect diode laser pan-retinal photocoagulation for type 1 ROP and anti-VEGF injection or pars-plana vitrectomy when indicated.

### *Statistical analysis*

Statistical analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL). Student t-test was used to compare the GA and BW of infants with no ROP, with ROP and type 1 ROP. The Chi-square test was used to compare categorical variables. To determine the appropriate GA and BW for screening of ROP and type 1 ROP, a receiver operating characteristic (ROC) plot was used. Different BW and GA thresholds were combined to establish sets of criteria and the sensitivity and specificity was determined in each setting. Also, the AAP, UK, and the national Iranian guidelines were applied to our patients to determine their efficacy. In the multivariate analysis, we used logistic models with the backward likelihood ratio method. All variables with significant levels less than 0.1 in multivariate analysis were entered in the logistic model. A p-value of less than 0.05 was considered to be statistically significant.

## Results

A total of 716 neonates with a GA of ≤37 weeks were enrolled in this study. The mean GA ± SD of the patients was 31.4 ± 2.8 weeks (range: 24-37 weeks). The mean BW of the patients was 1629 ± 502 grams (range: 600-3360 grams).

ROP was observed in either one or both eyes of 164 (22.9%) patients, of which 22 patients (13.41%) required treatment. There was no significant difference for type 1 ROP patients in terms of gender (45.5% female vs. 54.5% male, p-value=0.67).

GA and BW of patients with and without ROP and type 1 ROP are compared in Table I.

Systemic factors and potential risk factors for ROP were compared between patients presenting with and without treatment requiring ROP (Table II). According to logistic regression analysis none of the investigated variables showed a significant effect on the development of type 1 ROP requiring treatment.

ROC curve analysis on GA for ROP detection demonstrated that the area under the curve (AUC) was 0.815 (95% CI 0.779 to 0.852). ROC curve analysis on BW for ROP detection confirmed that the AUC was 0.798 (95% CI 0.760 to 0.837). Also, the AUC for type 1 ROP was 0.745 (95% CI 0.673 to 0.818) and 0.773 (95% CI 0.709 to 0.837) for GA and BW, respectively.

According to our data, when a screening threshold of BW ≤2000 grams and GA≤35 weeks was considered; 100% sensitivity for the diagnosis of ROP and type 1 ROP was reached.

When applying the current national screening threshold for ROP (BW ≤2000 grams and/or GA<34 weeks), 99.9% of ROP patients would be diagnosed without any cases of type 1 ROP requiring treatment being missed. This threshold would result in a very weak specificity (8.6% for ROP and 7% for treatment requiring ROP diagnosis) and 645 neonates who are not at risk of type 1 ROP requiring treatment are screened. This screening factor results in a high burden on the health care system.

On the other hand, following the screening recommendations of the AAP, would demonstrate 84.1% and 95.4% sensitivity for ROP and type 1 ROP requiring treatment, respectively. The AAP threshold would miss the diagnosis of one (4.5%) patient requiring ROP treatment. Also, using the UK's screening criteria one patient requiring ROP treatment would be missed.

In order to determine an appropriate screening threshold, we investigated several potential screening criteria in terms of sensitivity and specificity (Fig. 1). The best option was a threshold of GA≤32 weeks and/or BW ≤1600 grams which demonstrated a sensitivity of 95.7% and specificity of 33.6% for the diagnosis of any ROP and a sensitivity of 100% and specificity of 26.8% for type 1 ROP requiring treatment.

## Discussion

Improvement in neonatal care and the consequent rise in survival rates has led to an increase in the prevalence of ROP in middle-income countries. Early diagnosis and treatment of this disease is very important in preserving vision.[1,2,8-10]

**Table I.** Comparison of GA and BW of patients with and without ROP and type 1 ROP requiring treatment.

| | | Total | Group | | Difference | 95% CI | | p-value* |
|---|---|---|---|---|---|---|---|---|
| | | | No-ROP | ROP | | Lower | Upper | |
| GA | Mean ±SD | 31.4 ± 2.8 | 32.1±2.51 | 28.9±2.3 | 3.1 | 2.7 | 3.5 | < 0.001 |
| | Range | 24-37 | 25-37 | 24-35 | | | | |
| BW | Mean ±SD | 1629 ±502 | 1739±485 | 1257±362 | 482 | 401 | 562 | < 0.001 |
| | Range | 600-3360 | 600-3360 | 720-3000 | | | | |
| | | Total | Group | | | | | |
| | | | No-ROP | ROP | | | | |
| GA | Mean ±SD | 31.4±2.8 | 31.4±2.7 | 29.0±2.0 | 2.3 | 1.1 | 3.5 | < 0.001 |
| | Range | 24-37 | 25-37 | 24-32 | | | | |
| BW | Mean ±SD | 1629±502 | 1642±503 | 1202±239 | 439 | 107 | 228 | < 0.001 |
| | Range | 600-3360 | 600-3360 | 750-1600 | | | | |

*Student t-test
GA: gestational age; BW: birth weight

**Table II.** Comparison of associated risk factors in ROP requiring (Type 1 ROP) and not requiring treatment groups.

| | | Total | ROP requiring Treatment | | p-value* |
|---|---|---|---|---|---|
| | | | No | Yes | |
| Intubation | Yes | 63 (8.8) | 61 (8.8) | 2 (9.1) | 0.961 |
| | No | 653 (91.2) | 633 (91.2) | 20 (90.9) | |
| Transfusion | Yes | 131 (18.3) | 121 (17.4) | 10 (45.5) | 0.001 |
| | No | 585 (81.7) | 573 (82.6) | 12 (54.5) | |
| O2 Therapy | Yes | 476 (66.5) | 454 (65.4) | 22 (100) | 0.001 |
| | No | 240 (33.5) | 240 (34.6) | 0 (0) | |
| Phototherapy | Yes | 367 (51.3) | 349 (50.3) | 18 (81.8) | 0.004 |
| | No | 349 (48.7) | 345 (49.7) | 4 (18.2) | |
| ARDS | Yes | 459 (64.1) | 438 (63.1) | 21 (95.5) | 0.002 |
| | No | 257 (35.9) | 256 (36.9) | 1 (4.5) | |
| IVH | Yes | 32 (4.5) | 29 (4.2) | 3 (13.6) | 0.035 |
| | No | 684 (95.5) | 665 (95.8) | 19 (86.4) | |
| Apnea | Yes | 82 (11.5) | 76 (11) | 6 (27.3) | 0.018 |
| | No | 634 (88.5) | 618 (89) | 16 (72.7) | |
| Twin birth | Yes | 189 (26.4) | 185 (26.7) | 4 (18.2) | 0.37 |
| | No | 527 (73.6) | 509 (73.3) | 18 (81.8) | |

*Chi-square test
ARDS: acute respiratory distress syndrome, IVH: intraventricular hemorrhage

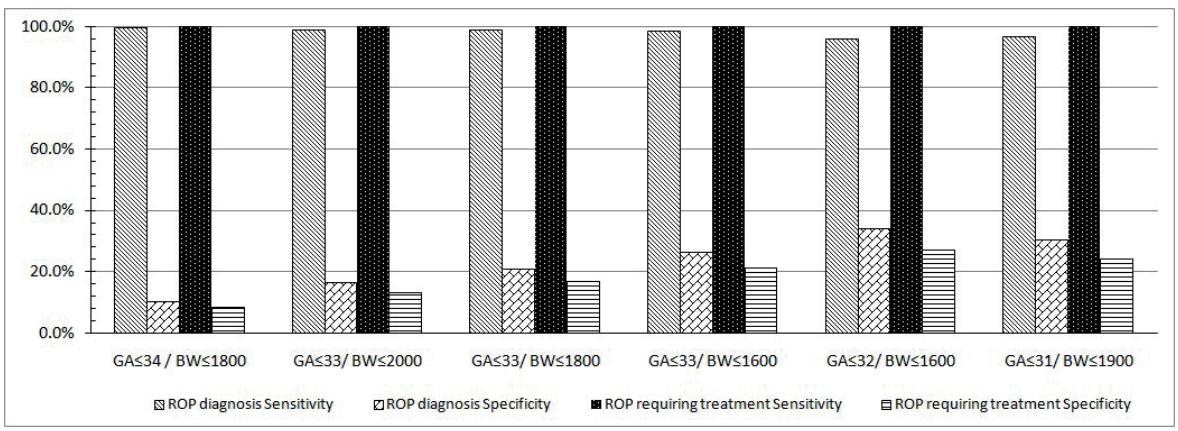



**Fig. 1.** Sensitivity and specificity for ROP and ROP requiring treatment diagnosis at different gestational age (GA) and birth weight (BW) thresholds using receiver operating characteristic curves.

The present study was conducted at the Amiralmomenin Hospital; the only tertiary hospital to specialize in ROP management in the Guilan province, north of Iran. The hospital is the main referral hospital for ROP in the north of Iran.

ROP was detected in 22.9% of the premature neonates. This rate was somewhat lower than the previous studies from Iran, reporting incidences of 33.3%, 42.1%, 37.2% and 26.2% from Tehran, Shiraz, Southern and North-east Iran, respectively.[11-14] We also noted lower rates compared to countries such as Turkey (27%), Oman (40.4%), Saudi Arabia (38.7%), India (25.3%), Egypt (34.4%) and Canada (40.4%).[9,15-19] In comparison lower incidence was reported

from China (15.9%), Bahrain (20.4%) and the United States (19.88%).[20-22]

Among neonates with ROP, the frequency of type 1 ROP requiring treatment in this study was 13.41%, which was higher than previous studies reported from Iran. Various studies from several provinces of Iran have reported rates from 7.5 to 11.1%.[11,12,23,24] The mean GA (29.0 ± 2.0 weeks) and BW (1202 ± 239 grams) of neonates with type 1 ROP in this study was similar to previous studies from Iran and in moderately developed countries.[11]

This difference between countries can be associated with genetics, NICU care facilities, socioeconomic status and the criteria set out in screening guidelines. Maintaining a balance between identifying all ROP infants in need of treatment and minimizing unnecessary examinations, as well as saving financial and human resources is very challenging.[9,25] Therefore, assessing screening criteria and determining criteria justified to each countries resources and requirements seems necessary.[8,19,26]

When applying the AAP and UK's guidelines to our data, we noticed that they would miss the diagnosis of one (4.5%) ROP infant in need of treatment. According to the latest regulations of the Ministry of Health and Medical Education of Iran, it is recommended that all infants with a GA of less than 34 weeks (33 weeks and 6 days or less) or a BW of 2000 grams or less, as well as infants born at birth more than or equal to 34 weeks gestation or weighing >2000 g if clinically unstable or are diagnosed as high-risk by a physician, should be examined for retinopathy. When we used our current national Ministry of Health Guidelines, all neonates with ROP in need of treatment were identified. However, when using these criteria, a large number of neonates (645) who are not at risk for type 1 ROP are being screened.

The most suitable ROP screening criteria would be one that does not miss any neonates with ROP that require treatment while limiting the burden on the health-care system and minimizing examinations of infants with mild or no ROP. By evaluating several potential screening criteria in terms of sensitivity and specificity, we reached a cut-off point of GA≤32 weeks and/or BW ≤1600 grams as the ideal criteria. This criterion has 100% sensitivity and 26.8% specificity for type 1 ROP. Roohipoor et al.[8] in 2016 showed that using the guidelines of other countries in Iran can lead to missing cases requiring treatment, so they suggested a cut-off point of G ≤32 weeks or BW≤2000 grams as the basis screening in Iran. A recent study from Tehran has proposed a cut-off point of GA≤32 weeks or BW≤1750 grams.[11] Findings of our study are in accordance with both mentioned studies and this suggests that national guidelines may need regular re-evaluation, especially in developing countries. In comparison to the two studies from Tehran, the lower cut-off BW reached in our study may be due to the larger number of patients referring to Tehran (a countrywide referral center), different socioeconomic status, different neonatal care and diagnostic facilities (e.g. ultrasound for accurate determination of GA and precision of weight scales).[27]

Evidence-based screening criteria are essential for ROP screening and are ongoing in various countries. A study in China found that using optimized criteria (GA <32 weeks or BW <1600 g) in comparison to China's Ministry of Health criteria (GA ≤34 weeks or a BW ≤1750 g) can reduce 43.2% of the examinations.[20] A study in Saudi Arabia showed that by using the Canadian criteria (GA ≤30 weeks or BW ≤1250 g) all ROP cases in need of treatment can be identified with 100% sensitivity and 13.6% specificity. These GA and BW values are lower than the values specified by the National Eye Health Program of the Saudi Ministry of Health (BW ≤1500 g or GA of ≤32 weeks).[9] A cohort study in Germany showed that in the absence of a specific risk factor, the risk of developing type 1 ROP in neonates with a GA ≥30 weeks is very low or zero.[10] This number is also lower than the current German national guideline (GA<32 weeks).[10] The newly defined criteria in these studies indicated a lower cut-off than the previously established values. The present

study and similar aforementioned studies indicate the need for periodic revision of criteria and even generating provincial specific criteria.

This study had some limitations, including the retrospective design and the relatively small sample size. Although our hospital is the primary referral center for ROP in the north of Iran, the short distance to the country's capital city medical centers may have limited the accuracy of populational demographics.

In conclusion, the most ideal ROP screening guideline is one that is very sensitive and does not miss any patients requiring treatment. Owing to different ethnic, diagnostic and therapeutic facilities and socioeconomic statuses in different countries and different regions of a country, it seems reasonable to determine local or even institutional evidence-based screening criteria. According to the present study for timely detection of type 1 ROP in this geographic region, it seems reasonable to screen for ROP at GA≤32 weeks or BW ≤1600 grams.

**Acknowledgement**

The authors would like to acknowledge Dr. Ehsan Kazemnezhad Leyli, Associate Professor of Biostatistics, and Dr. Hadi Shamsaei, ophthalmologist, for their great contribution to this study.

**Ethical approval**

The study was approved by our institutional ethics review board and was conducted in accordance with the tenets of the Declaration of Helsinki with the code of IR.GUMS. REC.1394.195.

**Author contribution**

The authors confirm contribution to the paper as follows: study concept and design: YA; data collection: YA, HB, MD, RSM, MA, AM, EA; analysis and interpretation of data: MD, ZM; writing the paper: MD. 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. Blencowe H, Lawn JE, Vazquez T, Fielder A, Gilbert C. Preterm-associated visual impairment and estimates of retinopathy of prematurity at regional and global levels for 2010. Pediatr Res 2013; 74: 35-49. https://doi.org/10.1038/pr.2013.205
2. Beden Ü, Demir S, Aygün C, Küçüködük S, Erkan D. Screening for retinopathy of prematurity (ROP) in the middle Black Sea region of Turkey. Turk J Pediatr 2012; 54: 223-229.
3. Karkhaneh R, Mousavi SZ, Riazi-Esfahani M, et al. Incidence and risk factors of retinopathy of prematurity in a tertiary eye hospital in Tehran. Br J Ophthalmol 2008; 92: 1446-1449. https://doi.org/10.1136/bjo.2008.145136
4. Gilbert C, Fielder A, Gordillo L, et al. Characteristics of infants with severe retinopathy of prematurity in countries with low, moderate, and high levels of development: implications for screening programs. Pediatrics 2005; 115: e518-e525. https://doi.org/10.1542/peds.2004-1180
5. Başmak H, Niyaz L, Şahin A, Erol N, Gürsoy HH. Retinopathy of prematurity: screening guidelines need to be reevaluated for developing countries. Eur J Ophthalmol 2010; 20: 752-755. https://doi.org/10.1177/112067211002000417
6. 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 2018; 142: e20183061. Pediatrics 2019; 143: e20183810. https://doi.org/10.1542/peds.2018-3810
7. Wilson CM, Ells AL, Fielder AR. The challenge of screening for retinopathy of prematurity. Clin Perinatol 2013; 40: 241-259. https://doi.org/10.1016/j.clp.2013.02.003

8. Roohipoor R, Karkhaneh R, Farahani A, et al. Retinopathy of prematurity screening criteria in Iran: new screening guidelines. Arch Dis Child Fetal Neonatal Ed 2016; 101: F288-F293. https://doi.org/10.1136/archdischild-2015-309137

9. Mgharbil E, Raffa LH, Alessa S, Alamri A. Screening premature infants for retinopathy of prematurity in a tertiary hospital in Saudi Arabia. Ann Saudi Med 2020; 40: 87-93. https://doi.org/10.5144/0256-4947.2020.87

10. Larsen PP, Müller A, Lagrèze WA, Holz FG, Stahl A, Krohne TU. Incidence of retinopathy of prematurity in Germany: evaluation of current screening criteria. Arch Dis Child Fetal Neonatal Ed 2021; 106: 189-193. https://doi.org/10.1136/archdischild-2020-319767

11. Khorshidifar M, Nikkhah H, Ramezani A, et al. Incidence and risk factors of retinopathy of prematurity and utility of the national screening criteria in a tertiary center in Iran. Int J Ophthalmol 2019; 12: 1330-1336. https://doi.org/10.18240/ijo.2019.08.15

12. Bayat-Mokhtari M, Pishva N, Attarzadeh A, Hosseini H, Pourarian S. Incidence and risk factors of retinopathy of prematurity among preterm infants in Shiraz/Iran. Iran J Pediatr 2010; 20: 303-307.

13. Afarid M, Hosseini H, Abtahi B. Screening for retinopathy of prematurity in South of Iran. Middle East Afr J Ophthalmo 2012; 19: 277-281. https://doi.org/10.4103/0974-9233.97922

14. Abrishami M, Maemori GA, Boskabadi H, Yaeghobi Z, Mafi-Nejad S, Abrishami M. Incidence and risk factors of retinopathy of prematurity in mashhad, northeast iran. Iran Red Crescent Med J 2013; 15: 229. https://doi.org/10.5812/ircmj.4513

15. Bas AY, Demirel N, Koc E, Isik DU, Hirfanoglu İM, Tunc T. 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

16. Reyes ZS, Al-Mulaabed SW, Bataclan F, et al. Retinopathy of prematurity: revisiting incidence and risk factors from Oman compared to other countries. Oman J Ophthalmol 2017; 10: 26-32. https://doi.org/10.4103/ojo.OJO_234_2014

17. Goyal A, Giridhar A, Gopalakrishnan M, Thachil T. Neonatal Intensive Care Unit-based screening program for retinopathy of prematurity and its treatment in an Indian population. Indian J Ophthalmol 2019; 67: 828-833. https://doi.org/10.4103/ijo.IJO_201_18

18. Hadi AMA, 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: 831-837. https://doi.org/10.2147/OPTH.S40136

19. Isaza G, Arora S, Bal M, Chaudhary V. Incidence of retinopathy of prematurity and risk factors among premature infants at a neonatal intensive care unit in Canada. J Pediatr Ophthalmol Strabismus 2013; 50: 27-32. https://doi.org/10.3928/01913913-20121127-02

20. Yang Q, Zhou X, Ni Y, et al. Optimised retinopathy of prematurity screening guideline in China based on a 5-year cohort study. Br J Ophthalmol 2021; 105: 819-823. https://doi.org/10.1136/bjophthalmol-2020-316401

21. Al Alawi EK, Al Omran MS, Al Bahrana EH. Incidence of retinopathy of prematurity in Bahrain, 2002-2011. Middle East Afr J Ophthalmol 2015; 22: 335-339. https://doi.org/10.4103/0974-9233.159750

22. Ludwig CA, Chen TA, Hernandez-Boussard T, Moshfeghi AA, Moshfeghi DM. The epidemiology of retinopathy of prematurity in the United States. Ophthalmic Surg Lasers Imaging Retina 2017; 48: 553-562. https://doi.org/10.3928/23258160-20170630-06

23. Feghhi M, Altayeb SMH, Haghi F, et al. Incidence of retinopathy of prematurity and risk factors in the south-western region of Iran. Middle East Afr J Ophthalmol 2012; 19: 101-106. https://doi.org/10.4103/0974-9233.92124

24. Ebrahim M, Ahmad RS, Mohammad M. Incidence and risk factors of retinopathy of prematurity in Babol, North of Iran. Ophthalmic Epidemiol 2010; 17: 166-170. https://doi.org/10.3109/09286581003734860

25. Luk AS, Yip WW, Lok JY, Lau HH, Young AL. Retinopathy of prematurity: applicability and compliance of guidelines in Hong Kong. Br J Ophthalmol 2017; 101: 453-456. https://doi.org/10.1136/bjophthalmol-2016-308900

26. Lee SK, Normand C, McMillan D, et al. Evidence for changing guidelines for routine screening for retinopathy of prematurity. Arch Pediatr Adolesc Med 2001; 155: 387-395. https://doi.org/10.1001/archpedi.155.3.387

27. Alizadeh Y, Zarkesh M, Moghadam RS, et al. Incidence and risk factors for retinopathy of prematurity in North of Iran. J Ophthalmic Vis Res 2015; 10: 424-428. https://doi.org/10.4103/2008-322X.176907

The Turkish Journal of Pediatrics 2022; 64: 228-238
https://doi.org/10.24953/turkjped.2021.83

**Original Article**

# Regulatory T and B cells in transient hypogammaglobulinemia of infancy

Ayça Emsen, Hülya Uçaryılmaz, Tuğba Güler, Hasibe Artaç

*Division of Immunology and Allergy, Department of Pediatrics, Selcuk University Faculty of Medicine, Konya, Turkey.*

## ABSTRACT

**Background.** Transient hypogammaglobulinemia of infancy (THI) is a heterogeneous disorder caused by an abnormal delay in reaching normal IgG levels in the first three years of life. Although THI is a common primary immune deficiency, its pathogenesis has not been fully elucidated. We aimed to investigate the role of regulatory T cells (Tregs) and B cells (Bregs) in the pathogenesis of THI.

**Methods.** T and B cell subsets were evaluated in 40 patients with THI aged 6–41 months and 23 healthy controls aged 6–51 months using flow cytometry. CD4 and interleukin-2 receptor-$\alpha$ alpha (CD25) expression and a lack of interleukin-7 receptor-$\alpha$ (CD127) were used for Treg identification. FoxP3 expression in Tregs was determined as a percentage and mean fluorescence intensity. B cell subsets (plasmablast, mature naive, primarily memory, new memory) and Bregs were defined according to CD19, CD38, and CD24 expressions.

**Results.** Patients with THI (15 females and 25 males; mean age: 18.8 ± 8.6 months) and controls (10 females and 13 males; mean age: 22.6 ± 13.1 months) participated in this study. While the proportion of Tregs of children with THI were significantly increased compared to the controls, primarily memory B cells were reduced. Additionally, the proportions of CD127 in $CD3^+$ and $CD3^+CD4^+$ T cells were significantly reduced in the patients with THI compared to the control. No significant difference was detected in the FoxP3 expression of Tregs and the frequency of Bregs in the children with THI.

**Conclusions.** Increased Tregs and decreased primarily memory B cells may cause antibody production delay in children with THI. Changes in the T and B cell compartments may be related to chronic immune activation and affected cellular immunity in THI. Further studies are needed to use T and B cell subsets in the prediction of IgG level recovery.

**Key words:** transient hypogammaglobulinemia of infancy, regulatory T cells, regulatory B cells.

Transient hypogammaglobulinemia of infancy (THI) is a primary immune deficiency disease that is a subclass of predominantly antibody deficiencies.[1] THI is characterized by prolongation of the physiological hypogammaglobulinemia period due to the delay in the production of immunoglobulin (Ig).[2] In THI, mainly IgG levels, prevalently IgA and IgM levels, are lower than two standard deviations (SDs).[3] Although the actual incidence of this disease is unknown because most patients are asymptomatic and severe infections are rare, different results have been reported in studies of various centers.[3-6] According to Kılıç et al.[5], 73.9% of primary immunodeficiencies were primary antibody deficiencies, and THI constituted 31% of primary antibody deficiencies (22.9% in primary immunodeficiencies) in Turkey.

Clinically, THI is characterized by recurrent lower respiratory tract infection (LRTI) and upper respiratory tract infection (URTI), urinary tract infections, gastroenteritis, and invasive infections.[3] THI can be distinguished from other immunodeficiencies by its intact cellular

✉ Hasibe Artaç
artachasibe@gmail.com

Received 14th January 2021, revised 6th May 2021, 27th July 2021, 9th October 2021, accepted 24th November 2021.

This study was presented as an oral presentation at the 1st International Rumi Pediatric Congress, 4-7 December 2019, Konya, Turkey.

immunity, protective antibody response, and clinical improvement in serum Ig levels.[6] Spontaneous clinical improvement occurs in the 9th–15th month, but normalization of IgG levels can be delayed up to 2-5 years of age.[7]

The cause of the immune system disorder leading to hypogammaglobulinemia has not been fully understood in THI.[8] Studies have reported reasons for low Ig levels in THI as abnormalities in the B cell compartment, decreased numbers of the CD19 complex and memory B cells, defects in T helper cell (Th) maturation and function, aberrant cytokine production, and increased number of regulatory T cells (Tregs) and myeloid-derived suppressor cells.[6,9-13]

In studies involving children with THI, it has been reported that antibody production deficiency is associated with delayed maturation of Th cells.[6] IL-2 receptor-$\alpha$ (CD25) and IL-7 receptor-$\alpha$ (CD127) play an essential role in T cell development and antigen-specific T cell responses. As a result of the binding of IL-7 to its receptor (CD127), antiapoptotic molecules upregulate in T cells, and the T cell receptor–mediated signaling pathway initiates, leading to IL-2 production and proliferation in newly activated T cells. After activation by antigens and IL-7 binding, T cells lose CD127 surface expression. Therefore, CD127 is used to distinguish between effector, memory, and regulatory T cells.[14] $CD25^{+}CD127^{low/-}$ cells (Tregs) have regulatory functions and play an essential role in suppressing excessive inflammatory responses, preventing reactions to harmless environmental molecules, and suppressing the autoantibody response to self-antigens.[15,16]

$CD19^{+}$ B cell subsets at different maturation stages have been defined according to their CD38 and CD24 expressions.[17] CD38 is a coreceptor in mature B cells and leads to apoptosis in early B cells while increasing survival in B cells derived from the lymph node germinal center.[18] CD24 is a cell surface receptor responsible for regulating the maturation stage of B cells in the bone marrow, similar to CD38.[19] Together with the B cell receptor, CD24 functions in the apoptosis of autoreactive B cells. CD24 can regulate B cell receptor–mediated B cell selection in the bone marrow, thereby producing and leading to the migration of regulatory B cells (Bregs).[20] Bregs ($CD19^{+}CD38^{hi}CD24^{hi}$ B cells) regulate T and B cell responses, including the maintenance of Tregs.[21,22] The functions of Bregs have been demonstrated in autoimmunity, allergy, infections, and cancer.[22-24] Inborn errors of immunity associated with Breg deficiency have not been described so far. However, a decrease in $CD19^{+}CD38^{hi}CD24^{hi}$ B cells (Bregs) of patients with common variable immune deficiency has been reported.[25,26]

Although B cells have often been investigated due to hypogammaglobulinemia in THI, the role of Bregs is unclear. In this study, we investigated Tregs and Bregs to elucidate the immunological mechanisms causing hypogammaglobulinemia in children with THI.

## Material and Methods

### *Study participants*

Forty patients diagnosed with THI aged 6–41 months and 23 healthy controls aged 6–51 months were included in this study. Informed consent was obtained from the parents of the children with THI before they were included in the study. This study was approved by the Ethical Committee of Selcuk University Medical Faculty (2015/265).

The diagnosis of THI was based on the following criteria: (i) low serum levels of mainly IgG (< 2 SDs below age-defined norms) with and/or without reduced IgA and IgM levels diagnosed in the first three years of life; (ii) normal vaccine responses, isohemagglutinins, peripheral blood lymphocyte subpopulations, and cellular immunity; and (iii) defined causes of hypogammaglobulinemia (such as other primary immunodeficiencies, genetic disorders, chromosomal abnormalities, and other systemic illnesses or drugs) were excluded.[3,27]

The initial indications for testing immunoglobulin levels were recurrent URTI and/or LRTI, recurrent otitis media, and gastroenteritis.[28] The patients' demographic data and clinical history (such as age, gender, types and frequency of infections in the last year, hospitalizations, and history of allergic diseases and other systemic diseases) were obtained from their medical records. Hemoglobin, leukocyte, lymphocyte, and platelet levels, as well as IgG, IgA, and IgM levels, were recorded on admission. Clinical observation was performed regularly every three months, and immunoglobulin levels were checked every six months. Infections and treatments (antibiotic prophylaxis, inhaled steroid, or intravenous immunoglobulin) of the patients were recorded during the follow-up.

### *Flow cytometric analysis*

Peripheral lymphocyte subsets, Tregs, and Bregs in ethylenediamine tetra-acetate anticoagulated peripheral blood samples of the patients with THI and the control group were analyzed using FACSAria III flow cytometer (Becton–Dickinson, CA, USA) and FACSDiva version 6.1.3 software package. Peripheral blood mononuclear cells were isolated from heparinized blood using Ficoll-Histopaque density gradient centrifugation, and flow cytometric analysis of these cells was performed on at least 50,000 acquired events. Treg staining was performed using CD3 (APC), CD4 (PerCP), CD25 (PE), FoxP3 (Alexa fluor 488), and CD127 (Alexafluor 700) monoclonal antibodies (Biolegend, San Diego, USA). In the flow cytometric analysis, lymphocytes were gated, and then $CD25^{+}CD127^{low/-}$ Tregs, $CD25^{low/-}CD127^{+}$ T, and $CD25^{-}CD127^{-}$ T cells were identified at the $CD3^{+}CD4^{+}$ Th gate (Fig. 1A and 1B).[15] FoxP3 expression of $CD25^{+}CD127^{low/-}$ Tregs was determined as a percentage and mean fluorescence intensity (MFI).

B cell subsets were determined according to their expression at maturation stages using CD19 (FITC), CD24 (APC), CD38 (Alexa fluor 700) monoclonal antibodies (Biolegend, San Diego, USA) (Fig. 2).[29] At the $CD19^{+}$ B cell gate in the lymphocyte population, the $CD38^{hi}CD24^{hi}$ Bregs, $CD38^{int}CD24^{int}$ mature naive B cells, $CD38^{hi}CD24^{-}$ plasmablast, $CD38^{-}CD24^{+}$ primarily memory B cells, and $CD38^{-}CD24^{-}$ new memory B cells were determined (Fig. 3A).[30]

### Statistical analysis

Statistical analysis of the data was performed using IBM SPSS for Windows 17.0 software package (IBM Corp, Armonk, NY, USA). Tregs and B cell subsets were determined and compared in the patients with THI and the control groups. Descriptive statistics such as the number of patients in the THI and control groups, geometric mean, arithmetic mean, minimum and maximum values, and mean ± 2 SD values were given for both groups. When

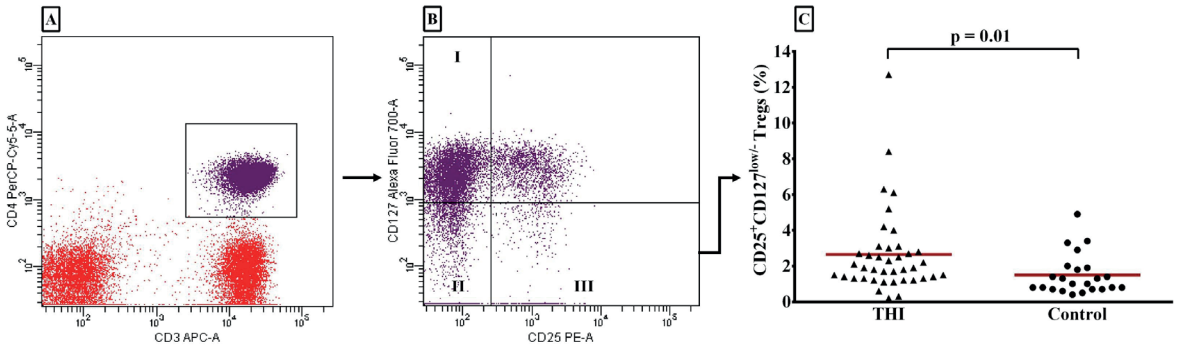



**Fig. 1.** Flow cytometric analysis of Tregs according to CD127 and CD25 expressions (A - B), statistically comparison of Tregs (C) in THI and control groups. $CD3^{+}CD4^{+}$ Th cells were classified as $CD25^{low/-}CD127^{+}$ T cells (I), $CD25^{-}CD127^{-}$ T cells (II) and $CD25^{+}CD127^{low/-}$ Tregs (III).

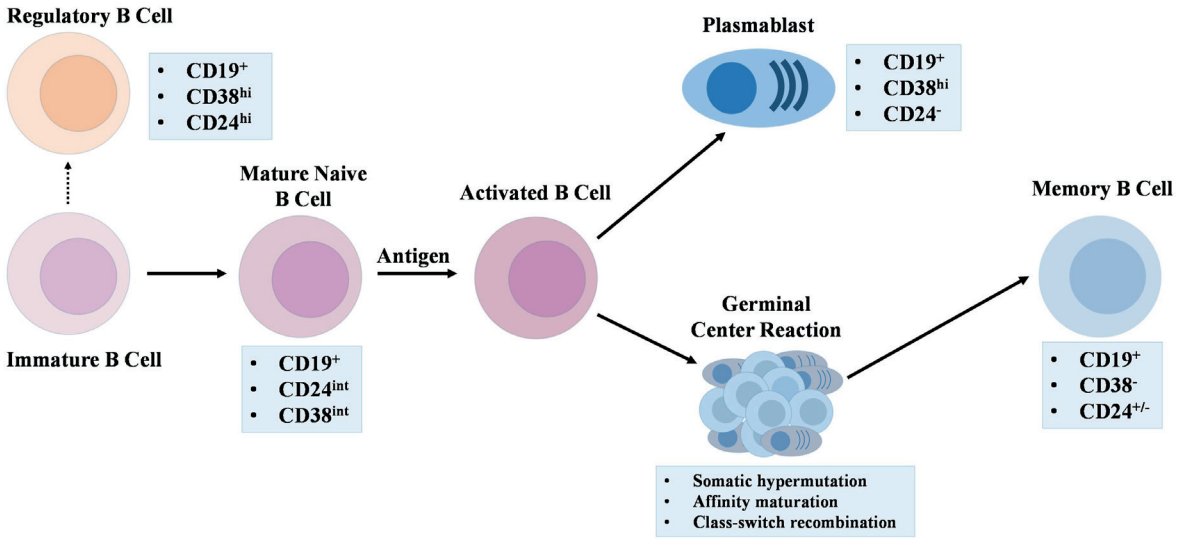



**Fig. 2.** B cell differentiation pathways and B cell subsets identified in this study. Immature B cells that develop in the bone marrow differentiate into regulatory B cells or mature naive B cells. Upon activation by antigen, mature naive B cells either give rise to plasmablasts or enter the germinal center reaction, in which somatic hypermutation, affinity maturation and class switch recombination to differentiate memory B cells.[29]

examining the differences in the comparison of means between two groups, the student t-test was used in independent groups for data showing normal distribution, while the Mann-Whitney U test was used for data without normal distribution. The significance level was accepted as $p < 0.05$.

## Results

### *Demographic characteristics and clinical features of the study population*

The THI group had 15 females and 25 males with a mean age of 18.8 ± 8.6 months (range: 6–41 months), while the control group consisted of 10 females and 13 males with a mean age of 22.6 ± 13.1 months (range: 6–51 months). There was no significant difference between the patient and control groups based on sex and age distribution.

When the clinical findings of the patients with THI were evaluated, the most common clinical presentations were recurrent URTIs in 24 patients (60%), LRTIs in 27 patients (67.5%), diarrhea in 10 patients (25%), urinary tract infection in 2 patients (5%), and allergic disease in 8 patients (20%). Twenty patients (50%) were hospitalized at least once due to infection. The main causes of hospitalization were acute bronchiolitis (70%), gastroenteritis (20%), and pneumonia (10%). Etiological agents were determined in 10 of 20 patients. *Rhinovirus*, *human metapneumovirus*, and *respiratory syncytial virus* were identified as causative pathogens of bronchiolitis cases. *Rotavirus* and *Streptococcus pneumonia* were detected as etiological agents for gastroenteritis and pneumonia cases, respectively. None of these patients had severe infections, including sepsis or meningitis. Four patients (10%) received intravenous IgG, and one of them had Kawasaki disease. Thirty-one patients with THI (77.5%) were taking antibiotic prophylaxis during months 2–29 (Table I). The serum levels for IgG reached age-matched normal values in the median age of 32 months (range: 24–58 months).

### *Laboratory studies*

The laboratory findings of the study groups are shown in Tables II and III. Serum IgG, IgA, and IgM levels at diagnosis were 406.4 ± 119.1, 19.2 ± 14.9, and 62.1 ± 22.8 mg/dL, respectively. Twenty-eight patients (70%) had low IgA, and

23 patients (57.5%) had low IgM titers. There was no significant difference between the patients in the THI and those in the control group based on leukocyte, lymphocyte, platelet, and hemoglobin levels (p > 0.05) (Table II).

$CD3^+$ Total T cell, $CD3^+CD4^+$T cells, $CD3^+CD8^+$ cytotoxic T cells, $CD19^+$ B cells, $CD16^+56^+$ natural killer cell counts, and CD4/CD8 T cell ratio were within normal ranges according to age in patients with THI.[31] There was no significant difference between the patients in the THI group and the control group based on lymphocyte subsets (p > 0.05) (Table II).

In the CD127 expression of T cells, although the percentage of CD127 in $CD3^+$ T and $CD3^+CD4^+$ T cells reduced (p = 0.00, p = 0.00, respectively), the CD127 MFI of these cells increased in patients

**Table I.** Clinical features and treatment of the patients with THI.

| | THI group (N = 40) | |
|---|---|---|
| | n | % |
| Clinical features | | |
| LRTI | 27 | 67.5 |
| URTI | 24 | 60 |
| Diarrhea | 10 | 25 |
| Allergic symptoms | 8 | 20 |
| UTI | 2 | 5 |
| Treatment | | |
| Antibiotic prophylaxis | 31 | 77.5 |
| Hospitalization | 20 | 50 |
| The use of IVIG | 4 | 10 |

THI: transient hypogammaglobulinemia of infancy, LRTI: lower respiratory tract infections, URTI: upper respiratory tract infections, UTI: urinary tract infection, IVIG: intravenous IgG

**Table II.** Serum immunoglobulin levels and lymphocyte subsets in THI and control groups.

| | THI | | Control | | p |
|---|---|---|---|---|---|
| | Mean ± SD | Min – Max | Mean ± SD | Min – Max | |
| IgG (mg/dl) | 406.4 ± 119.1 | 169 - 575 | 640.1 ± 163.9 | 358 – 950 | 0.000* |
| IgA (mg/dl) | 19.2 ± 14.9 | 6.7 – 81 | 48.9 ± 38.8 | 8.1 – 166 | 0.00** |
| IgM (mg/dl) | 62.1 ± 22.8 | 19.8 – 152 | 78.1 ± 35.6 | 30.1 – 209 | 0.02** |
| WBC ($x10^9$) | 9.1 ± 2.5 | 5.2 – 15.5 | 9.4 ± 2.7 | 6 – 16.4 | 0.76** |
| Lymphocyte | | | | | |
| % | 56.0 ± 9.9 | 29 – 74 | 53.6 ± 11.6 | 34 – 76 | 0.38* |
| Absolute ($x10^9$) | 5.1 ± 1.5 | 2.8 – 9 | 5.0 ± 1.7 | 2.6 – 8.9 | 0.55** |
| CD3 | | | | | |
| % | 65.9 ± 6.8 | 51 – 88 | 65.5 ± 7.3 | 49 – 77 | 0.91** |
| Absolute number | 3261.5 ± 1094.5 | 1711 – 6686 | 3246.0 ± 1266.5 | 1328 – 6590 | 0.89** |
| $CD3^+CD4^+$ T cells | | | | | |
| % | 42.3 ± 7.0 | 30.6 – 56.3 | 41.1 ± 9.2 | 26.1 – 59.1 | 0.49** |
| Absolute number | 2083.2 ± 719.8 | 1151 – 4090 | 2078.7 ± 1015.0 | 703 – 4717 | 0.63** |
| $CD3^+CD8^+$ T cells | | | | | |
| % | 19.9 ± 4.7 | 10.2 – 28 | 19.9 ± 4.4 | 10.4 – 26.8 | 0.96* |
| Absolute number | 1002.0 ± 436.9 | 399 – 2429 | 963.7 ± 349.2 | 357 – 1687 | 0.72* |
| $CD19^+$ B cells | | | | | |
| % | 23.3 ± 6.3 | 7.9 – 35.5 | 23.4 ± 6.3 | 14.1 – 36 | 0.68** |
| Absolute number | 1215.2 ± 707.7 | 288 – 4089 | 1137.8 ± 526.1 | 616 – 2601 | 0.62** |
| $CD16^+CD56^+$ NK Cells | | | | | |
| % | 6.7 ± 3.5 | 1.3 – 14.7 | 6.2 ± 4.3 | 0.5 – 18.0 | 0.27** |
| Absolute number | 333.2 ± 264.1 | 44 – 1669 | 313.4 ± 227.8 | 110 – 782 | 0.45** |

SD: standard deviation, IgG: immunoglobulin G, IgA: immunoglobulin A, IgM: immunoglobulin M, WBC: white blood cell, * Independent sample t-test **Mann–Whitney U test

**Table III.** T and B cell subsets in THI and control groups.

| | THI | | Control | | p |
|---|---|---|---|---|---|
| | Mean ± SD | Min – Max | Mean ± SD | Min – Max | |
| $CD3^+$ T cells | | | | | |
| CD127 (%) | 82.46 ± 10.20 | 43.4 – 96.3 | 89.97 ± 7.95 | 66.3 – 97.2 | 0.00** |
| CD127 (MFI) | 6574.3 ± 3471.3 | 1438 – 18537 | 5271.8 ± 3946.9 | 1458 – 20343 | 0.04** |
| $CD3^+CD4^+$ T cells | | | | | |
| CD127 (%) | 77.6 ± 13.0 | 34.5 – 96.6 | 87.9 ± 9.7 | 58.1 – 96.1 | 0.00** |
| CD127 (MFI) | 6114.8 ± 3207 | 1197 – 15962 | 4989.7 ± 3821 | 1335 – 19573 | 0.05** |
| $CD3^+CD4^+$T cell subsets (%) | | | | | |
| CD25+CD127low/- Tregs | 2.6 ± 2.3 | 0.2 – 12.7 | 1.5 ± 1.1 | 0.4 – 4.9 | 0.01** |
| CD25low/-CD127+ | 68.0 ± 13.6 | 29.1 – 89.6 | 77.4 ± 9.8 | 44.7 – 86.5 | 0.00** |
| CD25-CD127- | 19.4 ± 11.8 | 2.8 – 56.7 | 10.4 – 8.6 | 3.1 – 36.9 | 0.00** |
| $CD25^+127^{low/-}$ Tregs - FoxP3 Expression Level | | | | | |
| FoxP3 (%) | 29.0 ± 21.4 | 1.5 – 72.9 | 25.7 ± 13.4 | 2.4 – 49.2 | 0.79** |
| FoxP3 (MFI) | 300.4 ± 65.1 | 210 – 535 | 300.2 ± 113.3 | 207 – 764 | 0.30** |
| $CD19^+$ B cell subsets (%) | | | | | |
| $CD38^{hi}CD24^{hi}$ Bregs | 10.1 ± 3.8 | 2.7 – 23.5 | 9.0 ± 2.4 | 5.4 – 14.6 | 0.22* |
| $CD38^{hi}CD24^-$ Plasmablast | 2.4 ± 3.1 | 0.1 – 14.1 | 2.1 ± 2.1 | 0 – 8.4 | 0.96** |
| $CD38^{int}CD24^{int}$ Mature Naive B Cell | 58.7 ± 8.6 | 39.1 – 77.1 | 55.0 ± 9.8 | 38.1 – 73.6 | 0.13* |
| $CD38^-CD24^+$ Primarily Memory B Cell | 14.2 ± 5.6 | 5.7 – 26.0 | 18.9 ± 5.3 | 11.1 – 30.1 | 0.00* |
| $CD38^-CD24^-$ New Memory B Cell | 4.9 ± 3.6 | 0.1 – 21.1 | 6.4 ± 3.2 | 2.4 – 12.3 | 0.06** |

Treg: Regulatory T cells, Bregs: Regulatory B cells, * Independent sample t-test, **Mann–Whitney U test

with THI compared to the control group (p = 0.04, p = 0.05, respectively). In the analysis of $CD4^+$ Th cell subsets according to CD25 and CD127 expressions, the $CD4^+CD25^{low/-}CD127^+$ Th cells decreased (p = 0.00), while the $CD4^+CD25^-CD127^-$ Th cells and the $CD4^+CD25^+CD127^{-/low}$ Tregs increased in the THI group (p = 0.00, p = 0.01) (Table III) (Fig. 1).

Flow cytometric analysis of B cells showed that the $CD38^-CD24^+$ primarily memory B cells were reduced in the THI group (p = 0.00). There was no significant difference in the $CD38^{hi}CD24^{hi}$ Bregs, the $CD38^{int}CD24^{int}$ mature naive B cell, the $CD38^{bri}CD24^-$ plasmablast cells, and $CD38^-CD24^-$ new memory B cell subset (p > 0.05) (Table III) (Fig. 3).

Correlation analysis showed that there was no relationship between Tregs/Bregs and serum IgG, IgA, and IgM levels; frequency of infection; and intravenous IgG requirement. However, the ratio of FoxP3 in Tregs was negatively correlated with IgG recovery age.

## Discussion

In this study, to elucidate the pathophysiology of THI, we analyzed, for the first time, Th subsets in regards to CD127 and CD25 expressions and B cell subsets according to CD38 and CD24 expressions in 40 children with THI. This study demonstrated that patients with THI had some changes in the Th subsets, including $CD4^+CD25^+CD127^{-/low}$ Tregs and B cell subsets. While the percentage of CD127 of $CD3^+$ T and $CD3^+CD4^+$ Th cells decreased in children with THI, CD127 expression (MFI) increased. The children with THI had higher $CD4^+CD25^+CD127^{low/-}$ Tregs than the controls, but their $CD19^+CD38^-CD24^+$ primarily memory B cells were low.

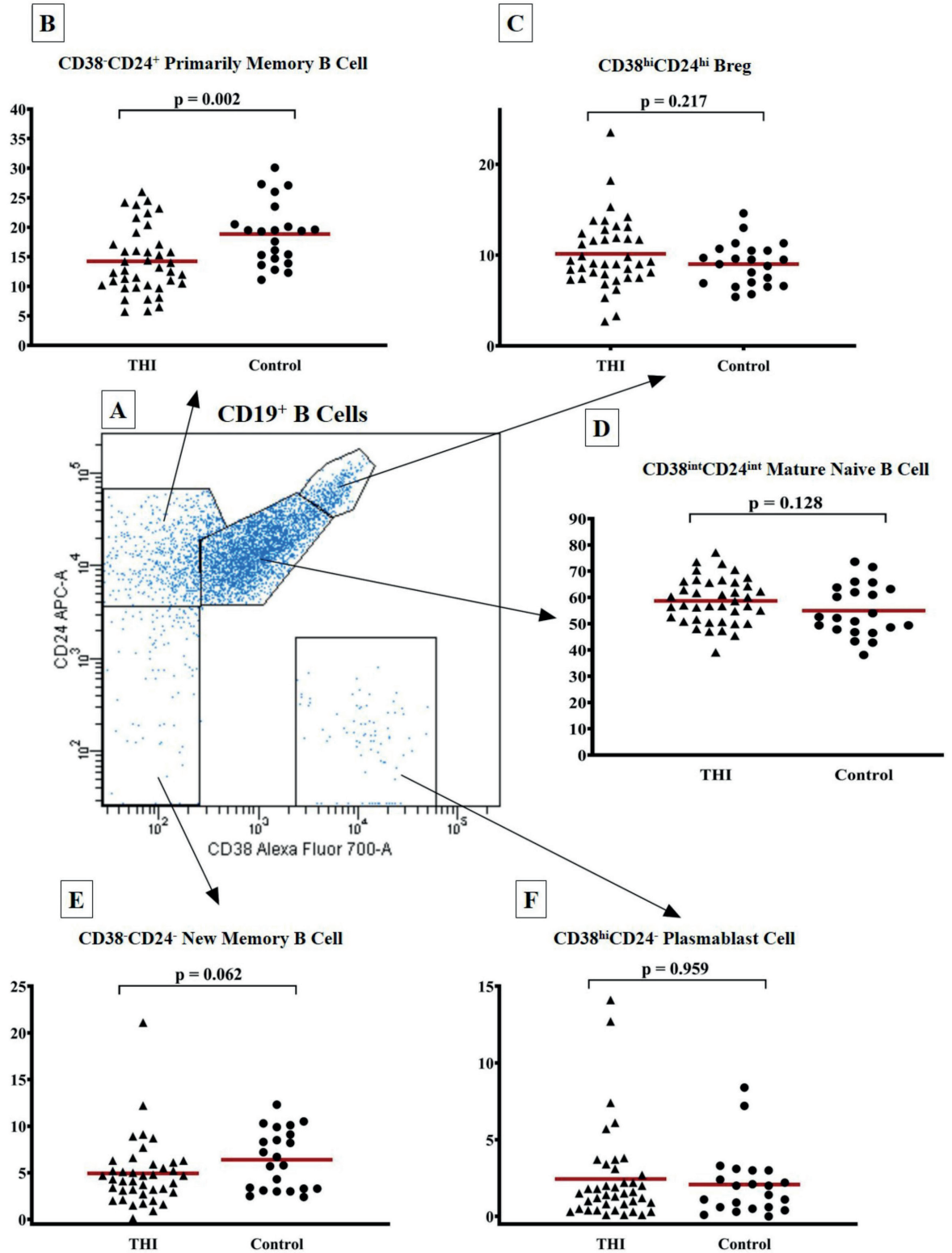



**Fig. 3.** Flow cytometric gating strategy of B cell subsets (A) in patients with THI and control groups (B-F). $CD19^+$ B cells were classified as $CD38^-CD24^+$ primarily memory B cell (B), $CD38^{hi}CD24^{hi}$ Bregs (C), $CD38^{int}CD24^{int}$ mature naive B cell (D), $CD38^-CD24^-$ new memory B cell (E), $CD38^{hi}CD24^-$plasmablast (F).

In our study, $CD4^+CD25^+CD127^{-/low}$ Tregs were found to increase in children with THI. $CD4^+CD25^+FoxP3^+$ Tregs in THI were first studied by Rutkowska et al.[11-13], and they reported elevated Treg numbers in children with THI[11], which is consistent with our findings. We found no significant difference in FoxP3 expression of Tregs in patients with THI compared to the control. However, the FoxP3 ratio of Tregs was negatively correlated with the IgG recovery age in THI.

Siegel et al.[6] reported that antibody production deficiency might be related to immature or delayed development of Th cells. In the current study, we found the Th cell count to be normal. However, IL-7 receptor-$\alpha$ (CD127) expression of total T and Th cells was reduced. In our study, the low percentage of CD127 in T cells may be expected to affect cellular immunity. However, the increased expression of CD127 (MFI) in T cells may be compensating for developmental and functional defects in these cells.

Dunham et al.[15], in their study of patients with AIDS, found that the $CD25^{low/-}CD127^+$ subset significantly decreased and the $CD25^-CD127^-$ subset increased in HIV–infected adult patients. A study by Shen et al.[32] among chronic patients with human hepatitis C virus (HCV) reported that all three Th cell subsets ($CD25^+CD127^{low/-}$, $CD25^{low/-}CD127^+$, and $CD25^-CD127^-$) increased compared to the healthy controls. In both studies among patients with HIV and HCV, the change in $CD4^+$ T cell subsets defined according to CD127 and CD25 expression profiles may be associated with mitigating chronic immune activation. Rutkowska et al.[12] reported that patients with THI had low serum IL-2 levels. These findings indicated that variations in different fractions of $CD4^+$ T cells and low efficiency of the immune response might be associated with infection susceptibility in children with THI.

In this study, Bregs and other B cell subsets according to CD38 and CD24 expressions were first investigated in THI. Primarily memory B cells significantly decreased in the THI group compared to the control. However, there was no significant difference in the percentage of Bregs, plasmablast, mature naive B cell, and new memory B cell subsets between the groups. In Eroglu et al.[28], memory B cell subsets were low in patients with THI, but there was no significant difference. Our previous study determined low immunoglobulin class switching and $IgM^+$ memory B cells, and the percentage of CD21 and CD81 that constituted the CD19 complex increased in THI.[10] In this study, CD38 expression in $CD19^+$ B cells was also low in children with THI. CD38 controls a signaling pathway involved in the growth, survival, and activation of lymphoid cells.[33,34] Deaglio et al.[35] reported that CD38-mediated signals are regulated at three distinct levels, and the CD19/CD81 complex mediates one of them. In vivo and ex vivo stimulation studies have revealed that B cells without CD81 have a hyperactive phenotype, and therefore, CD81 negatively regulates B cell activation.[35] The increased number of CD81 may suppress CD38 expression and cause insufficient B cell activation due to developed hypogammaglobulinemia in THI.

In our series, IgG levels reached normal levels at 24–58 months. Although the upper cutting age of normal IgG production is reported as 3–4 years, some studies indicate delayed recovery of IgG levels extending to 10 years.[3] Most children with THI spontaneously recover their IgG levels, which is consistent with our findings.[36,37] These studies showed that if these patients with THI were not suffering from recurrent or severe infections during their follow-up, IgG levels achieved normal levels within the expected time.[36,37]

Our study demonstrated that changes in the T cell compartment, including a decreased percentage of CD127 and increased CD127 expression in T cells, may be related to immune compensation mechanisms in THI. The higher percentage of $CD4^+CD25^+CD127^{-/low}$ Tregs and lower primary memory B cells may cause a delay in antibody production in children with THI. To summarize, our observations about changes in the T cell and B cell subsets may

contribute to improving our understanding of the pathogenesis of THI. Further studies are needed to determine whether the changes in the T cell compartment are associated with chronic immune activation caused by recurrent viral and bacterial stimulation.

### Ethical approval

This study was approved by the Ethical Committee of Selcuk University Medical Faculty (2015/265).

### Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: HA, AE; data collection: AE, HU, TG; analysis and interpretation of results: HA, AE; draft manuscript preparation: HA, AE, TG. All authors reviewed the results and approved the final version of the manuscript.

### Source of funding

This work was supported by the Scientific Research Projects Committee of Selcuk University (16401133).

### Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Walker AM, Kemp AS, Hill DJ, Shelton MJ. Features of transient hypogammaglobulinaemia in infants screened for immunological abnormalities. Arch Dis Child 1994; 70: 183-186. https://doi.org/10.1136/adc.70.3.183
2. Ballow M. Primary immunodeficiency disorders: antibody deficiency. J Allergy Clin Immunol 2002; 109: 581-591. https://doi.org/10.1067/mai.2002.122466
3. Dalal I, Reid B, Nisbet-Brown E, Roifman CM. The outcome of patients with hypogammaglobulinemia in infancy and early childhood. J Pediatr 1998; 133: 144-146. https://doi.org/10.1016/S0022-3476(98)70195-7
4. Yorulmaz A, Artaç H, Reisli I. Evaluation of patient follow-up with transient hypogammaglobulinemia in infancy diagnosis. J Contemp Med 2019; 9: 15-20. https://doi.org/10.16899/gopctd.534014
5. Kilic SS, Ozel M, Hafizoglu D, Karaca NE, Aksu G, Kutukculer N. The prevalences [correction] and patient characteristics of primary immunodeficiency diseases in turkey--two centers study. J Clin Immunol 2013; 33: 74-83. https://doi.org/10.1007/s10875-012-9763-3
6. Siegel RL, Issekutz T, Schwaber J, Rosen FS, Geha RS. Deficiency of T helper cells in transient hypogammaglobulinemia of infancy. N Engl J Med 1981; 305: 1307-1313. https://doi.org/10.1056/NEJM198111263052202
7. Ochs HD, Stiehm ER, Winkelstein JA. Antibody deficiencies. In: Ochs HD, Stiehm ER, Winkelstein JA (eds). Immunologic disorders in infants & children (5th ed). Philadelphia: Elsevier Saunders, 2004: 357-373.
8. McGeady SJ. Transient hypogammaglobulinemia of infancy. In: Sullivan KE, Stiehm ER (eds). Stiehm's Immune Deficiencies (1st ed). Amsterdam: Academic Press, 2014: 417-426. https://doi.org/10.1016/B978-0-12-405546-9.00018-2
9. Kowalczyk D, Baran J, Webster AD, Zembala M. Intracellular cytokine production by Th1/Th2 lymphocytes and monocytes of children with symptomatic transient hypogammaglobulinaemia of infancy (THI) and selective IgA deficiency (SIgAD). Clin Exp Immunol 2002; 127: 507-512. https://doi.org/10.1046/j.1365-2249.2002.01701.x
10. Artac H, Kara R, Gokturk B, Reisli I. Reduced CD19 expression and decreased memory B cell numbers in transient hypogammaglobulinemia of infancy. Clin Exp Med 2013; 13: 257-263. https://doi.org/10.1007/s10238-012-0200-y
11. Rutkowska M, Lenart M, Bukowska-Strakovà K, et al. The number of circulating CD4+ CD25high FoxP3+ T lymphocytes is transiently elevated in the early childhood of transient hypogammaglobulinemia of infancy patients. Clin Immunol 2011; 140: 307-310. https://doi.org/10.1016/j.clim.2011.04.003
12. Rutkowska M, Trzyna E, Lenart M, et al. The elevated number of circulating regulatory T cells in patients with transient hypogammaglobulinemia of infancy is not associated with any abnormalities in the genes encoding the TGF-β receptors. Clin Immunol 2013; 149: 83-85. https://doi.org/10.1016/j.clim.2013.06.008
13. Siemińska I, Rutkowska-Zapała M, Bukowska-Strakova K, et al. The level of myeloid-derived suppressor cells positively correlates with regulatory T cells in the blood of children with transient hypogammaglobulinaemia of infancy. Cent Eur J Immunol 2018; 43: 413-420. https://doi.org/10.5114/ceji.2018.81359

14. Fry TJ, Mackall CL. The many faces of IL-7: From lymphopoiesis to peripheral T cell maintenance. J Immunol 2005; 174: 6571-6576. https://doi.org/10.4049/jimmunol.174.11.6571

15. Dunham RM, Cervasi B, Brenchley JM, et al. CD127 and CD25 expression defines CD4+ T cell subsets that are differentially depleted during HIV infection. J Immunol 2008; 180: 5582-5592. https://doi.org/10.4049/jimmunol.180.8.5582

16. Liu W, Putnam AL, Xu-Yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 2006; 203: 1701-1711. https://doi.org/10.1084/jem.20060772

17. Buffa S, Pellicanò M, Bulati M, et al. A novel B cell population revealed by a CD38/CD24 gating strategy: CD38(-)CD24(-) B cells in centenarian offspring and elderly people. Age (Dordr) 2013; 35: 2009-2024. https://doi.org/10.1007/s11357-012-9488-5

18. Funaro A, Morra M, Calosso L, Zini MG, Ausiello CM, Malavasi F. Role of the human CD38 molecule in B cell activation and proliferation. Tissue Antigens 1997; 49: 7-15. https://doi.org/10.1111/j.1399-0039.1997.tb02703.x

19. Kay R, Rosten PM, Humphries RK. CD24, a signal transducer modulating B cell activation responses, is a very short peptide with a glycosyl phosphatidylinositol membrane anchor. J Immunol 1991; 147: 1412-1416.

20. Suzuki T, Kiyokawa N, Taguchi T, Sekino T, Katagiri YU, Fujimoto J. CD24 induces apoptosis in human B cells via the glycolipid-enriched membrane domains/rafts-mediated signaling system. J Immunol 2001; 166: 5567-5577. https://doi.org/10.4049/jimmunol.166.9.5567

21. Rosser EC, Mauri C. Regulatory B cells: Origin, phenotype, and function. Immunity 2015; 42: 607-612. https://doi.org/10.1016/j.immuni.2015.04.005

22. Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol 2012; 30: 221-241. https://doi.org/10.1146/annurev-immunol-020711-074934

23. van de Veen W, Stanic B, Wirz OF, Jansen K, Globinska A, Akdis M. Role of regulatory B cells in immune tolerance to allergens and beyond. J Allergy Clin Immunol 2016; 138: 654-665. https://doi.org/10.1016/j.jaci.2016.07.006

24. Jeske SS, Brand M, Ziebart A, et al. Adenosine-producing regulatory B cells in head and neck cancer. Cancer Immunol Immunother 2020; 69: 1205-1216. https://doi.org/10.1007/s00262-020-02535-6

25. Yesillik S, Agrawal S, Gollapudi SV, Gupta S. Phenotypic analysis of CD4+ Treg, CD8+ Treg, and Breg cells in adult common variable immunodeficiency patients. Int Arch Allergy Immunol 2019; 180: 150-158. https://doi.org/10.1159/000501457

26. Vlkova M, Ticha O, Nechvatalova J, et al. Regulatory B cells in cvid patients fail to suppress multifunctional IFN-γ+ TNF-α+ CD4+ T cells differentiation. Clin Immunol 2015; 160: 292-300. https://doi.org/10.1016/j.clim.2015.06.013

27. Bayram RO, Özdemir H, Emsen A, Türk Dağı H, Artaç H. Reference ranges for serum immunoglobulin (IgG, IgA, and IgM) and IgG subclass levels in healthy children. Turk J Med Sci 2019; 49: 497-505. https://doi.org/10.3906/sag-1807-282

28. Eroglu FK, Aerts Kaya F, Cagdas D, et al. B lymphocyte subsets and outcomes in patients with an initial diagnosis of transient hypogammaglobulinemia of infancy. Scand J Immunol 2018; 88: e12709. https://doi.org/10.1111/sji.12709

29. Oleinika K, Mauri C, Salama AD. Effector and regulatory B cells in immune-mediated kidney disease. Nat Rev Nephrol 2019; 15: 11-26. https://doi.org/10.1038/s41581-018-0074-7

30. Ucaryilmaz H, Koksal H, Emsen A, Kadoglou N, Dixon JM, Artac H. The role of regulatory T and B cells in the etiopathogenesis of idiopathic granulomatous mastitis. Immunol Invest 2022; 51: 357-367. https://doi.org/10.1080/08820139.2020.1832114

31. Ikincioğullari A, Kendirli T, Doğu F, et al. Peripheral blood lymphocyte subsets in healthy Turkish children. Turk J Pediatr 2004; 46: 125-130.

32. Shen T, Chen X, Xu Q, Lu F, Liu S. Distributional characteristics of CD25 and CD127 on CD4+ T cell subsets in chronic HCV infection. Arch Virol 2010; 155: 627-634. https://doi.org/10.1007/s00705-010-0626-z

33. Campana D, Suzuki T, Todisco E, Kitanaka A. CD38 in Hematopoiesis. In: Mehta K, Malavasi F (eds). Human CD38 and related molecules. Chem immunol. Basel: Karger, 2000: 169-188. https://doi.org/10.1159/000058768

34. Mallone R, Funaro A, Zubiaur M, et al. Signaling through CD38 induces NK cell activation. Int Immunol 2001; 13: 397-409. https://doi.org/10.1093/intimm/13.4.397

35. Deaglio S, Vaisitti T, Billington R, et al. CD38/CD19: A lipid raft-dependent signaling complex in human B cells. Blood 2007; 109: 5390-5398. https://doi.org/10.1182/blood-2006-12-061812

36. Keles S, Artac H, Kara R, Gokturk B, Ozen A, Reisli I. Transient hypogammaglobulinemia and unclassified hypogammaglobulinemia: 'Similarities and differences'. Pediatr Allergy Immunol 2010; 21: 843-851. https://doi.org/10.1111/j.1399-3038.2010.01010.x

37. Ozen A, Baris S, Karakoc-Aydiner E, Ozdemir C, Bahceciler NN, Barlan IB. Outcome of hypogammaglobulinemia in children: Immunoglobulin levels as predictors. Clin Immunol 2010; 137: 374-383. https://doi.org/10.1016/j.clim.2010.08.010

The Turkish Journal of Pediatrics 2022; 64: 239-245
https://doi.org/10.24953/turkjped.2021.1075

**Original Article**

# The effect of breast milk nesfatin-1 and ghrelin levels on growth in infants with SGA

Berna Eroğlu Filibeli[1], Melike Karabulut Bayraktar[2], Saliha Aksun[3], Gönül Çatlı[4], Jülide Gülizar Yıldırım[5], Bumin Nuri Dündar[4]

*[1]Divivion of Pediatric Endocrinology, [2]Department of Pediatrics, İzmir Medical Science University, Tepecik Training and Research Hospital, İzmir; Departments of [3]Biochemistry and [4]Pediatric Endocrinology, İzmir Katip Çelebi University Faculty of Medicine, İzmir; [5]Department of Public Health Nursing, İzmir Katip Çelebi University Faculty of Health Sciences, İzmir, Turkey.*

## ABSTRACT

**Background.** Current studies claim that peptides such as leptin, adiponectin, ghrelin, and nesfatin-1 found in breast milk may be responsible for the growth of infants. Therefore, we aimed to determine the association between breast milk total ghrelin and nesfatin-1 levels and anthropometric measurements of infants who were small for gestational age (SGA).

**Methods.** 20 SGA and 20 appropriate for gestational age (AGA) infants were enrolled in the study. Anthropometric measurements of infants were carried out at birth, 1st, and 4th months. In addition, total ghrelin and nesfatin-1 levels in the breast milk were concomitantly measured.

**Results.** Total ghrelin at the 4th month in breast milk waslower-level in the SGA group (p=0.015). In both groups, nesfatin-1 levels at the 4th month were lower than the values at the 1st month. Additionally, nesfatin-1 levels of SGA infants at the 4th month were higher (p=0.035).

**Conclusions.** Breast milk total ghrelin and nesfatin-1 levels differed in both groups, and it is probably referred to the growth discrepancy of these infants during the first months of life. Furthermore, we consider that higher breast milk nesfatin-1 levels at the 4th month may be a preventive against obesity in SGA infants who have potential risk for obesity in childhood and adulthood.

**Key words:** ghrelin, nesfatin-1, small for gestation age (SGA), appropriate for gestational age (AGA), breast milk.

The presence of many peptides such as leptin, ghrelin, adiponectin, obestatin, resistin, and nesfatin-1 has been shown in breast milk.[1] Ghrelin, leptin, and adiponectin molecules present in breast milk are effective in growing infants have also been reported.[2] Leptin hormone released from adipocytes plays a pivotal role in energy expenditure via the hypothalamus. In a previous study, leptin levels in small for gestational age (SGA) infants were found to be lower compared to appropriate for gestational age (AGA) and large for gestational age infants, and it has been suggested that this might contribute to faster growth of SGA infants.[3] As far as we know, it is the first research in the current literature regarding the relationship between breast milk ghrelin and nesfatin-1 levels and SGA infants' growth. Ghrelin is mainly synthesized from the stomach and is a growth hormone releaser that affects the hypothalamus-pituitary axis.[4] It exerts a stimulatory effect on food intake, and ghrelin cells have been found in the gastrointestinal tract.[5,6] It is related to the neuro-regulation of appetite, energy balance, and nutrient uptake.[7,8] Experimental studies have shown that ghrelin was found in the intestinal system, and it is associated with weight gain and calorie intake.[5,9] Ghrelin is also synthesized and released from breast tissue.[10] During the first four months of life, serum ghrelin levels of breastfed infants were higher

✉ Bumin Nuri Dündar
bumindundar@gmail.com

Received 30th April 2021, revised 18th July 2021, accepted 18th August 2021.

than formula-fed infants.[11] Cord blood ghrelin levels of SGA infants were significantly higher than AGA infants, and a negative correlation existed between anthropometric measurements and ghrelin levels in both groups.[12] A study has shown that serum ghrelin levels directly correlated with infants' age, weight, and height after the feeding period.[13]

Nesfatin-1 is also a peptide hormone with anorexigenic influences on the regulation of nutritional homeostasis.[14] In SGA infants, serum nesfatin-1 levels were higher than AGA infants, and a negative correlation had between serum nesfatin-1 levels and oral caloric intake.[15] Furthermore, nesfatin-1 has been detected in the breast milk of healthy and gestational diabetes mellitus women.[16] The role of nesfatin-1 levels in infant metabolism is not well-known.

This study aimed to investigate the relationship between breast milk total ghrelin (TGh) and nesfatin-1 levels and anthropometric measurements of SGA infants in the first four months of life.

## Material and Methods

Twenty SGA and 20 AGA infants were included in the study. SGA and AGA were defined as a birth weight below the 10th percentile and birth weight between the 10th and 90th percentile for gestational age and gender, respectively.[17] Anthropometric measurements of all infants, including weight, supine length, head circumference, chest circumference, mid-arm circumference, triceps skinfold thickness, were performed by the same researcher at birth, 1st, and 4th months. Weight was measured using an electronic scale (±5 g, EBSC 20, NECK), and the supine length was measured by a standard baby measuring board (± 0.1 cm). Body mass index (BMI; kg/m$^2$) was calculated by dividing the weight by the square of height. Head circumference was measured using a narrow non-stretch tape, passing it around the head, placing it on the most anterior protuberance of the forehead and the most posterior protuberance of the head's back with the nearest 0.1 cm. Chest circumference and mid-arm circumference measurements were recorded to the nearest 0.1 cm, using a non-elastic, flexible measuring tape. Chest circumference was measured at the level of the nipple during expiration. Mid-arm circumference was measured at the mid-point between the tip of the acromion process and the right upper arm's olecranon process. Triceps skinfold thickness was measured from the left side of the body to the nearest 0.1 mm, at the midpoint between the acromion and olecranon protrusions on the arm's posterior centerline using a skinfold caliper.

Infants with congenital malformations, chromosomal abnormalities, intrauterine infections, or fed with formula or mix were excluded from the study. In addition, mothers with preeclampsia, gestational diabetes mellitus, medications, or multiple pregnancies and those who did not sign the informed consent were excluded. The mothers of the infants were non-obese healthy postpartum women, and were on no medication. Five milliliters of breast milk were taken at the 1st and 4th months. Samples were collected in glass tubes and stored at -80°C until TGh and nesfatin-1 levels were assayed.

The local ethics committee of Tepecik Training and Research Hospital for Interventional Clinical Studies approved the study (Date:09.02.2017, Number:21). Informed verbal and written consent was obtained from the parents. The study was conducted by the principles of the Declaration of Helsinki.

### *Assays*

Breast milk TGh and nesfatin-1 were determined by enzyme-linked immunosorbent assay (ELISA). Nesfatin-1 levels were measured with Nesfatin-1 Phoenix kits ((1-82)/NUCB-2, Phoenix Pharmaceuticals, Inc., USA), and TGh levels were measured with Ghrelin Phoenix kits (Phoenix Pharmaceuticals, Inc., USA). Seven samples of standard materials with known concentrations for nesfatin-1 and ghrelin

molecules were studied with patient samples. The color intensity of all pieces in the plates was read as absorbance with the semi-automatic ELISA plate reader (Biotek, EL800, USA). Standard calibration curves were obtained by absorbance given by the standards. TGh and nesfatin-1 concentrations were calculated from the absorbance values in breast milk samples using standard calibration curves.

### *Statistical analysis*

Statistical analysis was performed using SPSS, version 25 (SPSS, Chicago, IL, USA). Measured values were expressed as mean ± SD (minimum-maximum). The Kolmogorov-Smirnov test evaluated the normal distribution of the data. The Chi-square test was used to compare the nominal variables between the groups. Mann Whitney-U and Wilcoxon signed-rank tests were used for non-normally distributed variables. Compatibility for normal distribution of the numeric measurement by independent groups was analyzed using the independent sample t-test. Pearson correlation test was applied to calculate the correlation between breast milk TGh and nesfatin-1 levels and infants' anthropometric measurements. A $p< 0.05$ value was considered statistically significant.

## Results

The SGA group's anthropometric measurements were lower than the AGA group at the birth and 1st month ($p<0.05$). In the 4th month, except for weight and chest circumference, the differences disappeared between the two groups ($p>0.05$) (Table I).

The increases in anthropometric measurements throughout four months were examined in all infants. There was no difference in the delta anthropometric measurements during the first month between SGA and AGA groups ($p>0.05$). However, in the fourth month, the increases in BMI, head circumference, chest circumference, mid-arm circumference, and triceps skinfold thickness were significantly higher in the SGA group ($p=0.003$, $p=0.006$, $p<0.001$, $p=0.007$, $p<0.001$).

**Table I.** SGA and AGA infants' anthropometric measurements at birth, 1st month and 4th month.

| | | Weight (g) | Body Mass index (kg/m²) | Head Circumference (cm) | Chest Circumference (cm) | Mid-arm Circumference (cm) | Triceps Skinfold Thickness (mm) |
|---|---|---|---|---|---|---|---|
| At birth | SGA (n=20) | 345.25±177.65 | 10.6±0.17 | 33±0.29 | 28.9±0.33 | 5.1±0.16 | 5.1±0.16 |
| | AGA (n=20) | 3361.25±315.08 | 13.1±0.21 | 34.85±0.93 | 32.63±1.32 | 6.49±0.74 | 6.4±0.73 |
| | *p* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| 1st month | SGA (n=20) | 3558±477.80 | 13.14±1.59 | 35.70±1.23 | 33.88±2.22 | 8.3±0.36 | 8.3±1.64 |
| | AGA (n=20) | 4607.50±471.04 | 14.67±1.18 | 37.3±1.09 | 37.07±1.47 | 9.5±0.28 | 9.55±1.28 |
| | *p* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| 4th month | SGA (n=20) | 6255.50±679.80 | 16.34±2.12 | 40.43±0.96 | 40.35±1.66 | 11.61±1.87 | 11.6±1.23 |
| | AGA (n=20) | 7057±521.04 | 16.95±1.51 | 41±1.03 | 41.45±1.41 | 11.55±1.58 | 11.5±1.58 |
| | *p* | **<0.001** | >0.05 | >0.05 | **<0.001** | >0.05 | >0.05 |

SGA: Small for gestational age, AGA: Appropriate for gestational age. Values were expressed as mean ± standard deviation.

**Table II.** The concentrations of breast milk total ghrelin in SGA and AGA infants.

| Total Ghrelin (pg/mL) | SGA (n=20) | AGA (n=20) | *p* |
|---|---|---|---|
| 1st month | 624.05±53.7 | 536.90±27.2 | >0.05[a] |
| 4th month | 521.80±45.7 | 596.45±26.9 | **0.015[a]** |
| *p* | >0.05[b] | >0.05[b] | |

SGA: Small for gestational age, AGA: Appropriate for gestational age
[a]Mann Whitney-U test, [b]Wilcoxon signed-rank test

**Table III.** The concentrations of breast milk nesfatin-1 in SGA and AGA infants.

| Nesfatin-1 (pg/mL) | SGA (n=20) | AGA (n=20) | *p* |
|---|---|---|---|
| 1st month | 447.4±86.5 | 375±35.4 | >0.05[a] |
| 4th month | 305.2±30.3 | 267±122.2 | **0.034[a]** |
| *p* | **0.006[b]** | **<0.001[b]** | |

SGA: Small for gestational age, AGA: Appropriate for gestational age
[a]Mann Whitney-U test, [b]Wilcoxon signed-rank test

**Table IV.** Correlation between anthropometric measurements and breast milk nesfatin-1 level in the SGA group (n=20).

| Anthropometric measurements | Nesfatin-1 at 1st month | | Nesfatin-1 at 4th month | |
|---|---|---|---|---|
| | r | *p* | r | *p* |
| Weight(g) | -0.436 | >0.05 | -0.330 | >0.05 |
| Body Mass index (kg/m²) | -0.043 | >0.05 | 0.115 | >0.05 |
| Head Circumference (cm) | -0.568 | **0.009** | -0.265 | >0.05 |
| Chest Circumference (cm) | -0.428 | >0.05 | 0.003 | >0.05 |
| Mid-arm Circumference (cm) | -0.425 | >0.05 | -0.144 | >0.05 |
| Triceps Skinfold Thickness (mm) | -0.210 | >0.05 | -0.263 | >0.05 |

**Table V.** Correlation between anthropometric measurements and breast milk total ghrelin level in the SGA group (n=20).

| Anthropometric measurements | Total ghrelin at 1st month | | Total ghrelin at 4th month | |
|---|---|---|---|---|
| | r | *p* | r | *p* |
| Weight(g) | -0.189 | >0.05 | 0.146 | >0.05 |
| Body Mass index (kg/m²) | -0.186 | >0.05 | 0.222 | >0.05 |
| Head Circumference (cm) | -0.159 | >0.05 | -0.014 | >0.05 |
| Chest Circumference (cm) | -0.415 | >0.05 | 0.069 | >0.05 |
| Mid-arm Circumference (cm) | 0.291 | >0.05 | -0.04 | >0.05 |
| Triceps Skinfold Thickness (mm) | -0.305 | >0.05 | -0.223 | >0.05 |

Breast milk TGh and nesfatin-1 levels were compared between SGA and AGA groups. There was no significant difference between the 1st and 4th-month breast milk TGh levels in both groups ($p$>0.05). While there was no difference between SGA and AGA groups in terms of the TGh levels at the 1st month, breast milk TGh levels at the 4th month were significantly higher in the AGA group ($p$=0.015) (Table II).

Breast milk nesfatin-1 levels at the 1st month were significantly higher than the 4th-month

levels in both groups ($p$=0.006, $p$<0.001). Breast milk nesfatin-1 levels at the 4th month were lower in the AGA group ($p$=0.034). Nevertheless, there were no differences in nesfatin-1 levels at 1st-month breast milk between groups ($p$>0.05) (Table III).

The relationship between anthropometric measurements of infants and breast milk TGh and nesfatin-1 levels was evaluated by Pearson correlation analysis. In the AGA group, there was no correlation between the anthropometric measurements of infants and breast milk TGh and nesfatin-1 levels (at 1st and 4th months) ($p$>0.05). While there was no correlation between anthropometric measurements of SGA infants and breast milk TGh levels, only head circumferences of SGA infants were negatively correlated with breast milk nesfatin-1 levels at the 1st month (r= -0.568, $p$= 0.009) (Table IV and Table V).

## Discussion

SGA infants grow faster and show a different growth pattern than AGA infants in the postnatal period.[18,19] Similarly, in our study, SGA infants overgrew and showed catch-up growth in many anthropometric measurements at the 4th month.

Studies related to ghrelin's effect on infants' growth have conflicting results. In SGA infants, serum ghrelin levels were significantly higher than AGA infants.[20] In another study performed on term and preterm infants, plasma ghrelin was inversely correlated with birth weight and body length in the term infants.[21] On the other hand, increases in ghrelin levels in colostrum, transition, and mature milk suggest that ghrelin levels in breast milk might also be parallel to infant growth.[16] Cesur et al.[22] showed that 4th month-breast milk active ghrelin levels positively correlated with term infants' weight gain. In our study, 4th month-breast milk TGh levels were lower in the SGA groups. Because the anthropometric parameters of the SGA infants at four months caught up, this decrease might be explained with a protective step from rapid growth by reducing appetite and calorie intake. However, no correlation between infants' anthropometric measurements and breast milk TGh levels in both groups was determined. Additionally, breast milk TGh levels in the 4th month were lower than in the 1st month, although not statistically significant. This decrease might be related to an increase in active ghrelin levels, like previously reported.[22] However, active ghrelin levels could not be evaluated in our study. Active ghrelin in breast milk may have a more pronounced effect than TGh on the catch-up period.

Serum nesfatin-1 level was not different among preterm and term infants, but it was higher in SGA infants than in AGA infants.[15] Also, in the same study, it was reported that serum nesfatin-1 levels were high at birth but decreased in the first seven days and increased after seven days over again.[15] In the current study, we found that breast milk nesfatin-1 level significantly reduced during the first four months of life, both in AGA and SGA infants. However, the breast milk nesfatin-1 level at the 4th month was higher in the SGA group than in the AGA group. One of the important health benefits of breast milk is its protective effect against obesity.[23] As we know, early rapid weight gain in SGA infants is associated with increased obesity risk in later life.[24] We suggest that higher breast milk nesfatin-1 levels in SGA infants at four months might help prevent early rapid weight gain in SGA infants that grow fast but have an increased risk of obesity in later life.[25] Only head circumference measurements at 1st month were negatively correlated with breast milk nesfatin-1 levels at 1st month. However, this correlation did not exist in the 4th month, and it might be explained by the small number of cases. These results supported that nesfatin-1 may also play an essential role in the catch-up period.

The effects of breast milk peptides on the growth of infants may occur directly or indirectly. Peptides are broken down in the intestinal

system. It has been shown that some peptides in breast milk resist proteolytic degradation in the gastrointestinal tract during the infantile period due to low gastric proteolytic activity and high permeability of intestine mucosa.[23,26] A long isoform of the leptin receptor was found in the human small intestines' enterocytes.[27] Experimental results have revealed that the expression of ghrelin mRNA in lambs' stomach increased rapidly in the early period and slowed down in the later period, and there was a significant linear correlation between this change and stomach weight in lambs.[9] The effects of ghrelin on the intestinal tract have not been resolved clearly; however, the results suggest that it is closely related to appetite, weight gain, and energy metabolism. However, there is no evidence that breast milk nesfatin-1 can pass into the systemic circulation or preserve biological function or has a receptor in the intestinal system.

There were some limitations to our study. The follow-up period was limited to four months which was relatively short for evaluating catch-up growth. We could not measure breast milk active ghrelin levels or serum levels of TGh and nesfatin-1. Another limitation was the small number of cases in the AGA and SGA groups. We also aimed to record the duration and frequency of feeding infants with breast milk included in the study, but data could not be collected due to incomplete records.

In conclusion, breast milk ghrelin and nesfatin-1 may play an important role in SGA infants' growth, and their effects on infant metabolism remain an undiscovered and controversial issue. Therefore, we believe that nesfatin-1 and ghrelin levels in both serum and breast milk should be investigated with a larger study population to reach a final judgment. Also, long-term follow-up studies are needed to better understand the effects of breast milk ghrelin and nesfatin-1 on growth, especially in SGA infants.

**Ethical approval**

The ethics committee of Tepecik Training and Research Hospital for Interventional Clinical Studies approved the study (Date: 09.02.2017, Number: 21).

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: BND, GÇ; data collection: MK, BEF; data analysis and interpretation: MK, BEF, SA, JGY; drafting of manuscript: BEF, MK; critical review of manuscript: BND, GÇ. All authors approve and take responsibility for the final version of the manuscript.

**Source of funding**

This study was supported by a grant from the Growing Child Association.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**REFERENCES**

1. Karatas Z, Durmus Aydogdu S, Dinleyici EC, Colak O, Dogruel N. Breastmilk ghrelin, leptin, and fat levels changing foremilk to hindmilk: is that important for self-control of feeding?. Eur J Pediatr 2011; 170: 1273-1280. https://doi.org/10.1007/s00431-011-1438-1
2. Çatlı G, Olgaç Dündar N, Dündar BN. Adipokines in breast milk: an update. J Clin Res Pediatr Endocrinol 2014; 6: 192-201. https://doi.org/10.4274/jcrpe.1531
3. Dundar NO, Anal O, Dundar B, Ozkan H, Caliskan S, Büyükgebiz A. Longitudinal investigation of the relationship between breast milk leptin levels and growth in breast-fed infants. J Pediatr Endocrinol Metab 2005; 18: 181-187. https://doi.org/10.1515/JPEM.2005.18.2.181
4. Colldén G, Tschöp MH, Müller TD. Therapeutic potential of targeting the ghrelin pathway. Int J Mol Sci 2017; 18: 798. https://doi.org/10.3390/ijms18040798

5. Blanco AM, Bertucci JI, Valenciano AI, Delgado MJ, Unniappan S. Ghrelin suppresses cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) in the intestine, and attenuates the anorectic effects of CCK, PYY and GLP-1 in goldfish (Carassius auratus). Horm Behav 2017; 93: 62-71. https://doi.org/10.1016/j.yhbeh.2017.05.004

6. Lin Y, Liang Z, He L, et al. Gut ghrelin regulates hepatic glucose production and insulin signaling via a gut-brain-liver pathway. Cell Commun Signal 2019; 17: 8. https://doi.org/10.1186/s12964-019-0321-y

7. Baldelli R, Bellone S, Broglio F, Ghigo E, Bona G. Ghrelin: a new hormone with endocrine and non-endocrine activities. Pediatr Endocrinol Rev 2004; 2: 8-14.

8. Savino F, Lupica MM, Liguori SA, Fissore MF, Silvestro L. Ghrelin and feeding behaviour in preterm infants. Early Hum Dev 2012; 88: 51-55. https://doi.org/10.1016/j.earlhumdev.2011.12.028

9. Wang W, Cheng L, Guo J, Ma Y, Li F. Expression of Ghrelin in gastrointestinal tract and the effect of early weaning on Ghrelin expression in lambs. Mol Biol Rep 2014; 41: 909-914. https://doi.org/10.1007/s11033-013-2935-2

10. Kierson JA, Dimatteo DM, Locke RG, Mackley AB, Spear ML. Ghrelin and cholecystokinin in term and preterm human breast milk. Acta Paediatr 2006; 95: 991-995. https://doi.org/10.1080/08035250600669769

11. Savino F, Fissore MF, Grassino EC, Nanni GE, Oggero R, Silvestro L. Ghrelin, leptin and IGF-I levels in breast-fed and formula-fed infants in the first years of life. Acta Paediatr 2005; 94: 531-537. https://doi.org/10.1111/j.1651-2227.2005.tb01934.x

12. Abdel Hakeem AH, Saed SZ, El Rehany MA, Yassin EE. Serum level of ghrelin in umbilical cord in small and appropriate for gestational age newborn infants and its relationship to anthropometric measures. J Clin Neonatol 2012; 1: 135-138. https://doi.org/10.4103/2249-4847.101694

13. Savino F, Grassino EC, Fissore MF, et al. Ghrelin, motilin, insulin concentration in healthy infants in the first months of life: relation to fasting time and anthropometry. Clin Endocrinol (Oxf) 2006; 65: 158-162. https://doi.org/10.1111/j.1365-2265.2006.02561.x

14. Dore R, Levata L, Lehnert H, Schulz C. Nesfatin-1: functions and physiology of a novel regulatory peptide. J Endocrinol 2017; 232: 45-65. https://doi.org/10.1530/JOE-16-0361

15. Cheng YY, Zhao XM, Cai BP, Ma LN, Yin JY, Song GY. Nesfatin-1 in newborns: relationship with endocrine and metabolic and anthropometric measures. J Pediatr Endocrinol Metab 2012; 25: 727-732. https://doi.org/10.1515/jpem-2012-0095

16. Aydin S. The presence of the peptides apelin, ghrelin and nesfatin-1 in the human breast milk, and the lowering of their levels in patients with gestational diabetes mellitus. Peptides 2010; 31: 2236-2240. https://doi.org/10.1016/j.peptides.2010.08.021

17. Lubchenco LO, Hansman C, Boyd E. Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 1966; 37: 403-408. https://doi.org/10.1542/peds.37.3.403

18. Karlberg J, Albertsson-Wikland K. Growth in full-term small-for-gestational-age infants: from birth to final height. Pediatr Res 1995; 38: 733-739. https://doi.org/10.1203/00006450-199511000-00017

19. Campisi SC, Carbone SE, Zlotkin S. Catch-Up growth in full-term small for gestational age infants: a systematic review. Adv Nutr 2019; 10: 104-111. https://doi.org/10.1093/advances/nmy091

20. Farquhar J, Heiman M, Wong AC, Wach R, Chessex P, Chanoine JP. Elevated umbilical cord ghrelin concentrations in small for gestational age neonates. J Clin Endocrinol Metab 2003; 88: 4324-4327. https://doi.org/10.1210/jc.2003-030265

21. Ng PC, Lee CH, Lam CW, Chan IH, Wong E, Fok TF. Ghrelin in preterm and term newborns: relation to anthropometry, leptin and insulin. Clin Endocrinol (Oxf) 2005; 63: 217-222. https://doi.org/10.1111/j.1365-2265.2005.02328.x

22. Cesur G, Ozguner F, Yilmaz N, Dundar B. The relationship between ghrelin and adiponectin levels in breast milk and infant serum and growth of infants during early postnatal life. J Physiol Sci 2012; 62: 185-190. https://doi.org/10.1007/s12576-012-0193-z

23. Badillo-Suárez PA, Rodríguez-Cruz M, Nieves-Morales X. Impact of metabolic hormones secreted in human breast milk on nutritional programming in childhood obesity. J Mammary Gland Biol Neoplasia 2017; 22: 171-191. https://doi.org/10.1007/s10911-017-9382-y

24. Monteiro PO, Victora CG. Rapid growth in infancy and childhood and obesity in later life--a systematic review. Obes Rev 2005; 6: 143-154. https://doi.org/10.1111/j.1467-789X.2005.00183.x

25. Hong YH, Chung S. Small for gestational age and obesity related comorbidities. Ann Pediatr Endocrinol Metab 2018; 23: 4-8. https://doi.org/10.6065/apem.2018.23.1.4

26. Baldi A, Ioannis P, Chiara P, Eleonora F, Roubini C, Vittorio D. Biological effects of milk proteins and their peptides with emphasis on those related to the gastrointestinal ecosystem. J Dairy Res 2005; 72: 66-72. https://doi.org/10.1017/S002202990500110X

27. Barrenetxe J, Villaro AC, Guembe L, et al. Distribution of the long leptin receptor isoform in brush border, basolateral membrane, and cytoplasm of enterocytes. Gut 2002; 50: 797-802. https://doi.org/10.1136/gut.50.6.797

The Turkish Journal of Pediatrics 2022; 64: 246-254
https://doi.org/10.24953/turkjped.2020.3003

**Original Article**

# Relation of serum irisin levels to obesity and non-alcoholic fatty liver disease

Gökçen Ulualan[1], Zeynep Küskü Kiraz[2], Birgül Kırel[3]

*Departments of [1]Pediatrics, [2]Medical Biochemistry and [3]Pediatric Endocrinology, Eskişehir Osmangazi University Faculty of Medicine, Eskişehir, Turkey.*

## ABSTRACT

**Background.** Irisin is a newly defined myokine which is induced by exercise, which stimulates white fat cells to have the characteristics of brown adipose tissue cell. It thereby causes thermogenesis, energy and weight loss and improvement in insulin sensitivity. These effects of irisin suggest that it may be associated with obesity, insulin resistance and non-alcoholic fatty liver disease (NAFLD).

**Methods.** The aim of the present study was to determine the relationship of serum irisin levels in obese children with NAFLD. A total of 60 pubertal obese adolescents (age range: 11-18 yrs) as well as age and sex matched 28 healthy children were included in the study. Thirty of obese patients had NAFLD.

**Results.** The median irisin levels were lower in the obese patients both with and without NAFLD when compared with the control group. NAFLD group had a higher BMI than obese controls, however, the irisin levels were not different between these groups. The irisin levels were negatively correlated with BMI, BMI SDS, waist, hip and arm circumferences, waist/hip ratio, triceps-biceps skinfold thickness and AST, ALT levels in the all study groups. However, it was positively correlated with BMI, BMI SDS and waist and hip circumference in the entire obese group and positively with BMI SDS in the NAFLD subgroup.

**Conclusions.** Consequently, circulating irisin levels are lower in obese adolescents and negatively correlated with body adiposity. In NAFLD patients, it may be related to steatosis and may decrease with liver damage.

**Key words:** irisin, obesity, non-alcoholic fatty liver disease, children.

Irisin is a myokine which was first described by Boström et al.[1] in 2012. The peroxisome proliferator-activated receptor-ɣ coactivator-1′ (PGC1)-α expression increases in the muscle cell during exercise, activates the Fibronectin type III domain-containing 5 (*FNDC5*) gene and forms the FNDC5 protein. As a result of the proteolysis of this protein, irisin is released into circulation. The circulating irisin increases the expression of uncoupling protein 1 (UCP1) mRNA in subcutaneous white adipose tissue (WAT) cells which is a characteristic of the brown adipose tissue (BAT) cells. The WAT cells gain features of BAT cells (browning). UCP1, a protein found in the inner membrane of the mitochondria, causes protons to escape from the intermembrane space to the matrix and results in heat generation during oxidative phosphorylation.[2] Irisin is thereby involved in thermogenesis and energy expenditure. Furthermore, increase of irisin levels in circulation was found to be protective against diet-induced weight gain and causes improvement of insulin resistance (IR).[1] The association of irisin levels with physical activity, parameters of glucose and lipid metabolism, obesity and obesity-related morbidities such as type 2 diabetes mellitus (DM), and metabolic

✉ Gökçen Ulualan
gokcen.ulualan@gmail.com

Received 17th October 2020, revised 31st January 2021, 30th August 2021, 8th November 2021, accepted 29th November 2021.

This article is based on thesis in medicine.

This manuscript has been presented as poster at the 20th European Congress of Endocrinology, Barcelona, Spain in 2018.

syndrome (MetS) have been investigated, and inconsistent results have been reported.[3-20]

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children and adolescents, with fat accumulation in the liver without any other diseases or alcohol and drug intake that could cause liver disease. The prevalence is increasing in parallel with the increasing prevalence of obesity.[21] Although the disease is generally detected as asymptomatic simple steatosis, it may slowly progress to steatohepatitis (NASH), characterized by liver cell damage and inflammation and may eventually lead to cirrhosis and rarely hepatocellular carcinoma through liver fibrosis. IR is primarily responsible for the etiopathogenesis of steatosis in the liver; obesity and dyslipidemia are the other main risk factors.[22,23] The prevalence of NAFLD is relatively higher in obese patients and patients with type 2 DM. Lifestyle changes such as diet and exercise are recommended in order to lose weight and increase insulin sensitivity for treatment of NAFLD.[24] It is known that hepatic steatosis and inflammation may be reduced by physical exercise without any weight loss.[25,26] As these lifestyle changes are generally not implemented completely, many pharmacological agents are used to prevent liver damage and to reduce the risk factors for NAFLD.[23,27]

Due to its similar relationship with exercise, obesity and IR which are the major risk factors in NAFLD development, irisin has been suggested to have a role in the development and prognosis of NAFLD by increasing energy expenditure and insulin sensitivity.[17] Contradictory results have been reported on the association between irisin and NAFLD in several previous studies.[17-20,28,29] To the best of our knowledge, there is no study investigating the relationship between circulating irisin levels and NAFLD in children. Therefore, we aimed to determine whether serum irisin levels are related to anthropometric measurements and metabolic and biochemical parameters in obese children with NAFLD.

## Material and Methods

Sixty pubertal patients with exogenous obesity (31 girls, 29 boys), between 11 and 18 years of age admitted to our pediatric endocrinology outpatient clinic were included in the study. Thirty of 60 obese patients had NAFLD. The control group consisted of 28 healthy pubertal children (14 girls, 14 boys) with similar age and gender. The patients with another disease or those using any drugs were excluded from the study.

The study was approved by Eskişehir Osmangazi University Clinical Researches Ethics Committee (Approval no: 148 – 03.06.2016). Children and their families were informed about the objective and methods of the study. Informed consent was obtained from the parents.

Physical examination was performed on all children. Puberty was determined according to the method of Tanner.[30,31] Body weight (BW) and height were measured. Body mass index (BMI) was calculated by BW (kg)/height $(m)^2$ and compared with the references according to age and gender.[32] Any BMI level at and above 95 percentile was considered as obese. The standard deviation scores (SDS) of BW, height and BMI were determined according to age and gender.[32] Subcutaneous fat thickness was measured from the triceps and biceps regions by a caliper. The arm circumference was measured from the mid-point of the arm between olecranon and acromion. The waist circumference was measured in the horizontal plane midway between the lowest rib and the iliac crest. The hip circumference was measured over the widest area of the hips. The waist-hip ratio was calculated.

After fasting for one night, two venous serum samples were collected for irisin level and biochemical analyses. Glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and

gamma-glutamyl transferase (GGT) levels were determined with photometric methods by Roche Cobas 8000 analyser, c702 module (Roche Diagnostics GmbH, Penzburg, Germany) autoanalyser. Insulin levels were determined through the electrochemiluminescence immunoassay (ECLIA) method by Roche Cobas 8000 c602 (Roche Diagnostics GmbH, Penzburg, Germany) autoanalyser.

Venous serum samples collected for irisin levels were stored at -80ºC until the analysis. Serum irisin levels were measured by commercial ELISA kits (RAG018R, BioVendor Inc., Candler, NC, USA) on VICTOR X3 (PerkinElmer, USA). The sensitivity of the method was 1 ng/ml; the intra-assay CV value was below 8.2%, and the inter-assay CV value was below 9.7%.

The homeostasis model assessment of IR (HOMA-IR) was calculated by using the following formula: fasting insulin level (uIU/ml) x fasting glucose (mg/dl) / 405. A HOMA-IR value above 5.22 in males and above 3.82 in females was considered as IR.[33]

A hepatobiliary USG was performed for NAFLD. The hepatosteatosis of the participants was graded as 1, 2, and 3 through the USG.[34]

Statistical analysis was performed by SPSS 23.0 program (IBM SPSS, Chicago, IL). Variables were expressed as mean ± standard deviations (SD), median (25%-75%), and percentage (%). All variables were assessed for normality and homogeneity of variance by the Shapiro Wilk test. The comparisons were performed through the t-test analysis and One-Way ANOVA when the variable was distributed normally or Mann-Whitney U test and Kruskal-Wallis H test when the variable was not distiributed normally. Pearson and Spearman correlation coefficients were conducted for the correlations. Chi-square analysis was used for the analysis of the cross tables. Any p-value below 0.05 was considered statistically significant.

**Results**

Clinical characteristics and laboratory data of all the study groups are shown in Table I. The BW, BW SDS, BMI, BMI SDS, waist, hip, arm circumference, waist-hip ratio, skinfold thicknesses (triceps and biceps), ALT, TG, insulin and HOMA-IR levels were higher in the obese patients with and without NAFLD when compared to the control group; however, HDL-C was lower ($p<0.05$). Furthermore BW, BW SDS, BMI, BMI SDS, waist, hip circumference, ALT and GGT levels were higher in the patients with NAFLD than the patients without NAFLD ($p<0.05$).

The median irisin levels in patients with both NAFLD [5.7 (4.6-6.5) μg/ml] and without NAFLD [5.05 (3.8-5.7) μg/ml] were also lower than in the control group [7.5 (6.5-9.08) μg/ml] ($p<0.05$). However, no significant difference was shown between obese patients with and without NAFLD ($p>0.05$) (Fig. 1).

The median irisin levels were not significantly different between the genders in all the study groups ($p>0.05$).

When the relationship between serum irisin level and other parameters were investigated, a negative correlation was detected between irisin and BMI (r=-0.53, p=0.001), BMI SDS (r=-0.346, p=0.001), BMI percentile (r=-0.3, p=0.000), waist circumference (r=-0.37, p=0.000), hip circumference (r=-0.3, p=0.000), waist-hip ratio (r=-0.374, p=0.000), skinfold thicknesses triceps (r=-0,36, p=0.001) and biceps (r=-0.389, p=0.000), arm circumference (r=-0.3, p=0.002), insulin (r=0.24, p=0.02), glucose/insulin ratio (r=-0.675 p=0.000), ALT (r=-0.4, p=0.000); and a positive corelation was detected between irisin and HDL-C (r=0.43, p=0.000) in the entire study group including obese and normal-weight group. However, the analysis performed in the subgroups revealed that irisin levels were positively correlated with skinfold thickness

**Table I.** Clinical characteristics and laboratory results of all study groups*.

| | Control Group (n=28) | Obese Patients with NAFLD (n=30) | Obese Patients without NAFLD (n=30) | p value |
|---|---|---|---|---|
| Gender (F/M) | 14/14 | 12/18 | 19/11 | |
| Age (Month) | 167 (145.5 - 192) | 166 (146.2 – 174.2) | 173 (154.7 – 186.7) | 0.49 |
| Height (cm) | 158.2 ± 10.3 | 159 (155.7- 163.5) | 158.2 (151.5 – 163) | 0.72 |
| Height SDS | -0.12 ± 0.69 | 0.67 ± 1.16 | -0.44 ± 1.13 | 0.22 |
| BW (kg) | 50.2 ± 9.5 | 81.1 ± 11.3[a] | 70.6 ± 11.5[b,c] | <0.001 |
| BW SDS | -0.32 ± 0.49 | 2.6 ± 0.91[a] | 1.94 ± 0.84[b,c] | <0.001 |
| BMI (kg/m²) | 19.7 (18.3 – 21) | 31.2 (29.1 – 34.7)[a] | 27.7 (26.1 – 29.5)[b,c] | <0.001 |
| BMI SDS | -0.27 (-0.47 – -0.02) | 2.44 (2.2 – 3.1)[a] | 2.09 (2 – 2.23)[b,c] | <0.001 |
| Waist (cm) | 70.4 ± 6.3 | 102.2 ± 9.6[a] | 94.1 ± 10.5[b,c] | <0.001 |
| Hip (cm) | 87.1 ± 7.8 | 109.4 ± 8[a] | 104 ± 10.3[b,c] | <0.001 |
| Waist/Hip Ratio | 0.81 (0.77 – 0.83) | 0.95 (0.89 – 0.98)[a] | 0.92 (0.84 – 0.96)[b] | <0.001 |
| Waist/Height Ratio | 0.44 (0.42 – 0.45) | 0.63 ± 0.06[a] | 0.59 ± 0.06[b,c] | <0.001 |
| Arm Circumference (cm) | 23 ± 2.6 | 31.3 ± 2.77[a] | 29.7 ± 3.62[b] | <0.001 |
| Skinfold Thickness (Triceps)(mm) | 1.1 ± 0.4 | 3.2 ± 0.87[a] | 3 ± 0.89[b] | <0.001 |
| Skinfold Thickness (Biceps)(mm) | 0.5 (0.4 – 0.8) | 2.3 ± 0.72[a] | 2.1 ± 0.89[b] | <0.001 |
| Glucose (mg/dl) | 84.5 (81 – 93.7) | 83.2 ± 8.56 | 87.6 ± 11.22 | 0.16 |
| Insulin (uIU/ml) | 8.5 (6.62 – 13.17 ) | 20.7 (14.68 – 27.88)[a] | 17.9 (12.6 – 23.87)[b] | <0.001 |
| Glucose/Insulin Ratio | 9.69 (6.74 – 13.04) | 3.7 (3.07 – 5.87)[a] | 5.21 (3.86 – 6.34)[b] | <0.001 |
| HOMA-IR | 1.76 (1.37 – 2.83) | 3.88 (2.59 – 5.72)[a] | 3.53 (2.7 – 5.95)[b] | <0.001 |
| AST (U/L) | 20 (17 – 23) | 22 (17.75 – 29) | 20.5 (17.75 – 25.25) | 0.59 |
| ALT (U/L) | 12 (8.2 – 14) | 23 (14 – 41.5)[a] | 15 (10.75 – 23.25)[b,c] | <0.001 |
| GGT (U/L) | 9.5 (6.5 – 12.5) | 17 (13.75 – 26)[a] | 12 (9 – 17)[c] | <0.001 |
| TC(mg/dl) | 148 ± 22.4 | 162.2 ± 32.71 | 154.9 ± 21.12 | 0.12 |
| TG (mg/dl) | 73.2 (49.2 – 100.2) | 122.3 ± 54.72[a] | 116.3 ± 55.75[b] | 0.005 |
| HDL-C (mg/dl) | 56.5 (48.7 – 63.7) | 43.5 (35.75 – 48.25)[a] | 44 (39.75 – 47)[b] | <0.001 |
| LDL-C (mg/dl) | 87.3 (65.9 – 102.8) | 104.6 ± 25.33[a] | 95.5 ± 17.91 | 0.009 |
| Irisin (μg/ml) | 7.5 (6.5-9.08) | 5.7 (4.6-6.5)[a] | 5.05 (3.8-5.7)[b] | <0.001 |

*Parameters with normal distribution were given as mean ± SD. Parameters without normal distribution were given as median (25% -75%). a: p<0.05, comparison between control group and obese patients with NAFLD. b: p<0.05, comparison between control group and obese patients without NAFLD. c: p<0.05, comparison between obese patients with and without NAFLD. NAFLD: non-alcoholic fatty liver disease, BW: body weight, SDS: standard deviation scores, BMI: body mass index, HOMA-IR: the homeostasis model assessment of insulin resistant, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase, TC: total cholesterol, TG: triglycerides, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol.

(triceps) (r=0.5, p=0.005) and negatively correlated with AST (r=-0.459, p=0.014) in the control group. In the entire obese group, there was a positive correlation between irisin levels and BMI (r=0.327, p=0.01), BMI SDS (r=0.376, p=0.003), waist circumference (r=0.27, p=0.03), hip circumference (r=0.26, p=0.04) and arm circumference (r=0.3, p=0.01). Moreover, the irisin levels were positively corelated with BMI SDS (r=0.39, p=0.034) and HDL-C (r=0.387, p=0.035) in the NAFLD group. No correlation was found between the irisin levels and other measured parameters in the obese patients without NAFLD (p>0.05).

Five patients with NAFLD had AST and ALT levels above 40 U/L. According to the severity of steatosis, there was grade 3 hepatosteatosis in

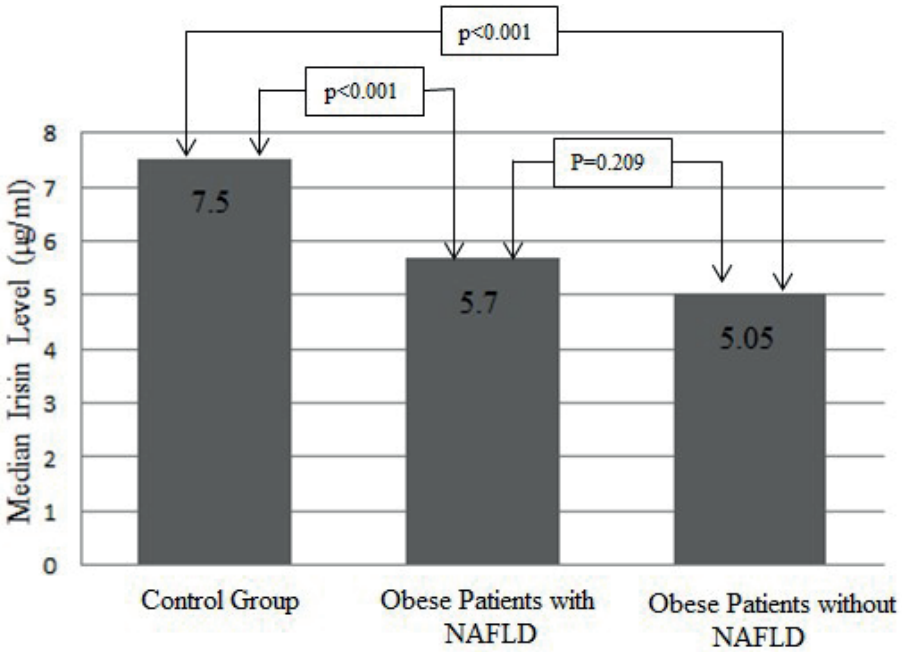



**Fig. 1.** Median irisin levels in all the study groups.

1 (n=1/30, 3.3%) patient, grade 2 hepatosteatosis in 5 (n=5/30, 16.7%) patients, and grade 1 hepatosteatosis in 24 (n=24/30, 80%) patients.

Insulin resistance was found in 25 patients (n=25/60, 41.7%) including 13 (n=13/60, 52%) patients with NAFLD. The serum irisin levels were not significantly different between the obese patients with and without IR (p>0.05).

## Discussion

In our study, serum irisin levels were lower in obese patients with and without NAFLD than the controls with normal body weight and negatively correlated with adiposity parameters in all study groups. It was reported in previous studies, that irisin levels in the circulation of obese adults and children were lower similar to our study, higher in some studies and did not change in others.[3-11] In addition, the irisin levels were negatively correlated with adiposity parameters in some studies and positively correlated in others.[3-8,13,15,35]

Boström et al.[1] first reported that irisin is secreted from muscle cells as the product of the *FNDC5* gene. It was later shown that irisin was synthesized in both muscle and adipose tissue.[9,12,36,37] However, *FNDC5* gene expression in adipose tissue was 100-200 times higher in muscle tissue than adipose tissue in some studies.[9,12] The elevated irisin levels with a positive correlation with adiposity markers in obese subjects in previous studies suggested that it may also be released from adipose tissue, or it may be a compensatory regulator for maintenance of the energy balance due to increased adipose tissue.[5,6,12,38] Palacios-Gonzales et al.[6] found that the irisin level was positively correlated with BMI in obese adults and suggested that circulating irisin in the basal metabolic state has arisen from adipose tissue, and irisin originated from the skeletal muscle is produced during exercise. Huh et al.[12] showed that lean body mass did not change in those who had bariatric surgery however, muscle FNDC5 mRNA and circulating irisin levels decreased along with the decrease in weight loss and BMI after the surgery. They also indicated that the amount of adipose tissue is a determinant of irisin. However, as in our study, Gonzales-Gil et al.[3] showed that circulating irisin levels were lower and negatively correlated with adiposity

markers in obese and MetS patients than those with normal-weight subjects. They found that circulating irisin is determined by the lean-fat ratio rather than the total amount of body muscle and fat mass.

In our study, serum irisin levels were negatively correlated with adiposity parameters in all the study groups and levels were lower in obese patients with and without NAFLD than the control group. These findings could be explained as a result or cause of the ratio of fat to muscle mass. However, the amount of muscle and adipose tissue was not determined. The lower irisin levels in obese patients may not be a result of obesity, but, also a cause of obesity. It is not possible to clarify the comment with this cross-sectional study plan.

Although serum irisin levels were positively correlated with adiposity parameters in the entire obese group including patients with and without NAFLD, levels were not different from obese patients without NAFLD from the patients with NAFLD. These results suggest that there may be a relationship between circulating irisin and steatosis, apart from the associations mentioned above between adipose tissue and irisin or obesity and irisin. It is suggested that there may be an adaptive response for the presence of steatosis. In addition to supportig this statistically insignificant comment, the irisin levels were numerically higher in NAFLD patients than obese controls without NAFLD. This finding may be related to relatively small size of the groups.

As in our study, Polyzos et al.[17] demonstrated that serum irisin levels were lower in obese patients with NAFLD and NASH diagnosed with biopsy and in obese controls than healthy controls. It was not different between the two patient groups. Circulating irisin levels did not correlate with ALT in this study. However, serum irisin levels were higher in patients with a higher degree of portal inflammation. They suggested that the irisin may have a preventive role in portal inflammation. Zhang et al.[18] found that circulating irisin levels are lower in Chinese obese adults with NAFLD than those without NAFLD. There was no healthy control group in this study. Serum irisin levels were negatively correlated with ALT and AST levels, and its levels increased in parallel with the increase in intrahepatic triglyceride content determined by MRI. Thus, the authors suggested that irisin could have a protective effect from hepatic steatosis.

Contrary to our study, Choi et al.[19] found that circulating irisin levels were higher in those with NAFLD than healthy controls, and those with mild NAFLD than those with severe NAFLD; however, no difference was detected between obese controls and healthy controls. In the NAFLD group, the irisin levels were not different between obese and non-obese patients. However, in the normal control group, serum irisin levels were lower in obese subjects than non-obese subjects. Furthermore, ALT levels were negatively correlated with irisin levels. Although the BMI was higher in patients with NAFLD, it was suggested that the increase of irisin was independent of BMI. Thus, increased irisin levels could be a protective factor for the early stage of NAFLD, and irisin levels decreased with steatosis severity.

Petta et al.[22] found in 593 patients with NAFLD who had a liver biopsy with a pre-diagnosis of NASH that neither the serum irisin nor hepatic irisin mRNA levels were not associated with the rs3480 A.G variant and any demographic, anthropometric and metabolic parameters. Both serum irisin levels and hepatic mRNA levels were found to be higher in those with grade 2-3 steatosis, those with NASH, and severe fibrosis. In contrast to these results, Choi et al.[19] reported that serum irisin levels were lower in patients with severe steatosis than those with mild steatosis. In the study by Petta et al.[22], it was found that hepatic cell fat accumulation in mice fed with high fat content was not affected by irisin, but it was related to the severity of NAFLD. In the invitro study conducted by these authors, it has been shown that irisin had an expression in hepatic stellate cells which is responsible for collagen synthesis and fibrosis,

and this expression was higher in patients with hepatic fibrosis. These findings indicated that irisin may be associated with extracellular fat accumulation and hepatic fibrogenesis.

NASH is considered as a further form of NAFLD. Furthermore, it is known that elevated serum ALT levels indicate liver cell destruction and are a favored marker indicating the presence of NASH.[21] In our study, the association between irisin and presence of NASH could not be evaluated because there were only five patients with elevated levels of ALT and AST. However, there was a negative correlation between ALT-AST levels and irisin levels as in previous studies.[18-20] This finding suggested that circulating irisin decreases with liver damage besides the relationships between irisin and steatosis. As mentioned above, it is suggested that irisin may be a protective factor for liver injury. However, in the study by Polyzos et al.[17], serum irisin levels were not correlated with ALT levels. On the other hand, Rizk et al.[29] found a positive correlation between serum irisin levels and ALT and AST levels. Furthermore, the irisin levels were higher in a group of MetS with normal liver enzymes and with both fatty liver disease and elevated liver enzymes than in the healthy control group and those with elevated liver enzymes compared to patients with normal liver enzyme levels in this study. Thus, they suggested that circulating irisin levels may be affected by hepatic clearance in these patients.

As a result of our study, obese adolescents with and without NAFLD have lower circulating irisin levels and the level decreases with body adiposity. In addition, irisin may be related to steatosis and may decrease with liver damage in NAFLD. On the other hand, NAFLD was not diagnosed through biopsy. This was a limitation of our study. These results as well as the studies mentioned above regarding serum irisin levels in patients with NAFLD, concluded that lower irisin levels may be a risk factor for NAFLD and may also have an association with the severity of NAFLD. In this context, it has been suggested that irisin may have a therapeutic role in obesity and type 2 DM and NAFLD.[27,39] Further comprehensive and detailed researches would be useful to clarify the issue.

## Ethical approval

The study was approved by Eskişehir Osmangazi University Clinical Researches Ethics Committee (Approval no: 148 – 03.06.2016).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BK, GU; data collection: GU; analysis and interpretation of results: BK, GU, ZKK; draft manuscript preparation: BK, GU. 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. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481: 463-468. https://doi.org/10.1038/nature10777
2. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. N Engl J Med 2009; 360: 1518-1525. https://doi.org/10.1056/NEJMoa0808949
3. Gonzalez-Gil AM, Peschard-Franco M, Castillo EC, et al. Myokine-adipokine cross-talk: potential mechanisms for the association between plasma irisin and adipokines and cardiometabolic risk factors in Mexican children with obesity and the metabolic syndrome. Diabetal Metab Syndr 2019; 11: 63. https://doi.org/10.1186/s13098-019-0458-2

4. Shim YS, Kang MJ, Yang S, Hwang IT. Irisin is a biomarker for metabolic syndrome in prepubertal children. Endocr J 2018; 65: 23-31. https://doi.org/10.1507/endocrj.EJ17-0260

5. Binay Ç, Paketçi C, Güzel S, Samancı N. Serum irisin and oxytocin levels as predictors of metabolic parameters in obese children. J Clin Res Pediatr Endocrinol 2017; 9: 124-131. https://doi.org/10.4274/jcrpe.3963

6. Palacios-González B, Vadillo-Ortega F, Polo-Oteyza E, et al. Irisin levels before and after physical activity among school-age children with different BMI: a direct relation with leptin. Obesity (Silver Spring) 2015; 23: 729-732. https://doi.org/10.1002/oby.21029

7. Jang HB, Kim HJ, Kang JH, Park SI, Park KH, Lee HJ. Association of circulating irisin levels with metabolic and metabolite profiles of Korean adolescents. Metabolism 2017; 73: 100-108. https://doi.org/10.1016/j.metabol.2017.05.007

8. Reinehr T, Elfers C, Lass N, Roth CL. Irisin and its relation to insulin resistance and puberty in obese children: a longitudinal analysis. J Clin Endocrinol Metab 2015; 100: 2123-2130. https://doi.org/10.1210/jc.2015-1208

9. Moreno-Navarrete JM, Ortega F, Serrano M, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab 2013; 98: E769-E778. https://doi.org/10.1210/jc.2012-2749

10. Elizondo-Montemayor L, Silva-Platas C, Torres-Quintanilla A, et al. Association of irisin plasma levels with anthropometric parameters in children with underweight, normal weight, overweight, and obesity. Biomed Res Int 2017; 2017: 2628968. https://doi.org/10.1155/2017/2628968

11. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity-correlation with body mass index. Peptides 2013; 39: 125-130. https://doi.org/10.1016/j.peptides.2012.11.014

12. Huh JY, Panagiotou G, Mougios V, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism 2012; 61: 1725-1738. https://doi.org/10.1016/j.metabol.2012.09.002

13. Park KH, Zaichenko L, Brinkoetter M, et al. Circulating irisin in relation to insulin resistance and the metabolic syndrome. J Clin Endocrinol Metab 2013; 98: 4899-4907. https://doi.org/10.1210/jc.2013-2373

14. Yan B, Shi X, Zhang H, et al. Association of serum irisin with metabolic syndrome in obese Chinese adults. PLoS One 2014; 9: e94235. https://doi.org/10.1371/journal.pone.0094235

15. Choi YK, Kim MK, Bae KH, et al. Serum irisin levels in new-onset type 2 diabetes. Diabetes Res Clin Pract 2013; 100: 96-101. https://doi.org/10.1016/j.diabres.2013.01.007

16. Liu JJ, Wong MD, Toy WC, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications 2013; 27: 365-369. https://doi.org/10.1016/j.jdiacomp.2013.03.002

17. Polyzos SA, Kountouras J, Anastasilakis AD, Geladari EV, Mantzoros CS. Irisin in patients with nonalcoholic fatty liver disease. Metabolism 2014; 63: 207-217. https://doi.org/10.1016/j.metabol.2013.09.013

18. Zhang HJ, Zhang XF, Ma ZM, et al. Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. J Hepatol 2013; 59: 557-562. https://doi.org/10.1016/j.jhep.2013.04.030

19. Choi ES, Kim MK, Song MK, et al. Association between serum irisin levels and non-alcoholic fatty liver disease in health screen examinees. PLoS One 2014; 9: e110680. https://doi.org/10.1371/journal.pone.0110680

20. Shanaki M, Moradi N, Emamgholipour S, et al. Lower circulating irisin is associated with nonalcoholic fatty liver disease and type 2 diabetes. Diabetes Metab Syndr 2017; 11: S467-S472. https://doi.org/10.1016/j.dsx.2017.03.037

21. Nobili V, Alisi A, Valenti L, et al. NAFLD in children: new genes, new diagnostic modalities and new drugs. Nat Rev Gastroenteral Hepatol 2019; 16: 517-530. https://doi.org/10.1038/s41575-019-0169-z

22. Petta S, Valenti L, Marra F, et al. MERTK rs4374383 polymorphism affects the severity of fibrosis in non-alcoholic fatty liver disease. J Hepatol 2016; 64: 682-690. https://doi.org/10.1016/j.jhep.2015.10.016

23. Tailleux A, Wouters K, Staels B. Roles of PPARs in NAFLD: potential therapeutic targets. Biochim Biophys Acta 2012; 1821: 809-818. https://doi.org/10.1016/j.bbalip.2011.10.016

24. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. J Hepatol 2017; 67: 829-846. https://doi.org/10.1016/j.jhep.2017.05.016

25. Arias-Loste MT, Ranchal I, Romero-Gómez M, Crespo J. Irisin, a link among fatty liver disease, physical inactivity and insulin resistance. Int J Mol Sci 2014; 15: 23163-23178. https://doi.org/10.3390/ijms151223163

26. Hallsworth K, Fattakhova G, Hollingsworth KG, et al. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut 2011; 60: 1278-1283. https://doi.org/10.1136/gut.2011.242073

27. Boutari C, Perakakis N, Mantzoros CS. Association of adipokines with development and progression of nonalcoholic fatty liver disease. Endocrinol Metab (Seoul) 2018; 33: 33-43. https://doi.org/10.3803/EnM.2018.33.1.33

28. Hu J, Ke Y, Wu F, et al. Circulating ırisin levels in patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenteral Res Pract 2020; 2020: 881891. https://doi.org/10.1155/2020/8818191

29. Rizk FH, Elshweikh SA, Abd El-Naby AY. Irisin levels in relation to metabolic and liver functions in Egyptian patients with metabolic syndrome. Can J Physiol Pharmacol 2016; 94: 359-362. https://doi.org/10.1139/cjpp-2015-0371

30. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969; 44: 291-303. https://doi.org/10.1136/adc.44.235.291

31. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970; 45: 13-23. https://doi.org/10.1136/adc.45.239.13

32. Neyzi O, Furman A, Bundak R, Gunoz H, Darendeliler F, Bas F. Growth references for Turkish children aged 6 to 18 years. Acta Paediatr 2006; 95: 1635-1641. https://doi.org/10.1080/08035250600652013

33. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu M, Kendirici M, Keskin M, Kondolot M. Insulin resistance in obese children and adolescents: HOMA-IR cut-off levels in the prepubertal and pubertal periods. J Clin Res Pediatr Endocrinol 2010; 2: 100-106. https://doi.org/10.4274/jcrpe.v2i3.100

34. Kim SH, Lee JM, Kim JH, et al. Appropriateness of a donor liver with respect to macrosteatosis: application of artificial neural networks to US images-initial experience. Radiology 2005; 234: 793-803. https://doi.org/10.1148/radiol.2343040142

35. Pardo M, Crujeiras AB, Amil M, et al. Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. Int J Endocrinol 2014; 2014: 857270. https://doi.org/10.1155/2014/857270

36. Roca-Rivada A, Castelao C, Senin LL, et al. FNDC5/irisin is not only a myokine but also an adipokine. PLoS One 2013; 8: e60563. https://doi.org/10.1371/journal.pone.0060563

37. Kurdiova T, Balaz M, Vician M, et al. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. J Physiol 2014; 592: 1091-1107. https://doi.org/10.1113/jphysiol.2013.264655

38. Crujeiras AB, Zulet MA, Lopez-Legarrea P, et al. Association between circulating irisin levels and the promotion of insulin resistance during the weight maintenance period after a dietary weight-lowering program in obese patients. Metabolism 2014; 63: 520-531. https://doi.org/10.1016/j.metabol.2013.12.007

39. Leung PS. The potential of irisin as a therapeutic for diabetes. Future Med Chem 2017; 9: 529-532. https://doi.org/10.4155/fmc-2017-0035

The Turkish Journal of Pediatrics 2022; 64: 255-264
https://doi.org/10.24953/turkjped.2021.1437

**Original Article**

# Elevated neurotensin levels among obese adolescents may be related to emotion dysregulation and impulsivity: a cross-sectional, case-control study

Gonca Özyurt[1], Gülten Cingöz[2], Yusuf Öztürk[3], Tuncay Küme[4], Bumin Nuri Dündar[5], Ali Evren Tufan[3], Gönül Çatlı[5]

*Departments of [1]Child and Adolescent Psychiatry and [5]Pediatric Endocrinology, İzmir Katip Çelebi University Faculty of Medicine, İzmir; [2]Department of Pediatrics, Sağlık Bilimleri University, Tepecik Training and Research Hospital, İzmir; [3]Department of Child and Adolescent Psychiatry, Bolu Abant İzzet Baysal University Faculty of Medicine, Bolu; [4]Department of Biochemistry, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey.*

## ABSTRACT

**Background.** In this study, we aimed to evaluate the serum neurotensin (NT) levels and their relationships with self-reported anxiety, emotion regulation skills and impulsivity in healthy and obese adolescents.

**Methods.** Adolescents who gained weight between 12- 17 years of age and who were above the 95th percentile (p) for body mass index (BMI)>95p were compared with age- and gender-matched healthy adolescents with a BMI of 3-85 p. Anthropometric measurements were performed, and serum NT levels were analyzed with ELISA method in all participants. Barrat Impulsivity Scale-11 (BIS-11), Screen for Child Anxiety Related Disorders (SCARED) and Difficulties in Emotion Regulation Scale (DERS) were used for evaluating self-reported impulsivity, anxiety and emotion regulation. MANOVA with follow-up univariate ANOVAs (Bonferroni corrected) were used for group comparisons. P was set at 0.05 (two-tailed).

**Results.** Sixty-five obese and 65 healthy adolescents were included in the study. In the obese group, NT levels were significantly elevated compared to the control group. Self-reported emotion-regulation difficulties, anxiety and impulsivity were significantly elevated among obese adolescents. Serum NT levels among the obese group were positively correlated with emotion dysregulation and impulsivity scores.

**Conclusions.** In this study, we found emotional dysregulation, anxiety, impulsivity, and serum NT levels were significantly elevated among obese adolescents compared to controls. NT levels in the obese group correlated with impulsivity and emotion dysregulation. Further studies should evaluate the potential role of NT in the etiology of psychopathology among adolescents who are obese.

**Key words:** adolescent, anxiety, emotion regulation, neurotensin, obesity.

Obesity can affect 5.0 % of children worldwide, especially among the economically disadvantaged.[1] It may affect children across all age groups and there seems to be a temporal trend of increase within the last 40 years.[1,2] The current consensus is that pediatric obesity may arise due to interactions between biological, developmental, behavioral, genetic and environmental factors.[3] The ongoing COVID-19 pandemic may also contribute to the emergence and persistence of pediatric obesity.[4]

Neurotensin (NT) is a 13 amino acid peptide secreted from the enteroendocrine cells in the small intestine and the central nervous system.[5] It may modulate the dopaminergic, serotonergic and glutamatergic function in the nigro-striatal and meso-cortical limbic systems and may have an anorexigenic effect via the lateral hypothalamic region.[6,7] Various

✉ Yusuf Öztürk
yusuf26es@hotmail.com

Received 4th May 2021, revised 6th July 2021, 26th July 2021, 16th August 2021, accepted 22nd August 2021.

pre-clinical studies suggest that it may have a role in anorexia as a response to stress, emergence of anxiety- like behavior, hedonic eating, reward/ reinforcement and memory.[8-11] A recent review suggested that NT may have therapeutic potential[12] and another study suggested that elevated levels of its precursor may predict weight gain and associated metabolic abnormalities among children.[13] Butler et al. (2015) found that plasma NT levels were elevated among children with Prader-Willi syndrome characterized by hyperphagia via decreasing gastric motility.[14] Available studies suggest that pediatric obesity at least in a subgroup of patients may be associated with elevated levels of anxiety, impulsivity and emotional eating.[15-17]

Despite the importance of NT functioning in those constructs, no study up to now has evaluated the relationships between NT levels and anxiety, impulsivity and emotion regulation among obese children.

Therefore; in this study, we aimed

a) to compare serum NT levels among obese and healthy adolescents
b) to compare self-reported emotion regulation, anxiety and impulsivity scores among obese and healthy adolescents, and
c) to investigate the relation of NT with emotional regulation, anxiety and impulsivity among obese adolescents.

## Material and Methods

### *Study design, center and time frame*

The study was designed as a uni-center, cross-sectional, case-control study and obese and healthy adolescents aged between 12-17 years were enrolled between April 2017 and April 2018.

### *Inclusion Criteria and Exclusion Criteria*

Patients with a body mass index (BMI) percentile of > 95 according to the WHO criteria[18] formed the obese group. Obese adolescents with a body mass index (BMI) >95 percentile and healthy adolescents with a BMI between 3 and 85 percentiles, according to the data of Turkish National Growth Charts [A], who had similar age and gender distribution and admitted for routine control were enrolled in the study.[19] Patients with underlying endocrine (hypothyroidism, Cushing syndrome, etc.) or non-endocrine (hypothalamic dysfunction, drug use, syndromic diseases) pathologies were excluded from the study. Psychiatric disorders including neurodevelopmental disorders were excluded with semi structered clinical interview via Schedule for Affective Disorders and Schizophrenia for School Age Children Present and Life-time Version (KIDDIE-SADS-PL)[20] and Diagnostic and Statistical Manual of Mental Disorders (DSM–5). Both life-time and acute psychopathology (within two months) were excluded. Ninety eight obese adolescents were evaluated with K-SADS-PL and DSM-5 criteria. 33 adolescents were excluded, 21 of the adolescents had attention deficit and hyperactivity disorder; 8 of them had anxiety disorder; 2 of the adolescents had major depressive disorder and 2 of them had obsessive compulsive disorder. The same child and adolescent psychiatrist evaluated all the adolescents in one year. She gave new appointments to adolescents for psychiatric evaluation after pediatric endocrinologic and pediatric examination. Patients with a history of intracranial operation, syndromic obesity findings (Prader Willi, Alström, Laurence–Moon-Biedle syndrome, etc.) or a genetic cause for monogenic obesity (leptin gene defect, leptin receptor defect, etc.), an active infection, evidence of hypothalamic dysfunction, a history of pregnancy or lactation that can affect OXT release, and who were not willing to participate in the study were also excluded.

Adolescents who applied to the general pediatrics outpatient clinic for any complaints, who were similar to the obese group in terms of age and gender, without chronic diseases, and whose BMI percentile were between 3-85 were included in the healthy control group.

### *Anthropometric Evaluation*

Height (cm), body weight (kg) and waist circumference (cm) of all cases included in the study were measured after an overnight fast in the morning. The height was measured with the Harpenden stadiometer (Holtain Ltd., Crosswell, Wales, UK) with a measurement accuracy of 0.1 cm, and the body weight was measured with a SECA scale (SECA Medizinische Messsysteme und Waagen, Hamburg, Deutschland) with a measurement sensitivity of 0.1 kg after all clothes were removed except underwear.

The BMI is calculated by dividing body weight in kilograms (kg) by height in meters squared (m2) and it is expressed as kg/ m2. The website www.ceddcozum.com, developed by the Turkish Pediatric Endocrinology and Diabetes Association, was used to calculate percentile and standard deviation scores for weight, height, head circumference, and BMI according to Olcay Neyzi[19], CDC and WHO references. BMI SDS was calculated for all children aged 12-17 years included in the study.

### *Blood Samples*

Blood samples were taken after a minimum of 12 hours of fasting. Serum NT levels were studied using the firm's original reactives with standardized methods on Architect AU5800 (Beckman Coulter, Brea, CA, USA) analyzer on serum samples kept at -80°C. Serum was analyzed with NT (Neurotensin (Human, Rat, Mouse)- EIA Kit, 96 wells, CAT No:EK-048-03, Phoenix Pharmaceuticals) using enzyme-linked immunosorbent assay (ELISA) method.

### *Psychiatric Evaluation*

**Sociodemographic Data Form:** This form was prepared to collect information about sociodemographic characteristics of children and parents and completed by the clinicians.

**Schedule for Affective Disorders and Schizophrenia for School Age Children Present and Life-time Version (KIDDIE-SADS-PL):** It is a semi-structured interview form which was developed by Kauffman et al. in order to examine present and life-time psychopathology in children and adolescents aged between 6-18 years.[20] Turkish translation and reliability and validity study of KIDDIE-SADS-PL were carried out by Gökler et al., in 2004.[21]

**Difficulties in Emotion Regulation Scale (DERS):** Gratz and Roemer developed DERS in order to measure difficulties in emotion regulation.[22] The scale has six subscales including awareness, clarity, non-acceptance, strategies, impulse, and goals. Higher scores indicate the existence of difficulty in the regulation of stronger emotions. Turkish adaptation, reliability and validity study of the scale was conducted by Rugancı et al., in 2010.[23] Adolescents completed the DERS forms in this study. Confirmatory Factor Analyses of the DERS was evaluated in Turkish adolescents.[24] This scale has been used in various studies in Turkish adolescents.[25,26]

**The Screen for Anxiety-Related Emotional Disorders (SCARED):** This instrument consists of 41 Likert-type items evaluating symptoms of anxiety over the previous three months and has parent/ caregiver and child versions.[27] The Turkish reliability and validity study was conducted by Çakmakçı, in 2004.[28] Both child and parent's report were used in this study.

**Barratt Impulsiveness Scale-11 (BIS-11):** BIS-11 was developed by Barratt to evaluate motor, attentional and cognitive facets of impulsivity. Elevated scores denote greater impulsivity.[29] Turkish validity and reliability study of BIS-11 was conducted by Güleç et al., in 2008.[30] Adolescents completed BIS-11 in this study. It has been used in various studies on adolescents in Turkey.[31,32]

### *Ethics Approval*

IRB approval was granted by the İzmir Katip Çelebi University Faculty of Medicine Clinical Research local Ethics Board (Date:13.09.2017 Approval Number:193). Written informed

consent of adolescents and their parents were procured prior to study participation and all study procedures were in accordance with the Declaration of Helsinki and local laws and regulations.

### *Sampling size (Power analysis)*

G*Power Version 3.0.10 was used for statistical power analysis.[33] Since there were no similar studies in literature, regarding the difference between two mean values at a moderate level and taking effect size as 0.5 (using cohen criteria), alpha as 0.05 and power as 0.80, the total sample size was determined as 128 adolescents with equal number of obese youth and controls.

### *Statistical analysis*

Statistical analyses were conducted with SPPS 24.0 (IBM Inc., ChicaArmonk, NY, USA) program. Assumptions of normality were evaluated with Kolmogorov-Smirnov test. Quantitative variables were summarized as means and standard deviations. Comparison of multiple dependent variables across groups was conducted with MANOVA (via Pillai's trace) followed with univariate ANOVAs (Bonferroni corrected). DERS, BIS-11 and SCARED domains of adolescents with obesity and control adolescents along with NT levels were compared with MANOVA. Due to non-normal distribution, lack of equality of error variances for several subscales (Levene's test, DERS-nonacceptance p=0.001, DERS-impulsivity p=0.004, DERS-strategy p=0.03, DERS-clarity p<0.001, all BIS-11 subscales, SCARED-child form and neurotensin p<0.001), Pillai's trace was used to evaluate results. Chi-square test was used for the comparison of nominal variables across groups. Spearman's rank order correlation analyses were conducted to evaluate relationships between quantitative variables. Partial correlations were used to control for effects of age, gender and BMI. P was set at 0.05 (two-tailed).

## Result

A total of 65 obese (mean age 14.6 ± 1.4 years, 32 female) and 65 healthy adolescents (control group) (mean age 14.6 ± 1.5 years, 32 females) were included in the study. The groups did not differ significantly in terms of gender and mean age. BMI, BMI- SDS and NT levels of obese youth and the controls are shown in Table I (for each, p<0.001).

DERS, BIS-11 and SCARED scores according to groups is illustrated in Table II. In MANOVA, the effect of diagnosis (F = 52.179, p= 0.000, partial η2= 0.84] was significant while results for univariate ANOVAs are presented in Table III.

For pair-wise comparisons, adolescents with obesity had significantly elevated scores in DERS-clarity (p= 0.000, 95% CI= 1.6-3.5), DERS-goal (p=0.000, 95% CI= 3.5-6.0), DERS-strategy (p=0.000, 95% CI= 6.7-10.7), DERS-impulsivity (p=0.000, 95% CI= 5.5-8.7), DERS-nonacceptance (p=0.000, 95% CI= 2.9-6.2), DERS-awareness (p=0.000, 95% CI= 2.5-4.6), DERS-total score

**Table I.** Demographic characteristics, BMI and neurotensin levels between obese and healthy adolescents.

| | Obese Group *(n=65)* mean± SD | Control Group *(n=65)* mean± SD | Statistics (p value) |
|---|---|---|---|
| Age (years) | 14.6±1.4 | 14.6±1.5 | 0.976 |
| Sex (male/ female) | 32/33 | 32/33 | 1.000 |
| BMI (kg/m$^2$) | 35.5±4.4 | 20.8±2.1 | <0.001 |
| BMI SDS | 3.1±0.6 | -0.02±0.7 | <0.001 |
| Neurotensin (ng/ml) | 0.61±0.39 | 0.40±0.11 | <0.001 |

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

**Table II.** Comparisons of DERS, BIS, SCARED subscale scores of obese and healthy adolescents.

| | Obese Group *(n=65)* mean± SD | Control Group *(n=65)* mean± SD |
|---|---|---|
| DERS subscales | | |
| Clarity | 12.36±2.69 | 9.72±2.67 |
| Goal | 17.24±3.45 | 12.50±3.48 |
| Strategy | 20.81±6.53 | 12.04±4.03 |
| Impulsivity | 19.29±5.18 | 12.23±3.32 |
| Non-acceptance | 15.33±5.17 | 10.80±3.62 |
| Awareness | 16.07±3.51 | 12.40±1.59 |
| Total score | 101.14±16.52 | 69.70±9.27 |
| BIS-11 subscales | | |
| Attentional impulsivity | 19.24±3.75 | 11.86±2.86 |
| Motor impulsivity | 24.52±2.67 | 17.09±5.03 |
| Non planning | 23.40±2.22 | 15.89±4.13 |
| Total Impulsivity | 67.16±5.30 | 44.84±10.30 |
| SCARED scores | | |
| SCARED child | 26.90±10.07 | 6.66±4.53 |
| SCARED parent | 26.37±10.70 | 6.38±5.14 |

DERS: difficulties in emotion regulation scale, SCARED: the screen for anxiety-related emotional disorders, BIS-11: barratt impulsiveness scale-11

**Table III.** Effect of obesity diagnosis on domains of emotion regulation difficulties, impulsivity, neurotensin levels and severity of anxiety symptoms.

| Independent variables | Dependent Variables | Univariate F | dF | P* | Partial η2 |
|---|---|---|---|---|---|
| Obese vs. Control | DERS Clarity | 27.8 | (1,119) | 0.000 | 0.190 |
| | DERS Goal | 54.8 | (1,119) | 0.000 | 0.315 |
| | DERS Strategy | 74.3 | (1,119) | 0.000 | 0.384 |
| | DERS Impulsivity | 76.3 | (1,119) | 0.000 | 0.391 |
| | DERS Nonacceptance | 30.2 | (1,119) | 0.000 | 0.203 |
| | DERS Awareness | 48.8 | (1,119) | 0.000 | 0.291 |
| | DERS Total score | 157.4 | (1,119) | 0.000 | 0.569 |
| | BIS Attentional impulsivity | 143.0 | (1,119) | 0.000 | 0.546 |
| | BIS Motor impulsivitiy | 94.1 | (1,119) | 0.000 | 0.442 |
| | BIS Non planning | 151.6 | (1,119) | 0.000 | 0.560 |
| | BIS Total Impulsivity | 214.2 | (1,119) | 0.000 | 0.643 |
| | Neurotensin | 17.1 | (1,119) | 0.000 | 0.126 |
| | SCARED-Child form | 199.7 | (1,119) | 0.000 | 0.627 |

DERS: difficulties in emotion regulation scale, SCARED: the screen for anxiety-related emotional disorders, BIS-11: barratt impulsiveness scale-11.
*Bonferroni corrected.

(p=0.000, 95% CI= 26.3-36.2), BIS-11-attentional impulsivity (p=0.000, 95% CI= 6.2-8.6), BIS-11-motor impulsivity (p=0.000, 95% CI= 5.7-8.6), BIS-11-non planning (p=0.000, 95% CI= 6.3-8.7), BIS-11-total impulsivity (p=0.000, 95% CI= 19.0-25.0), neurotensin (p=0.000, 95% CI= 0.1-0.3) and SCARED-child form (p=0.000, 95% CI= 17.4,23.0; all Bonferroni corrected).

In obese and control groups, serum NT level was found to be positively correlated with all BIS and DERS subscales and total scores ($p < 0.05$). After adjustment for age, gender, and BMI, the positive correlation among NT and BIS attentional, non-planning and total impulsivity scores, DERS strategy, impulsivity and total scores persisted; however, the relationship between serum NT level and BIS motor impulsivity, DERS goal, clarity, non-acceptance, awareness disappeared (Table IV).

## Discussion

This uni-center, cross-sectional, case-control study evaluated NT levels along with self and parent–reported anxiety, self-reported impulsivity and emotion regulation problems and the relationships among those constructs in adolescents with obesity and age and gender-matched controls. As a result, NT levels were found to be significantly elevated along with impulsivity, anxiety and emotion regulation problems among adolescents with obesity. NT levels correlated significantly with cognitive and attentional impulsivity and impulsivity while trying to regulate emotions after adjusting for BMI, age and gender.

Various pre-clinical and clinical studies suggest an important role for NT in emergence of obesity.[10,12,34] Butler and colleagues reported that plasma NT levels were elevated among children with Prader-Willi syndrome characterized by hyperphagia[14] and obesity while Barchetta and colleagues (2020) reported that plasma pro-NT levels may predict weight gain and associated metabolic abnormalities among children.[13] A previous study by the same group suggested that NT may also be a biomarker of insülin-resistance and problems in metabolism.[35] Our results support those reported previously and suggest that elevations in NT levels may be associated with adolescent obesity. However, as NT levels were also elevated among children

**Table IV.** Correlations of serum NT levels (ng/mL) with self-reported impulsivity, self- and parent- reported anxiety, self-reported emotion regulation and anthropometric parameters.

| | All subjects *(n=130)* | | All subjects *(n=130)* | |
|---|---|---|---|---|
| | *Spearman's Rho* | **p* | *Partial Correlation* | ***p* |
| Age (years) | -0.088 | 0.322 | | |
| Gender | 0.041 | 0.643 | | |
| BMI (kg/m$^2$) | 0.293 | *0.001* | | |
| BIS Attentional impulsivity | 0.523 | *<0.001* | 0.472 | *<0.001* |
| BIS Motor impulsivity | 0.303 | *<0.001* | 0.142 | 0.128 |
| BIS Non planning | 0.387 | *<0.001* | 0.262 | 0.004 |
| BIS Total Impulsivity | 0.447 | *<0.001* | 0.346 | *<0.001* |
| DERS Clarity | 0.178 | *0.043* | 0.050 | 0.590 |
| DERS Goal | 0.308 | *<0.001* | 0.172 | 0.064 |
| DERS Strategy | 0.344 | *<0.001* | 0.202 | *0.029* |
| DERS Impulsivity | 0.375 | *<0.001* | 0.249 | *0.007* |
| DERS Non-acceptance | 0.286 | *0.001* | 0.163 | 0.079 |
| DERS Awareness | 0.241 | *0.006* | 0.097 | 0.300 |
| DERS Total score | 0.413 | *<0.001* | 0.286 | *0.002* |
| SCARED Child score | 0.171 | 0.061 | -0.080 | 0.390 |
| SCARED Parent score | 0.105 | 0.074 | -0.163 | 0.078 |

DERS: difficulties in emotion regulation scale, SCARED: the screen for anxiety-related emotional disorders, BIS-11: barratt impulsiveness scale-11

*Spearman's correlation analysis; Serum NT level as dependent variable

** Partial correlation coefficient; controlling for age, gender and BMI.

with coeliac disease and among patients with disrupted renal functioning[36,37], elevations in NT may be due to increased gastro-intestinal permeability, low grade inflammation or changes in renal function, rather than obesity *per se*. Further studies on obese adolescents may also evaluate renal functioning and inflammatory markers or use gastro-intestinal endoscopy along with NT levels to elucidate the contribution of those factors.

Previous studies suggest that pediatric obesity at least in a subgroup of patients may be associated with elevated levels of anxiety, impulsivity, emotion regulation problems and emotional eating.[15-17] Supporting those views, Sezer Efe and colleagues found that social anxiety and emotional eating were elevated and displayed positive correlations among obese adolescents.[38] Yilmaz Kafali and colleagues found that although emotion regulation problems were elevated among obese adolescents, unhealthy life-style practices such as internet addiction and emotional eating mediated the effects of those problems on obesity.[39] Sönmez and colleagues found that inattention, hyperactivity and impulsivity symptoms were elevated among obese children and adolescents.[40] A study employing ecological momentary assessment suggested that impulsivity may contribute to dysregulated eating among over-weight and obese youth.[41] The results of our study also support those reported previously and suggest that emotion regulation problems, impulsivity and self- and parent-reported anxiety were significantly elevated among obese adolescents. Due to a lack of evaluating life-style practices and emotional eating patterns among our sample and due to the cross-sectional design of our study we could not offer hypotheses on causality and mediation. Further studies may employ larger samples and prospective designs to evaluate the differential contributions of those constructs to the emergence and persistence of pediatric obesity.

Pre-clinical studies suggest that NT may have a role in regulation of stress and anxiety, hedonic eating, reward/ reinforcement and memory.[8-11] Furthermore, recent studies suggest that NT may be involved in cognitive changes associated with obesity.[42,43] Despite those promising studies, no study up to now evaluated the relationships between NT levels and anxiety, impulsivity and emotional dysregulation among obese adolescents. We found that NT levels correlated significantly with emotion dysregulation and all facets of impulsivity. NT levels correlated significantly with cognitive and attentional impulsivity and impulsivity while trying to regulate emotions after adjusting for BMI, age and gender. The correlations between serum NT level and BIS motor impulsivity, DERS goal, clarity, non-acceptance, awareness disappeared after adjusting for age, gender and BMI, while others remained unchanged. Although the cross-sectional nature of our study precludes hypotheses about causality, those findings may suggest a role of BMI, age and gender in NT levels. Also, effects of NT on motor impulsivity, DERS-goal, clarity, non-acceptance and awareness may partially overlap with emotional eating and binging. Our results may support a role of NT in emotional and cognitive symptoms associated with obesity which may be primarily due to impulsivity. Previous reports on association of impulsivity with changes in dopaminergic functioning and inhibition of D2R signaling by neurotensin may also support this hypothesis.[44,45] However, this hypothesis should be evaluated with further pre-clinical and clinical studies evaluating the role of NT and its precursors in impulsivity and obesity.

Our results should be evaluated within their limitations. Firstly, the precursor of NT, pro-NT is more stable and has a longer half-life than NT and our results may be further enriched had we measured this precursor along with NT. Secondly; our results are valid only for adolescents evaluated within the specified time-frame at the study centers who were free of endocrine and genetic etiologies and they may not be generalized to other patients or obese adolescents in the community. Thirdly, NT levels may interact with growth

hormone levels and may change during puberty and our results sohuld be replicated with obese children and adults.[46] Fourth, we did not evaluate the role of NT in emotional eating and further studies on the role of NT in pediatric obesity may use age-appropriate questionnaires (e.g. Child-Three Factor Eating Questionnaire) to evaluate it.[47] Fifth, NT levels may also depend on renal function, integrity of the gastro-intestinal mucosa and low grade inflammation[36,37] and further studies are needed to evaluate the contributions of those factors to elevations of NT in pediatric obesity. Sixth, we did not evaluate for the effects of exercise, diet and binge eating.[48,49] Seventh; there may be distinct patterns of pediatric obesity according to change in BMI through development and the relative contribution of NT may differ across those groups.[50] Eighth, while determining the exclusion criteria, some psychiatric disorders were diagnosed according to DSM-IV (with help of K-DSADS-PL) and some disorders (e.g., neurodevelopmental disorders) according to DSM-5 criteria. Lastly, although the DERS and BIS-11 scales are used in many studies in adolescents, there is no validity and reliability study in the adolescent age group. Longitudinal studies may illustrate the role of NT in developmental subgroups of pediatric obesity.

Regardless of its limitations, our results suggest that circulating NT levels may be elevated among obese adolescents along with anxiety, impulsivity and emotion dysregulation. Also, NT levels may correlate significantly with various facets of impulsivity.

Overall, NT signaling could be an important target for pharmacotherapeutic interventions for psychiatric problems in obesity.

## Ethical approval

IRB approval was granted by the İzmir Katip Çelebi University Faculty of Medicine Clinical Research Local Ethics Board (Date:13.09.2017 Approval Number:193).

## Author contribution

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

## Source of funding

This work was supported by the scientific research project comittee of İzmir Katip Çelebi Universityby grant number 2017-TDU-TIPF-0037.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, et al. Health effects of overweight and obesity in 195 countries over 25 years. N Engl J Med 2017; 377: 13-27. https://doi.org/10.1056/NEJMoa1614362
2. Kansra AR, Lakkunarajah S, Jay MS. Childhood and adolescent obesity: a review. Front Pediatr 2021; 8: 581461. https://doi.org/10.3389/fped.2020.581461
3. Qasim A, Turcotte M, de Souza RJ, et al. On the origin of obesity: identifying the biological, environmental and cultural drivers of genetic risk among human populations. Obes Rev 2018; 19: 121-149. https://doi.org/10.1111/obr.12625
4. Tester JM, Rosas LG, Leung CW. Food insecurity and pediatric obesity: a double whammy in the era of COVID-19. Curr Obes Rep 2020; 9: 442-450. https://doi.org/10.1007/s13679-020-00413-x
5. Boules M, Li Z, Smith K, Fredrickson P, Richelson E. Diverse roles of neurotensin agonists in the central nervous system. Front Endocrinol (Lausanne) 2013; 4: 36. https://doi.org/10.3389/fendo.2013.00036
6. Opland D, Sutton A, Woodworth H, et al. Loss of neurotensin receptor-1 disrupts the control of the mesolimbic dopamine system by leptin and promotes hedonic feeding and obesity. Mol Metab 2013; 2: 423-434. https://doi.org/10.1016/j.molmet.2013.07.008

7. Ferraro L, Tomasini MC, Mazza R, et al. Neurotensin receptors as modulators of glutamatergic transmission. Brain Res Rev 2008; 58: 365-373. https://doi.org/10.1016/j.brainresrev.2007.11.001

8. Azevedo EP, Tan B, Pomeranz LE, et al. A limbic circuit selectively links active escape to food suppression. Elife 2020; 9: e58894. https://doi.org/10.7554/eLife.58894

9. Li B, Chang LL, Xi K. Neurotensin 1 receptor in the prelimbic cortex regulates anxiety-like behavior in rats. Prog Neuropsychopharmacol Biol Psychiatry 2021; 104: 110011. https://doi.org/10.1016/j.pnpbp.2020.110011

10. Torruella-Suárez ML, Vandenberg JR, Cogan ES, et al. Manipulations of central amygdala neurotensin neurons alter the consumption of ethanol and sweet fluids in mice. J Neurosci 2020; 40: 632-647. https://doi.org/10.1523/JNEUROSCI.1466-19.2019

11. Lénárd L, László K, Kertes E, et al. Substance P and neurotensin in the limbic system: Their roles in reinforcement and memory consolidation. Neurosci Biobehav Rev 2018; 85: 1-20. https://doi.org/10.1016/j.neubiorev.2017.09.003

12. Kan Yeung AW, Georgieva MG, Kirilov K, et al. Neurotensins and their therapeutic potential: research field study. Future Med Chem 2020; 12: 1779-1803. https://doi.org/10.4155/fmc-2020-0124

13. Barchetta I, Bertoccini L, Sentinelli F, et al. Circulating pro-neurotensin levels predict bodyweight gain and metabolic alterations in children. Nutr Metab Cardiovasc Dis 2021; 31: 902-910. https://doi.org/10.1016/j.numecd.2020.11.025

14. Butler MG, Nelson TA, Driscoll DJ, Manzardo AM. High plasma neurotensin levels in children with Prader-Willi syndrome. Am J Med Genet A 2015; 167: 1773-1778. https://doi.org/10.1002/ajmg.a.37103

15. Kang NR, Kwack YS. An update on mental health problems and cognitive behavioral therapy in pediatric obesity. Pediatr Gastroenterol Hepatol Nutr 2020; 23: 15-25. https://doi.org/10.5223/pghn.2020.23.1.15

16. O'Hara VM, Curran JL, Browne NT. The co-occurrence of pediatric obesity and ADHD: an understanding of shared pathophysiology and implications for collaborative management. Curr Obes Rep 2020; 9: 451-461. https://doi.org/10.1007/s13679-020-00410-0

17. Hemmingsson E. Early childhood obesity risk factors: socioeconomic adversity, family dysfunction, offspring distress, and junk food self-medication. Curr Obes Rep 2018; 7: 204-209. https://doi.org/10.1007/s13679-018-0310-2

18. WHO obesity criteria. 2021. Available at: https://www.who.int/toolkits/growth-reference-data-for-5to19-years (Accessed on February 11, 2021).

19. Neyzi O, Bundak R, Gokcay 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

20. Kaufman J, Birmaher B, Brent D, et al. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 1997; 36: 980-988. https://doi.org/10.1097/00004583-199707000-00021

21. Gökler B, Ünal F, Pehlivantürk B, Kültür EÇ, Akdemir D, Taner Y. Reliability and validity of schedule for affective disorders and schizophreni a for school age children-present and lifetime version-Turkish Version (K-SADS-PL-T). Turk J Child Adolesc Ment Health 2004; 11: 109-116.

22. Gratz KL, Roemer L. Multidimensional assessment of emotion regulation and dysregulation: Development, factor structure, and initial validation of the difficulties in emotion regulation scale. J Psychopathol Behav Assess 2004; 26: 41-54. https://doi.org/10.1023/B:JOBA.0000007455.08539.94

23. Rugancı RN, Gençöz T. Psychometric properties of a Turkish version of the Difficulties in Emotion Regulation Scale. J Clin Psychol 2010; 66: 442-455. https://doi.org/10.1002/jclp.20665

24. Sarıtaş-Atalar D, Gençöz T, Özen A. Confirmatory factor analyses of the Difficulties in Emotion Regulation Scale (DERS) in a Turkish adolescent sample. Eur J Psychol Assess 2015; 31: 12-19. https://doi.org/10.1027/1015-5759/a000199

25. Yazici KU, Yazici IP. Decreased theory of mind skills, increased emotion dysregulation and insight levels in adolescents diagnosed with obsessive compulsive disorder. Nord J Psychiatry 2019; 73: 462-469. https://doi.org/10.1080/08039488.2019.1652341

26. Percinel I, Ozbaran B, Kose S, et al. Increased deficits in emotion recognition and regulation in children and adolescents with exogenous obesity. World J Biol Psychiatry 2018; 19: 112-118. https://doi.org/10.1080/15622975.2016.1265147

27. Birmaher B, Khetarpal S, Brent D, et al. The Screen for Child Anxiety Related Emotional Disorders (SCARED): scale construction and psychometric characteristics. J Am Acad Child Adolesc Psychiatry 1997; 36: 545-553. https://doi.org/10.1097/00004583-199704000-00018

28. Çakmakçı FK. Validity and reliability study of the Screen for Child Anxiety Related Disorders. Turk J Child Adolesc Ment Health 2004; 11: 2.

29. Barratt ES. Anxiety and impulsiveness related to psychomotor efficiency. Percept Mot Skills 1959; 9: 191-198. https://doi.org/10.2466/pms.1959.9.3.191

30. Güleç H, Tamam L, Yazici M, et al. Psychometric properties of the Turkish Version of the Barratt Impulsiveness Scale-11. J Clin Psychopharmacol 2008; 18: 251-258.

31. Akın E, Berkem M. İntihar girişiminde bulunan ergenlerde öfke ve dürtüsellik. Marmara Medical Journal 2012; 25: 148-52.

32. Çakmak S, Gedikli H, Demirkol ME, et al. Effects of parental divorcement on impulsivity in adolescence. Klinik Psikiyatri 2018; 21: 137-147. https://doi.org/10.5505/kpd.2018.44127

33. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007; 39: 175-191. https://doi.org/10.3758/BF03193146

34. Li J, Song J, Zaytseva YY, et al. An obligatory role for neurotensin in high-fat-diet-induced obesity. Nature 2016; 533: 411. https://doi.org/10.1038/nature17662

35. Barchetta I, Cimini FA, Capoccia D, et al. Neurotensin is a lipid-induced gastrointestinal peptide associated with visceral adipose tissue inflammation in obesity. Nutrients 2018; 10: 526. https://doi.org/10.3390/nu10040526

36. Iorfida D, Montuori M, Trovato CM, Tiberti C, Sansone A, Cucchiara S. Fasting neurotensin levels in pediatric celiac disease compared with a control cohort. Gastroenterol Res Pract 2020; 2020: 1670479. https://doi.org/10.1155/2020/1670479

37. Tönjes A, Hoffmann A, Kralisch S, et al. Pro-neurotensin depends on renal function and is related to all-cause mortality in chronic kidney disease. Eur J Endocrinol 2020; 183: 233-244. https://doi.org/10.1530/EJE-20-0087

38. Efe YS, Özbey H, Erdem E, Hatipoğlu N. A comparison of emotional eating, social anxiety and parental attitude among adolescents with obesity and healthy: a case-control study. Arch Psychiatr Nurs 2020; 34: 557-562. https://doi.org/10.1016/j.apnu.2020.09.007

39. Yilmaz Kafali H, Uçaktürk SA, Mengen E, Akpinar S, Erguven Demirtas M, Uneri OS. Emotion dysregulation and pediatric obesity: investigating the role of Internet addiction and eating behaviors on this relationship in an adolescent sample. Eat Weight Disord 2021; 26: 1767-1779. https://doi.org/10.1007/s40519-020-00999-0

40. Sönmez AÖ, Yavuz BG, Aka S, Semiz S. Attention-deficit hyperactivity disorder symptoms and conduct problems in children and adolescents with obesity. Sisli Etfal Hastan Tip Bul 2019; 53: 300-305. https://doi.org/10.14744/semb.2019.09475

41. Goldschmidt AB, Smith KE, Lavender JM, Engel SG, Haedt-Matt A. Trait-level facets of impulsivity and momentary, naturalistic eating behavior in children and adolescents with overweight/obesity. J Psychiatr Res 2019; 110: 24-30. https://doi.org/10.1016/j.jpsychires.2018.12.018

42. Fazzari G, Zizza M, Di Vito A, et al. Reduced learning and memory performances in high-fat treated hamsters related to brain neurotensin receptor1 expression variations. Behav Brain Res 2018; 347: 227-233. https://doi.org/10.1016/j.bbr.2018.03.015

43. Saiyasit N, Sripetchwandee J, Chattipakorn N, Chattipakorn SC. Potential roles of neurotensin on cognition in conditions of obese-insulin resistance. Neuropeptides 2018; 72: 12-22. https://doi.org/10.1016/j.npep.2018.09.002

44. Binder EB, Kinkead B, Owens MJ, Nemeroff CB. Neurotensin and dopamine interactions. Pharmacol Rev 2001; 53: 453-486.

45. Mole TB, Irvine MA, Worbe Y, Collins P, Mitchell SP, Bolton S. Impulsivity in disorders of food and drug misuse. Psychol Med 2014; 45: 771-782. https://doi.org/10.1017/S0033291714001834

46. Bozzola M, Thome AN, Giraldi E, Lhiaubet AM, Schimpff RM. Plasma neurotensin levels in prepubertal children and adults: possible involvement in the regulation of growth hormone secretion. J Pediatr Endocrinol Metab 1998; 11: 615-621. https://doi.org/10.1515/JPEM.1998.11.5.615

47. Demir D, Bektaş M, Bektaş İ, Demir Ş, Bryant EJ. Psychometric properties of the Turkish version of the Child Three-Factor Eating Questionnaire for primary and secondary school students. Public Health Nutr 2021; 24: 427-435. https://doi.org/10.1017/S1368980020001767

48. Bulbul S. Exercise in the treatment of childhood obesity. Turk Pediatri Ars 2020; 55: 2-10. https://doi.org/10.14744/TurkPediatriArs.2019.60430

49. Turan S, Sarioglu FC, Erbas IM, et al. Altered regional grey matter volume and appetite-related hormone levels in adolescent obesity with or without binge-eating disorder. Eat Weight Disord 2021; 26: 2555-2562.

50. O'Connor TG, Williams J, Blair C, Gatzke-Kopp LM, Francis L, Willoughby MT. Predictors of developmental patterns of obesity in young children. Front Pediatr 2020; 8: 109. https://doi.org/10.3389/fped.2020.00109

The Turkish Journal of Pediatrics 2022; 64: 265-273
https://doi.org/10.24953/turkjped.2020.2371

**Original Article**

# Predictors of febrile urinary tract infection caused by extended-spectrum beta-lactamase-producing bacteria

Eren Soyaltın[1], Gökçen Erfidan[1], Mustafa Kavruk[2], Seçil Arslansoyu Çamlar[1], Nisel Yılmaz[3], Demet Alaygut[1], Fatma Mutlubaş[1], Belde Kasap Demir[1,4]

*[1]Division of Pediatric Nephrology, Departments of [2]Pediatrics and [3]Clinical Microbiology, University of Health Sciences, İzmir Tepecik Training and Research Hospital İzmir; [4]Department of Pediatric Nephrology and Rheumatology, İzmir Katip Çelebi University Medical Faculty, İzmir, Turkey.*

## ABSTRACT

**Background.** We aimed to evaluate the predictability of extended-spectrum beta-lactamase (ESBL)-producing bacteria (PB) with inflammation markers and hemogram parameters as neutrophil-lymphocyte-ratio (NLR), platelets-lymphocyte-ratio (PLR) and mean-platelet-volume (MPV) in infants with febrile urinary tract infection until the urine cultures are resulted.

**Methods.** Infants between 2-24 months hospitalized for the first febrile urinary tract infections were grouped as those infected with ESBL-PB and non-ESBL-PB. The demographic and laboratory data (inflammation markers and hemogram parameters) and the ultrasonographical findings were compared between the two groups.

**Results.** A total of 232 patients were included in the study. The mean age was 8.82 ± 5.68 (2-23) months and 114 (49%) of them were female. *Escherichia coli* was the most common isolated bacteria (79%) followed by *Klebsiella pneumoniae* (15.5%) in urine cultures. There were 88 patients in ESBL-PB infected group and 144 patients in the non-ESBL-PB group. The hematologic parameters such as white blood cell count (WBC) count, NLR, PLR, MPV and procalcitonin (PCT) were similar between the two groups. Only the rate of ultrasonographic abnormalities was significantly higher in infants infected with ESBL-PB (p=0.012). The risk of ESBL-PB positivity in urine cultures increased with age (OR 1.068, 95% CI 1.002-1.139, p=0.045), PCT (OR 1.094, 95% CI 1.011-1.184, p=0.025), and ultrasonographic abnormalities (OR 3.981, 95% CI 1.792-8.845, p=0.001).

**Conclusions.** Platelet counts, WBC, MPV, NLR, PLR, and PCT were not reliable markers, however having an ultrasonographic abnormality is the most important independent risk factor for prediction of infection with ESBL-PB.

**Key words:** urinary tract infection, extended-spectrum beta-lactamase-producing bacteria, platelet-to-lymphocyte ratio , neutrophil-to-lymphocyte ratio.

Urinary tract infections (UTI) have been noted as the most common bacterial infections among young children.[1] If UTI is not diagnosed and treated correctly and adequately in children, it can lead to complications such as chronic kidney disease, hypertension, and even end-stage renal failure due to scar formation in kidneys.[1-3] It has been reported that 91% of bacteria causing UTIs in children are Gram-negative bacteria.[4] According to recent reports, *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* were isolated among the most resistant bacteria in children with UTIs.[5] Especially in the past 10 years, *E. coli* has increased dramatically worldwide as the cause of UTIs.[6] The production of β-lactamase is the main mechanism of resistance against the action of β-lactam antibiotics in Gram-negative bacteria.[7] Besides, extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria also have resistance to trimethoprim/sulfamethoxazole, fluoroquinolones, and aminoglycosides. This

✉ Eren Soyaltın
erensoyaltin@hotmail.com

Received 10th August 2020, revised 9th January 2021, 18th April 2021, accepted 8th October 2021.

makes infection management caused by these pathogens even more complicated.[8,9] The increased resistance to antimicrobial agents results in inappropriate empirical therapy.[9] To avoid this, it is very important to predict the bacteria until urine culture results in UTIs caused by ESBL producing bacteria (ESBL-PB).[10] However, there are a limited number of studies on the presence of a rapid marker in UTIs caused by ESBL-PB.

Recent studies have reported the predictors of upper UTIs, which include systemic inflammatory markers. A significant high C-reactive protein (CRP) has been reported in patients with UTIs caused by *E. coli, Proteus spp., K .pneumoniae, Staphylococcus aureus,* and others, than those without UTI.[11-13] White blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), and procalcitonin (PCT) were all reported to be correlated with upper UTI as well as CRP.[14] However, it has not been stated which marker is predictive for ESBL-PB.

The NLR and platelet-to-lymphocyte ratio (PLR) have been proposed as new markers of systemic inflammation.[15] Several studies reported that NLR is a measure of systemic inflammation and it has been used as a guide in the prognosis of bacterial diseases, ischemic heart disease, and several types of cancer.[16] Besides NLR and PLR; the mean platelet volume (MPV) has been evaluated as a biochemical marker in chronic and/or acute inflammatory disorders, including bacterial and rheumatologic diseases.[17,18]

In this study, we aimed to evaluate the predictability of ESBL-PB with inflammation markers including WBC, CRP, PCT, NLR, PLR, and MPV in infants with febrile UTI until the urine culture and antibiogram results are reported. To the best of our knowledge, this is the first study evaluating these associations.

## Material and Methods

Patients aged between 2-24 months who were admitted to our pediatrics polyclinic and pediatric emergency unit with fever, diagnosed and hospitalized for the first febrile UTI between 2016 - 2020 were retrospectively analyzed. The diagnosis of febrile UTI was based on the following criteria: 1) body temperature ≥38°C; 2) a positive urine culture collected by catheterization (bacterial growth of ≥100,000 cfu/ml of a single uropathogen). The indications for hospitalization were; age <3 months; having toxic or septic state; having symptoms of urinary obstruction or significant underlying disease and inability to tolerate adequate oral fluids or medications. The families were asked whether the patients had any medical history of urinary system anomalies. Patients with no ultrasonographic imaging in their medical history and those who had ultrasonographic imaging with normal urinary system findings were recorded as patients without a pathological urinary system history. The demographical data of the patients including age, gender, personal medical history about renal pathologies [hydronephrosis (urinary distention of the renal pelvi-calyceal system with/without obstruction to the urinary outflow distal to the renal pelvis), vesicoureteral reflux (VUR), ureteropelvic or ureterovesical obstructions or stenosis, neurogenic bladder, renal hypo-dysplasia, and posterior urethral valve (PUV)], urine dipstick analysis including pyuria (presence of ≥5 white blood cells/hpf in centrifuged urine sample) and nitrite positivity, complete blood count (CBC) including white blood cell (WBC), platelet, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), mean platelets volume (MPV), C-reactive protein (CRP), procalcitonin (PCT) and the ultrasonographical findings at the admission to hospital due to UTI were recorded.

Culture plates were incubated in incubators at a mean ambient temperature of 35±2°C, and under normal atmospheric conditions for 18-24 hours; culture plates with bacterial growth of $\geq 10^5$ cfu/ml of a single species on their surfaces were examined. Cultures with mixed growths were excluded. The isolates obtained were identified at a species level using conventional methods,

and fully automated bacterial identification system (Phoenix TM 100, Becton Dickinson, MD, USA). Antimicrobial susceptibilities of isolates were determined using Kirby-Bauer disc diffusion system and fully automated systems in compliance with the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST).[19] The presence of extended-spectrum beta-lactamase (ESBL) was determined using ceftazidime, ceftazidime-clavulanic acid, and cefotaxime-cefotaxime clavulanic acid discs. As a control ATCC 25923 strain of *E. coli* was used.

Patients were grouped as those infected with ESBL-PB and those with non-ESBL-PB. Urine analysis results (pyuria, nitrite positivity), NLR, PLR, MPV, CRP, PCT, and the ultrasonographical findings were compared between the two groups.

The study has been approved by the Ethics Committee of Tepecik Training and Research Hospital (no: 2020/5-8).

### *Statistical analysis*

Analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used to evaluate the normal distribution of continuous variables between groups. Continuous parameters with normal distribution were compared by the Student's t-test and shown in mean ± standard deviation, whereas those without normal distribution were evaluated by Mann-Whitney U test and defined as median (25th – 75th percentile). Categorical variables between groups were compared using the Chi-square test. We also performed a multivariate statistical analysis of factors related to the ESBL-PB positivity in urine cultures by using a logistic regression model and Odds ratios were evaluated after adjustment for other factors. A p value of <0.05 was considered significant in all statistical evaluations.

## Results

A total of 232 patients were included in the study. The demographic data of the patients including age, gender, personal medical history about renal pathologies, pyuria and nitrite positivity, renal ultrasonography findings during febrile UTI are presented in Table I. While 57 cases had a known urinary tract abnormality, there was no defined urinary system pathology in the remaining 175 cases (Table I). The underlying bacterial pathogens are shown in Table I, with *E. coli* being the most common (79%) followed by *K. pneumoniae* with a 15.5% isolation rate. Among patients, 88 (38%) had ESBL-PB and 144 (62%) had non-ESBL-PB yielded in urine cultures (Table I).

When demographic and laboratory findings of patients were compared between the ESBL-PB and non-ESBL-PB groups, none of the demographic findings, urine, or blood parameters were significantly different between the groups (p>0.05). The hematologic parameters such as WBC count, NLR, PLR, MPV, and CRP were similar between the two groups. Only the rate of patients with renal ultrasonographic abnormalities during hospitalization for febrile UTI regardless of their medical history; was significantly higher in infants infected with ESBL-PB (p=0.012, Table II).

Age, PCT levels, and presence of ultrasonographic abnormalities were adopted as confounders in the logistic regression model for the multivariate analysis (Table III). All three variables were found to be independently associated with ESBL-PB positivity in urine cultures of infants with febrile UTI (Table III).

## Discussion

Febrile UTI is one of the most common bacterial infections in children and usually causes irreversible renal damage when not diagnosed and treated early. Timely medication and

**Table I.** Demographical, clinical and microbiological data of patients with febrile urinary tract infection (N=232).

| | Results |
|---|---|
| Age (month), mean ± SD (minimum-maximum) | 8.82 ± 5.68 (2-23) |
| Gender, n (%) | |
| Female | 114 (49.1) |
| Male | 118 (50.9) |
| History of renal pathology, n (%) | |
| None | 175 (75.4) |
| Hydronephrosis | 19 (8.2) |
| Vesicoureteral reflux | 14 (6.0) |
| Posterior ureteral valve | 7 (3.0) |
| Renal stone | 7 (3.0) |
| Renal hypoplasia | 3 (1.3) |
| Neurogenic bladder | 3 (1.3) |
| Ureteropelvic obstruction | 3 (1.3) |
| Ureterovesical obstruction | 1 (0,4) |
| Pyuria | 211 (90.9) |
| Nitrite positivity | 91 (39.1) |
| Bacteria | |
| *Escherichia coli*, n (%) | 184(78.9) |
| ESBL (+) | 69 (37.5) |
| ESBL (-) | 115 (62.5) |
| *Klebsiella pneumoniae*, n (%) | 36 (15.5) |
| ESBL (+) | 19 (52.7) |
| ESBL (-) | 17 (47.3) |
| Other ESBL (-) bacteria, n (%) | |
| *Pseudomonas aeruginosa* | 8 (3.4) |
| *Proteus mirabilis* | 2 (0.9) |
| *Enterococcus cloacae* | 1 (0.4) |
| *Citrobacter koseri* | 1 (0.4) |
| Ultrasonography findings during UTI, n (%) | |
| Normal | 161 (69.0) |
| Hydronephrosis | 57 (24.6) |
| Renal stone | 8 (3.4) |
| Renal hypoplasia | 4 (1.7) |
| Renal cysts | 2 (0.9) |

ESBL: extended-spectrum beta-lactamase, UTI: urinary tract infection

adequate antibiotic duration can considerably improve the disease outcome.[1,2] In the current study, we evaluated characteristics and inflammation markers of 232 children admitted with febrile UTI aged between 2-24 months and their association with ESBL-PB. We found that having ultrasonographic urinary system abnormalities was the most significant independent risk factor for ESBL-PB associated UTI, increasing the risk up to almost 4-fold. This result was in accordance with some recent studies, in which UTIs with ESBL-PB were found to be significantly higher in patients with urinary system anomalies in USG.[10,20,21]

**Table II.** Comparison of demographic and laboratory data of infants infected with ESBL-PB (extended-spectrum beta-lactamase producing bacteria) and non-ESBL-PB.

| Variables | Patients infected with ESBL-PB (n=88) | Patients infected with non-ESBL-PB (n=144) | p |
|---|---|---|---|
| Age (month) | 9.88 (4-15) | 7 (4-10) | 0.056 |
| Gender (female), n (%) | 43 (49) | 71 (49) | 1.000 |
| Urological abnormality in medical history, n (%) | 41 (47) | 51 (35) | 0.160 |
| Pyuria, n (%) | 85 (96) | 127 (88) | 0.102 |
| Nitrite positivity, n (%) | 35 (39) | 8 (40) | 0.996 |
| White blood cell (/mm$^3$) | 13,900 (10,400-19,675) | 14,700 (10,375-19,250) | 0.463 |
| C-reactive protein (mg/dl) | 33.60 (10-89.25) | 30.50 (9.83-95.75) | 0.893 |
| Procalcitonin (ng/ml) | 0.39 (0.18-4.47) | 0.16 (0.11-1.03) | 0.078 |
| NLR (neutrophil-to-lymphocyte ratio) | 1.68 (0.77-2.54) | 1.41 (0.76-2.59) | 0.603 |
| PLR (platelets-to-lymphocyte ratio) | 79.44 (60.38-126.62) | 77.75 (53.08-115.6) | 0.564 |
| Mean platelet volume (μm$^3$) | 7.8 (7.3-8.4) | 7.6 (7.13-8.3) | 0.252 |
| Platelets count (× 10$^3$/ml) | 383 (315-458) | 380 (297-471) | 0.733 |
| Renal ultrasonographic abnormality, n (%) | 44 (50) | 46 (32) | 0.012 |

Continuous variables are presented as median (25th – 75th percentile).

**Table III.** Results of multivariate analysis of variables related to extended-spectrum beta-lactamase producing bacteria positivity in urine culture.

| Variables | Unit | Odds ratio | 95% CI | p |
|---|---|---|---|---|
| Age | 1 month | 1.068 | 1.002-1.139 | 0.045 |
| Procalcitonin | 1 ng/ml | 1.094 | 1.011-1.184 | 0.025 |
| Ultrasonographic abnormality | Yes | 3.981 | 1.792-8.845 | 0.001 |

In many studies, inflammation markers such as WBC, CRP, PCT, NLR, PLR were evaluated in patients with UTIs. These markers were used to differentiate lower UTIs and acute pyelonephritis and to predict renal complications with non-invasive and widely used biomarkers rather than invasive screening methods such as dimercaptosuccinic acid scintigraphy (DMSA) or voiding cystourethrography in most of the studies.[14,22-24] In our study, we evaluated these inflammation markers to predict infections with ESBL-PB, which have been known to have a higher risk for renal complications, to initiate the appropriate treatment, until the urine culture results are out.

Some recent studies have reported that platelets have an important role in the pathogenesis of inflammatory diseases and they can be referred to as an indicator of UTIs.[25] Not only platelet count, but also MPV has been reported as a useful index in the diagnosis of inflammatory diseases, tuberculosis, acute pyelonephritis, and UTIs in some studies.[26-29] MPV has also been reported at high levels in UTIs caused by Gram-positive bacteria.[25,30] Conversely, we have found similar MPV values between the two groups.

The NLR is a substitute marker of inflammation and it has been reported to be useful for the discrimination of systemic bacterial infection and also to predict the outcomes in studies.[31-34] In this study, we aimed to present the NLR as a practical biomarker to predict ESBL-PB in febrile UTI. Han et al.[31] evaluated NLR to predict acute pyelonephritis and they found a significant correlation between elevated NLR and DMSA defect of acute pyelonephritis. Similar results were detected in the study by Lee et al.[35] about the prediction of renal cortical

defect and scar using NLR in children. Gökhan et al.[36] also reported that NLR was significantly higher in patients with a UTI compared to healthy subjects. Contrary to these studies, Kazımoğlu et al.[37] reported that there was no significant difference in NLR between infected and non-infected kidney transplant patients. But they found a significant difference in the NLR ratio between the patients infected with *E. coli* and others.[37] There is no previous study evaluating NLR in patients infected with ESBL-PB and non-ESBL-PB, and in our study, we could not define any difference in NLR levels between the groups.

Platelets to lymphocyte ratio is another inflammation marker and it is used to predict disease activity, prognosis, and survival rates in systemic inflammatory diseases as rheumatologic diseases, cancers, and bacterial and bloodstream infections.[38] The PLR is a new hematological marker related to systemic inflammation result syndrome. It has been reported as a predictor of severe infections, malignity, coronary artery disease, and inflammatory rheumatic diseases in large cohort studies.[39-42] However, PLR has not been reported as predictive as NLR in some studies.[43-45] Similar to those studies, we could not detect differences in PLR between ESBL-PB infected and non-ESBL-PB infected patients.

Many recent studies have reported that inflammation markers could not constantly distinguish renal damage.[24,46,47] Gervaix et al.46 and Smolkin et al.[47] reported that CRP was not sufficient as a predictive marker, with a sensitivity of 83% and 100%, respectively, but a specificity of 18.5%. Shaikh et al.[48] reported a comprehensive review of 24 studies about the usefulness of CRP, ESR, and PCT for renal damage in UTI and they found that none of the tests were accurate enough to allow for detecting renal scarring. They have reported the sensitivity of CRP (cut-off 20 mg/L) and PCT in predicting pyelonephritis is high (94% and 86%, respectively), but specificity varies (39% and 74%, respectively).[48] In contrast, Yi Han et al.[31] reported high CRP levels correlated with renal scarring on a positive DMSA scan. Mushi et al.[3] evaluated CRP levels to predict Gram-negative bacterial UTI and patients with Gram-negative bacterial infections had 3.54 times higher Odds of having positive CRP values. Additionally, many studies have reported high CRP values in patients with UTIs caused by Gram-negative bacteria.[49-51] In our study, we compared CRP values between patients with UTIs caused by ESBL-PB and non-ESBL-PB and we could not find any significant differences between the two groups (p=0.893).

PCT has developed as a favorable marker for diagnosing bacterial infections because of its higher levels in patients infected with bacteria than viruses or non-specific inflammatory diseases. In recent studies, increased levels of PCT have been reported in patients infected with Gram-negative bacteria which are explained by the fact that these pathogens produce exotoxins and cause more inflammation.[52-55] Moreover, Watanabe et al. reported that ESBL-BP positive cases presented higher levels of PCT than cases infected with non-ESBL-PB and they specified that PCT might be a useful marker for detecting patients infected with ESBL-PB and facilitating rapid and appropriate antibiotic treatment.[55] We could not find any difference in PCT levels between the groups. In multivariate analyses, age and PCT emerged as statistically significant risk factors for infection with ESBL-PB, however, we thought that was practically insignificant since the Odds ratios were quite low.

Our study has some limitations. The retrospective nature of the study was one limitation. To rule out false-positive reactions and contamination in the urine culture, only the cases whose urine samples for culture were collected with a urinary catheter were selected. Another limitation was that this group of patients might not represent the whole pediatric population who had a febrile UTI between 2 to 24 months.

In conclusion, this is the first study evaluating the use of easily accessible and cheap parameters including WBC, platelet counts, MPV, NLR,

PLR to predict UTIs caused by ESBL-PB in infants. We could not demonstrate that they were reliable markers for the prediction of ESBL- PB positivity. One possible explanation for these differences can be the small number of UTIs with ESBL-PB in our study. However, we have demonstrated that having renal ultrasonographic abnormality is the most important independent risk factors. Further studies in larger populations would help to decide the predictivity of the inflammation markers and ultrasonographic findings for UTIs caused by ESBL-PB since the number of studies is limited.

## Ethical approval

The study has been approved by the Ethics Committee of Tepecik Training and Research Hospital (no: 2020/5-8).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BKD; data collection: ES, MK, GE; analysis and interpretation of results: SAÇ, GE, NY, DA; draft manuscript preparation: ES, FM, BKD. 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. Festo E, Kidenya BR, Hokororo A, Mshana SE. Predictors of urinary tract infection among febrile children attending at Bugando Medical Centre Northwestern, Tanzania. Arch Clin Microbiol 2011; 2: 2.
2. Ahmed M, Moremi N, Mirambo MM, et al. Multi-resistant gram negative enteric bacteria causing urinary tract infection among malnourished underfives admitted at a tertiary hospital, northwestern, Tanzania. Ital J Pediatr 2015; 41: 44. https://doi.org/10.1186/s13052-015-0151-5
3. Mushi MF, Alex VG, Seugendo M, Silago V, Mshana SE. C - reactive protein and urinary tract infection due to Gram-negative bacteria in a pediatric population at a tertiary hospital, Mwanza, Tanzania. Afr Health Sci 2019; 19: 3217-3224. https://doi.org/10.4314/ahs.v19i4.45
4. Weinstein RA, Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis 2005; 41: 848-854. https://doi.org/10.1086/432803
5. Edlin RS, Shapiro DJ, Hersh AL, Copp HL. Antibiotic resistance patterns of outpatient pediatric urinary tract infections. J Urol 2013; 190: 222-227. https://doi.org/10.1016/j.juro.2013.01.069
6. Chong Y, Shimoda S, Shimono N. Current epidemiology, genetic evolution and clinical impact of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol 2018; 61: 185-188. https://doi.org/10.1016/j.meegid.2018.04.005
7. Hu FP, Guo Y, Zhu D, et al. Antimicrobial resistance profile of clinical isolates in hospitals across China: report from the CHINET surveillance program, 2017. Chinese Journal of Infection and Chemotherapy 2018; 18: 241-251.
8. Blomberg B, Manji KP, Urassa WK, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. BMC Infect Dis 2007; 7: 43. https://doi.org/10.1186/1471-2334-7-43
9. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis 2006; 42(Suppl 2): S82-S89. https://doi.org/10.1086/499406
10. Dayan N, Dabbah H, Weissman I, Aga I, Even L, Glikman D. Urinary tract infections caused by community-acquired extended- spectrum β-lactamase- producing and nonproducing bacteria: a comparative study. J Pediatr 2013; 163: 1417-1421. https://doi.org/10.1016/j.jpeds.2013.06.078
11. Khan F, Malik M, Afzal K, Malik A, Khalid M. Renal biometry and serum c-reactive protein levels in the evaluation of urinary tract infections. Indian J Nephrol 2004; 14: 10-14.
12. Honkinen O, Jahnukainen T, Mertsola J, Eskola J, Ruuskanen O. Bacteremic urinary tract infection in children. Pediatr Infec Dis J 2000; 19: 630-634. https://doi.org/10.1097/00006454-200007000-00009

13. Galloway A, Green H, Windsor J, Menon K, Gardner B, Krishnan K. Serial concentrations of C-reactive protein as an indicator of urinary tract infection in patients with spinal injury. J Clin Pathol 1986; 39: 851-855. https://doi.org/10.1136/jcp.39.8.851

14. Koufadaki AM, Karavanaki KA, Soldatou A, et al. Clinical and laboratory indices of severe renal lesions in children with febrile urinary tract infection. Acta Paediatr 2014; 103: 404-409. https://doi.org/10.1111/apa.12706

15. Wang J, Zhu QW, Cheng XY, et al. Assessment efficacy of neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio in preeclampsia. J Reprod Immunol 2019; 132: 29-34. https://doi.org/10.1016/j.jri.2019.02.001

16. Lowsby R, Gomes C, Jarman I, et al. Neutrophil to lymphocyte count ratio as an early indicator of blood stream infection in the emergency department. Emerg Med J 2015; 32: 531-534. https://doi.org/10.1136/emermed-2014-204071

17. Shemirani AH, Nagy B Jr, Takáts AT, et al. Increased mean platelet volume in primary Raynaud's phenomenon. Platelets 2012; 23: 312-316. https://doi.org/10.3109/09537104.2011.618563

18. Bilici S, Sekmenli T, Goksu M, Melek M, Avci V. Mean platelet volume in diagnosis of acute appendicitis in children. Afr Health Sci 2011; 11: 427-432.

19. European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameters Version 5.0, valid from 2015-01-01.

20. Topaloglu R, Er I, Dogan BG, et al. Risk factors in community-acquired urinary tract infections caused by ESBL-producing bacteria in children. Pediatr Nephrol 2010; 25: 919-925. https://doi.org/10.1007/s00467-009-1431-3

21. Kizilca O, Siraneci R, Yilmaz A, et al. Risk factors for community-acquired urinary tract infection caused by ESBL-producing bacteria in children. Pediatr Int 2012; 54: 858-862. https://doi.org/10.1111/j.1442-200X.2012.03709.x

22. Dotis J, Printza N, Marneri A, Gidaris D, Papachristou F. Urinary tract infections caused by extended-spectrum betalactamase-producing bacteria in children: a matched casecontrol study. Turk J Pediatr 2013; 55: 571-574.

23. Leroy S, Fernandez-Lopez A, Nikfar R, Romanello C, Bouissou F, Gervaix A, et al. Association of procalcitonin with acute pyelonephritis and renal scars in pediatric UTI. Pediatrics 131; 2013: 870-879. https://doi.org/10.1542/peds.2012-2408

24. Bouguila J, Khalef I, Charfeddine B, et al. Comparative study of C-reactive protein and procalcitonin in the severity diagnosis of pyelonephritis in children. Pathol Biol (Paris) 2013; 61: 93-98. https://doi.org/10.1016/j.patbio.2013.01.010

25. Zayed KMS, Abdelhakeem AM, Gafar HS, Eldahshan TAEK. Diagnostic of platelet parameters versus interleukin-6 in children with urinary tract infection. Egypt Paediatr Assoc Gaz 2016; 64: 142148. https://doi.org/10.1016/j.epag.2016.04.002

26. Nassaji M, Ghahremanfard F, Mirmohammadkhani M, Tamadon MR. Mean platelet volume and other platelet indices in adults patients with acute pyelonephritis. Asian J Pharm Health Sci 2014; 4: 1097-1101.

27. Sert A, Aypar E, Odabas D. Mean platelet volume in acute rheumatic fever. Platelets 2013; 24: 378-382. https://doi.org/10.3109/09537104.2012.701029

28. Gunluoglu G, Yazar EE, Veske NS, Seyhan EC, Altin S. Mean platelet volume as an inflammation marker in active pulmonary tuberculosis. Multidiscip Respir Med 2014; 9: 11-15. https://doi.org/10.1186/2049-6958-9-11

29. Tekin M, Konca C, Gulyuz A, Uckardes F, Turgut M. Is the mean platelet volume a predictive marker for the diagnosis of acute pyelonephritis in children?. Clin Exp Nephrol 2015; 19: 688-693. https://doi.org/10.1007/s10157-014-1049-z

30. Srinivasa S, Dhingra P. Platelet indices in children with urinary tract infection. Int J Contemp Pediatr 2018; 5: 953-957. https://doi.org/10.18203/2349-3291.ijcp20181520

31. Han SY, Lee IR, Park SJ, Kim JH, Shin JI. Usefulness of neutrophil-lymphocyte ratio in young children with febrile urinary tract infection. Korean J Pediatr 2016; 59: 139-144. https://doi.org/10.3345/kjp.2016.59.3.139

32. Yoon NB, Son C, Um SJ. Role of the neutrophil-lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community-acquired pneumonia. Ann Lab Med 2013; 33: 105-110. https://doi.org/10.3343/alm.2013.33.2.105

33. de Jager CP, van Wijk PT, Mathoera RB, de Jongh-Leuvenink J, van der Poll T, Wever PC. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. Crit Care 2010; 14: R192. https://doi.org/10.1186/cc9309

34. Zahorec R. Ratio of neutrophil to lymphocyte counts: rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy 2001; 102: 5-14.

35. Lee JW, Park SJ, Park KB, Yoo GH, Kim SS, Lee SM. Prediction of renal cortical defect and scar using neutrophil to lymphocyte ratio in children with febril urinary tract infection. Nuklearmedizin 2017; 56: 109-114. https://doi.org/10.3413/Nukmed-0878-17-01

36. Gökhan Ş, Tanrıverdi F, Pamukçu Günaydın G, et al. Importance of thiol/ disulfide homeostasis and the neutrophil-to-lymphocyte ratio in diagnosing of urinary tract ınfections in the emergency department. Cyprus J Med Sci 2019; 4: 19-23. https://doi.org/10.5152/cjms.2019.704

37. Kazimoglu H, Uysal E, Dokur M, Gunerkan HR. Evaluation of the relationship between neutrophil lymphocyte ratio and the most common bacterial urinary tract infections after transplantation. Bratisl Med J 2019; 120: 161-165. https://doi.org/10.4149/BLL_2019_026

38. Loonen AJ, de Jager CP, Tosserams J, et al. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One 2014; 9: e87315. https://doi.org/10.1371/journal.pone.0087315

39. Acar E, Özcan Ö, Deliktaş H, et al. Laboratory markers has many valuable parameters in the discrimination between acute appendicitis and renal colic. Ulus Travma Acil Cerrahi Derg 2016; 22: 17-22. https://doi.org/10.5505/tjtes.2015.69488

40. Kawamura Y, Takeshita S, Kanai T, Yoshida Y, Nonoyama S. The combined usefulness of the neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in predicting intravenous immunoglobulin resistance with Kawasaki disease. J Pediatr 2016; 178: 281-284.e1. https://doi.org/10.1016/j.jpeds.2016.07.035

41. Qin B, Ma N, Tang Q, et al. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were useful markers in assessment of inflammatory response and disease activity in SLE patients. Mod Rheumatol 2016; 26: 372-376. https://doi.org/10.3109/14397595.2015.1091136

42. Kim HA, Jung JY, Suh CH. Usefulness of neutrophil-to-lymphocyte ratio as a biomarker for diagnosing infections in patients with systemic lupus erythematosus. Clin Rheumatol 2017; 36: 2479-2485. https://doi.org/10.1007/s10067-017-3792-5

43. Kim DS, Shin D, Lee MS, et al. Assessments of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in Korean patients with psoriasis vulgaris and psoriatic arthritis. J Dermatol 2016; 43: 305-310. https://doi.org/10.1111/1346-8138.13061

44. Özer S, Yılmaz R, Sönmezgöz E, et al. Simple markers for subclinical inflammation in patients with Familial Mediterranean Fever. Med Sci Monit 2015; 21: 298-303. https://doi.org/10.12659/MSM.892289

45. Kelesoglu FM, Aygun E, Okumus NK, et al. Evaluation of subclinical inflammation in familial Mediterranean fever patients: relations with mutation types and attack status: a retrospective study. Clin Rheumatol 2016; 35: 2757-2763. https://doi.org/10.1007/s10067-016-3275-0

46. Gervaix A, Galetto-Lacour A, Gueron T, et al. Usefulness of procalcitonin and C-reactive protein rapid tests for the management of children with urinary tract infection. Pediatr Infect Dis J 2001; 20: 507-511. https://doi.org/10.1097/00006454-200105000-00007

47. Smolkin V, Koren A, Raz R, Colodner R, Sakran W, Halevy R. Procalcitonin as a marker of acute pyelonephritis in infants and children. Pediatr Nephrol 2002; 17: 409-412. https://doi.org/10.1007/s00467-001-0790-1

48. Shaikh N, Borrell JL, Evron J, Leeflang MM. Procalcitonin, C-reactive protein, and erythrocyte sedimentation rate for the diagnosis of acute pyelonephritis in children. Cochrane Database Syst Rev 2015; 1: CD009185. https://doi.org/10.1002/14651858.CD009185.pub2

49. Yildiz B, Poyraz H, Cetin N, Kural N, Colak O. High sensitive C-reactive protein: a new marker for urinary tract infection, VUR and renal scar. Eur Rev Med Pharmacol Sci 2013; 17: 2598-2604.

50. Yen C, Chen D. Urinary tract infection in children. J Microbiol Immunol Infect 1999; 32: 199-205.

51. Naseri M. Alterations of peripheral leukocyte count, erythrocyte sedimentation rate, and C-reactive protein in febrile urinary tract infection. Iran J Kidney Dis 2008; 2: 137-142.

52. Yamamoto N, Takegawa R, Seki M, et al. Pneumorachis associated with multiorgan infection due to Citrobacter koseri. Diagn Microbiol Infect Dis 2013; 77: 370-372. https://doi.org/10.1016/j.diagmicrobio.2013.08.017

53. Yamaguchi K, Ishii Y, Tateda K, et al. Nationwide surveillance of parenteral antibiotics containing meropenem activities against clinically isolated strains in 2012. Jpn J Antibiot 2014; 67: 73-107.

54. Hawkey PM. Multidrug-resistant Gram-negative bacteria: a product of globalization. Jsp Infect 2015; 89: 241-247. https://doi.org/10.1016/j.jhin.2015.01.008

55. Watanabe Y, Oikawa N, Hariu M, Fuke R, Seki M. Ability of procalcitonin to diagnose bacterial infection and bacteria types compared with blood culture findings. Int J Gen Med 2016; 9: 325-331. https://doi.org/10.2147/IJGM.S115277

The Turkish Journal of Pediatrics 2022; 64: 274-284
https://doi.org/10.24953/turkjped.2021.415

**Original Article**

# Evaluation of nutritional status and related factors in children with cystic fibrosis

Aylin Yücel[1], Sevgi Pekcan[2], Beray Selver Eklioğlu[3], Hasan Ali Yüksekkaya[1], Gökçen Ünal[2], Aslı İmran Yılmaz[2]

*Departments of [1]Pediatric Gastroenterology, Hepatology and Nutrition, [2]Pediatric Chest Diseases and [3]Pediatric Endocrinology, Necmettin Erbakan University, Meram Medical Faculty, Konya, Turkey.*

## ABSTRACT

**Background.** This study aimed to evaluate the nutritional status and body composition in children with cystic fibrosis (CF), in accordance with the new nutritional targets defined by European Society for Clinical Nutrition and Metabolism (ESPEN), the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Cystic Fibrosis Society (ECFS) 2016.

**Methods.** In this cross-sectional study, data were collected prospectively in a single centre. A record was made for a total of 95 patients with CF of clinical data. Anthropometric data were evaluated using the World Health Organization growth standards. The bone mineral density (BMD) z-score was adjusted for height by measuring dual-energy X-ray absorptiometry (DXA). The speed of sound z-score values were measured with quantitative ultrasound (QUS).

**Results.** The nutritional status was normal in 37.9% of patients aged <2 years and 33.3% of patients aged 2-18 years. When the DXA BMD z-score values were corrected for height, it was determined that the BMD deficit was less. The calcaneus QUS SOS z-score mean value was lower than the mean height for age z-score adjusted BMD ($BMD_{HAZ}$).

**Conclusions.** The malnutrition rates of CF patients were higher than the rates previously reported in literature. As there are insufficient nutritional data in Turkey, there is a need for multi-centre studies to determine the frequency of malnutrition according to the new classifications. It is clear that QUS measurements cannot replace DXA in the diagnosis of osteopenic bone disease. However, when low values are determined with QUS as the first recommended measurement in the screening of bone status, it can be considered appropriate to confirm the status with DXA.

**Key words:** bone densitometry, cystic fibrosis, malnutrition, nutritional status, quantitative ultrasound, height for age z-score adjusted BMD.

Cystic fibrosis (CF) is an autosomal recessive, multi-systemic disorder which forms as a result of mutation in the protein encoding gene known as cystic fibrosis transmembrane conductance regulator (CFTR). The two most significant problems determining survival are malnutrition and pulmonary disease.[1] The nutritional status in CF is related to insufficient macro/micronutrient intake, maldigestion/malabsorption, increased energy requirement, and genotype.[2] Maldigestion and malabsorption associated with exocrine pancreatic failure cause loss of energy. This loss may be increased in conditions such as accompanying intestinal inflammation, small intestinal bacterial overgrowth, insulin resistance and impaired liver functions. In addition, total and resting energy expenditure (TEE, REE) are increased

✉ Aylin Yücel
ayucel82@hotmail.com

Received 8th February 2021, revised 24th March 2021, 12th June 2021, accepted 28th July 2021.

This study was presented at the 12th National Pediatric Gastroenterology, Hepatology and Nutrition Congress, 18-21 April 2018, İzmir, Turkey.

in CF patients, and CF-related infections and persistent pulmonary inflammation cause a further increase in energy requirement. In addition to all these problems, loss of appetite because of infections, gastrointestinal problems or side-effects of medications and inability to obtain sufficient calorie intake have been found to contribute to malnutrition.[3]

Under-nutrition leads to retarded growth development, impaired respiratory muscle function, reduced exercise tolerance, immunological disorders and increased sensitivity to infections.[4] Severe nutritional deficiency in infancy and childhood can result in a significant deterioration in respiratory functions, short life expectancy and impairments in cognitive functions.[3] Correction of the nutritional status improves quality of life.[4]

In 2002, the European and US nutrition consensus reports for CF patients were published. Subsequently, it was reported that the ideal body weight percentage (IBW%) could ignore malnutrition in children of short height and could exaggerate the severity of malnutrition in tall children.[5] The nutritional classifications for CF patients were updated in 2016 by the European Society for Clinical Nutrition and Metabolism (ESPEN), the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Cystic Fibrosis Society (ECFS). The parameters recommended for use in nutritional status evaluation and the target values defined for use in the determination of a sufficient nutritional status has changed with these reports. According to this consensus, IBW% is no longer used. Malnutrition classification is made according to length and weight percentile in infants aged < 2years and according to body mass index percentile (BMIp) in children and adolescents aged 2-18 years. Normal nutritional status is defined as ≥50th percentile of these parameters, impaired nutritional status as 10th-50th percentile, and persistent under-nutrition as <10th percentile.

Another factor related to nutritional status is bone mineralisation, and the incidence of osteopenic bone disease in adolescents and adults with CF is extremely high.[6] In the 2016 ESPEN- ESPGHAN-ECSF nutrition consensus report in accordance with the 2011 European Cystic Fibrosis Bone Mineralisation guidelines, it is recommended for CF patients that bone mineral density (BMD) measurements are taken with dual-energy X-ray absorptiometry (DXA) at specific intervals starting with the first measurements at age 8-10 years. In the presence of risk factors for osteopenia, it has been stated that first measurements can be taken earlier. In the presence of short stature, the BMD z-score should be corrected for height, since the deficit in BMD will seem overestimated. Even though a sufficient nutritional status can currently be obtained at increasing rates in CF patients, under-nutrition remains an important problem and is a risk factor for osteopenic bone disease.[3,7]

BMD measurements made with quantitative ultrasound (QUS) is a rapid, easy to apply method and has the advantage of no radiation exposure.[8] Studies of adults on this subject and the few studies conducted on children with CF have reported that it can be used for screening purposes in CF patients with a sufficient nutritional status, but no consensus has been reached on this subject as yet.

There are insufficient data related to the nutritional status of CF patients in Turkey. The aim of this study was to determine the incidence of malnutrition in a cystic fibrosis centre in Turkey with anthropometric measurements made in accordance with the new nutritional classifications defined in 2016 by ESPEN, ESPGHAN and ECSF, and to evaluate the relationship of the nutritional status with clinical, laboratory and genetic characteristics and bone mineralisation values.

## Material and Methods

### *Ethical statement*

Ethical approval for the study has been obtained from the Ethics Committee of Necmettin Erbakan University Meram Medical Faculty (code number: 2017/990). In addition, written informed consent was obtained from parents or legal guardians of all children prior to any study-related procedure in the study.

### *Study Design*

This single-centre, cross-sectional study included patients diagnosed with CF according to the 2016 consensus criteria who attended regular follow-up examinations in the period between 2017-2018, and for whom measurements were taken and recorded.[9] Patients were excluded from the study if they were aged >18 years, or if <18 years were determined with mutations not defined in the Cystic Fibrosis Mutation database following all gene DNA analysis, or were receiving systemic steroid treatment. For patients with hospitalisation within the previous month, pulmonary exacerbation, or antibiotic use, measurements were taken at the follow-up visit after clinical stabilisation was obtained. A total of 95 patients who met the study criteria and consented to participate, were included in the study.

### *Clinical parameters*

A record was made for each patient of age, gender, gestational age, birthweight, number of hospitalisations, whether or not enteral nutrition support was administered, medical treatments and mutations. The mutations were separated into 3 groups according to the ECSF Patient Registry Annual Data Report 2015. Group 1 was classified as F508del homozygote, Group 2 as F508del heterozygote, and Group 3 as other mutations. For patients aged >5 years, the highest values from 2 consecutive spirometry measurements were selected. If there was a difference of >5% between the FEV1 and FVC values of the two spirometric measurements, it was applied for a third time. Patients without an acceptable spirometry value were not included in the study. In the evaluation of the nutritional status, the serum markers recommended in the 2015 consensus were used (blood count, iron status, plasma fat-soluble vitamin levels, serum liver function tests, and electrolytes). For the evaluation of body composition, total body DXA was applied and the BMD z-scores were recorded. Since the children included in our study were shorter in height for age, BMD measurements were also ajusted according to the height for age z-score (HAZ). The BMD z-scores below -2 are considered to be CF-related low BMD. Calcaneus QUS was applied using a Hologic Sahara bone sonometer device (35 Crosby Drive, Bedford, MA 01730). The z-score values of the QUS speed of sound (SOS) measurements were recorded.

### *Anthropometric measurements*

Body weight, height, mid-upper arm circumference (MUAC), triceps skin fold (TSF) and subscapular skin fold (TSF, SSF) measurements were taken and recorded for each patient. Height measurements were taken with a stadiometer, standing for patients aged >2 years and lying down for patients <2 years. Body weight measurements were recorded using a 10-gram-sensitive infant scale for infants, who were weighed naked, and a 100-gram-sensitive digital adult scale for children aged >2 years. The MUAC was measured from the middle of the acromion notch and the olecranon notch of the left arm with the elbow joint in mild flexion. The TSF and SSF were measured by a single person using a Holtain Skinfold Caliper, and the average of 3 measurements was recorded for the analyses. Using the measured parameters, the body mass index ($g/m^2$) (BMI), weight-for-age (WA), height-for-age (HA), weight-for- height (WH) percentile and z-score values were calculated using the World Health Organisation (WHO) growth standards and Anthro/AnthroPlus (version 3.2.2, January 2011) software. In accordance with the recommendations of the new nutritional consensus, we used WA, HA

and WH percentile (WAp, HApandWHp) for nutritional classification in children younger than 2 years old, and BMI (g / $m^2$) percentile (BMIp) for children older than 2 years old. The non-adjusted real weight and height values were used in the analysis. Calculations not in Anthro/AnthroPlus program (TSF, SSF percentile, z-score calculations for children >5 years) were made using the Center for Disease Control and Prevention (CDC) growth standards. The z-scores of the anthropometric measurements were compared. Normal nutritional status was accepted as ≥50th percentile of the WAp and HAp in patients up to the age of 2 years and of the BMIp in those aged >2 years, impaired nutritional status was accepted as 10th-50th percentile and persistent under-nutrition as <10th percentile.[3]

### *Statistical analysis*

Data obtained in the study were analysed statistically using SPSS 20.0 software (IBM Inc, Chicago, IL, USA). By calculating descriptive measurements, categorical variables were stated as frequency and percentage and numerical variables as mean ± standard deviation (SD) or median (interquartile range, IQR), as appropriate. Conformity of continuous variables to normal distribution was assessed with the Kolmogorov-Smirnov test. The variables were seen not to have normal distribution so the Mann-Whitney U-test and the Kruskal Wallis test were used in the comparisons. To determine correlations between categorical variables, Chi-square analysis with Monte Carlo correction was used and for correlations between numerical variables, Spearman's Rho correlation analysis was applied. Significant results were demonstrated with graphs. Taking the type-1 error value as 5% for the whole study, a value of $p<0.05$ was accepted as statistically significant.

## Results

The 95 patients with CF included in the study comprised 51 (53.7%) girls and 44 (46.3%) boys with a mean age of 79 months (79.26 ± 61.18) and 66 (69.5%) patients were aged >2 years. According to gestational age, 13.7% were preterm. In almost half (45.3%) of the patients, there was parental consanguinity, and in 40% there was a familial history of CF. The median (IQR) number of hospitalisations was 2 (35). Other clinical characteristics are shown in Table I.

All anthropometric measurements of patients were evaluated according to the z-scores. The mean z-score values of the anthropometric measurements are shown as a box-plot graph in Figure 1.

When the nutritional classifications were examined in the 29 infants aged ≤2 years, normal nutritional status was determined in 27.6% (n=8) according to W-Ap, in 41.4% (n=12) according to H-Ap, and in 37.9% (n=11) according to W-Hp. In the 66 patients aged 2-18 years, normal nutritional status was determined in 33.3% (n=22) (Table II).

The nutritional classifications were compared with FEV1, FVC, BMD z-score, $BMD_{HAZ}$, SOS z-score and mutation classifications. Patients aged <2 years were only compared with mutation groups as BMD and spirometry values were not measured. A statistically significant correlation was determined between the presence of Delta F508 heterozygote mutation and normal and impaired nutritional status according to W-Ap (p=0.017). None of the patients with F508 homozygote mutation had normal nutritional status. Of the patients in the normal nutritional status group, 75% had other mutations, 54.5% of patients in the impaired nutritional status group had F508 del heterozygote mutation and 50% of the patients in the persistent under-nutrition group had F508del homozygote mutation. No statistically significant correlation was determined between the mutation groups and the H-Ap and W-Hp nutritional classifications (Table III).

In patients aged >2 years, the nutritional classifications made according to BMIp were compared with the clinical and laboratory parameters. In the 56 patients aged >5 years,

**Table I.** Clinical characteristics of 95 patients with cystic fibrosis.

| Patients characteristics | Results |
|---|---|
| Age (month) | 79.26 ± 61.18 |
| Age at diagnosis (month) | 19.12 ± 36.87 |
| Height (cm) | 108.95 ± 34.10 |
| Weight (kg) | 21.18 ± 14.30 |
| Mutation, n (%) | |
| F508del homozygous | 22 (23.2) |
| F508del heterozygous | 17 (17.9) |
| Other mutations | 56 (58.9) |
| Comorbidities, n (%) | |
| No | 91 (95.8) |
| Hypothyroid | 2 (2.1) |
| Phenylketonuria | 1 (1.1) |
| Down's syndrome | 1 (1.1) |
| Clinical presentation, n (%) | |
| Neonatal screening | 24 (25.3) |
| Malnutrition | 9 (9.5) |
| Cough | 12 (12.6) |
| Recurrent pulmonary infections | 7 (7.4) |
| Malnourishment/nausea | 5 (5.3) |
| Chronic diarrhea | 2 (2.1) |
| Pseudo-Bartter Syndrome | 10 (10.5) |
| Nasal polyps | 2 (2.1) |
| Elevated liver enzymes /cholestasis | 4 (4.2) |
| Chronic/Recurrent pancreatitis | 3 (3.2) |
| Meconium ileus | 4 (4.2) |
| Respiratory distress/cyanosis | 13 (13.7) |
| Medical treatments, n (%) | |
| PERT | 85 (89.5) |
| Enteral nutrition supplementation | |
| Polymeric | 34 (35.8) |
| Oligomeric | 15 (15.8) |
| Fat-soluble vitamin | 78 (82.1) |
| Proton pump inhibitor | 23 (24.2) |
| Dornase alpha | 89 (93.7) |
| Inhaler beta-2 agonist | 41 (43.2) |
| Inhaler corticosteroids | 26 (27.4) |
| Laboratory findings, mean ± SD | |
| Hemoglobin (g/dl) | 12.81 ± 1.6 |
| Serum iron (μg/dl) | 64.72 ± 33.98 |
| Iron-binding capacity(μg/dl) | 262.3 ± 74.89 |
| Ferritin (ng/ml) | 57.29 ± 78.49 |
| Vitamin A (μg/dl) | 247.2 ± 180.45 |
| Vitamin D (ng/ml) | 27.28 ± 26.19 |

PERT: pancreatic enzyme replacement treatment

**Table I.** Continued.

| Patients characteristics | Results |
|---|---|
| Laboratory findings, mean ± SD | |
| Vitamin E (µg/ml) | 13.02 ± 7.44 |
| Vitamin B12 (pg/ml) | 520.96 ± 254.74 |
| Folic acid (ng/ml) | 17.19 ± 6.11 |
| Zinc (µmol/l) | 19.38 ± 34.7 |
| Albumin (g/dl) | 4.27 ± 0.46 |
| Sodium (meq/L) | 138.16 ± 3.91 |
| Calcium (mg/dl) | 9.68 ± 0.62 |
| FEV1 (%) | 86.36 ± 21.6 |
| FVC (%) | 81.33 ± 19.01 |
| DXA/BMD z-score | 0.32 ± 0.83 |
| QUS/SOS z-score | -0.129 ± 1.21 |
| $BMD_{HAZ}$ | 0.548 ± 0.698 |

BMD: bone mineral density, BMDHAZ: height for age z-score adjusted BMD, DXA: dual-energy X-ray absorptiometry, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, PERT: pancreatic enzyme replacement treatment, QUS: quantitative ultrasound, SOS: speed of sound.

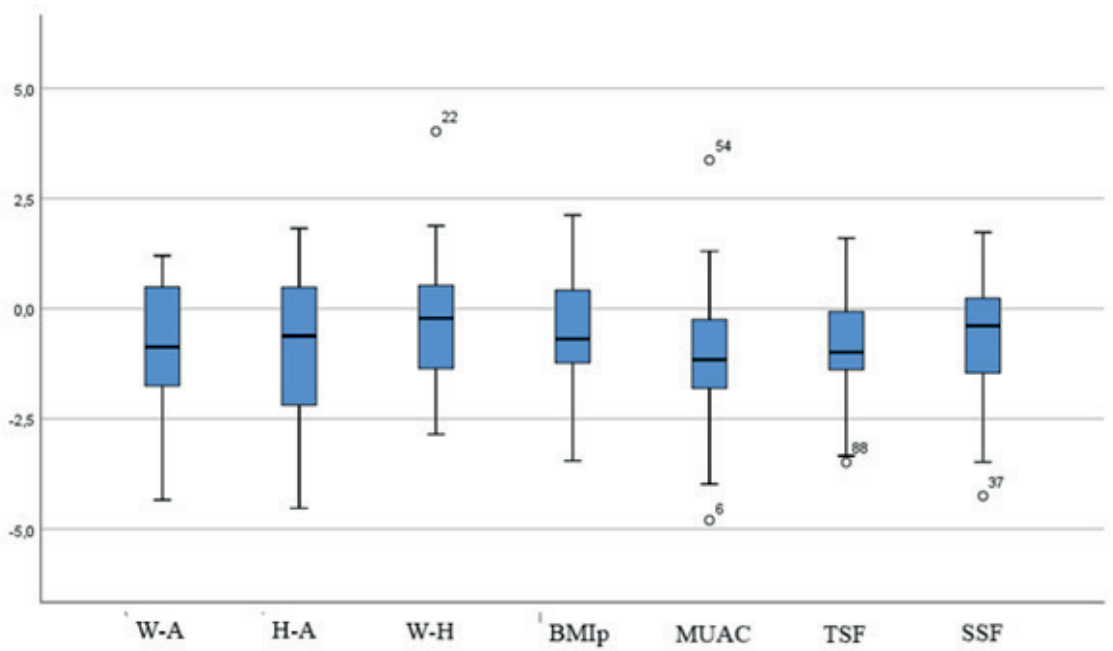



**Fig. 1.** Box-plots of z-scores of anthropometric measurements. BMI: body mass index, H-A: height-for-age, MUAC: mid-upper arm circumference, TSF: triceps skin fold, SSF: subscapular skin fold, W-A: weight-for-age, W-H: weight-for-height.

when the respiratory function tests, DXA and calcaneus QUS results were compared, the QUS SOS z-score value (p=0.041) and FVC (p=0.040) were found to be lower in the impaired nutritional status and persistent under-nutrition groups according to BMIp. When nutritional classifications according to BMIp were compared with BMD z-score and height for age z-score adjusted BMD ($BMD_{HAZ}$), although both parameters were measured quite

**Table II.** Nutritional classification based on age-specific anthropometric measurements.

| Anthropometric parameters | | n (%) |
|---|---|---|
| W-A percentile | Normal nutritional status (≥ 50th percentile) | 8 (27.6) |
| (Infants ≤ 2 years) | Impaired nutritional status (10-50th percentile) | 11 (37.9) |
| | Persistent under-nutrition (<10th percentile) | 10 (34.5) |
| H-A percentile | Normal nutritional status (≥ 50th percentile) | 12 (41.4) |
| (Infants ≤ 2 years) | Impaired nutritional status (10th-50th percentile) | 9 (31) |
| | Persistent under-nutrition (<10th percentile) | 8 (27.6) |
| W-H percentile | Normal nutritional status (≥ 50th percentile) | 11 (37.9) |
| (Infants ≤ 2 years) | Impaired nutritional status (10-50th percentile) | 9 (31) |
| | Persistent under-nutrition (<10th percentile) | 9 (31) |
| BMI percentile | Normal nutritional status (≥ 50th percentile) | 22 (33.3) |
| (Children 2-18 years) | Impaired nutritional status (10-50th percentile) | 27 (40.9) |
| | Persistent under-nutrition (<10th percentile) | 17 (25.8) |

W-A: weight-for-age, H-A: height-for-age, W-H: weight-for-height, BMI: body mass index.

**Table III.** The mutation results of patients (< 2 years) according to nutritional classification system.

| Mutation groups according to nutritional classification system | | Nutritional status, n (%) | | | P value |
|---|---|---|---|---|---|
| | | Normal | Impaired | Persistent undernutrition | |
| W-A percentile | | (n=8) | (n=11) | (n=10) | |
| Mutation | F508del homozygous | 0 | 3 (27.3) | 5 (50) | 0.017* |
| | F508del heterozygous | 2 (25)[a] | 6 (54.5)[b] | 2 (20) | |
| | Other mutations | 6 (75) | 2 (18.2) | 3 (30) | |
| H-A percentile | | (n=12) | (n=9) | (n=8) | |
| Mutation | F508del homozygous | 4 (33.3) | 1 (11.1) | 3 (37.5) | 0.871 |
| | F508del heterozygous | 3 (25) | 5 (55.6) | 2 (25) | |
| | Other mutations | 5 (41.7) | 3 (33.3) | 3 (37.5) | |
| W-H percentile | | (n=11) | (n=9) | (n=9) | |
| Mutation | F508del homozygous | 2 (18.2) | 3 (33.3) | 3 (33.3) | 0.443 |
| | F508del heterozygous | 3 (27.3) | 5 (55.6) | 2 (22.2) | |
| | Other mutations | 6 (54.5) | 1 (11.1) | 4 (44.4) | |

* $p < 0.05$
a, b: percentages denoted by a different letter indicate significant differences between groups.
W-A: weight-for-age, H-A: height-for-age, W-H: weight-for-height.

low in the persistent undernutrition group, there was no significant difference between the groups (p=0.086, p=0.730) (Table IV).

The z-scores of the anthropometric measurements were compared with the BMD measurements and the spirometry results. A moderate positive correlation was determined between the W-A z-score and the BMD z-score (DXA), FEV1 and FVC. A significant correlation was determined between the H-A z-score and the BMD z-score (DXA) and FEV1. There was a negative correlation between $BMD_{HAZ}$ and H-A z-scores, however it was not statistically significant. A low-level positive correlation was determined between the BMI z-score and FEV1 and FVC. Significant correlations were determined between the MUAC z-score and FEV1, between the TSF z-score and the BMD z-score (DXA) and FEV1, and between the SSF z-score and all the variables (Table V).

**Table IV.** The bone mineral density, spirometry and mutation results of patients (>2 years) according to the nutritional classifications.

| Variables | BMIp Nutritional Classification | | | P value |
|---|---|---|---|---|
| | Normal (n=17) | Impaired (n=22) | Persistent undernutrition (n=17) | |
| DXA BMD z- score | 0.54 ± 0.52 | 0.44 ± 0.50 | -0.09 ± 1.23 | 0.086 |
| BMDHAZ | 0.67 ± 0.36 | 0.66 ± 054 | 0.27 ± 1.02 | 0.730 |
| QUS SOS z- score | 0.27 ± 1.18 | 0.001 ± 0.99 | -0.74 ± 1.34[a] | 0.041* |
| FEV1 (%) | 95.12 ± 17.17 | 87.45 ± 18.20 | 76.70 ± 26.18 | 0.063 |
| FVC (%) | 87.02 ± 15.22 | 84.68 ± 16.24 | 71.58 ± 22.52[a] | 0.040* |
| Mutation, n (%) | | | | |
| F508del homozygous | 5 (22.7) | 7 (25.9) | 2 (11.8) | 0.298 |
| F508del heterozygous | 3 (13.6) | 3 (11.1) | 1 (5.9) | |
| Other mutations | 14 (63.6) | 17 (63) | 14 (82.4) | |

Data are parented as mean ± standard deviation unless otherwise specified.
BMDHAZ: height for age z-score adjusted BMD, BMIp: body mass index percentile, DXA BMD: dual-energy x-ray absorptiometry bone mineral density, FVC: forced vital capacity, FEV1: forced expiratory volume in 1 second, QUS SOS: quantitative ultrasound speed of sound.
* p<0.05

**Table V.** Relationships between the z-scores of the anthropometric measurements and the spirometry and bone mineral density values.

| Measurements | | BMD (DXA) z-score | $BMD_{HAZ}$ | SOS (QUS) z-score | FEV1 (%) | FVC (%) |
|---|---|---|---|---|---|---|
| W-A z-score | R | 0.340 | -0.109 | 0.223 | 0.355 | 0.310 |
| | p | 0.010* | 0.424 | 0.095 | 0.008* | 0.021* |
| H-A z-score | R | 0.284 | -0.217 | 0.060 | 0.317 | 0.218 |
| | p | 0.033* | 0.109 | 0.659 | 0.019* | 0.111 |
| W-H z-score | R | 0.900 | 0.200 | -0.200 | 0.500 | 0.500 |
| | p | 0.037* | 0.800 | 0.747 | 0.667 | 0.667 |
| BMI z-score | R | 0.253 | -0.003 | 0.307 | 0.306 | 0.289 |
| | p | 0.057 | 0.981 | 0.020 | 0.023* | 0.033* |
| MUAC z-score | R | 0.199 | -0.041 | 0.204 | 0.326 | 0.253 |
| | p | 0.139 | 0.765 | 0.127 | 0.015* | 0.062 |
| TSF z-score | R | 0.288 | 0.068 | 0.175 | 0.295 | 0.257 |
| | p | 0.030* | 0.621 | 0.192 | 0.029* | 0.058 |
| SSF z-score | R | 0.352 | 0.091 | 0.331 | 0.398 | 0.325 |
| | p | 0.007* | 0.507 | 0.012* | 0.003* | 0.016* |

BMI: body mass index, H-A: height-for-age, MUAC: mid-upper arm circumference, TSF: triceps skin fold, SSF: subscapular skin fold, W-A: weight-for-age, W-H: weight-for-height.
* p<0.05

## Discussion

The recommended parameters and target values for use in the evaluation of nutritional status in patients with CF were changed with the most recent guidelines published in 2016 by the ECSF and the Cystic Fibrosis Foundation. In the 2002 consensus reports, height for age <5%, ideal body weight < 90% and BMIp<10% were defined as nutritional deficiency. "At-risk nutritional status" was defined as 10-25% of weight-for-length percentile in infants <2 years

and as 10-25% of BMIp for children >2 years. According to the most recent consensus, the 50th percentile was defined as the nutritional target, in other words, it was stated that to be able to be said to have a sufficient nutritional status, children with CF should have similar growth as their healthy peers. For acceptance of a population as mostly healthy, the mean z-score should be close to zero.[3,10]

According to the ECSF 2015 Patient Registry Annual Data Report, which included the data of 42,054 CF patients from 29 countries, including Turkey, in 20,196 patients aged ≤18 years, the mean W-A z-score ranged from -0.3 to -0.7, and the mean H-A score from -0.2 to -0.4. However, in the 2015 data, only 95 patients were reported from Turkey, and of those, height and weight measurements were available in only 35.[11] In the current study, the z-score values of all the anthropometric measurements of all 95 patients were negative (W-A z-score -1.016±1.36; H-A z-score -0.862±1.38; W-H z-score -0.313±1.41; BMI z-score -0.706±1.35; MUAC z-score -1.116±1.44; TSF z-score -0.752±1.13; SSF z-score -0.458±1.18).

In the evaluation of the current study according to nutritional classifications, 31% of the patients aged <2 years, and 25.8% of those aged >2 years were determined with persistent under-nutrition. In a 2016 study by Bahreyn et al.[12] which evaluated 109 CF patients, 72% were determined as malnourished. In a small study conducted in Brazil by Silva Pinta et al.[13] malnutrition was determined at the rate of 33.3%, and in a multi-centre study in Italy in 2009, Lucidi et al.[14] determined malnutrition in 12.9% of patients aged <2 years and in 20.9% of those aged >2 years.

Although the malnutrition rates in the current study were lower than those reported from other Asian countries, they were higher than the rates reported from European countries. In the current study population, a sufficient nutritional status was determined in only 37.9% (W-Hp>50%) of patients aged <2 years, and in 33.3% (BMIp>50%) of older children and adolescents. Lucidi et al.[14] reported a sufficient nutritional status in 64.4% (W-Hp>25%) of patients aged <2 years and in 69.5% (BMIp>25%) of children >2 years, although when BMIp >50% was used as the nutritional target, a sufficient nutritional status was reported in only 45.6%. Thus it can be seen that some patients who were accepted as having a normal nutritional status according to the old classifications should now be accepted as impaired nutritional status.

In the current study, it was observed that there was a proportional deterioration of the BMD (DXA, QUS) values with the spirometric parameters with progression from normal nutritional status towards persistent under-nutrition. There was a positive correlation between nutritional status and BMD in CF patients. Although there was no statistically significant relationship between $BMD_{HAZ}$ and nutritional parameters, we found that $BMD_{HAZ}$ values were better than BMD z-score values corrected for age and gender. BMD measurements are affected by height. In particular, conditions such as inflammation, immobilization, and malabsorption negatively affect both bone mineralization and linear growth.[15] In cases where linear growth is impaired, BMD z-score values adjusted for only age and sex may not reflect the truth and may overestimate the BMD deficit. In our study, we found that BMD deficit was less when BMD z-score values were corrected for height, however the difference was not statistically significant. Andrea Kelly et al.[16] compared BMD z-scores measured by DXA with height-adjusted BMD z-scores in 82 patients with cystic fibrosis and 322 healthy children and they found that BMD deficits were less when BMD values were adjusted for height. They stated that BMD deficits in children with cystic fibrosis can be attributed to the change in linear growth.[16] According to the 2011 European Cystic Fibrosis Bone Mineralisation Guidelines, DXA is the gold standard method for BMD evaluation in CF patients. In children z-score values corrected for height should be used. Those with a BMD z-score of <-2 can be accepted as CF-related low BMD. The guideline recommends that the first

measurement is taken at the age of 8-10 years, then repeated once every 5 years if the z-score is >-1, once every 2 years if between -1 and -2, and once a year if <-2, and if there is a risk factor for low BMD, the first measurement should be taken at a younger age.[7] In a study that compared the DXA and QUS methods in the BMD evaluations of adult CF patients, it was concluded that even if QUS was used instead of DXA, screening of these patients could be useful.[8] In the first study (2009) that evaluated the efficacy of QUS in BMD evaluations of children with CF, the QUS results of 29 relatively healthy CF patients with a sufficient nutritional status were found to be similar to those of a healthy control group. Similar results were reported in another study of 35 CF patients in Spain.[17,18]

In the current study group, which had higher rates of malnutrition compared to reports in literature, QUS and DXA were applied to the 56 patients aged >5 years and the QUS values were lower than the mean $BMD_{HAZ}$ ($BMD_{HAZ}$ 0,548±0,698; QUS/BMD z-score -0.129±1.21). Thus, the BMD was shown to be lower with QUS measurements than with DXA measurements. Considering that QUS has lower sensitivity and specificity than DXA, we suggest that screening QUS in CF patients and when a low BMD is detected confirming it by DXA may be an option to reduce the radiation exposure of patients.

Increasing awareness and determining the current nutritional status are the first steps to be taken in reaching nutritional targets in CF patients. When the new nutritional classifications are used, it is clear that fewer patients can be accepted as having a normal nutritional status. As there are insufficient nutritional data of CF patients in Turkey, there is a need for multi-centre studies using the new classifications.

QUS measurements cannot replace DXA in the diagnosis of osteopenic bone disease in CF patients. However, if low BMD is determined following QUS as the first recommended measurement in the screening of bone health, confirmation with DXA can be considered a better choice to be able to reduce radiation exposure.

## Ethical approval

Ethical approval for the study has been obtained from the the Ethics Committee of Necmettin Erbakan University Meram Medical Faculty (code number: 2017/990).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AY, SP, BSE, HAY; data collection: AY, GU, AIY; analysis and interpretation of results: AY; draft manuscript preparation: AY. 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. Culhane S, George C, Pearo B, Spoede E. Malnutrition in cystic fibrosis: a review. Nutr Clin Pract 2013; 28: 676-683. https://doi.org/10.1177/0884533613507086
2. Gaskin KJ. Nutritional care in children with cystic fibrosis: are our patientsbecoming better?. Eur J Clin Nutr. 2013; 67: 558-564. https://doi.org/10.1038/ejcn.2013.20
3. Turck D, Braegger CP, Colombo C, et al. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and adults with cystic fibrosis. Clin Nutr 2016; 35: 557-577. https://doi.org/10.1016/j.clnu.2016.03.004
4. Morton AM. Symposium 6: young people, artificial nutrition and transitional care. The nutritional challenges of the young adult with cystic fibrosis: transition. Proc Nutr Soc 2009; 68: 430-440. https://doi.org/10.1017/S0029665109990176

5. Hirche TO, Hirche H, Jungblut S, et al. Statistical limitations of percent ideal body weight as measure for nutritional failure in patients with cystic fibrosis. J Cyst Fibros 2009; 8: 238-244. https://doi.org/10.1016/j.jcf.2009.03.004

6. De Schepper J, Roggen I, Van Biervliet S, et al. Comparative bone status assessment by dual energy X-ray absorptiometry, peripheral quantitative computed tomography and quantitative ultrasound in adolescents and young adults with cystic fibrosis. J Cyst Fibros 2012; 11: 119-224. https://doi.org/10.1016/j.jcf.2011.10.004

7. Sermet-Gaudelus I, Bianchi ML, Garabédian M, et al. European cystic fibrosis bone mineralisation guidelines. J Cyst Fibros 2011; 10: 16-23. https://doi.org/10.1016/s1569-1993(11)60004-0

8. Flohr F, Lutz A, App EM, Matthys H, Reincke M. Bone mineral density and quantitative ultrasound in adults with cystic fibrosis. Eur J Endocrinol 2002; 146: 531-536. https://doi.org/10.1530/eje.0.1460531

9. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998; 132: 589-595. https://doi.org/10.1016/S0022-3476(98)70344-0

10. Sullivan JS, Mascarenhas MR. Nutrition: Prevention and management of nutritional failure in Cystic Fibrosis. J Cyst Fibros 2017; 16: 87-93. https://doi.org/10.1016/j.jcf.2017.07.010

11. Zolin A, Orenti A, Naehrlich L, et al. ECFSPR annual data report 2015. Karup, Denmark: European Cystic Fibrosis Society; 2017.

12. Isa HM, Al-Ali LF, Mohamed AM. Growth assessment and risk factors of malnutrition in children with cystic fibrosis. Saudi Med J 2016; 37: 293-298. https://doi.org/10.15537/smj.2016.3.13476

13. Silva Pinto IC, Silva CP, Britto MC. Nutritional, clinical and socioeconomic profile of patients with cystic fibrosis treated at a referral center in northeastern Brazil. J Bras Pneumol 2009; 35: 137-143. https://doi.org/10.1590/S1806-37132009000200006

14. Lucidi V, Alghisi F, Raia V, et al. Growth assessment of paediatric patients with CF comparing different auxologic indicators: a multicentre Italian study. J Pediatr Gastroenterol Nutr 2009; 49: 335-342. https://doi.org/10.1097/MPG.0b013e31818f0a39

15. Zemel BS, Leonard MB, Kelly A, et al. Height adjustment in Assessing DEXA Measurements of Bone Mass and Density in Children. Clin Endocrinol Metab 2010; 95: 1265-1273. https://doi.org/10.1210/jc.2009-2057

16. Kelly A, Schall JI, Stallings VA, Zemel BS. Deficits in bone mineral content in children and adolescents with cystic fibrosis are related to height deficits. J Clin Densitom 2008; 11: 581-589. https://doi.org/10.1016/j.jocd.2008.07.002

17. Sood M, Hambleton G, Super M, Fraser WD, Adams JE, Mughal MZ. Bone status in cystic fibrosis. Arch Dis Child 2001; 84: 516-520. https://doi.org/10.1136/adc.84.6.516

18. Lopez-Rodriguez MJ, Lavado-Garcia JM, Canal-Macias ML, Calderon-Garcia JF, Moran JM, Pedrera-Zamorano JD. Quantitative ultrasound in Spanish children and young adults with cystic fibrosis. Biol Res Nurs 2012; 15: 280-284. https://doi.org/10.1177/1099800412441500

The Turkish Journal of Pediatrics 2022; 64: 285-292
https://doi.org/10.24953/turkjped.2021.4217

**Original Article**

# Episcleral Iodine-125 radioactive plaque brachytherapy as a salvage treatment for retinoblastoma in the era of intra-arterial chemotherapy

Ahmet Murat Sarıcı[1], Tülin Tiraje Celkan[2], Bilge Batu Oto[1], Atilla Şahin[3], Ömer Uzel[4], Didar Uçar[1]

*Departments of [1]Ophthalmology, [2]Pediatrics and [4]Radiation Oncology, Cerrahpasa University Faculty of Medicine, İstanbul; [3]Department of Ophthalmology, Tokat Turhal State Hospital, Tokat, Turkey.*

## ABSTRACT

**Background.** Retinoblastoma shows high rates of recurrence after initial chemotherapy (systemic or intra-arterial). Our aim was to evaluate the effectiveness of iodine-125 radioactive plaque brachytherapy as a salvage treatment with globe-preserving attributes after initial chemotherapy in patients with intraocular retinoblastoma.

**Methods.** The effect of brachytherapy was investigated retrospectively in 17 eyes of 17 patients who were followed up due to retinoblastoma between May 2012 and June 2018 and who received iodine-125 radioactive plaque brachytherapy as a salvage treatment after systemic or intra-arterial chemotherapy. The regression, ocular toxicity, and enucleation rates were evaluated at the end of the follow-up period.

**Results.** The tumor locations were post equator, macular, anterior to the equator, and peripapillary in 5, 3, 7, and 2 patients, respectively. Regression was initially and rapidly observed in 17 of the 17 eyes that underwent brachytherapy. Enucleation was performed in 5 (29.42%) of these patients due to recurrence with diffuse tumor involvement, and 4 of the tumors were located anterior to the equator. In 12 (70.58%) patients, the eyes were protected from enucleation following local brachytherapy.

**Conclusions.** Radioactive plaque brachytherapy can be applied as an effective salvage therapy with successful results in retinoblastoma patients who have received initial systemic or intra-arterial chemotherapy. Post equator-located solitary tumors have the highest success rate.

**Key words:** retinoblastoma, cancer, eye, brachytherapy, iodine-125.

Retinoblastoma, which can occur unilaterally or bilaterally, is the most common intraocular malignancy of childhood, constituting 11% of all cancers in the first year following birth and 3–4 % of all pediatric cancers.[1-3] The annual global incidence of retinoblastoma is 1 in 15,000–20,000 live births, and about 8000 new cases occur each year.[2] A study in the United States determined a 10-year survival rate of 90.3% in patients with bilateral retinoblastoma and 96.1% in those with unilateral retinoblastoma.[4] In a study conducted in Turkey, the 10-year survival rate of patients with unilateral retinoblastoma were found to be 90.74%, with 87.35% in bilateral cases.[5] Another study from a tertiary referral center in Turkey reported a 96.1% survival rate in a 20-year period.[6] In less developed countries, the 5-year survival rate is lower as 60.2%.[7]

The treatment methods include chemotherapy (intravenous, intra-arterial, intravitreal, periocular), focal therapies (thermotherapy, photocoagulation, cryotherapy, brachytherapy), external beam radiotherapy, and enucleation. With recent increases in treatment options, eye protection has become an important goal.[8,9] Selecting the appropriate treatment is determined by considering the size of the tumor,

✉ Ahmet Murat Sarıcı
ahmetsarici@gmail.com

Received 17th August 2021, revised 12th September 2021, 15th October 2021, accepted 7th November 2021.

whether it is unilateral or bilateral, macular involvement, the tumor's relationship with other tissues such as the optic disc, choroid, and sclera, the patient's age and general health status, and the family's wishes.[10,11] In recent years, recurrences after previous systemic or intra-arterial chemotherapy are generally managed by intra-arterial chemotherapy as a salvage treatment.[12,13] However, vascular complications from intra-arterial chemotherapy can cause total visual loss and can therefore be devastating, particularly in patients where this is the only seeing eye.[14]

Our aim was to evaluate the effectiveness of iodine-125 plaque brachytherapy as a salvage treatment in patients with recurrences after systemic or intra-arterial chemotherapy and to emphasize its role, particularly in patients' whose parents are concerned about the vascular complications of intra-arterial chemotherapy.

## Material and Methods

Following the approval of the local ethics committee (Cerrahpaşa School of Medicine Ethic Committee reference no.: E-83045809-604.01.02-35245), the effect of brachytherapy was investigated retrospectively in 17 eyes of 17 patients who were followed-up due to intraocular retinoblastoma at a tertiary clinic between May 2012 and June 2018. The patients' files were reviewed, and the patients who had received iodine-125 radioactive plaque brachytherapy as a salvage treatment for tumor recurrence after the completion of primary systemic or intra-arterial chemotherapy were enrolled in the study. The data were collected from the patients' files and included the patient's gender, age at the time of brachytherapy, laterality of the tumor, and tumor classification according to the International Classification of Retinoblastoma. Any treatments prior to brachytherapy were noted. The tumor response (regression or recurrence), need for enucleation, radiation-related complications, metastasis, and fatal events were evaluated at the end of the follow-up period. Recurrences were defined as the progression of the main tumor and the occurrence of a new tumor (aside from the main tumor) that could not be managed by cryotherapy or laser. Regression was defined as a decrease in the tumor basal diameter and thickness and the inactivation of the tumor. The regression and enucleation rates evaluated at the end of the follow-up period were the main outcomes.

An individualized brachytherapy plan was generated for each patient. Tumors adjacent to the optic disc (peripapillary; Figures 1 and 2) were managed with notched plaques. The maximum tumor diameter and distance from the inner sclera were used for treatment planning. The prescription dose was 45–50 Gy to the prescription point, which was defined as the tumor distance from the inner sclera plus 1 mm to account for scleral thickness. The mean dose was 70.04 cGy per hour, and the mean duration of the brachytherapy was 68.8 hours. The plaque diameter was chosen based on the largest diameter of the tumor and included a lateral margin of 2 mm around the target. All episcleral plaques were inserted under general anesthesia following careful tumor localization by indirect ophthalmoscopy and temporary disinsertion of the extraocular muscles, if required. The plaques were temporarily affixed to the globe with nonabsorbable sutures and subsequently removed under general anesthesia after delivery of the prescribed radiation dose based on the calculated treatment time. The iodine-125 sources (Eckert & Ziegler BEBIG, Berlin, Germany) were loaded in Collaborative Ocular Melanoma Study type plaques (Eckert & Ziegler BEBIG, Berlin, Germany).

Before and after the application of the radioactive plaque, the patients were regularly examined under general anesthesia in operating room conditions at intervals of 3–4 weeks, and the images were recorded using a Retcam® (Clarity Medical Systems, Inc., Pleasanton, CA). The radioactive plaque treatments were all performed by the same surgeon (AS), who also conducted the regular examinations.

The statistical analysis was performed using SPSS version 21.0. The descriptive statistics were expressed as mean ± standard deviation for the continuous data, and the categorical variables were presented as percentages. The globe salvage rates were assessed using the Kaplan–Meier survival analysis method.

## Results

The 17 patients who received plaque brachytherapy included 11 (64.7%) boys and 6 (35.3%) girls with a mean age of 32 ± 10.3 months (range, 24–56 months) at the time of the radioactive plaque application. The mean interval between the first diagnosis and the radioactive plaque application was 21 ± 10.83 months (range: 10–48 months). The mean post-brachytherapy follow-up time was 23 ± 17.11 months (range, 6–50 months). The tumor locations were post equator, macular, anterior to the equator, and peripapillary in 5, 3, 7, and 2 patients, respectively. The tumors were classified as group B in 5 (30%) eyes, C in 1 (5%) eye, and D in 11 (65%) eyes (Table I). Thirteen (76%) of the 17 patients had bilateral retinoblastoma, and all these patients had previously received systemic chemotherapy. Four of the 13 patients with bilateral retinoblastoma had had the other eye enucleated. Nine patients had received intra-arterial chemotherapy as a salvage treatment before the plaque treatment. Two patients underwent plaque brachytherapy for the same eye for different tumors. Initial regression was observed in 17 of the 17 eyes that underwent brachytherapy (Figures 1-4). Enucleation was performed in 5 (29,42 %) of these patients due to diffuse tumor recurrence. In total, globe salvage was ensured in 12 (70.58%) eyes among the 17 patients after brachytherapy (Figures 1-4). The local control rate was 70.58 % at 2 years (Figure 5). In all the patients who required enucleation, the surgery was performed within the first 6 months of therapy. Two (11.76%) patients developed cataract, and 2 (11.76%) had radiation maculopathy after treatment. One patient who

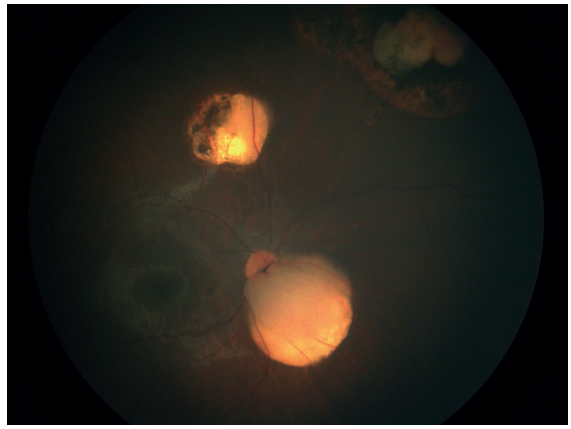


**Fig. 1.** Patient 1 peripapillary tumor recurrences after systemic chemotherapy.

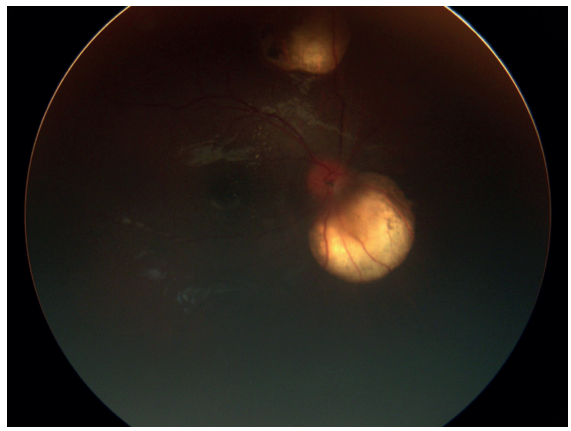


**Fig. 2.** The tumor was successfully treated by notched iodine-125 plaque brachytherapy.

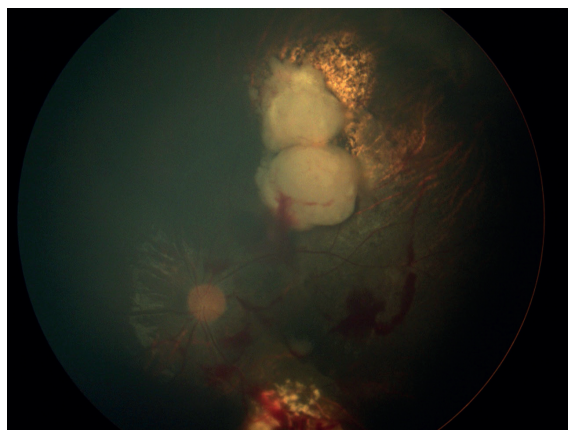


**Fig. 3.** Patient 12, tumor recurrences beneath to the previous scar. The fellow eye was enucleated.

**Table I.** Tumors demographic features, previous treatments and brachytherapy results case by case.

| Patients' demographics | | | | | | | Treatment | | | Follow-up (mo) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient no | Age @treatment (mo) | Gender | OD / OS / OU | Stage of treated eye | Stage of fellow eye ICRB | Tumor location | Systemic chemotherapy | IAC | IVC, no | Brachytherap time from dx | Post op | Enucleation |
| 1 | 24 | M | OU | OD/D | B | peripapillary | yes | no | no | 19 | 42 | - |
| 2 | 22 | F | OU | OD/B | B | macular | yes | no | no | 16 | 24 | - |
| 3 | 38 | M | OU | OD/D | enucleated | post equatorial | yes | no | no | 28 | 44 | - |
| 4 | 32 | F | OS | OS/B | Normal | peripapillary | yes | yes | no | 26 | 16 | - |
| 5 | 56 | F | OU | OD/D | B | pre equatorial | yes | yes | yes ,2 | 48 | 4 | + |
| 6 | 58 | M | OD | OD/C | Normal | pre equatorial | yes | no | yes ,2 | 46 | 18 | - |
| 7 | 36 | M | OU | OS/D | B | post equatorial | yes | yes | yes,2 | 14 | 6 | + |
| 8 | 32 | M | OD | OD/D | Normal | pre equatorial | yes | yes | yes,3 | 18 | 16 | - |
| 9 | 24 | F | OU | OD/B | B | macular | yes | yes | no | 14 | 24 | - |
| 10 | 30 | M | OU | OD/D | B | pre equatorial | yes | yes | yes,3 | 10 | 4 | + |
| 11 | 36 | M | OU | OD/B | D | post equatorial | yes | no | no | 24 | 46 | - |
| 12 | 24 | M | OU | OS/D | enucleated | post equatorial | yes | yes | yes,1 | 14 | 34 | - |
| 13 | 30 | F | OU | OD/D | B | pre equatorial | yes | no | yes,3 | 16 | 4 | + |
| 14 | 28 | M | OU | OS/B | enucleated | macular | yes | yes | no | 18 | 46 | - |
| 15 | 26 | M | OU | OD/D | enucleated | pre equatorial | yes | no | no | 16 | 50 | - |
| 16 | 26 | F | OD | OD/D | Normal | pre equatorial | yes | yes | yes,4 | 14 | 4 | + |
| 17 | 34 | M | OU | OS/D | B | post equatorial | yes | yes | yes,2 | 16 | 12 | - |

OD: Right eye, OS: Left eye, OU: Both eyes mo: months
ICRB: intraocular classification of retinoblastoma; IAC: intra-arterial chemotherapy, IVC: intravitreal chemotherapy, RAP: radioactive plaque brachytherapy Post op: Post operation after plaque brachytherapy, Enuc: enucleation

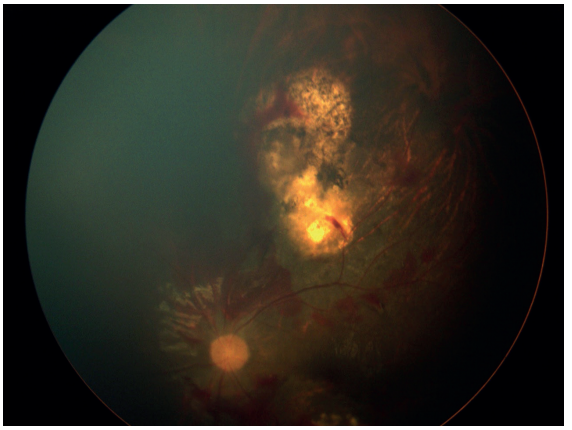


**Fig. 4.** The tumor totally regressed after iodine-125 plaque brachytherapy.

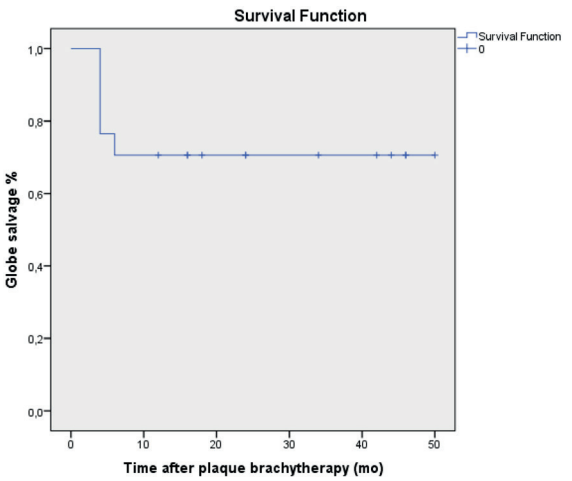



**Fig. 5.** Kaplan-Meier analysis of globe salvage.

had high risk factors pathologically was lost during follow-up after enucleation. They had subsequently developed metastasis and died 6 months after enucleation.

## Discussion

The primary goal in the treatment of retinoblastoma is to ensure survival and, if possible, to protect the eye. The success of globe-protecting treatment has increased with the introduction of localized therapies such as intra-arterial chemotherapy and intravitreal chemotherapy following reduction with systemic chemotherapy.[10,11]

In their 1993 study, Hernandez et al. mentioned plaque brachytherapy as an effective primary treatment modality as well as in cases where other modalities had failed.[15] In recent years, several reports have shown that brachytherapy is still on the agenda of ocular oncologists in the era of intra-arterial chemotherapy. In the study conducted by Echegaray et al. in 2019, only 2 of their 11 patients who received brachytherapy had recurrence.[16] In that study, both recurrent eyes were identified as Group D tumors. No recurrences were observed in the Group A, B, or C patients. In our study, no recurrence was observed in either the Group B or C tumors, whereas recurrence was seen in the Group D patients. Eye preservation was achieved with brachytherapy in 12 (70.58%) eyes of the 17 patients in our study, all of whom had already received intra-arterial chemotherapy or systemic chemotherapy. In their study, Francis et al. reported their brachytherapy results as a salvage/adjuvant following intra-arterial chemotherapy for intraocular retinoblastoma and concluded that brachytherapy was effective after intra-arterial chemotherapy.[17]

We had a high success rate with the group B and C tumors in our study. Recurrences after plaque brachytherapy were seen in the group D tumors located anterior to the equator and were eventually enucleated. Solitary tumors with no previous intravitreal chemotherapy (IVC) or tumors that had received limited IVC had a higher success rate. We observed that the failed group consisted of tumors that responded very well to the initial therapy but had a diffuse endophytic recurrence early within the first year following brachytherapy. The other risk factor was previous IVC. We concluded that the eyes that had produced vitreous or subretinal seedings were more prone to failure after brachytherapy.

We preferred brachytherapy over intra-arterial chemotherapy in the patients whose fellow eyes were already enucleated and had solitary recurrences. Stathopoulos et al. reported a

17% risk of acute choroidal ischemia after intra-arterial chemotherapy, and one third of these patients in their study developed total visual loss.[14] Shields et al. also detected retinal vascular abnormalities at a rate of 13% and choroidal vascular abnormalities at a rate of 11% by fluorescein angiography after intra-arterial chemotherapy.[18] Our main concern was potential severe vascular (e.g., total ophthalmic artery occlusion) complications as a result of intra-arterial chemotherapy in the only seeing eye. In our opinion, radiation complications can be more easily managed than severe vascular complications following intra-arterial chemotherapy in patients who have only one functional eye. Although it is very well known that external beam radiotherapy increases secondary cancer risk in retinoblastoma patients, there have been no reports of secondary cancer after radioactive plaque brachytherapy.[19,20] In our study, we did not observe any secondary cancer following brachytherapy.

Among our patients, two (11.76%) developed cataract, and two (11.76%) had radiation maculopathy. Abouzeid et al. reported only one case of radiation retinopathy in their patients who received an average dose of 50 Gy to the tumor apex using Ru-106.[21] Echegaray et al. confirmed a rate of 18% for non-proliferative retinopathy and 9% for cataract after a mean apical dose of 44 Gy.[16] Our apical radiation dose (45–50 Gy) was similar to that used in those studies, with similar results. Clinicians should therefore follow up if the development of radiation retinopathy side effects is suspected in these patients.

The weakness of our study was its retrospective design. However, in the management of retinoblastoma, the treatment modality is decided individually and based on many different parameters, such as patient age, the condition of the fellow eye, and previous treatment responses. Because of this, even if a treatment protocol has been established, the tumor response can affect the treatment choice dynamically.

A strength of our study was that each patient's follow-up was undertaken by the same experienced ocular oncologist and recorded in detail to allow the collection of homogenous data that could be analyzed without any bias. This further provided a standard evaluation of each patient. An additional strength was the relatively long mean follow-up (almost 2 years) after radioactive plaque treatment.

In conclusion, radioactive plaque brachytherapy can be applied as an effective treatment option with successful results in retinoblastoma patients who have received systemic or intra-arterial chemotherapy prior to brachytherapy. Our results were similar to those of previous reports. Plaque brachytherapy can be used not only in Group A and B tumors, but also in advanced tumors as a globe-saving procedure. Tumors located in the post-equatorial region in our study responded with a high success rate. Eyes with anteriorly located recurrences and eyes that had previously had multiple IVC to control seedings had a lower chance of treatment success with brachytherapy. Solitary tumors located posteriorly were the best candidates for brachytherapy, and brachytherapy can thus be recommended to patients' parents who are concerned about the vascular complications of intra-arterial chemotherapy.

## Ethical approval

Following the approval of the local ethics committee (Cerrahpaşa School of Medicine Ethic Committee reference no.: E-83045809-604.01.02-35245).

## Author contribution

The authors contribution to the paper as follows: study conception and design: AMS, AŞ, ÖU; data collection: BBO, AŞ; analysis and interpretation of results: AMS, TTC, DU; draft manuscript preparation: AMS, AŞ, TTC, ÖU. 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. AlAli A, Kletke S, Gallie B, Lam WC. Retinoblastoma for Pediatric Ophthalmologists. Asia Pac J Ophthalmol (Phila) 2018; 7: 160-168. https://doi.org/10.22608/apo.201870
2. Foix-L'Hélias L, Aerts I, Marchand L, et al. Are children born after infertility treatment at increased risk of retinoblastoma?. Hum Reprod 2012; 27: 2186-2192. https://doi.org/10.1093/humrep/des149
3. Dimaras H, Corson TW, Cobrinik D, et al. Retinoblastoma. Nat Rev Dis Prim 2015; 27: 15021. https://doi.org/10.1038/nrdp.2015.21
4. Andreoli MT, Chau FY, Shapiro MJ, et al. Epidemiological trends in 1452 cases of retinoblastoma from the Surveillance, Epidemiology, and End Results (SEER) registry. Can J Ophthalmol 2017; 52: 592-598. https://doi.org/10.1016/j.jcjo.2017.05.012
5. Özkan A, Pazarli H, Celkan T, et al. Retinoblastoma in Turkey: survival and clinical characteristics 1981-2004. Pediatr Int 2006; 48: 369-373. https://doi.org/10.1111/j.1442-200X.2006.02223.x
6. Gündüz AK, Mirzayev I, Temel E, et al. A 20-year audit of retinoblastoma treatment outcomes. Eye (Lond) 2020; 34: 1916-1924. https://doi.org/10.1038/s41433-020-0898-9
7. Waddell K, Matua M, Bidwell C, et al. A ten-year study of retinoblastoma in Uganda: an approach to improving outcome with limited resources. Cancer Epidemiol. 2021; 71: 101777. https://doi.org/10.1016/j.canep.2020.101777
8. Soliman SE, Racher H, Zhang C, MacDonald H, Gallie BL. Genetics and molecular diagnostics in retinoblastoma--an update. Asia Pac J Ophthalmol (Phila) 2017; 6: 197-207. https://doi.org/10.22608/apo.201711
9. Mendoza PR, Grossniklaus HE. Therapeutic options for retinoblastoma. Cancer Control 2016; 23: 99-109. https://doi.org/10.1177/107327481602300203
10. Shields CL, Shields JA. Retinoblastoma management: advances in enucleation, intravenous chemoreduction, and intra-arterial chemotherapy. Curr Opin Ophthalmol 2010; 21: 203-212. https://doi.org/10.1097/ICU.0b013e328338676a
11. Carcaboso AM, Moulin A, Schaiquevich P, et al. Conservative management of retinoblastoma: challenging orthodoxy without compromising the state of metastatic grace. "Alive, with good vision and no comorbidity". Prog Retin Eye Res 2019; 73: 100764. https://doi.org/10.1016/j.preteyeres.2019.05.005
12. Shields CL, Say EA, Pointdujour-Lim R, et al. Rescue intra-arterial chemotherapy following retinoblastoma recurrence after initial intra-arterial chemotherapy. J Fr Ophtalmol 2015; 38: 542-549. https://doi.org/10.1016/j.jfo.2015.03.004
13. Shields CL, Kaliki S, Al-Dahmash S, et al. Management of advanced retinoblastoma with intravenous chemotherapy then intra-arterial chemotherapy as alternative to enucleation. Retina 2013; 33: 2103-2109. https://doi.org/10.1097/IAE.0b013e318295f783
14. Stathopoulos C, Bartolini B, Marie G, et al. Risk factors for acute choroidal ischemia after intra-arterial melphalan for retinoblastoma: the role of the catheterization approach. Ophthalmology 2021; 128: 754-764. https://doi.org/10.1016/j.ophtha.2020.09.021
15. Hernandez JC, Brady LW, Shields CL, Shields JA, DePotter P. Conservative treatment of retinoblastoma. The use of plaque brachytherapy. Am J Clin Oncol 1993; 16: 397-401. https://doi.org/10.1097/00000421-199310000-00005
16. Echegaray JJ, Al-Zahrani YA, Singh A. Episcleral brachytherapy for retinoblastoma. Br J Ophthalmol 2020; 104: 208-213. https://doi.org/10.1136/bjophthalmol-2019-313985
17. Francis JH, Barker CA, Wolden SL, et al. Salvage/adjuvant brachytherapy after ophthalmic artery chemosurgery for intraocular retinoblastoma. Int J Radiat Oncol Biol Phys 2013; 87: 517-523. https://doi.org/10.1016/j.ijrobp.2013.06.2045
18. Bianciotto C, Shields CL, Iturralde JC, Sarici A, Jabbour P, Shields JA. Fluorescein angiographic findings after intra-arterial chemotherapy for retinoblastoma. Ophthalmology 2012; 119: 843-849. https://doi.org/10.1016/j.ophtha.2011.09.040
19. Imhof SM, Mourits MP, Hofman P, et al. Quantification of orbital and mid-facial growth retardation after megavoltage external beam irradiation in children with retinoblastoma. Ophthalmology. 1996; 103: 263-268. https://doi.org/10.1016/S0161-6420(96)30706-9

20. Wong FL, Boice JD Jr, Abramson DH, et al. Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. JAMA 1997; 278: 1262-1267. https://doi.org/10.1001/jama.1997.03550150066037

21. Abouzeid H, Moeckli R, Gaillard MC, et al. Ruthenium brachytherapy for retinoblastoma. Int J Radiat Oncol Biol Phys 2008; 71: 821-828. https://doi.org/10.1016/j.ijrobp.2007.11.004

The Turkish Journal of Pediatrics 2022; 64: 293-301
https://doi.org/10.24953/turkjped.2020.1714

**Original Article**

# Developing growth reference charts for the head circumference of Pakistani children aged 6 to 18 years

Muhammad Asif[1], Muhammad Aslam[2], Tariq Ismail[3], Akasha Rahman[2], Nasir Saleem[2]

*[1]Department of Statistics, Govt. Degree College, Qadir Pur Raan, Multan, Pakistan; [2]Department of Statistics and [3]Institution of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan.*

## ABSTRACT

**Background.** Head circumference (HC) measurement is a significant measure of brain volume. It is also considered a powerful predictor in the evaluation of developmental and neurological disorders in children. This study aims to develop smoothed reference curves for HC of the Pakistani children of age 6 to 18 years.

**Methods.** A cross-sectional dataset, consisting of 9194 school-going children of age 6-18 years, were obtained using a multi-ethnic anthropometric survey. For the measurement of HC (cm), the standard procedure was adopted. For both sexes, the smoothed centile curves of HC were developed by using the lambda-mu-sigma (LMS) statistical approach. Moreover, we compared our 50th percentile curves to those produced for few other countries.

**Results.** The centiles curves of both sexes indicated that the HC increased with age. Until the age of 10 years, the boys had larger HC percentiles than those of the girls. From the age of 11 years, upper percentiles (90th, 95th and 97th) of the girls were higher than those of the boys. The comparison of our 50th percentile data for the HC with the data from the United States (US) and Turkish children revealed that the Pakistani children of both genders had smaller head sizes in all ages when compared to those reported for the latter stated countries.

**Conclusions.** Our results show the larger disparity of HC percentiles in different countries. This comprehensive study suggests that the references from the US Centers for Disease Control and Prevention data and other populations are not suitable for Pakistani children. Therefore, each country is required to create its own HC reference curves, separately.

**Key words:** head circumference, lambda-mu-sigma method, Pakistani children, growth reference curves.

Measuring the head circumference (HC) is one of the most crucial tasks during a pediatric physical examination. The HC of infants and children correlates with cognitive functions, intracranial volume, and brain volume.[1-3] Therefore, pediatricians and neurologists frequently use the HC standard charts as a valuable tool to trace the brain development in children and diagnose neurological diseases, if any.

✉ Muhammad Asif
asifmalik722@gmail.com

Received 22nd June 2020, revised 8th September 2020, 18th March 2021, 25th April 2021, accepted 23rd May 2021.

In 2000, the HC growth charts for US children were produced by the Centers for Disease Control and Prevention (CDC).[4] In this research, only children aged birth to 36 months were studied. In 2002, the US epidemiological researchers found that the HC was shown to be an "important magnitude to predict the brain volume in children aged 7 to 16 years (r = 0.67)" as well as a "excellent predictor of brain volume in children aged 1.7 to 6 years (r = 0.93).[1] Similar correlation was also reported for adults aged 17 to 42 years (r = 0.69).[1] After that, various studies in different countries have reported the HC charts for the justification of monitoring head growth beyond 36 months. For example, Rollins et al.[5] included the US pediatric population

aged birth to 21 years for presenting the HC growth reference charts and Neyzi et al.[6] in 2015, produced HC growth reference curves for the sample of Turkish children, aged birth to 18 years. Another study[7] established the HC reference charts for 5 to 18 years old Turkish children. Zaki et al.[8] presented the HC data by using a sample of 27,826 healthy Egyptian children and adolescents.

The reference data on head size for children of different developing countries is very limited. To the best of our knowledge, one study[9] from Iran presented the HC standards for school-going children aged 6.5-11.5 years using a sample of 2237 children during the years, 2002-3 and a more recent study[10] during 2016 from Pakistan, presented the HC charts for children of 2 to 5 years. However, the HC reference data for children aged 6 to 18 years are still not that much available. There is also a significant disparity in head sizes among the children of different countries as well as among different ethnicities of the same country[6-9], suggesting that growth references for each country should be established separately. Therefore, we present a study with the developed HC growth reference curves for 6 to 18 years Pakistani children. This data would be helpful not only for the child neurologists in Pakistan but also for neurologists of the region.

## Material and Methods

### *Study population and design*

Our study enrolled 9194 school-going children who participated in a multi-ethnic anthropometric survey (MEAS) conducted in 2016. The MEAS was conducted in three densely populated cities of the Punjab province (i.e., Lahore, located in central Punjab; Rawalpindi, located in North of Punjab and Multan, located in south Punjab) and the capital city, Islamabad. The main reasons for choosing these cities were their growing educational & health facilities and sufficient job opportunities in the public and private sectors. For these reasons, families have more commonly migrated to these cities from the other regions of the country. Furthermore, the Pakistan National Human Development Report (PNHDR-2017) also placed these cities in the medium to high human development category.[11] The mixed- ethnic population of these cities can be expected to be a representative of Pakistan's pediatric population. In previous studies[12,13], further information on the children's selection in this multi-ethnic survey may be found. Briefly, the studied children aged 6 to 18 years were sampled from different schools. On demand of an investigator, Punjab, and the Federal Department of Education (Schools) provided a grade-by-grade complete list of schools (i.e., elementary, secondary, and higher secondary schools) in the designated cities, and schools were picked using simple random sampling from the lists. Each school's classes were chosen at random, and all the children present on the day of data collection were invited to take part in the study. In order to obtain the HC measurement, a written informed consent was taken from both the school's heads and children's parents. The authors claim that all procedures used in this study adhere to the Helsinki Declaration and the ethical norms of relevant national and institutional committees on human experimentation (2013). The Departmental Ethics Committee of Bahauddin Zakariya University, Multan. Pakistan approved this study (Approval Date: 13 March 2017, IRB #: Stat-271/2017).

Although in the MEAS, different measurements were collected by the data collection team members, but the measurement of HC is of concern here. The HC was measured using a non-stretch tape after following the standard techniques.[14] During measurement, the children was instructed to look straight, and tape was placed over the child's head in the distance from above the eyebrows and ears and around the back of the head to get the maximum circumference. The HC measures were collected by the same experts to avoid possible bias (for further details about the data collection, see Aslam et al.[10] and Asif et al.[12]).

For this study, inclusion criteria were: (a) agreeing to participate in the study, (b) age between 6 and 18 years, (c) absence of congenital disorders that may affect the HC (confirmed based on information from the parents). Whereas exclusion criteria were (a) having a chronic, disease such as renal failure, cystic fibrosis, Celiac disease, and non-idiopathic epilepsy etc. (b) history of premature delivery.

### *Statistical analysis*

The descriptive analyses for quantitative variables, age (years) and HC (cm) are described as mean with standard deviation (SD). The LMS statistical method, proposed by Cole[15,16], was used to estimate the age and gender specific smoothed set of centiles "3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th" of HC. This technique represents the evolving HC distribution in terms of three curves: the Box-Cox power (L) to remove skewness from the data by age, the median (M), and the coefficient of variation (CV) (S). The smooth spline function in R was used to smooth these three curves. The needed centile (C) value for a given age was calculated as C= M (1+LSZ) 1/L, where L, M, and S are the fitted curve values, and Z is the normalized distribution's Z-score. Z was substituted as -1.88, -1.645, 0.00, 1.645, and 1.88, respectively, for predicting the 3rd, 5th, 50th, and 95th and 97th percentile values.[15] The software "Statistical Package for Social Sciences (SPSS) version 21.0" and R version 3.2.0 were used to analyze the data.

## Results

Mean, standard deviation (SD), the frequency distribution of subjects by age and age-and-sex-specific smoothed percentile values of HC are listed in Table I. Fig. 1(a) and Fig. 1(b) displayed the HC percentile curves for both boys and girls, respectively. The centiles curves of both sexes indicated that HC increase with age. Sex-wise notable differences in data of the HC were also seen. The lower percentiles (3rd, 5th, 10th and 25th) of boys were higher than girls except between the ages of 11 and 12 years. It was noted that the girls' HC during 11- and 12-years of age was larger than those of boys. The upper percentiles (90th, 95th and 97th) of girls were also higher than of boys after 13 years of age. The LMS-derived median (50th) percentile curves were compared with those for the data from the US[5] and Turkish children[6] through Fig. 2 (a + b). The HC values for the Pakistani children of both genders were lower than the reference values from the latter stated studies.

## Discussion

Growth monitoring during infancy and childhood age is very crucial for pediatric care and growth charts of length /height, weight and HC are commonly used for this purpose. The gathering of reference data for the study of growth in HC among children of similar ethnic backgrounds is a fundamental goal of auxologic investigations.[17] The HC growth charts of children are considered as powerful anthropometric tools for monitoring brain growth and diagnosing neurological disorders, because the size of HC is tightly connected to cognitive function, intracranial volume, and brain volume.[1,3,17,18] Furthermore, microcephaly and macrocephaly may be linked to a variety of medical issues, including different syndromes.[17] According to Winter and Baraitser[19], there are 114 syndromes that are linked to macrocephaly. Among these, macrocephaly perseveres into adulthood and the most common of which is Fragile X syndrome.[20] Some intrauterine infections may also be related to both microcephaly and macrocephaly.[17] All of these emphasize the need for some valid HC reference charts for higher ages also.

Up to 3 years of age, head size reaches approximately 90% of the adult size and clinicians usually do not recommend the routine follow-up of the HC growth after this age for normal developing children. However, some neurological disorders and genetic syndromes may appear after 3 years of age among abnormally developing children and any significant reduction in the HC found

**Table I.** Age-and- gender-specific Mean, SD and smoothed HC (cm) percentiles (3rd to 97th) for the Pakistani boys and girls, aged 6-18 years.

| Age (years) | Smoothed Percentiles | | | | | | | | | | Mean (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 3rd | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 97th | |
| Boys (n=4972) | | | | | | | | | | | |
| 6 | 292 | 46.79 | 47.14 | 47.69 | 48.64 | 49.74 | 50.88 | 51.95 | 52.61 | 53.05 | 49.77 (1.66) |
| 7 | 279 | 47.09 | 47.45 | 48.02 | 48.98 | 50.09 | 51.23 | 52.30 | 52.95 | 53.38 | 50.17 (1.73) |
| 8 | 273 | 47.20 | 47.59 | 48.19 | 49.21 | 50.33 | 51.46 | 52.49 | 53.31 | 53.66 | 50.31 (1.58) |
| 9 | 247 | 47.43 | 47.86 | 48.57 | 49.48 | 50.57 | 51.76 | 52.92 | 53.37 | 54.05 | 50.71 (1.71) |
| 10 | 420 | 47.56 | 48.05 | 48.74 | 49.68 | 50.79 | 51.97 | 53.10 | 53.61 | 54.02 | 50.84 (1.74) |
| 11 | 439 | 47.69 | 48.14 | 48.82 | 49.92 | 51.09 | 52.22 | 53.19 | 53.76 | 54.12 | 51.06 (1.67) |
| 12 | 675 | 48.18 | 48.59 | 49.22 | 50.27 | 51.42 | 52.56 | 53.58 | 54.18 | 54.57 | 51.43 (1.79) |
| 13 | 593 | 48.80 | 49.16 | 49.74 | 50.72 | 51.84 | 53.00 | 54.08 | 54.74 | 55.17 | 51.80 (1.63) |
| 14 | 563 | 49.16 | 49.60 | 50.27 | 51.35 | 52.51 | 53.62 | 54.59 | 55.15 | 55.51 | 52.50 (1.75) |
| 15 | 546 | 49.72 | 50.16 | 50.83 | 51.90 | 53.04 | 54.13 | 55.07 | 55.62 | 55.97 | 53.01 (1.68) |
| 16 | 381 | 50.26 | 50.65 | 51.24 | 52.23 | 53.31 | 54.39 | 55.34 | 55.91 | 56.28 | 53.32 (1.59) |
| 17 | 169 | 50.69 | 51.02 | 51.54 | 52.43 | 53.44 | 54.50 | 55.49 | 56.10 | 56.50 | 53.44 (1.53) |
| 18 | 95 | 51.10 | 51.41 | 51.88 | 52.71 | 53.68 | 54.71 | 55.68 | 56.29 | 56.70 | 53.76 (1.46) |
| Girls (n=4222) | | | | | | | | | | | |
| 6 | 405 | 46.00 | 46.36 | 46.94 | 47.93 | 49.09 | 50.31 | 51.48 | 52.20 | 52.68 | 49.17 (1.80) |
| 7 | 381 | 46.39 | 46.77 | 47.38 | 48.41 | 49.58 | 50.80 | 51.94 | 52.63 | 53.09 | 49.61 (1.66) |
| 8 | 376 | 47.00 | 47.40 | 48.02 | 49.08 | 50.28 | 51.50 | 52.63 | 53.10 | 53.56 | 50.30 (1.90) |
| 9 | 336 | 47.28 | 47.68 | 48.30 | 49.37 | 50.57 | 51.79 | 52.92 | 53.60 | 53.76 | 50.60 (1.77) |
| 10 | 459 | 47.47 | 47.88 | 48.51 | 49.57 | 50.78 | 52.00 | 53.12 | 53.80 | 54.24 | 50.82 (1.71) |
| 11 | 325 | 48.12 | 48.54 | 49.18 | 50.27 | 51.49 | 52.72 | 53.84 | 54.52 | 54.96 | 51.45 (1.85) |
| 12 | 436 | 48.39 | 48.80 | 49.45 | 50.54 | 51.75 | 52.99 | 54.11 | 54.78 | 55.22 | 51.80 (1.90) |
| 13 | 460 | 48.68 | 49.11 | 49.78 | 50.89 | 52.12 | 53.34 | 54.44 | 55.10 | 55.52 | 52.17 (1.86) |
| 14 | 341 | 48.98 | 49.45 | 50.17 | 51.34 | 52.59 | 53.80 | 54.86 | 55.48 | 55.87 | 52.50 (1.84) |
| 15 | 257 | 49.23 | 49.71 | 50.44 | 51.62 | 52.87 | 54.08 | 55.12 | 55.73 | 56.12 | 52.83 (1.81) |
| 16 | 191 | 49.56 | 50.00 | 50.67 | 51.78 | 53.01 | 54.22 | 55.31 | 55.95 | 56.37 | 52.92 (2.04) |
| 17 | 129 | 49.95 | 50.31 | 50.87 | 51.86 | 53.03 | 54.29 | 55.50 | 56.27 | 56.78 | 53.13 (1.67) |
| 18 | 126 | 50.26 | 50.56 | 51.03 | 51.90 | 53.01 | 54.32 | 55.73 | 56.73 | 57.45 | 53.22 (1.77) |

HC: head circumference, SD: standard deviation

in malnourished children may have serious implications for their future performance and achievement.[18] A colossal literature have also demonstrated that serial HC measurements taken in infancy or prepubertal period are a reliable predictor of brain volume and may be used to map the course of brain growth, hence predicting cognitive performance later in life.[1,3,21,22] A Helsinki Birth Cohort Study (HBCS) has demonstrated that infancy, childhood, and adolescent periods are critical for the development of intellectual abilities and slow HC growth during infancy may continue to appear in childhood and adolescents that extend widespread consequences on mental health throughout the lifespan.[23] In a prospective study of children aged 9 to 10 years old from Southern India, HC was found to be positively linked with learning and visio-spatial abilities.[22,24] Thus, population-specific HC references are needed for clinical evaluations and early detection of HC overgrowth and undergrowth.

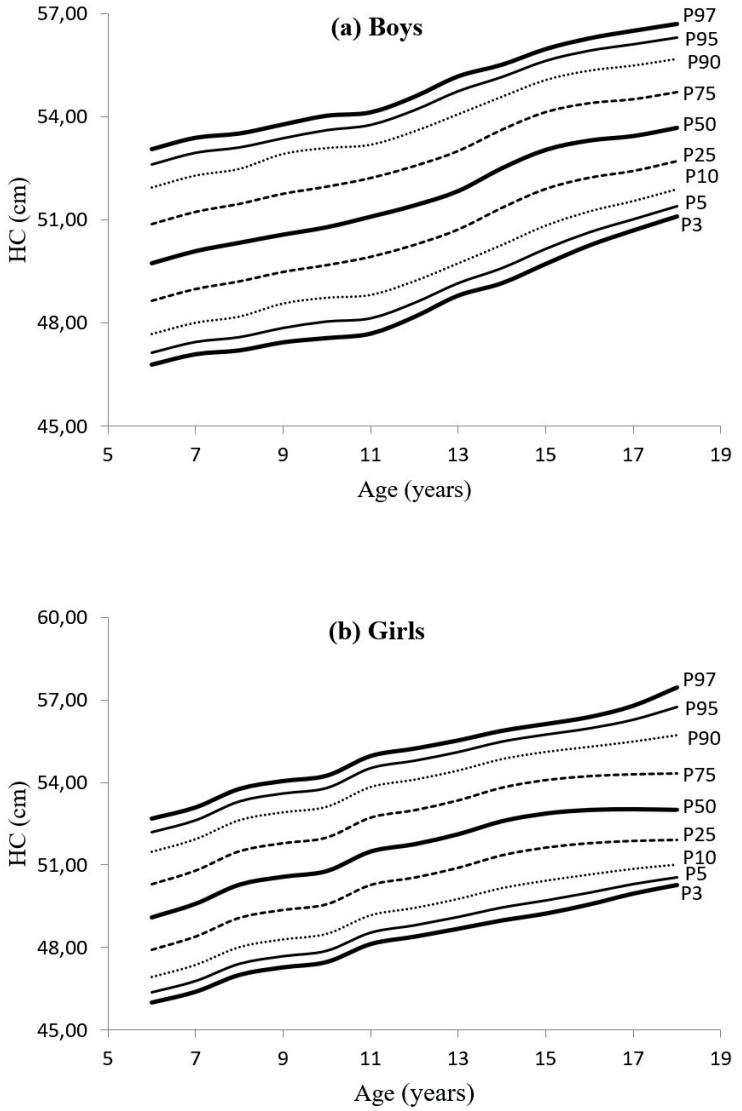



**Fig. 1. (a) and (b):** Smoothed head circumference growth curves for the Pakistani boys and girls using the LMS method.

To the authors' best knowledge, after an Iranian research[9], this is the first comprehensive study in the developing nations that presented the reference values of HC for 6 to 18 years aged children using a standardized measurement technique. As a result, we attempted to compare our findings to those of Iranian[9] and other international investigations.[5-7] Our findings revealed that, except for the ages 11 to 13 years, boys have higher mean HC values than. A study with Iranian children[9] found parallel findings for the mean values of HC and the mean HC of girls was higher around 11 years age. In our study, the mean differences in HC between boys and girls decreases before puberty, become negative around pubertal age, and increases after puberty. These findings were also consistent with the Iranian study.[9] The most recent study by Kara et al.[7] also accounted for the lower differences in pubertal age (around 0.54 to 0.59cm) and after puberty, the difference gradually increases. This lower difference in pubertal age may be due to the fact that pubertal growth spurt occurs earlier in girls than boys.

Similar to the previous studies[5-7,9], the percentile values (3rd to 97th) of HC increased in both sexes with age. Sex-related differences in HC percentiles indicated that all percentile values among boys were larger than girls till the age of 10 years. From 11 years of age, upper percentiles (90th, 95th and 97th) of girls were higher than boys that were consistent with a study of Iranian children.[9] Another study with the Turkish children[25] aged between 6 and 12 years also report a higher percentile (98th) value for girls at the age of 12 years.

Several studies[26,27] confirmed the disparity in the head sizes of children belongs to different countries as well as in the children of different ethnic groups of the same country. Recent data from Turkish children collected by Kara et al.[7] greatly differed from the US children and Iranian data by Ayatollahi and Shayan[9] also highly differed from the Japanese, Turkish, Irish and US children. We also compared our 50th percentile values for HC with the data from the US[5] and Turkish studies.[6] Our HC reference values were moving parallel to those of other countries. However, they were considerably smaller than the corresponding international data (see Fig 2. (a) and (b). It is important to discuss here some visible factors that may associate with the low HC percentile values in children. Children grow differently over the globe[28], and we know that the growth and body shape of children is assessed by different anthropometric parameters in the form of height for age, weight for age, and body mass index (BMI) for age etc. The studies[29,30] with the Pakistani children already showed that Pakistani boys and girls had lower height, weight and BMI as compared to the WHO and US CDC references population. A study by Bushby et al.[31] emphasized that the HC was closely related to the height of the individual. Another study by Nguyen et al.[32] with Canadian male adults also found a significant positive correlation between HC and height and weight. This could explain the results of our study in which we indicated that HC 50th percentile of Pakistani children and adolescents was significantly smaller than the US population. Moreover, multiple environmental factors also have a great influence on the head size of children including maternal education and diet, maternal intelligent quotient, maternal BMI, socio-economic profile, child birth weight, exclusive breast feeding, maternal smoking and maternal reproductive history etc.[33] These factors are very common among Pakistani women and may have caused the disparity in HC references values in different populations and emphasize the need for every population to develop their own HC standards.

In Pakistan, WHO HC growth standards[34] (birth to 5 years) are used by child neurologists to see the growth in the brain of children. But there are no HC standards over 5 years of age children. The major strength of our study is that we developed HC reference data by using a sample of healthy Pakistani children aged 6 to 18 years that are free from any neurological disorders. Our developed reference data are satisfactory for local use than for use in other countries. This information can be used to identify neurological abnormalities in children whose head size are beyond the normal ranges and these abnormal population of children can be referred to neurologists for further neurological diagnoses and monitoring as mentioned in an earlier study.[9] Using the LMS method, we presented HC-for-age growth reference curves based on a comprehensive sample of Pakistani children from 6 to 18 years of age which is the first for the Pakistani pediatric population of a wide age range. It was reported that boys had a larger average head size than girls except for pubertal age. The results showed a significant disparity between our centiles and centiles of the other populations. Based on these findings and to avoid wrong interpretation, it is recommended that each country should produce its own HC growth charts. Since the current and earlier local published study[10] did not include the HC data of newborn children or children up to 2 years of age and the results of this study suggest that this gap might be filled because a sufficient increase in head size is shown during this age.

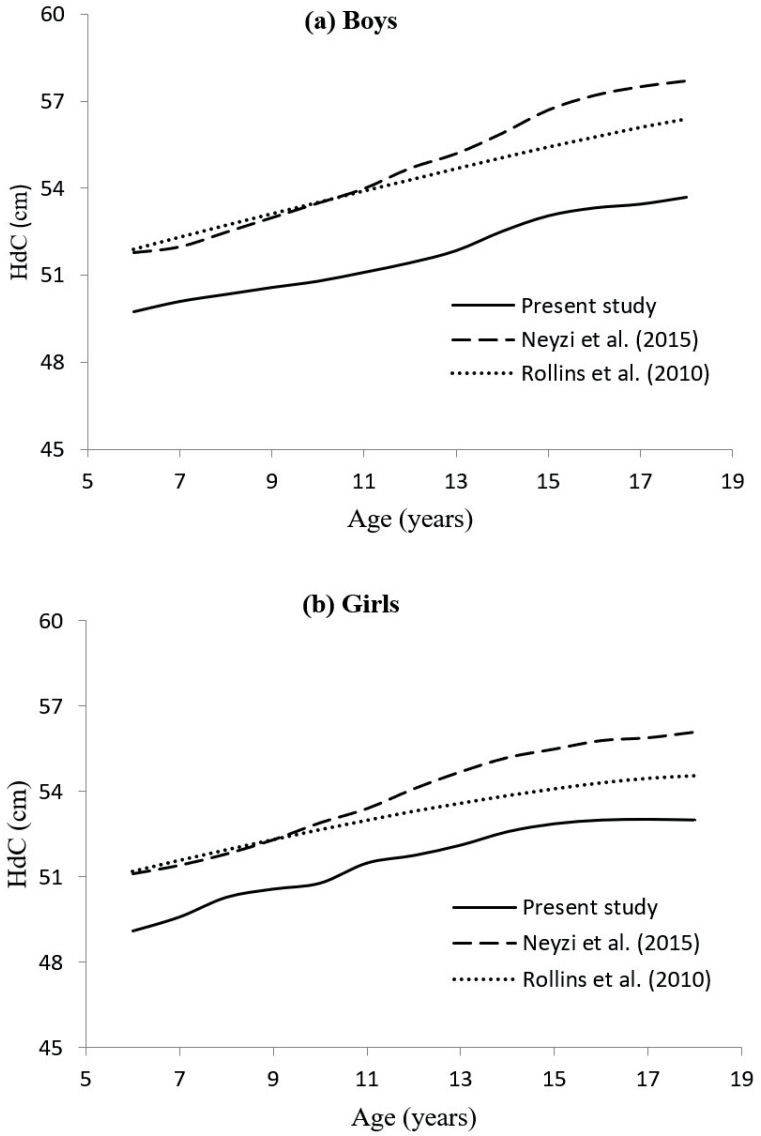



**Fig. 2. (a) and (b):** Comparison of median (50th) head circumference (cm) percentiles for the Pakistani boys and girls with the US (Rollins et al., 2010) and Turkish children (Neyzi et al., 2015).

**Acknowledgements**

The authors would like to thank school heads and the parents and guardians of children who cooperated with our data collection team during this study. We are also thankful to all data collection team members who took a keen interest in data collection and enthusiastically took measurements using standard procedures. We are grateful to Justyna Wyszyńska, a Polish medical researcher who also studied it critically and gave her expert opinion in finalizing the manuscript.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: MA and TI and MA; data collection: MA, AR, NS; analysis and interpretation of results: MA, MA, NS; drafting of manuscript: MA, TI, NA,

AR; critical review of manuscript: MA, AR, and TI. All authors reviewed the results and approved the final revision of the manuscript as submitted.

**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. Bartholomeusz HH, Courchesne E, Karns CM. Relationship between head circumference and brain volume in health normal toddlers, children, and adults. Neuropediatrics 2002; 33: 239-241. https://doi.org/10.1055/s-2002-36735
2. Holden KR. Heads you win, tails you lose: measuring head circumference. Dev Med Child Neurol 2014; 56: 705. https://doi.org/10.1111/dmcn.12449
3. Gale CR, O'Callaghan FJ, Bredow M, Martyn CN; Avon Longitudinal Study of Parents and Children Study Team. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics 2006; 118: 1486-1492. https://doi.org/10.1542/peds.2005-2629
4. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC growth charts for the United States: methods and development. Vital Health Stat 2002; 11: 1-190.
5. Rollins JD, Cllins JS, Holden KR. United States head circumference growth reference charts: birth to 21 years. J Pediatr 2010; 156: 907-913.e2. https://doi.org/10.1016/j.jpeds.2010.01.009
6. Neyzi O, Bundak R, Gokcay G, et al. Reference values of 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
7. Kara B, Etiler N, Uncuoglu AA, et al. Head circumference charts for Turkish children aged five to eighteen years. Arch Neuropsychiatr 2016; 53: 55-62. https://doi.org/10.5152/npa.2015.10202
8. Zaki ME, Hassan NE, El-Masry SA. Head circumference reference data for Egyptian children and adolescents. Eastern Maditer Health J 2008; 14: 69-81.
9. Ayatollahi SMT, Shayan Z. Head circumference standards for school children of Shiraz, Iran. J Trop Pediatr 2006; 52: 406-410. https://doi.org/10.1093/tropej/fml039
10. Aslam M, Asif M, Joiya SJ, Altaf S, Bhatti SH. Establishing growth reference charts for head circumference of the Pakistani children, using the Lambda-Mu-Sigma (LMS) Statistical Method. Iran J Pediatr 2019; 29: e84970. https://doi.org/10.5812/ijp.84970
11. Pakistan National Human Development Report 2017. Unleashing the Potential of a Young Pakistan. United Nations Development Programme (UNDP), Pakistan. Available at: http://hdr.undp.org/en/content/national-human-development-report-2017-pakistan (Accessed on May 07, 2018).
12. Asif M, Aslam M, Mustafa S, Altaf S. Age-specific differences, and interrelation between anthropometric variables in Pakistani children aged 2 to 19 years. Rawal Med J 2018; 43: 164-169.
13. Asif M, Aslam M, Altaf S. Evaluation of anthropometric parameters of central obesity in Pakistani children aged 5-12 years, using receiver operating characteristic (ROC) analysis. J Pediatr Endocrinol Metab 2018; 31: 971-977. https://doi.org/10.1515/jpem-2018-0193
14. Hall JG, Allanson JE, Gripp KW, Slavotinek AM. Handbook of Physical Measurements (2nd ed). Oxford University Press: New York, 2007.
15. Cole TJ. Fitting smoothed centile curves to reference data. J R Stat Soc Ser 1988; 151: 385-418. https://doi.org/10.2307/2982992
16. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44: 45-60.
17. Elmali F, Altunay C, Mazicioglu MM, et al. Head circumference growth reference charts for Turkish children aged 0-84 months. Pediatr Neurol 2012; 46: 307-311. https://doi.org/10.1016/j.pediatrneurol.2012.02.016
18. Oyedeji GA, Olamijulo SK, Osinaike Al, Esimai VC, Odunusi EO, Aladekomo TA. Head circumference of rural Nigerian children-the effect of malnutrition on brain growth. Cent Afr J Med 1997; 43: 264-268.
19. Winter RM, Baraitser M. London dysmorphology database. Oxford: Oxford University Press, 1996.

20. Williams CA, Dagli A, Battaglia A. Genetic disorders associated with macrocephaly. Am J Med Genet A 2008; 146A: 2023-2037. https://doi.org/10.1002/ajmg.a.32434

21. Lindley AA, Benson JE, Grimes C, Cole TM 3rd, Herman AA. The relationship in neonates between clinically measured head circumference and brain volume estimated from head CT-scans. Early Hum Dev 1999; 56: 17-29. https://doi.org/10.1016/S0378-3782(99)00033-X

22. Sindhu KN, Ramamurthy P, Ramanujam K, et al. Low head circumference during early childhood and its predictors in a semi-urban settlement of Vellore, Southern India. BMC Pediatrics 2019; 19: 1-11. https://doi.org/10.1186/s12887-019-1553-0

23. Räikkönen K, Forsén T, Henriksson M, et al. Growth trajectories and intellectual abilities in young adulthood: The Helsinki Birth Cohort study. Am J Epidemiol 2009; 170: 447-455. https://doi.org/10.1093/aje/kwp132

24. Veena SR, Krishnaveni GV, Wills AK, et al. Association of birthweight and head circumference at birth to cognitive performance in 9-to 10-year-old children in South India: prospective birth cohort study. Pediatr Res 2010; 67: 424-429. https://doi.org/10.1203/PDR.0b013e3181d00b45

25. Karabiber H, Durmaz Y, Yakıncı C, et al. Head circumference measurement of urban children aged between 6 and 12 in Malatya, Turkey. Brain Dev 2001; 23: 801-804. https://doi.org/10.1016/S0387-7604(01)00318-7

26. Meredith HV. Human head circumference from birth to early adulthood: racial, regional and sex comparisons. Growth 1971; 35: 233-251.

27. Palti H, Peritz E, Flug D, Gitlin M, Adler B. Comparison of head circumference in an Israeli child population with United States and British standards. Ann Hum Biol 1983; 10: 195-198. https://doi.org/10.1080/03014468300006351

28. Eveleth PB, Tanner JM. Worldwide Variation in Human Growth (2nd ed). Cambridge: Cambridge University Press, 1990. https://doi.org/10.1017/CBO9780511629105

29. Mushtaq MU, Gull S, Mushtaq K, et al. Height, weight and BMI percentiles and nutritional status relative to the international growth references among Pakistani school-aged children. BMC Pediatr 2012; 12: 31. https://doi.org/10.1186/1471-2431-12-31

30. Asif M, Aslam M, Wyszynska J, Altaf S. Establishing body mas index growth charts for Pakistani children and adolescents using the Lambda-Mu-sigma (LMS) and quantile regression method. Minerva Pediatr 2020; Online ahead of print. https://doi.org/10.23736/S0026-4946.20.05745-X

31. Bushby KM, Cole T, Matthews JN, Goodship JA. Centiles for adult head circumference. Arch Dis Child 1992; 67: 1286-1287. https://doi.org/10.1136/adc.67.10.1286

32. Nguyen AKD, Simard-Meilleur AA, Berthiaume C, Godbout R, Mottron L. Head circumference in Canadian male adults: development of a normalized chart. Int J Morphol 2012; 30: 1474-1480. https://doi.org/10.4067/S0717-95022012000400033

33. Brantsaeter AL, Birgisdottir BE, Meltzer HM, et al. Maternal seafood consumption and infant birth weight, length and head circumference in the Norwegian mother and child cohort study. Br J Nutr 2012; 107: 436-444. https://doi.org/10.1017/S0007114511003047

34. WHO Child Growth Standards. Head Circumference-for-age, Arm Circumference-for-age, Triceps Skinfolds-for-age, and Subscapular Skinfolds-for-age. Methods and Development. Geneva: World Health Organization, 2007.

The Turkish Journal of Pediatrics 2022; 64: 302-311
https://doi.org/10.24953/turkjped.2020.2558

**Original Article**

# Epileptic encephalopathy with electrical status epilepticus during slow sleep: evaluation of treatment response from a tertiary center

Betül Kılıç, Mecit Acar, Yasemin Topçu, Güzide Turanlı

*Department of Pediatric Neurology, İstanbul Medipol University Faculty of Medicine, İstanbul, Turkey.*

## ABSTRACT

**Background.** This study aimed to evaluate the clinical, electrophysiological, etiological features, and treatment response in children with epileptic encephalopathy with electrical status epilepticus during slow sleep (ESES).

**Methods.** Clinical data, records of electroencephalograms (EEG), and brain magnetic resonance imaging (MRI) findings of 33 patients with ESES who were treated, and followed up for at least one year were retrospectively analyzed.

**Results.** Of all patients, 57.6% were male, and 42.4% were female. The mean age was 10.45 ± 2.88 years. At first admission, 90% of patients had seizures, and 10% had only school failure. Twelve patients had childhood focal epileptic syndrome. In etiology, asphyxia (n=6), hydrocephalus (n=2), polymicrogyria (n=1), and mesial temporal sclerosis (n=1) were determined. Neurological examination was abnormal in 27.2%, and brain MRI findings were pathological in 36.3% of the patients. During the ESES phase, the spike-wave index (SWI) on the non-rapid eye movement (NREM) sleep EEG was >85% in 16 patients and 50-85% in 17 patients. Only one patient received one, and the others had at least two antiseizure medications. Benzodiazepines were found to be the most effective treatment. In the two-year follow-up, 24 patients (72.7%) were seizure-free, and nineteen patients (57.5%) had complete recovery of SWI on their NREM sleep EEG. There was a significant correlation with reduction of the SWI on the EEG and seizure control ($p < 0.001$). In addition, a significant correlation was found between neurocognitive and behavioral scores scored before and after treatment, seizure control, and EEG recovery.

**Conclusions.** ESES is an epileptic encephalopathy that can be treated safely with antiseizure medications. Neurocognitive examinations and follow-up of EEG findings are valuable in terms of the treatment response. Benzodiazepines were found to be very effective in additional treatment.

**Kew words:** electrical status epilepticus during slow sleep, EEG findings, antiseizure treatment.

Epileptic encephalopathy with electrical status epilepticus during slow sleep (ESES) is characterized by an electrographic pattern of encephalopathy, neurocognitive regression, and different behavioral disorders, with typical electroencephalogram (EEG) findings as continuous epileptic slow-spike wave activity in non-rapid eye movement (NREM) sleep.[1-3]

✉ Betül Kılıç
betulklc82@gmail.com

Received 25th August 2020, revised 11th November 2020, 10th January 2021, 21st April 2021, accepted 13th June 2021.

Tassinari et al.[1] first used the term status epilepticus in sleep in 1977, but the same group termed this phenomenon as ESES in 2000 because it did not always appear with seizures. In the literature, it is used synonymously with continuous spikes and waves during slow sleep (CSWS).[4]

ESES is an age-related epileptic syndrome. Therefore, patients may be in various clinical stages at admission. The average time for the first seizure is 2-4 years, and neurocognitive regression occurs in 5-6 years. Different types of seizures may occur in completely normal

children or may occur following neurocognitive regression.[5-7]

There is no consensus in the literature on ESES diagnostic criteria. Typical EEG findings are 1-3 Hz focal or generalized spike-wave activities (SWI), taking on 85% of NREM sleep.[2,3] However, some authors have accepted lower cut off rates in clinically similar patients with SWI <85%.[8-10]

Clinical seizures tend to resolve spontaneously during adolescence. ESES EEG pattern also disappears in all cases, on average by the age of eleven years.[1] The disappearance of the seizures and the ESES pattern on the EEG may be simultaneous, or the seizures may disappear before or after the disappearance of the ESES pattern on the EEG.[2]

It is currently recommended that ESES syndrome should be treated as soon as possible.[7] Although many antiseizure medications can be used in treatment, different data have been obtained regarding their efficacy, so there is a wide range of treatment strategies and combinations.[10]

Our study aimed to evaluate the clinical, electrophysiological, etiological characteristics and treatment responses of ESES patients.

## Material and Methods

The files of pediatric patients with ESES followed at Istanbul Medipol University Faculty of Medicine Pediatric Neurology Clinic between 2014 and 2017 were reviewed. Demographic data, age at the time of first seizure and ESES diagnosis, consanguineous marriage, family history of febrile convulsion and epilepsy, seizure type, neurological examination, cognitive evaluation, sleep and wakefulness EEG findings, ESES characteristics, antiseizure treatments, comorbid psychiatric findings, brain magnetic resonance imaging (MRI) findings, etiological causes, two-year treatment efficacy, and the follow-up period of the patients were evaluated.

All patients underwent long-term sleep and awake EEG (at least one hour), and 13 patients underwent all-night video EEG in addition to routine EEG recordings. Long-term EEGs were repeated at least twice a year. SWI (%) was obtained as the total number of minutes of all spike- and slow-wave activities divided by the total number of minutes of NREM and multiplied by 100.[11] The diagnosis of ESES was made with calculated SWI, occupying ≥50% of NREM sleep EEG tracing.

Neurological examination and brain MRI were performed for all patients. Neuropsychological evaluation could only be performed in five patients with the WISC-R test. In our study, neurocognitive tests could not be performed to all patients due to inability to reach the examination and presence of intellectually disabled patients. Parents, teachers, and caregivers were interviewed about the patients' neurocognitive status regarding the deterioration and recovery, and they were also evaluated by clinical judgements. Accordingly, neurocognitive abnormalities were graded between 1-5 points according to school success, learning difficulties, intellectual deterioration, memory deficits, behavioral changes (e.g., hyperactivity, aggressiveness, anxiety, lying, nail-biting, shyness, encopresis), and expressive language disturbances. Neurocognitive and behavioral evaluations performed before and after treatment were compared according to seizure outcome and EEG improvement.

The estimated onset of ESES was determined as the regression onset, the end of the ESES period as the time of seizure control, and cognitive improvement time with the disappearance of ESES pattern on the NREM sleep EEG. Response to antiseizure medications was evaluated as the complete disappearance of the ESES pattern, more than 50% reduction of the SWI, less than 50% reduction of the SWI, and unresponsiveness. The clinical response was graded as complete disappearance of the clinical findings observed during the ESES phase, partial recovery, and nonresponsive.

### *Statistical analysis*

Non-measurable values were expressed as mean ± standard deviation. Pearson chi-square and Fisher's exact test were performed for categorical variables. The correlations between cognitive improvement, and SWI change, also seizure reduction were calculated. SPSS for Windows v.17 (SPSS, Chicago, IL, USA) was used for the analysis. A *p*-value <0.05 was considered to indicate significance.

The study was conducted in accordance with the Helsinki Declaration, and the study protocol was approved by the Ethics Committee of Istanbul Medipol University Faculty of Medicine (08.01.2020/09).

## Results

### *General clinical features/seizure and EEG findings before the onset of ESES*

A total of the 33 patients (19 boys and 14 girls) diagnosed with ESES syndrome were evaluated in the study. The mean age of the patients was 10.45 ± 2.88 (min. 5, max. 17) years. In all, 12.1% of the patients had a consanguineous marriage of parents, 36.3% had a family history of epilepsy, and 3% had febrile seizures. A total of 90% of patients had seizures at first admission, and 10% had school failure. One of the three patients who presented with school failure had night terrors as an additional complaint, and the other had headaches.

Twelve (36.3%) patients had childhood focal epileptic syndrome, 11 were benign childhood epilepsy with centrotemporal spikes, and one was early-onset childhood occipital epilepsy. Six patients (18%) had a history of asphyxia, two had hydrocephalus, one had polymicrogyria, and one had mesial temporal sclerosis. Before ESES period, nine patients had mental retardation, one had attention deficit hyperactivity disorder, one had bipolar disorder, and one had migraine.

Neurological examination was abnormal in 27.3%, and brain MRI findings were pathological in 36.3% of the patients. Abnormal brain MRI findings of the patients are summarized in Table I.

Thirty of 33 patients had seizures before ESES, and the mean age at first seizure was 5.06 ± 3.01 (1-13 years). The period between the age of seizures onset with ESES onset age was 2.7 ± 2.2 years. The seizure type was generalized in 33.3% (eight patients had tonic-clonic, two had myoclonic seizures), focal in 56.7%, combined generalized, and focal in 10.0%.

EEG findings before ESES period were focal in 29 (87.9%), multifocal in 9.1%, and generalized in only one patient (3.1%). In 12 (41.4%) of these patients, focal EEG findings were dominant in the left hemisphere and 58.6% (17/29) in the right hemisphere. While 97% of the focal epileptic discharges on the EEG originated from the anterior regions (frontal, frontocentral, or frontotemporal), only 3% were observed on the posterior regions (posterior temporal, temporo-occipital, or occipital).

### *Seizure and EEG characteristics in ESES*

The mean age at initial ESES diagnosis was 7.93 ± 2.65 years (4–12 years), and the mean follow-up period was 29.1 ± 14.2 months (12-36 months). In the ESES phase, there were 16 (48.5%) patients with SWI >85% and 17 patients (51.5%) with 50-85% SWI on the NREM sleep EEG. The cognitive development of our patients was found to be abnormal in 45.5%. There was no significant relationship between SWI on the EEG and neurocognitive status (p = 0.07).

**Table I.** Magnetic resonance imaging (MRI) findings of ESES patients.

| Findings | n (%) |
|---|---|
| Periventricular leukomalacia | 4 (12.1) |
| Thalamus involvement | 4 (12.1) |
| Hydrocephalus | 2 (6.1) |
| Polymicrogyria | 1 (3.0) |
| Mesial temporal sclerosis | 1 (3.0) |

Hemi ESES was detected in 30.3% of patients (n = 10/33), and was higher in patients with pathological brain MRI (p=0.02). Neurocognitive abnormalities were detected in 17 patients during ESES phase (51.5%). A statistically significant correlation was found between the difference in neurocognitive and behavioral scores graded before and after treatment with EEG recovery (r=0.53, p=0.001; Fig. 2), but not with seizure control (r=0.1, p=0.54; Fig. 1).

### *Response to treatment*

Among all patients, 97% received polytherapy, and 3% had monotherapy. Initially, 13 patients were treated with valproic acid, 16 patients with carbamazepine, two with clonazepam, and two with phenobarbital. Only one of them continued treatment with monotherapy. The remaining 32 patients used two or more antiseizure medications. In additional therapy, benzodiazepines were found to be the most effective treatment (in 20 patients). Also, additional therapy included sulthiame in six patients, and levetiracetam in one patient was found to be effective (Table II). None of the patients received steroids during the follow-up period. ESES recovery time on the EEG was found to be 9.09 ± 6.9 months. At the end of at least two-year follow-up, a clinically significant response was obtained in 90.9% of patients (24 seizure-free, partial response in 6 patients), and only three patients were clinically unresponsive. Nineteen patients (57.6%) had complete disappearance of the ESES pattern on the EEG, three patients (9.1%) had more than 50% improvement of the SWI, four patients (12.1%) had less than 50% improvement of the SWI, and unresponsiveness was observed in 7 (21.2%) patients. There was a significant relationship between reduction of the SWI on the EEG and a seizure control (p <0.001). Only seven patients with structural lesions in etiology did not have a significant improvement in neurocognitive functions during follow-up. Especially in childhood focal epileptic syndromes, significant improvement in neurocognitive functions was observed before and after treatment.

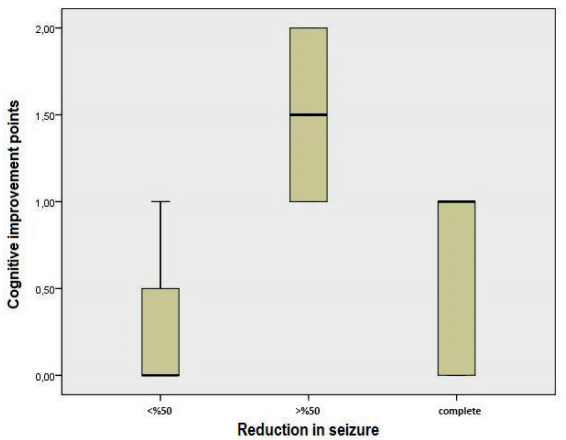



**Fig. 1.** The relationship between seizure reduction and cognitive improvement.

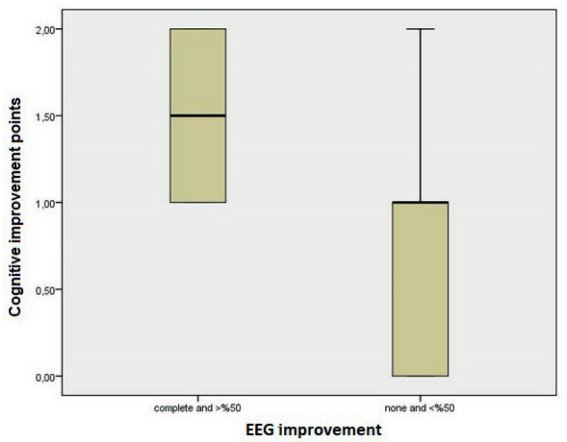



**Fig. 2.** The relationship between reduction of the SWI on NREM EEG and cognitive improvement.

Gender, age, consanguinity, family history of epilepsy and febrile convulsion, age at the time of ESES, ESES duration, and SWI on the EEG at the diagnosis did not significantly differ in treatment response. In follow-up with treatment, there was a significant difference in seizure control only according to etiology. Family history of epilepsy, pre-ESES seizure type, abnormal brain MRI, abnormal neurological examination, neurocognitive retardation, etiology, and the type of additional therapy were statistically significant in the reduction of SWI on the NREM sleep EEG. Despite the lack of significant difference in treatment of seizure control, benzodiazepines were found to be the most statistically significant treatment in terms

**Table II.** Demographic and clinical characteristics of patients and response to treatment of seizure.

| Characteristics | | Seizure response to treatment, n (%) | | | p |
|---|---|---|---|---|---|
| | | Seizure free | >50% reduction | <50% reduction | |
| Gender | Female | 13 (92.9) | 1 (7.1) | 0 (0) | 0.059 |
| | Male | 11 (57.9) | 5 (26.3) | 3 (15.8) | |
| Abnormal neurological examination | Yes | 4 (44.4) | 4 (44.4) | 1 (11.1) | 0.056 |
| | No | 20 (83.3) | 2 (8.3) | 2 (8.3) | |
| Neurocognitive retardation | Yes | 10 (58.8) | 4 (23.5) | 3 (17.6) | 0.139 |
| | No | 14 (87.5) | 2 (12.5) | 0 (0.0) | |
| Abnormal brain MRI | Yes | 10 (83.3) | 1 (8.3) | 1 (8.3) | 0.49 |
| | No | 16 (76.2) | 4 (19.0) | 1 (4.8) | |
| Etiology | | | | | 0.02 |
| Childhood focal epileptic syndrome | | 11 (91.7) | 0 (0.0) | 1 (8.3) | |
| Structural | | 4 (40.0) | 4 (40.0) | 2 (20) | |
| Unknown | | 9 (81.8) | 2 (18.2) | 0 (0) | |
| SWI on the EEG at the diagnosis | | | | | 0.162 |
| >85% | | 14 (87.5) | 1 (6.3) | 1 (6.3) | |
| 50-85% | | 10 (58.8) | 5 (29.4) | 2 (11.8) | |
| Additional treatment | | | | | 0.14 |
| Benzodiazepines | | 16 (80.0) | 3 (15.0) | 1 (5.0) | |
| Sulthiame | | 5 (83.3) | 1(16.7) | 0 (0) | |
| Levetiracetam | | 1 (100) | 0 (0.0) | 0 (0) | |

SWI: spike-wave index ,EEG: electroencephalogram, MRI: magnetic resonance imaging

of reduction of the SWI on the NREM sleep EEG (p = 0.009; Table III, Fig. 3).

## Discussion

In our study, age, gender, age at the first seizure, and age at ESES were found to be consistent with the literature. It has been reported in the literature that the age of onset of seizures in patients with ESES is younger than in other epileptic patients.[12,13] In our cases, while no patient was diagnosed before the age of 4, only one patient was diagnosed at the age of 16. The time between the age of first seizure and the diagnosis of ESES was approximately two years.

Although the classical ESES definition suggests that the SWI occurs between 85 to 100% of NREM sleep, in later studies, lower threshold values were accepted in the ESES definition since patients with an SWI <85% also showed neuropsychological regression.[6,8,10,14-18] In our study, no correlation was found between SWI and psychomotor evaluation (p = 0.07). Also, we did not find a significant statistical relationship between SWI on the EEG at the diagnosis and treatment response.

The certain cause of ESES is unknown. However, in most patients, structural lesions of the brain that begin at an early stage are shown, and genetic factors have been described. There is a notable group of patients whose etiology remains unknown.[4,5,13,19,20]

Abnormal brain MRI findings were found in 36.3% of our cases. The most common abnormal findings were thalamic involvement in four patients (33.3%) and periventricular leukomalacia in four patients (33.3%). In the literature, the incidence of structural brain abnormalities has been reported as 20- 50%, thalamic involvement in 29%, and periventricular leukomalacia in approximately

**Table III.** Demographic and clinical characteristics of the patients and their SWI on the EEG after treatment.

| Characteristics | | EEG: reduction in spike–wave index, n (%) | | p |
|---|---|---|---|---|
| | | Complete disappearance + >50%reduction | <50% reduction + no response | |
| Gender | Female | 11 (78.6) | 3 (21.4) | 0.21 |
| | Male | 11 (57.9) | 8 (42.1) | |
| Focal seizures | Yes | 16 (76.2) | 5 (23.8) | 0.04 |
| | No | 4 (40.0) | 6 (60.0) | |
| Abnormal neurological examination | Yes | 3 (33.3) | 6 (66.7) | 0.01 |
| | No | 19 (79.2) | 5 (20.8) | |
| Neurocognitive retardation | Yes | 8 (47.1) | 9 (52.9) | 0.01 |
| | No | 14 (87.5) | 2 (12.5) | |
| Abnormal brain MRI | Yes | 6 (50.0) | 6 (50.0) | 0.04 |
| | No | 16 (76.2) | 5 (23.8) | |
| Hemi ESES | Yes | 8 (80.0) | 2 (20.0) | 0.28 |
| | No | 14 (60.9) | 9 (39.1) | |
| SWI on the EEG at the diagnosis | | | | 0.085 |
| >85% | | 13 (81.3) | 3 (18.8) | |
| 50-85% | | 9 (52.9) | 8 (47.1) | |
| Etiology | | | | 0.001 |
| Childhood focal epileptic syndrome | | 10 (83.3) | 2 (16.7) | |
| Structural | | 2 (20.0) | 8 (80.0) | |
| Unknown | | 10 (90.9) | 1 (9.1) | |
| Additional treatment | | | | 0.009 |
| Benzodiazepines | | 15 (75.0) | 5 (25.0) | |
| Sulthiame | | 5 (83.3) | 1 (16.7) | |
| Levetiracetam | | 1 (100.0) | 0 (0.0) | |

ESES: electrical status epilepticus during slow sleep SWI: spike-wave index ,EEG: electroencephalogram, MRI: magnetic resonance imaging

25% of patients.[3,13,17,21-23] The presence of thalamic lesion alone can lead to epilepsy and ESES, with impaired neurodevelopment. An impaired thalamocortical circuit leads to the propagation of sudden wave discharges.[24] Polymicrogyria and hydrocephalus are the most commonly reported cortical malformations in the etiology of ESES.[6,25] The presence of polymicrogyria allows rapid propagation of sudden wave discharges.[26] In our series, only one patient had polymicrogyria, and two patients had hydrocephalus.

Family history of epilepsy and consanguineous marriage were significantly higher in our patients. These results suggest that the possible genetic causes of ESES may be higher in our patients. However, genetic studies could not be performed on our patients due to economical problems.

In previous studies, it was reported that the rate of moderate-to-severe neurocognitive impairment was 53% in ESES, and neurocognitive decline was observed in two-thirds of the patients.[3,6,27] Arhan et al.[10] reported that cognitive impairment was 40.6%. The present study determined neurocognitive impairment in 51.5% of our patients. In addition, the first complaint of 10% of our patients was school failure without seizures. Attention deficit hyperactivity disorder, hyperkinetic behavior, expressive language disorders, and learning disability have also been reported in the ESES period.[6]

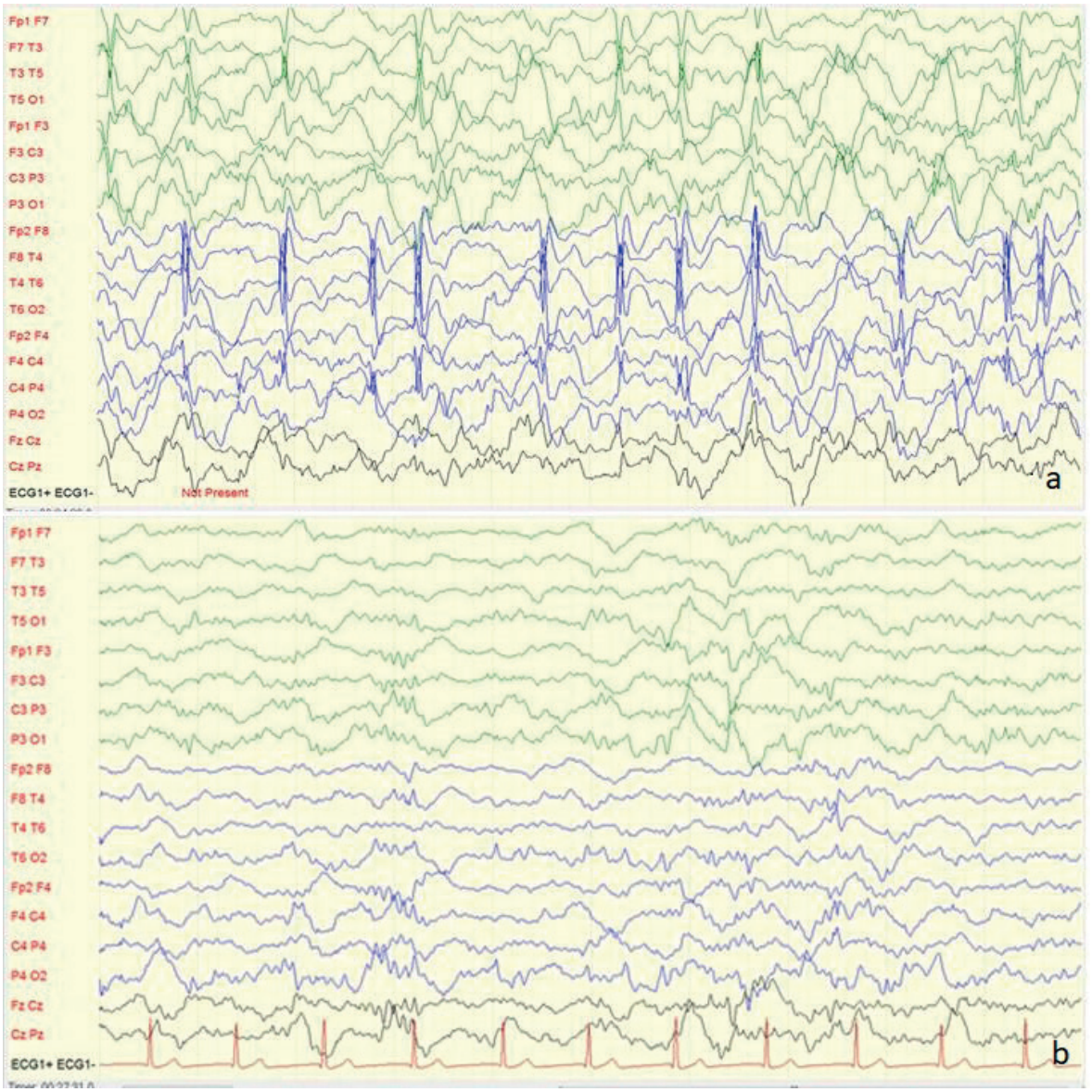



**Fig. 3 . (a)** ESES pattern (SWI> 85%) is seen in NREM sleep of an 8-year-old female patient with atypical benign rolandic epilepsy treated with valproic acid; **(b)** after the addition of benzodiazepine to the treatment of the same patient, the ESES pattern on the EEG shows complete disappearance.

Therefore, it can be considered that patients with various neurocognitive psychiatric symptoms should be evaluated in terms of ESES. van den Munckhof et al.[22] showed that in children with ESES, cognitive improvement after treatment was strongly associated with reduction of the SWI, but there was no significant increase in intelligence quotient (IQ). Previous studies have found that cognitive performance and prognosis are related to the location, severity, and duration of EEG abnormalities.[6,7,10,22] In our study, improvement in EEG abnormality and reduction of the SWI showed a significant correlation with cognitive improvement. However, it was found that decrease of seizures and cognitive recovery did not correlate (Figs. 1-2).

Seizures in our patients were mostly focal. While seizures were initially uniform and less frequent, it was reported that seizures varied,

and the incidence and duration of seizures increased in resistant cases. In a retrospective study, 98% of 117 patients, and in another study 77% of 21 cases had focal seizures.[8,9] It has been reported that seizures are generally focal in ESES syndrome, and seizures mostly start as focal during sleep in patients with generalized seizures.[8,9,13]

Most of our patients had focal EEG discharges before the ESES period. The epileptic focus in the EEG was mostly on anterior regions of the hemisphere (97%). Other ESES studies have also reported that epileptic activities are more common on anterior regions of the brain.[9,13]

The electrographic activity in ESES may occur diffusely in both hemispheres or predominantly asymmetric in one hemisphere or limited hemispheric in only one hemisphere. Caraballo et al.[9] reported that there was no significant relationship between the asymmetric electroencephalographic findings of ESES, and the clinical presentation of the patients regarding the percentage of the SWI. All patients with asymmetric ESES were found with symptomatic/structural etiology. In our study, 30.3% of the patients (n = 10/33) had limited EEG findings in the form of hemi-ESES in only one hemisphere, and there was a significant relationship between hemi-ESES and brain MRI abnormalities (p = 0.02). It was observed that the presence of hemi-ESES did not make a significant difference in terms of the reduction of the SWI after treatment.

Although many antiseizure medications can be used in the treatment of seizures, different data have been obtained regarding their efficacy. Therefore, a wide range of treatment priorities and combinations are proposed, and there is still no consensus on the treatment of ESES. It is also reported that immunomodulatory treatments, ketogenic diet, and surgical treatment are effective in selected cases.[18]

In a study that analyzed 112 articles and 950 treatments administered to 575 patients, antiseizure medications were found to be effective in 49% of patients (n=495), benzodiazepines in 68% (n=171), and steroids in 81% (n=166), as cognitive or EEG improvements. Surgical treatment resulted in improvement in 90% of patients (n=62). In a subgroup analysis of sequentially reported patients (585 treatments in 282 patients), antiseizure medications improved 34% of the patients, benzodiazepines 59%, and steroids 75%. The postoperative recovery rate was 93% in selected cases. Before the onset of ESES, normal development and the absence of structural abnormalities have been observed to affect the treatment rate positively.[18]

The efficacy of antiseizure medications (including benzodiazepines) that we used in our study was quite high (90.9%) without steroid use. Benzodiazepines were the most effective treatment in additional therapy. Although it was not statistically significant in patients receiving sulthiame for additional treatment, it was observed to have a clinically and electrographically significant effect. In a study, it was reported that sulthiame (5-30 mg/kg/day) given in additional treatment had a significant effect on seizure control and EEG abnormalities in children with ESES syndrome.[28]

The limitations of our study were the small number of patients, the retrospective nature of the study, and the lack of neurocognitive tests due to inability to reach the examination. A larger number of more comprehensive studies are needed in terms of etiology determination and treatment efficacy.

This study confirms that ESES is an epileptic encephalopathy over a wide SWI range. Patients with SWI >50% on the NREM EEG should be followed up regularly with neuropsychological evaluations. The etiology and duration of ESES can be considered to significantly affect long-term prognosis. In our study, benzodiazepines, and sulthiame were found to be effective in combination with other antiepileptic drugs or alone.

### Ethical approval

The study was conducted in accordance with the Helsinki Declaration, and the study protocol was approved by the Ethics Committee of Istanbul Medipol University Faculty of Medicine (08.01.2020/09).

### Author contribution

Study conception and design: GT, YT, BK; data collection: BK, MA; analysis and interpretation of results: BK, MA, YT, GT; draft manuscript preparation: BK, YT, 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.

### Conflicts of interest

The authors declare that there is no conflict of inretest.

## REFERENCES

1. Tassinari CA, Rubboli G, Volpi L, et al. Encephalopathy with electrical status epilepticus during slow sleep or ESES syndrome including the acquired aphasia. Clin Neurophysiol 2000; 11(Suppl 2): S94-S102. https://doi.org/10.1016/S1388-2457(00)00408-9
2. Bureau M. Continuous spikes and waves during slow sleep (CSWS): definition of the syndrome. In: Beaumanoir A, Bureau M, Deonna T, Mira L, Tassinari CA (Eds). Continuous Spikes and Waves during Slow Sleep. Electrical Status Epilepticus during Slow Sleep: Acquired Epileptic Aphasia and Related Conditions. London: John Libbey, 1995: 17-26.
3. Fernandez IS, Chapman KE, Peters JM, et al. The Tower of Babel: survey on concepts and terminology in electrical status epilepticus in sleep and continuous spikes and waves during sleep in North America. Epilepsia 2013; 54: 741-750. https://doi.org/10.1111/epi.12039
4. Scheltens-de Boer M. Guidelines for EEG in encephalopathy related to ESES/CSWS in children. Epilepsia 2009; 50(Suppl 7): 13-17. https://doi.org/10.1111/j.1528-1167.2009.02211.x
5. De Negri M. Electrical status epilepticus during sleep (ESES). Different clinical syndromes: towards a unifying view? Brain Dev 1997; 19: 447-451. https://doi.org/10.1016/S0387-7604(97)00058-2
6. Raha S, Shah U, Udani V. Neurocognitive and neurobehavioral disabilities in Epilepsy with Electrical Status Epilepticus in slow sleep (ESES) and related syndromes. Epilepsy Behav 2012; 25: 381-385. https://doi.org/10.1016/j.yebeh.2012.08.028
7. Holmes GL, Lenck-Santini PP. Role of interictal epileptiform abnormalities in cognitive impairment. Epilepsy Behav 2006; 8: 504-515. https://doi.org/10.1016/j.yebeh.2005.11.014
8. Fortini S, Corredera L, Pastrana AL, Reyes G, Fasulo L, Caraballo RH. Encephalopathy with hemi-status epilepticus during sleep or hemi-continuous spikes and waves during slow sleep syndrome: a study of 21 patients. Seizure 2013; 22: 565-571. https://doi.org/10.1016/j.seizure.2013.04.006
9. Caraballo RH, Veggiotti P, Kaltenmeier MC, et al. Encephalopathy with status epilepticus during sleep or continuous spikes and waves during slow sleep syndrome: a multicenter, long-term follow-up study of 117 patients. Epilepsy Res 2013; 105: 164-173. https://doi.org/10.1016/j.eplepsyres.2013.02.010
10. Arhan E, Serdaroglu A, Aydin K, Hırfanoglu T, Soysal AS. Epileptic encephalopathy with electrical status epilepticus: an electroclinical study of 59 patients. Seizure 2015; 26: 86-93. https://doi.org/10.1016/j.seizure.2015.01.008
11. Patry G, Lyagoubi S, Tassinari CA. Subclinical "electrical status epilepticus" induced by sleep in children. A clinical and electroencephalographic study of six cases. Arch Neurol 1971; 24: 242-252. https://doi.org/10.1001/archneur.1971.00480330070006
12. Saltik S, Uluduz D, Cokar O, Demirbilek V, Dervent A. A clinical and EEG study on idiopathic partial epilepsies with evolution into ESES spectrum disorders. Epilepsia 2005; 46: 524-533. https://doi.org/10.1111/j.0013-9580.2005.45004.x
13. Sánchez Fernández I, Loddenkemper T, Peters JM, Kothare SV. Electrical status epilepticus in sleep: clinical presentation and pathophysiology. Pediatr Neurol 2012; 47: 390-410. https://doi.org/10.1016/j.pediatrneurol.2012.06.016
14. Van Hirtum-Das M, Licht EA, Koh S, Wu JY, Shields WD, Sankar R. Children with ESES: variability in the syndrome. Epilepsy Res 2006; 70(Suppl 1): S248-S258. https://doi.org/10.1016/j.eplepsyres.2006.01.020

15. Kramer U, Sagi L, Goldberg-Stern H, Zelnik N, Nissenkorn A, Ben-Zeev B. Clinical spectrum and medical treatment of children with electrical status epilepticus in sleep (ESES). Epilepsia 2009; 50: 1517-1524. https://doi.org/10.1111/j.1528-1167.2008.01891.x

16. Caraballo RH, Cejas N, Chamorro N, Kaltenmeier MC, Fortini S, Soprano AM. Landau-Kleffner syndrome: a study of 29 patients. Seizure 2014; 23: 98-104. https://doi.org/10.1016/j.seizure.2013.09.016

17. Değerliyurt A, Yalnizoğlu D, Bakar EE, Topçu M, Turanli G. Electrical status epilepticus during sleep: a study of 22 patients. Brain Dev 2015; 37: 250-264. https://doi.org/10.1016/j.braindev.2014.04.007

18. van den Munckhof B, van Dee V, Sagi L, et al. Treatment of electrical status epilepticus in sleep: a pooled analysis of 575 cases. Epilepsia 2015; 56: 1738-1746. https://doi.org/10.1111/epi.13128

19. Galanopoulou AS, Bojko A, Lado F, Moshe SL. The spectrum of neuropsychiatric abnormalities associated with electrical status epilepticus in sleep. Brain Dev 2000; 22: 279-295. https://doi.org/10.1016/S0387-7604(00)00127-3

20. Singhal NS, Sullivan JE. Continuous spike-wave during slow wave sleep and related conditions. ISRN Neurol 2014; 2014: 619079. https://doi.org/10.1155/2014/619079

21. Yilmaz S, Serdaroglu G, Akcay A, Gokben S. Clinical characteristics and outcome of children with electrical status epilepticus during slow wave sleep. J Pediatr Neurosci 2014; 9: 105-109. https://doi.org/10.4103/1817-1745.139266

22. van den Munckhof B, Alderweireld C, Davelaar S, et al. Treatment of electrical status epilepticus in sleep: clinical and EEG characteristics and response to 147 treatments in 47 patients. Eur J Paediatr Neurol 2018; 22: 64-71. https://doi.org/10.1016/j.ejpn.2017.08.006

23. Bartolini E, Falchi M, Zellini F, et al. The syndrome of polymicrogyria, thalamic hypoplasia, and epilepsy with CSWS. Neurology 2016; 86: 1250-1259. https://doi.org/10.1212/WNL.0000000000002526

24. Steriade M, Contreras D. Spike-wave complexes and fast components of cortically generated seizures. I. Role of neocortex and thalamus. J Neurophysiol 1998; 80: 1439-1455. https://doi.org/10.1152/jn.1998.80.3.1439

25. Caraballo R, Cerso'simo R, Fejerman N. A particular type of epilepsy in children with congenital hemiparesis associated with unilateral polymicrogyria. Epilepsia 1999; 40: 865-871. https://doi.org/10.1111/j.1528-1157.1999.tb00792.x

26. Guerrini R, Genton P, Bureau M, et al. Multilobar polymicrogyria, intractable drop attack seizures, and sleep-related electrical status epilepticus. Neurology 1998; 51: 504-512. https://doi.org/10.1212/WNL.51.2.504

27. Liukkonen E, Kantola-Sorsa E, Paetau R, Gaily E, Peltola M, Granstrom ML. Longterm outcome of 32 children with encephalopathy with status epilepticus during sleep, or ESES syndrome. Epilepsia 2010; 51: 2023-2032. https://doi.org/10.1111/j.1528-1167.2010.02578.x

28. Fejerman N, Caraballo R, Cersósimo R, Ferraro SM, Galicchio S, Amartino H. Sulthiame add-on therapy in children with focal epilepsies associated with encephalopathy related to electrical status epilepticus during slow sleep (ESES). Epilepsia 2012; 53: 1156-1161. https://doi.org/10.1111/j.1528-1167.2012.03458.x

The Turkish Journal of Pediatrics 2022; 64: 312-321
https://doi.org/10.24953/turkjped.2021.1644

**Original Article**

# A feasibility study of risk prediction modelling for vaso-occlusive crisis in children with sickle cell disease

Merve Türkegün Şengül[1], Bahar Taşdelen[1], Selma Ünal[2], Veysi Akbey[3]

*Departments of [1]Biostatistics and Medical Information and [2]Child Health and Diseases, Mersin University Faculty of Medicine, Mersin; [3]Mersin Su Hospital, Mersin, Turkey.*

## ABSTRACT

**Background.** The availability of a selection of biomarkers that includes information about disease risk is very important in the treatment of sickle cell disease (SCD). We used the predictiveness curve (PC), which classifies diseased individuals according to low- and high-risk thresholds, for this purpose. Our aim was to define this new statistical method and to determine the biomarkers that predict vaso-occlusive crisis (VOC) in children with SCD to guide preventive treatment.

**Methods.** Thirty-eight pediatric patients with SCD were included in this feasibility study. Leucocytes (WBC), C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor (TNF-$\alpha$), and YKL-40 were studied in patients with VOC and without VOC. The patient group with a low or high risk of VOC was assessed using the PC. Risk prediction and classification performance were evaluated using the PC and receiver operating characteristic (ROC) curve. Results. According to the PC, patients with a high risk of VOC could be detected via TNF-$\alpha$, IL-6, and WBC, and TNF-$\alpha$ was the best risk prediction marker (TPF = 0.67).

**Conclusions.** The PC provides disease risk information by comparing more than one biomarker and can thereby help clinicians determine appropriate preventive treatments. This is the first study to evaluate biomarkers to predict VOC risk in SCD patients.

**Key words:** predictiveness curve, risk prediction, classifying, risk threshold, vaso-occlusion crisis.

In personalized medicine, clinicians can decide on the appropriate treatment for a patient based on the individual's demographic and genetic characteristics and/or biomarker measures so that the right treatment can be provided at the right time to the right patient.[1,2] Biomarker selection is currently the starting point for research on the diagnosis, identification, and treatment of many diseases.[3] Determining the biomarkers that provide the most beneficial information about disease risk is an important phase in the treatment process of complex diseases such as sickle cell disease (SCD).[4] Vaso-occlusive crisis (VOC) is the hallmark of SCD and is associated with various complications, including acute chest syndrome, multi-organ failure, and sudden death. VOC is both a cause of death and responsible for the majority of SCD patient hospitalizations.[5] It is important to understand the differences between VOC pain and other pain syndromes such as bone infarction, avascular necrosis, and leg ulcers in SCD patients so that the appropriate treatment can be determined.[6] No specific biomarker(s) are available for VOC diagnosis in SCD patients. This makes the treatment process challenging.[7] The prediction and classification of VOC risk would therefore be useful in treating these patients.

Biomarkers are used to classify or predict the risk according to the purpose of the research. Statistical processes are necessary to describe and select biomarkers.[8,9] The diagnostic classification performance of biomarkers is usually evaluated by a receiver operating

✉ Merve Türkegün Şengül
merveturkegun@gmail.com

Received 31st May 2021, revised 11th August 2021, accepted 5th October 2021.

characteristic (ROC) curve, sensitivity, and specificity criteria. These statistics can classify individuals as completely diseased or healthy; however, they do not provide information about future disease risk.[10] The risk of contracting a disease can be modelled as a function of the biomarkers using prediction models such as logistic or Cox regression.[11] Both methods are frequently used in biomarker selection in personalized medicine; however, individual differences should be considered when choosing a biomarker based on a diagnostic test because, main aim of personalized medicine is to determine which biomarker or biomarker combination can be used to separate patients into subgroups so that relevant treatment is offered to each patient.[1,2] The predictiveness curve (PC), which is a new prediction technique, offers an alternative to the classic statistical methods. PC visually presents population distribution of disease risk predicted by a continuous marker or risk model.[11]

In this study, we present a new graphic method, the PC, to calculate VOC risk. Our aim was to classify SCD patients based on their VOC risk. In doing so, we aimed to help clinicians develop patient-specific treatment plans and prevent the complications of this disease in the early stages so that treatment can be managed easily and fast.

Several candidate inflammatory biomarkers were identified in this feasibility study to predict the risk of VOC in a cohort of pediatric patients with SCD, namely, leucocytes (WBC) and C-reactive protein (CRP), which are routine follow-up markers, and the inflammation markers interleukin-6 (IL-6), tumor necrosis factor (TNF-$\alpha$), and YKL-40 (also called chitinase-3-like-1)

## Material and Methods

### *Predictiveness curve*

The most common method for classifying patient risk is the ROC curve, which is obtained by pointing the false positive ratio (1–specificity) of the diagnostic test at different cut points against the true positive ratio (sensitivity). The minimum value for the sum of the false positive and false negative ratios is considered to be the appropriate threshold to indicate disease[12], but the obtained results only provide information only about disease status, not about disease risk. The PC plays an important role in the treatment management process for chronic diseases as it can be used to determine the population distribution of the predicted disease risk and the low-and high- risk groups.[13]

The PC models a biomarker's capacity of risk prediction and visually presents the distribution of the risk levels for the population from which the cohort is selected. It is used to determine disease risk for both the population and each individual and it illustrates absolute risk probabilities against the expected absolute disease risk values of individuals.[14] The most striking feature of the PC is the creation of a common scale that facilitates comparisons between biomarkers or risk models because the original scale is different and cannot be used for comparison purposes.[13]

The disease risk indicated by the biomarker ($Y$) is calculated using Equation 1, where $D$ is the binary outcome variable related to disease ($D$=1 [present], $D$=0 [absent]) and $Y$ is the biomarker value as the continuous variable:

$$Risk\ (Y) = P(D = 1 \mid Y = y) \qquad \text{Equation 1}$$

The PC is then drawn as the function of the disease risk versus cumulative risk percentage ($v$). The PC is a curve of R($v$) versus to $v$ and is indicated by Equation 2:[11]

$$R\ (v) = \mathrm{P}(D = 1 \mid Y = F^{-1}\ (v)) \qquad \text{Equation 2}$$

The inverse functions are easier to interpret than risk percentages. The inverse function $R^{-1}(p)$ of the risk percentage $R(v)$ gives a cumulative distribution of *risk* ($Y$) in the population, and it is the proportion of the population that has a risk less than or equal to p ($R^{-1}(p) = P[risk\ (Y) \leq p]$).[13]

To obtain the inverse functions, the low-and high-risk thresholds of the disease should be determined. These thresholds are detected by the trajectory of disease, the type of treatment, and the general prevalence of the disease. $P_L$ and $P_H$ represent the low- and high-risk thresholds, respectively. 1 - $R^{-1}$ ($p_H$) is the ratio for a high-risk population, and $R^{-1}$ ($p_L$) is the ratio for a low-risk population. This determines the percentage of the patient population that is below the low-risk threshold and above the high-risk threshold. The PC can be interpreted easily and quickly using the inverse functions. The percentage of the cohort between the low- and high-risk thresholds are unclear and are calculated using $R^{-1}$ ($p_H$) − $R^{-1}$ ($p_L$).[11,13,14] This risk class can be termed "the equivocal risk range."[15] We have defined this equivocal risk range as the "gray area".

Two or more risk models can be compared with the PC. When evaluating biomarkers, both the percentage of patients in the gray area and the true positive fraction (TPF) and false positive fraction (FPF) of the classification performance statistics are considered.[11,16]

### *Data collection*

We designed a retrospective feasibility study and reviewed the medical records of hospitalized pediatric patients diagnosed with SCD who were admitted to the Department of Child Hematology, Mersin University Faculty of Medicine, Research Center Hospital, Mersin, between May 2016 and May 2017. The study group consisted of SCD patients between 3 and 17 years of ages. Patients who had been followed up regularly for a year, were not receiving erythrocyte transfusions, had not had VOC at last one month prior to the study, and did not have an inflammatory disease aside from SCD were included in the study.

The protocol was approved by the Mersin University Clinical Research Ethics Committee (protocol number of Mersin University-2018/258). All patient's parent was well informed about the protocols of study, and written informed consent was obtained.

The patients were divided into two groups: those who had had VOC for at least one year and the steady-state SCD patients who reported no VOC episodes for at least one year. The WBC, CRP, and inflammation parameters of IL-6, TNF-$\alpha$, and serumYKL-40 were used to predict VOC risk in the steady-state patients. During the steady state period, 5 ml of blood samples was collected from the patients for a complete blood count and to measure the CRP, IL-6, TNF-$\alpha$ and serumYKL-40 levels. The blood samples were kept at −20°C until the study was complete.

### *Statistical methods*

#### *Univariate analysis*

The Shapiro–Wilk test was used to check the normal distribution. We presented continuous variables as mean and standard deviation (mean ± SD). Frequencies (n) and percentages (%) were used for categorical data Student's *t*-test was used to compare age means between the steady-state and VOC patients. A chi-square test was performed to determine the relationships between the VOC and gender groups. McNemar's test was performed for the categorical data. Agreement between the new risk groups and the VOC groups was calculated using the Kappa statistic. Statistical significance was set at $p < 0.05$ for all the comparisons.

#### *Multiple analysis and predictiveness curve*

A multiple logistic regression (MLR) model was used to calculate age-and gender-adjusted risk thresholds for all the biomarkers. The risk predictions were recorded for each patient with the MLR analysis. The mean of the predicted values and the 95% confidence interval (95% CI) of the mean were then calculated. The lower and upper limits of the CI were defined as the low- and high- risk thresholds for the biomarkers, thereby establishing the risk groups. The low- and high-risk thresholds of VOC for a randomly selected patient were determined visually using the PC model. To select the biomarkers that could detect VOC risks in SCD patients, TPF ≥

0.60 and FPF ≤ 0.30 criteria and CIs not including 0 were considered statistically significant.

ROC analysis was conducted twice. First, we studied the performance of the biomarkers in discriminating between the VOC and steady-state patients. Second, we assessed the performance of the biomarkers in discriminating between the low- and high-risk groups. The area under the curve (AUC), accuracy, positive predictive value (PPV), negative predictive value (NPV) and 95% CIs of these values were then calculated.

### *Software*

STATISTICA Version 13.5.0.17 (TIBCO Software Inc., Hillview Avenue Palo Alto, CA) was used for the univariate statistics. Stata/MP 11.0 (Stata Corp, College Station, TX) and its risk prediction package *predcurve* was used for PC analyses.[17,18]

## Results

### *The diagnostic performances of biomarkers*

In this study, the mean age of the patients was 12.6 ± 4.2 years. In the steady-state group, 55% of the patients were girls (n = 11), and 45% were boys (n = 9). In the VOC group, 27.8% of patients were girls (n = 5), and 72.2% were boys (n = 13). Both the gender ($p$ = 0.090) and age ($p$ = 0.941) distributions of the groups were similar (age means, 12.5 ± 4.2 years, 12.8 ± 4.3 years, respectively).

The AUC values of the WBC, IL-6, YKL-40 and TNF-$\alpha$ markers were calculated as less or equal to 0.50 (AUC= 0.434, 0.391, 0.535, 0.358, respectively). These biomarkers failed to distinguish the patients with VOC and those with steady-state disease. Although only CRP was greater than 0.50, it was not statistically significant (AUC=0.616, p=0.20).

### *Evaluation of the risk thresholds using prediction model*

All the biomarkers were modeled together with adjusted age and gender to assess their risk prediction performance for VOC. Based on the MLR, the risk prediction model was:

*(VOC risk) = 2.089 − 0.082 (WBC) + 0.130 (CRP) + 0.001 (YKL-40) − 0.008 (IL-6) − 0.028 (TNF-α) − 0.014 (age) − 1.43 (gender).*

The risk predictions were recorded for each patient using MLR. The mean of the predicted values and the 95% CI of the mean were calculated. The mean of the risk predictions was calculated as 0.47 ± 0.28, and the 95% CI was 0.38 – 0.55. For VOC in SCD patients, the low-risk threshold was 0.38, and the high-risk threshold was 0.55. This meant that the patients whose risk estimation value was below 0.38 had a slight possibility of experiencing a VOC episode and those whose risk estimation value was above 0.55 had a higher possibility of experiencing a VOC episode. The contribution of biomarkers to risk estimation was evaluated visually by plotting the PC for each biomarker along with these thresholds. When the TNF-$\alpha$, IL-6, and WBC markers were evaluated together with the covariate variables, we observed that they could be used to classify patients with a high risk of VOC. As shown in Table I, TNF-$\alpha$ had maximum TPF of 0.67 and minimum FPF of 0.20 Among the patients in the cohort, 31.6% were at or below the low-risk threshold. The patients with a VOC risk of 0.55 or greater comprised 39.5% of the cohort. TNF-$\alpha$ levels accurately predicted that 67% of the patients had a high risk of VOC (TPF = 0.67). 28.9% of the patients could not be classified into either the low- or high-risk groups, and allocated to the gray area based on their TNF-$\alpha$ levels (Table I).

The PC for TNF-$\alpha$ (Fig. 1) provided a more efficient predictive performance than the other biomarkers. The PC was interpreted in two ways without considering the risk thresholds. First, we evaluated the cumulative distributions using the $x$-axis. The results showed that 80% of the population had a VOC risk of 0.73 and lower, while 20% of the population had a VOC risk higher than 0.73. Second, we interpreted the cumulative distribution versus the risks on the $y$-axis. It could therefore be said that the

**Table I.** Summary statistics for the biomarkers according to the predictiveness curve.

| | Low risk threshold ($p_L$)=0.38 (n=14) | | | High risk threshold ($p_H$)=0.55 (n=16) | | | GA (n=8) |
|---|---|---|---|---|---|---|---|
| | TPF (95% CI) | FPF (95% CI) | Risk percentile $R^{-1}(0.38)$ | TPF (95% CI) | FPF (95% CI) | 100-Risk percentile $1-R^{-1}(0.55)$ | Risk percentile |
| TNF- $\alpha$ | 0.89 (0.63-1.00) | 0.55 (0.16-0.88) | 31.60% | 0.67 (0.23-0.91) | 0.20 (0.04-0.50) | 39.50% | *28.90%* |
| IL-6 | 0.71 (0.64-1.00) | 0.60 (0.18-0.85) | 34.20% | 0.67 (0.00-0.93) | 0.25 (0.00-0.58) | 41.10% | 23.70% |
| WBC | 0.72 (0.53-1.100) | 0.55 (0.21-1.00) | 39.50% | 0.66 (0.00-0.88) | 0.30 (0.00-0.56) | 44.70% | 15.80% |
| CRP | 0.78 (0.45-1.00) | 0.60 (0.21-1.00) | 34.20% | 0.39 (0.07-0.91) | 0.25 (0.00-0.57) | 28.90% | 36.90% |
| YKL-40 | 0.78 (0.50-1.00) | 0.40 (0.20-1.00) | 42.10% | 0.72 (0.00-0.90) | 0.45 (0.00-0.57) | 55.30% | 2.60% |

WBC: leucocyte, TNF-α: tumor necrosis factor, IL-6: interleukin-6, CRP: C-reactive protein,YKL40:chitinase-3-like-1,pL: low-risk threshold, pH: high-risk threshold, $R^{-1}(0.38)$: the ratio for a low-risk population, $1-R^{-1}(0.55)$ : the ratio for a high-risk population,GA: Grey Area; ($R^{-1}(0.55)- R^{-1}(0.38)$), TPF: true positive fraction, FPF: false positive fraction, 95% CI: 95% confidence interval for TPF and FPF.

TPF > 0.60 and FPF < 0.30 criteria and confidence intervals not including zero were considered statistically significant. For IL-6, TPF was 0.67 and FPF was 0.25 and 41.1 percent of patients were in the high-risk group or above for IL-6. Since TPF and FPF confidence intervals included zero, IL-6 was evaluated as not statistically significant.)

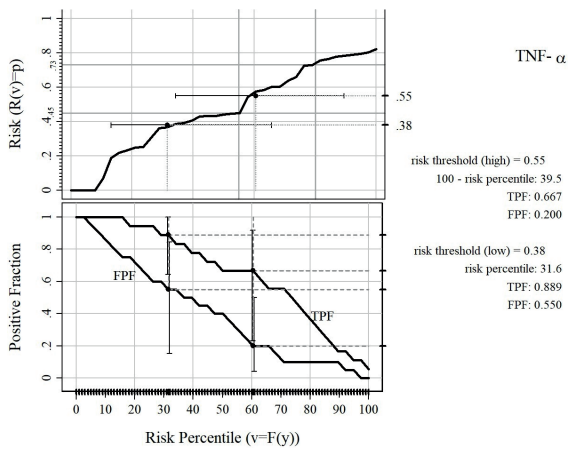



**Fig. 1.** Predictivenes curve for TNF- $\alpha$

(TNF-$\alpha$ is predictive of low risk in $R^{-1}(0.38)$ = 31.6% of the population, of high risk in $1-R^{-1}(0.55)$ = 39.5% and it leaves 28.9% of patients in the gray area.)

patients whose VOC risk was 0.45 comprised 55% of the cohort.

For IL-6, the TPF was 0.67 and the FPF was 0.25 Accordingly, 41.1% of the patients were in the high-risk group or above. Since both CIs included 0, IL-6 was evaluated as not statistically significant. On the other hand, the other biomarkers were excluded from evaluation because of a low TPF, a high FPF, or a CI of 0 (Table I).

The biomarkers did not identify the low-risk patients as successfully as the high-risk patients. The TPF values were calculated as above 0.60 for all the biomarkers, while the FPF values were over 0.30 (Table I). Even though the low-risk threshold of the population was known, not all the biomarkers could accurately identify the patients at low risk of VOC.

### *Differences between the VOC and risk groups*

According to the risk model, the risk predictions of 14 patients were below the low threshold, while the risk predictions of 16 patients were above the high threshold. Notwithstanding, eight patients' risk predictions were unclear (in the gray area), and these patients were not considered in the analysis. We constituted new risk groups based on the low- and high-risk thresholds using individual risks calculated by applying MLR.

The agreement between the new predicted risk groups and the observed VOC groups was investigated (Table II). No statistically significant differences were noted between the observed VOC and the predicted VOC risk groups when applying McNemar's test ($p > 0.05$) (Table II). This meant that the new prediction technique was agreement with the gold standard test results. The new technique can therefore be used for the prediction of VOC risk. The classification performance statistics were also calculated for these groups. The new risk groups distinguished the VOC and steady state patients with 70% accuracy (95% CI 50.60 - 85.26). The risk groups identified 62.5% of those at high risk of having a VOC episode (PPV = 62.5%) and 78.57% of those at low risk of having steady state (NPV = 78.57). Moderate agreement was also observed between the new risk groups and crisis groups' results (Kappa = 0.405 95% CI 0.086% – 0.725%).

The discrimination of power in the observed and predicted VOC groups was assessed for all the biomarkers using ROC analysis (Table III). The patients in the gray area were not included in this analysis. The discrimination power of TNF-$\alpha$, IL-6, and WBC was uninterpretable for the VOC and steady-state patients in the observed VOC groups (AUC <0.50). CRP and YKL-40 had weak discrimination power and were not statistically significant. While TNF-$\alpha$ and IL-6 had excellent discrimination power in the low-and high-risk VOC groups, the discrimination power of WBC was acceptable in the predicted VOC risk groups.

## Discussion

The most common reason of hospitalization among SCD patients is acute VOC episodes. Different risk factors trigger VOC episodes, which are sometimes unpredictable. VOC can cause a variety of complications, including acute chest syndrome, multi-organ failure and sudden death.[5] Treatments for VOC have focused on the symptomatic management of painful episode like to reduce pain, but it is currently not possible to prevent the painful episodes.[19] We therefore focused on developing

**Table II.** Differences between and observed and predicted groups.

| Predicted VOC groups | Observed VOC groups | | p |
|---|---|---|---|
| | VOC | Steady-state | |
| High risk (>0.55) | 10 (76.9) | 6 (35.3) | |
| Low risk (<0.38) | 3 (23.1) | 11 (64.7) | 0.50 |
| Total | 13 (100) | 17 (100) | |

VOC: vaso-occlusion crisis. Data are shown as n (%) and were compared by McNemar test. PPV: positive predictive value, NPV: negative predictive value. 95% CI: 95% confidence interval for PPV and NPV. PPV is 62.50% (95% CI (45.05%-77.20%]) NPV is 78.57% (95% CI (56.13%-91.31%]). Accuracy is 70% (95% CI (50.60%-85.26%]))

**Table III.** ROC analysis results for observed and predicted groups for biomarkers.

| | Observed crisis groups | | | Predicted risk groups | | |
|---|---|---|---|---|---|---|
| | Crisis (n=13) vs steady state (n=17) | | | High risk (n=16) vs Low risk(n=14) | | |
| | AUC (95%CI) | p | Power | AUC (95%CI) | p | Power |
| TNF-$\alpha$ | 0.335 (0.138-0.531) | 0.32 | 0.13 | 0.835 (0.685-0.985) | <0.001 | 0.94 |
| IL-6 | 0.385 (0.179-0.591) | 0.18 | 0.39 | 0.929 (0.773-0.990) | <0.001 | 0.99 |
| WBC | 0.439 (0.228-0.650) | 0.08 | 0.57 | 0.710 (0.520-0.900) | 0.03 | 0.53 |
| CRP | 0.683 (0.490-0.876) | 0.38 | 0.09 | 0.650 (0.450-0.850) | 0.14 | 0.30 |
| YKL-40 | 0.606 (0.389-0.824) | 0.16 | 0.32 | 0.585 (0.377-0.793) | 0.42 | 0.11 |

AUC: area under the curve, 95% CI: 95% confidence interval for AUC, Power: statistical power for AUC. If risk is ≥ 0.55, it is high risk. If risk is ≤0.38, it is low risk.

a useable PC method to help clinicians prevent the complications of SCD in the early stages using predictive risk biomarkers and risk groups. Our study results are not generalizable to the entire SCD population because our sample size was small. However, we used our data to introduce a new prediction technique and obtained reasonable results. Nevertheless, risk models like PC should be calculated using a large cohort that is representative of the target population.[11] The use of a large cohort could provide greater confidence in the results and be generalizable. Patients could then be classified with high accuracy, and percentage of grey area patients would decrease. We aimed to demonstrate that this new prediction technique could be applicable for use in patients with this important chronic disease However, we did not consider the patients' genotypes and other important clinical factors. The simultaneous evaluation of genotypes and such clinical factors in a large sample size may have a considerable effect on the results of future studies.

The hematologic changes that appear in the steady state or during the VOC are very important for SCD treatment.[20] WBC, CRP, IL-6, and TNF-$\alpha$ levels are known to increase during VOC episodes in SCD patients.21 Although many biomarkers have been identified to assist in the diagnosis of SCD, none is a reliable and certain indicator of VOC risk.[7] Although high LDH, PCT, and WBC levels are statistically associated with VOC risk on admission, WBC and PCT are not used as risk markers for VOC. When red blood cell transfusion or exchange is considered during VOC episode but the clinical indication is unclear, LDH level may help in making a decision. But this result for LDH does not guarantee predict VOC risk.[22,23] Studies have shown a strong correlation between CRP and WBC levels and hospitalization with VOC episodes.[24,25] These studies have suggested that WBC and CRP levels could be used as predictive biomarkers for VOC by applying a univariate statistical analysis to compare the means of the biomarkers between groups or a correlation analysis. We aimed to determine the best biomarkers to predict VOC risk. Accordingly, we classified patients at risk of VOC based on their biomarker values using the new statistical technique, PC.

ROC analysis is insufficient for risk prediction, because ROC analysis only distinguishes people as diseased or healthy.[26,27] The proposed PC indicates the capacity of a biomarker to predict disease risk. It also provides information about the performance of these predictive models while estimating risk in the population.[13] The PC can further be used to display the population distribution of disease risk as predicted by the chosen biomarker.[15] The PC provides important information about risk, which cannot be shown using the ROC curve, by classifying patients who have low- and high disease risk based on the biomarker values. By applying thresholds to classify patients as high or low risk, the value of a continuous biomarker can be evaluated by estimating the PPV and the NPV.[28]

An acceptable low- and high-risk threshold or risk intervals should be determined in the research, development, and selection of studies for biomarkers.[11] Risk thresholds depend on the context of the disease and include weighing the expected costs against benefits associated with a high-risk findding.[13] However, alternative methods can be used to determine risk thresholds because such analyses are difficult and time-consuming.[27] In our study, we calculated the low- and high-risk thresholds using a MLR risk model. By applying the PC model and risk thresholds, we were able to obtain the VOC risks for children with SCD.

The probability of VOC is estimated according to the threshold value of a biomarker calculated using the PC and can thereby help to start, continue, or complete treatment for a clinical study.[29,30] A perfect marker is indicated if the biomarker has a TPF = 1 and a FPF = 0. Even if the TPF and the FPF are not equal to 1 and 0, respectively, or are less, the marker can still be beneficial. The criteria to decide which biomarker will be useful depends on the study purpose. The general approach is that the TPF

and the FPF should be balanced.[16] Biomarkers can be classified as fitting the optimal balance of TPF and FPF if the *risk value > risk threshold* rule, which is often used as a clinical decision criterion.[11] In our study, we considered the clinical status of the patients, calculated their risk thresholds, and then used this status to determine the biomarker selection criteria; TPF ≥ 0.60 was the ratio of detecting patients with VOC, while a FPF ≤ 0.30 was the ratio indicated to show the steady-state patients with VOC. Our results showed that all the biomarkers were insufficient to classify low-risk patients.

If the risk of the disease is close to 0 or 1, the drug dose, variety of treatment, and duration of treatment can easily be determined. However, if the risk of disease is in the gray area, the decision becomes more difficult.[11] If a biomarker assigns a large number of individuals to the low- and high-risk groups and a smaller number of individuals to the gray area, it is accepted that the risk biomarker is strong or best.[14,15] In our study, five patients with VOC and three steady-state patients were in the gray area. In such instances, the question of whether such individuals should be evaluated as low- or high-risk patients is important. The status of patients who cannot not be assigned to any risk class and remain in the gray area is an important topic as this could lead to these patients being over- or under-treated. For patients in the gray area, genetic and environmental risk factors and compliance with treatment should therefore be investigated retrospectively.

According to the PC results, TNF-$\alpha$ was the best biomarker for classifying patients at a high risk of VOC (Table I). TNF-$\alpha$, IL-6, and WBC were found to discriminate between the low- and high-risk groups when the PC risk model was used with the ROC analysis (Table III). The PC and ROC analysis results were consistent. On the other hand, the classic statistic results showed that classifications based on the performance of the biomarkers for steady-state patients and those with VOC were not good. AUC values were above 0.60 for all biomarkers. These results mean that making comparisons according to risk categories using the PC method can provide more accurate results in critical diseases like SCD.

It is a feasibility study. So, we evaluated statistical power of study in the table III. The statistical power for AUCs was lower than 0.80 and an unacceptable level for the observed groups. Once the low- and high- risk groups had been predicted, AUC values and the statistical power increased for TNF-$\alpha$, IL-6, and WBC. Notably, the statistical powers were higher than 0.80 for the TNF-$\alpha$ and IL-6 (Table III). Although our sample size was small, agreement was established between the predicted and observed results.

The findings of our study showed that classifying patients by their VOC risk group and managing their treatment accordingly are extremely important. Group comparisons of biomarkers should be done in line with the low and high VOC risk groups in SCD patients. Attention should be paid to the treatment of patients whose VOC risk is unclear.

## Ethical approval

The protocol was approved by the Mersin University Clinical Research Ethics Committee by protocol number of MEU-2018/258.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BT, MTŞ; data collection: SÜ, VA; analysis and interpretation of results: MTŞ, BT; draft manuscript preparation: MTŞ. 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. Zhao Y, Zeng D. Recent development on statistical methods for personalized medicine discovery. Front Med 2013; 7: 102-110. https://doi.org/10.1007/s11684-013-0245-7
2. Li W, Chen C, Li X, Beckman RA. Estimation of treatment effect in two-stage confirmatory oncology trials of personalized medicines. Stat Med 2017; 36: 1843-1861. https://doi.org/10.1002/sim.7272
3. Antoniou M, Jorgensen AL, Kolamunnage-Dona R. Biomarker-Guided adaptive trial designs in phase II and phase III: A methodological review. PLoS One 2016; 11: 1-30. https://doi.org/10.1371/journal.pone.0149803
4. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet 2013; 381: 142-151. https://doi.org/10.1016/S0140-6736(12)61229-X
5. Ballas SK, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: frequency, etiology, and prognostic significance. Am J Hematol 2005; 79: 17-25. https://doi.org/10.1002/ajh.20336
6. Darbari DS, Sheehan VA, Ballas SK. The vaso-occlusive pain crisis in sickle cell disease: Definition, pathophysiology, and management. Eur J Haematol 2020; 105: 237-246. https://doi.org/10.1111/ejh.13430
7. Tumblin A, Tailor A, Hoehn GT, et al. Apolipoprotein A-I and serum amyloid a plasma levels are biomarkers of acute painful episodes in patients with sickle cell disease. Haematologica 2010; 95: 1467-1472. https://doi.org/10.3324/haematol.2009.018044
8. Lipkovich I, Dmitrienko A. Strategies for identifying predictive biomarkers and subgroups with enhanced treatment effect in clinical trials using SIDES. J Biopharm Stat 2014; 24: 130-153. https://doi.org/10.1080/10543406.2013.856024
9. Gosho M, Nagashima K, Sato Y. Study designs and statistical analyses for biomarker research. Sensors (Basel) 2012; 12: 8966-8986. https://doi.org/10.3390/s120708966
10. Huang Y, Pepe MS. Semiparametric methods for evaluating the covariate-specific predictiveness of continuous markers in matched case-control studies. J R Stat Soc Ser C Appl Stat 2010; 59: 437-456. https://doi.org/10.1111/j.1467-9876.2009.00707.x
11. Pepe MS, Feng Z, Huang Y, et al. Integrating the predictiveness of a marker with its performance as a classifier. Am J Epidemiol 2008; 167: 362-368. https://doi.org/10.1093/aje/kwm305
12. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982; 143: 29-36. https://doi.org/10.1148/radiology.143.1.7063747
13. Huang Y, Pepe MS, Feng Z. Evaluating the predictiveness of a continuous marker. Biometrics 2007; 63: 1181-1188. https://doi.org/10.1111/j.1541-0420.2007.00814.x
14. Huang Y, Pepe MS. Assessing risk prediction models in case-control studies using semiparametric and nonparametric methods. Stat Med 2010; 29: 1391-1410. https://doi.org/10.1002/sim.3876
15. Huang Y, Pepe MS. A parametric ROC model-based approach for evaluating the predictiveness of continuous markers in case-control studies. Biometrics 2009; 65: 1133-1144. https://doi.org/10.1111/j.1541-0420.2009.01201.x
16. Pepe MS, Janes H, Longton G, Leisenring W, Newcomb P. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am J Epidemiol 2004; 159: 882-890. https://doi.org/10.1093/aje/kwh101
17. STATISTICA (data analysis software system). Version 13.5.0.17. Hillview Avenue, CA; USA: TIBCO Software Inc; 2017.
18. Stata/MP (Statistical Software) Release 11. College Station, Texas; USA: StataCorp LP; 2009.
19. Osunkwo I, Manwani D, Kanter J. Current and novel therapies for the prevention of vaso-occlusive crisis in sickle cell disease. The Adv Hematol 2020; 11: 1-15. https://doi.org/10.1177/2040620720955000
20. Zempsky WT. Evaluation and treatment of sickle cell pain in the emergency department: paths to a better future. Clin Pediatr Emerg Med 2010; 11: 265-273. https://doi.org/10.1016/j.cpem.2010.09.002
21. Rees DC, Gibson JS. Biomarkers in sickle cell disease. Br J Haematol 2011; 156: 433-445. https://doi.org/10.1111/j.1365-2141.2011.08961.x
22. Stojanovic KS, Steichen O, Lefevre G, et al. High lactate dehydrogenase levels at admission for painful vaso-occlusive crisis is associated with severe outcome in adult SCD patients. Clin Biochem 2012; 45: 1578-1582. https://doi.org/10.1016/j.clinbiochem.2012.07.114
23. Onyekwelu KC, Ufelle SA, Ikekpeazu JE, Ezeh RC, Uche CZ, Udoh IP. Changes during vaso-occlusive crisis (VOC) and normal state in sickle cell disease patients. Med Case Rep Rev 2019; 2: 1-4. https://doi.org/10.15761/MCRR.1000122

24. Krishnan S, Setty Y, Betal SG, et al. Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell vasocclusive crises. Br J Haematol 2010; 148: 797-804. https://doi.org/10.1111/j.1365-2141.2009.08013.x

25. Al-Basheer JG, Abdelgadir O, Mustafa AEM, Muddathir ARM, Abdelgadir RE. C - reactive protein level and WBC count as biomarkers for vaso-occlusive crisis among patients with sickle cell disease. American Journal of Medicine and Medical Sciences 2015; 5: 283-286.

26. Gail MH, Pfeiffer RM. On criteria for evaluating models of absolute risk. Biostatistics 2005; 6: 227-239. https://doi.org/10.1093/biostatistics/kxi005

27. Pepe MS, Feng Z, Gu JW. Comments on 'Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond' by M. J. Pencina et al., Statistics in Medicine. Stat Med 2008; 27: 173-181. https://doi.org/10.1002/sim.2991

28. Sachs MC, Zhou XH. Partial summary measures of the predictiveness curve. Biom J 2013; 55: 589-602. https://doi.org/10.1002/bimj.201200146

29. Empereur-mot C, Guillemain H, Latouche A, Zagury JF, Viallon V, Montes M. Predictiveness curves in virtual screening. J Cheminform 2015; 7: 52. https://doi.org/10.1186/s13321-015-0100-8

30. Janes H, Pepe MS, Bossuyt PM, Barlow WE. Measuring the performance of markers for guiding treatment decisions. Ann Intern Med 2011; 154: 253-259. https://doi.org/10.7326/0003-4819-154-4-201102150-00006

The Turkish Journal of Pediatrics 2022; 64: 322-331
https://doi.org/10.24953/turkjped.2020.3343

**Original Article**

# Evaluation of nocturnal blood pressure changes and urinary electrolyte excretion in children with enuresis

Zeynep Şengül Emeksiz[1], Pınar Işık Ağras[2], Serhat Emeksiz[1], Yıldız Bilge Dallar[1]

*Departments of [1]Pediatrics and [2]Pediatric Nephrology, Ministry of Health Ankara Training and Research Hospital, Ankara, Turkey.*

## ABSTRACT

**Background.** Monosymptomatic nocturnal enuresis (MNE) is defined as involuntary nighttime urination of children over five years of age without any congenital or acquired defect in the central nervous system. Many factors, mainly nocturnal polyuria, sleep disorders, decreased bladder capacity, and bladder dysfunctions play a role in the etiology of MNE.

**Methods.** Eighty-three children diagnosed with MNE were included in the study. Complete blood cell count, blood biochemistry, renin, and aldosterone levels of all children were obtained. Twenty-four-hour urine samples were collected separately daytime and nighttime and urinary electrolytes were evaluated. Also, 24-hour ambulatory blood pressure monitoring (ABPM) was performed for each patient. The results were evaluated by comparing both enuretic children vs. control group and enuretic children with polyuria vs. without polyuria.

**Results.** When we compared the enuretic children and the control group in terms of urinary electrolytes, the fractional excretion of sodium (FENa) and fractional excretion of potassium (FEK) values of the enuretic group were higher than the control. The evaluation of the 24-hour ABPM findings revealed no significant difference in terms of the mean arterial pressure (MAP) and diastolic blood pressure (DBP) during the daytime and nighttime measurements. The daytime systolic blood pressure (SBP), however, was significantly lower in the enuretic group. When enuretic children with and without polyuria and the control group were compared, the nighttime, FENa, FEK, as well as nighttime urinary excretion of calcium and protein were significantly higher in enuretic children with polyuria. No difference was detected on the MAP, SBP, or DBP values.

**Conclusions.** In conclusion, the nighttime urinary solute excretion of enuretic children was found to be higher and this condition may especially be associated with pathogenesis of nighttime polyuria. In enuretic children, nighttime blood pressure changes were not influential in the etiopathogenesis in all patient groups and multiple mechanisms may play a role in the pathogenesis of enuresis.

**Key words:** enuresis, ambulatory blood pressure, urinary electrolyte excretion.

Monosymptomatic nocturnal enuresis (MNE) is involuntary urination only during sleep which is not accompanied by any bladder symptoms and with completely normal daytime urination pattern. Its prevalence is about 10% in seven-year-olds and about 5% in 10-year-olds.[1,2]

Numerous factors influence the etiology of primary MNE. The least implicated among these factors are nocturnal polyuria, sleep disorders, decreased bladder capacity and bladder dysfunction. However, the same pathophysiological mechanisms do not apply to each patient and it is considered that different mechanisms could be responsible for different wet nights of the same patient.[2,3]

It is well established that sodium and potassium excretion of normal children present a diurnal rhythm and that excretion is reduced during

✉ Zeynep Şengül Emeksiz
drzeynep83@hotmail.com

Received 10th November 2020, revised 3rd May 2021, accepted 5th October 2021.

nighttime but this rhythm is impaired in enuretic children.[4] Increased solute excretion accompanying tubular reabsorption of water can also explain this situation. In the literature, there are studies suggesting that the pathogenesis of enuresis is affected by disturbances in distal renal tubules.[5]

In enuretic children; our knowledge on the relationship between nocturnal blood pressure changes and nocturnal polyuria is still limited. In this study, it is aimed to reveal the correlation between the daytime – nighttime blood pressure changes and electrolyte excretion in children with primary MNE.

## Material and Methods

In this study; 83 children diagnosed with MNE were included. The control group was constituted by 62 patients with same age and gender characteristic. Children with MNE with minimum three wet nights a week were included in the patient group before any medical or behavioral treatment were started. The control group consisted of children who applied to outpatient clinics with complaints other than enuresis, including acute mild respiratory tract infection or for routine check-up.

Complete blood cell counts, biochemical parameters, renin and aldosterone levels of all children were worked on venous blood samples that were taken at 08:00 a.m. For determination of renin and aldosterone levels, RAI-DSL kit (Active® RIA DSL; Diagnostic Systems Laboratories, Inc., Webster, TX) was used. The samples were employed in the Ministry of Health Ankara Training and Research Hospital Biochemistry and Hematology laboratories.

During the following 24-hour following sampling, urine samples were collected separately in nighttime and daytime for evaluating sodium, potassium, creatinine, phosphorous, calcium and protein values.

For night time urine collection, the child was awakened once more than the usual nightly incontinence frequency, taken to the toilet and urine was collected for the whole night including the first morning urine sample.

FENa, FEK, tubular phosphorous reabsorption (TPR) was calculated with following formulae:

FENa (%) = [(urinary sodium / urinary creatinine) x (serum creatinine / serum sodium)] x 100

FEeK (%) = [(urinary potassium / urinary creatinine) x (serum creatinine / serum potassium)] x 100

TPR (%) = [(1- (urinary phosphorous / urinary creatinine) x (serum creatinine / serum phosphorous)] x 100

Urinary protein excretion was calculated as "mg/m²/h" while urinary calcium excretion was calculated as "mg/kg/day".

The bladder capacity of the enuretic children was calculated by the formula: 30 + [age (years) x 30] ml formula. Children with a nighttime urine volume exceeding 130% of the expecting bladder capacity were acknowledged as polyuric enuretic children. The results were compared in two stages: enuretic children vs. control group and enuretic children with/without polyuria vs. control group.

Simultaneously with urine collection, the ambulatory blood pressures of all children were measured with Mobil – O – Graph NG (I.E.M. GmbH, Germany) device at intervals of 15 minutes during daytime and 30 minutes on average during nighttime while children were conducting their normal daily activities. During ambulatory blood pressure measurement, sleeping and waking up times were recorded. Day and night time measurements were defined and evaluated according to these data. Blood pressure percentiles according to age, gender and height were used for evaluation of measurements.[6]

Written informed consent was obtained for each child included in the study. The study was approved by the Ethics Committee of Ministry

of Health Ankara Training and Research Hospital (no: 0398/2010).

### *Statistical analysis*

The data obtained from this study were evaluated by means of SPSS 22 package software. Descriptive statistics were indicated as mean ± standard deviation (minimum – maximum) or as median (IQR, 25th–75th percentile) while nominal variables were indicated as observation count and (%). For double group comparisons Student's t-test, for triple group comparison Kruskall-Wallis test; for comparison of paired variables, the Wilcoxon tests were used. For correlation analysis, Pearson correlation analysis method was used. The significance level was accepted <0.05.

## Results

Among 83 enuretic children, 33 (39.8%) were girls and 50 (60.2%) were boys with average age of 9.2 ± 2.6 years. In the control group, 32 children out of 62 (51.7%) were girls while 30 (48.35) were boys with an average age of 9.9 ± 2.2 years. In terms of age and gender, the children with enuresis were of similar characteristics with the control group (p>0.05).

### *Comparison of enuretic children with the control groups*

When the results of complete blood cell count of patients were assessed, no significant difference (p>0.05) was found between the children with enuresis and the control group in terms of hemoglobin, white blood cell count and platelet counts. However, hematocrit values were lower in the control group (p<0.05).

Among biochemical parameters, urea, creatinine, albumin, sodium, potassium, calcium and phosphorous were evaluated. The values of phosphorous were determined to be lower in the control group (p<0.05). No significant difference in terms of other biochemical parameters was detected between two groups (p>0.05). There was no significant difference in terms of serum renin and aldosterone levels. (p>0.05) (Table I)

The mean nighttime urine volume of enuretic group was distinctively higher (1.1 ± 0.7 ml/kg/h) than control group (0.9 ± 0.5 ml/kg/h) (p<0.05). Daytime urine volumes were similar in both groups (Table II).

The mean FENa of the enuretic group, calculated in the nighttime urine samples was found to be significantly higher than that of

**Table I.** Laboratory findings of children included in the study.

| Variables | Enuretic children (N=83) | Control group (N=62) | P value |
|---|---|---|---|
| Hemoglobin (gr/dl) | 13.2 ± 0.9 | 12.9 ±1.2 | >0.05 |
| Hematocrit (%) | 39.0 ± 2.7 | 37.7 ± 3.6 | <0.05* |
| White blood cell count (/mm$^3$) | 7938 ± 2864 | 7740 ± 2563 | >0.05 |
| Platelet count (/mm$^3$) | 302,554 ± 82,209 | 286,916 ± 66,685 | >0.05 |
| Urea (mg/dl) | 24.9 ± 7.3 | 25.1 ± 7.3 | >0.05 |
| Creatinine (mg/dl) | 1.5 ± 0.2 | 0.6 ± 0.1 | >0.05 |
| Albumin (gr/dl) | 4.5 ± 0.2 | 4.6 ± 0.4 | >0.05 |
| Sodium (mEq/L) | 137.9 ± 13.8 | 139.3 ± 2.3 | >0.05 |
| Potassium(mEq/L) | 4.4 ± 0.3 | 4.2 ± 0.6 | >0.05 |
| Calcium (mg/dl) | 9.9±0.4 | 9.9 ± 0.4 | >0.05 |
| Phosphorus (mg/dl) | 4.7±0.5 | 4.4 ± 0.6 | <0.05* |
| Renin (pg/ml) | 30.2 ± 18.5 | 25.7 ± 12.0 | >0.05 |
| Aldosterone (pg/ml) | 211.3 ± 136.8 | 176.1 ± 129.0 | >0.05 |

Data are presented as mean ± standard deviation.
*p <0.05

the control group (p<0.05). Yet there was no significant difference between enuretic children and control group regarding the mean FENa calculated in daytime urine samples (p>0.05) (Table II).

FEK value calculated in nighttime urine samples was higher in enuretic children compared to control group. No significant difference was determined regarding the daytime values of the same parameters (p>0.05). When comparing enuretic children with the control group in terms of daily urinary excretion of calcium and protein, no significant difference was determined (p> 0.05) (Table II).

TPR values were comparable between the two groups (p>0.05) (Table II).

Evaluation of ambulatory blood pressure values showed that daytime mean systolic blood pressure of enuretic children (104.1± 6.5 mm Hg) was lower compared to that of the control group (107.2± 8.4 mm Hg) (p<0.05). When systolic daytime blood pressure load was analyzed; enuretic children showed lower results (5.1± 5.9 mm Hg) than the control group (8.7± 10.1 mm Hg) (p<0.05). No significant difference was determined between the two groups in terms of other ambulatory blood pressure parameters (p>0.05; Table III).

### *Comparison of enuretic children with vs. without polyuria and control group*

Children with polyuria had significantly higher mean FENa and FEK in nighttime urine samples (p <0.05); but in daytime urine samples, there were no differences. Similarly, the urinary calcium and protein excretion in nighttime urine samples were distinctly higher in the group of enuretic children with polyuria, compared to enuretic children without polyuria and the control group (p<0.05; Table IV). Mean TPR values were comparable.

Comparison of 24-hour ABPM parameters of the children included in the study showed only one significant difference which was the systolic blood pressure load. Daytime systolic blood pressure load was significantly higher in enuretic children with polyuria compared to those enuretic children without polyuria

**Table II.** 24-hour urine findings of enuretic children and the control group.

| Variables | Enuretic Children (N=83) | Control Group (N=62) | P value |
|---|---|---|---|
| Daytime urine volume (ml/kg/h) | 1.3 ± 0.8 | 1.1 ± 0.5 | >0.05 |
| Nighttime urine volume (ml/kg/h) | 1.1 ± 0.7 | 0.9 ± 0.5 | <0.05* |
| FENa in daytime urine (%) | 1.2 ± 1.8 | 0.9 ± 0.7 | >0.05 |
| FENa in nighttime urine (%) | 1.0 ± 1.4 | 0.5 ± 0.3 | <0.05* |
| FEK in daytime urine (%) | 6.3 ± 5.4 | 7.9 ± 1.9 | >0.05 |
| FEK in nighttime urine (%) | 2.2 ± 2.9 | 1.5 ± 1.5 | <0.05* |
| Daytime urinary calcium excretion (mg/kg/day) | 1.2 ± 1.1 | 1.2 ± 1.2 | >0.05 |
| Nighttime urinary calcium excretion (mg/kg/day) | 0.7 ± 0.6 | 0.5 ± 0.4 | >0.05 |
| Daytime urinary protein excretion (mg/m²/h) | 4.6 ± 3.5 | 5.4 ± 4.5 | >0.05 |
| Nighttime urinary protein excretion (mg/m²/h) | 5.1 ± 4.9 | 5.3 ± 5.5 | >0.05 |
| TPR daytime (%) | 95.2 ± 3.8 | 93.6 ± 11.2 | >0.05 |
| TPR nighttime (%) | 94.6 ± 3.1 | 94.9 ± 5.8 | >0.05 |

Data are presented as mean ± standard deviation.
FENa: fractional excretion of sodium, FEK: fractional excretion of potassium, TPR: tubular phosphorus reabsorption.

**Table III.** 24-hour blood pressure monitoring data of enuretic and control group.

| Variables | Enuretic Children (N=83) | Control Group (N=62) | P value |
|---|---|---|---|
| MAP daytime (mmHg) | 81.3 ± 5.0 | 83.1 ± 6.7 | >0.05 |
| MAP nighttime (mmHg) | 72.2 ± 5.6 | 73.4 ± 4.7 | >0.05 |
| SBP daytime (mmHg) | 104.1 ± 6.5 | 107.2 ± 8.4 | <0.05* |
| SBP nighttime (mmHg) | 94.7 ± 7.4 | 97.1 ± 7.1 | >0.05 |
| DBP daytime (mmHg) | 62.0 ± 4.8 | 63.2 ± 6.8 | >0.05 |
| DBP nighttime (mmHg) | 53.2 ± 5.0 | 53.9 ± 4.4 | >0.05 |

Data are presented as mean ± standard deviation.
*p < 0.05
DBP: mean diastolic pressure, MAP: mean arterial pressure, SBP: mean systolic pressure.

**Table IV.** 24-hour urine findings of enuretic children with/without polyuria and control group.

| Parameters | Enuretic children with polyuria (N=24) | | Enuretic children without polyuria (N=59) | | Control Group (N=62) | |
|---|---|---|---|---|---|---|
| | Daytime | Nighttime | Daytime | Nighttime | Daytime | Nighttime |
| FENa (%) | 1.7 ± 3.3 | 1.4 ± 1.9 *, a | 1.0 ± 0.8 | 0.7 ± 0.4 *, a | 0.9 ± 0.7 | 0.5 ± 0.3 *, a |
| FEK (%) | 7.8 ± 5.2 | 3.8 ± 2.8 *, a | 5.9 ± 5.5 | 1.6 ± 2.7 *, a | 8.0 ± 1.9 | 1.5 ± 1.5 *, a |
| Protein excretion (mg/m2/h) | 5.5 ± 3.1 | 7.9 ± 7.9 *, a | 4.3 ± 3.6 | 4.2 ± 2.9 *, a | 5.5 ± 4.5 | 5.4 ± 5.5 *, a |
| Calcium excretion (mg/m2/h) | 1.2 ± 0.7 | 0.9 ± 0.5 *, a | 1.2 ± 1.2 | 0.6 ± 0.7 *, a | 1.2 ± 1.3 | 0.5 ± 0.4 *, a |
| TPR (%) | 94.6 ± 2.5 | 94.0 ± 2.4 | 95.4 ± 4.2 | 94.8 ± 3.3 | 93.6 ± 11.3 | 94.9 ± 5.8 |

Data are presented as mean ± standard deviation.
FEK= fractional excretion of potassium, FENa: fractional excretion of sodium, TPR: tubular phosphorus reabsorption.
*p < 0.05
a: nighttime FENa, FEK, protein, and Ca excretion were significantly higher in enuretic children with polyuria, compared to enuretic children without polyuria and the control group.

**Table V.** 24-hour blood pressure monitoring data of enuretic children with/without polyuria and the control group.

| Parameters | Enuretic children with polyuria (N=24) | | Enuretic children without polyuria (N=59) | | Control Group (N=62) | |
|---|---|---|---|---|---|---|
| | Daytime | Nighttime | Daytime | Nighttime | Daytime | Nighttime |
| SBP (mm Hg) | 105.7 ± 6.7 | 94.8 ± 5.6 | 103.5 ± 6.7 | 94.5 ± 7.3 | 106.8 ± 8.1 | 96.8 ± 6.9 |
| SBP-SDS | 0.0±0.8 | -0.1 ± 0.7 | -0.2 ± 0.8 | -0.1 ± 0.9 | 0.1 ± 1.0 | 0.1 ± 0.9 |
| DBP (mm Hg) | 63. 3± 4.2 | 53.3 ± 4.3 | 61.7 ± 4.8 | 53.0 ± 4.0 | 62.8 ± 6.5 | 53.8 ± 4.4 |
| DBP-SDS | 0.1 ± 0.7 | -0.0 ± 0.9 | -0.1 ± 0.8 | -0.1 ± 0.8 | 0.0 ± 1.1 | 0.0 ± 0.9 |
| MAP (mm Hg) | 82.5 ± 4.9 | 72.3±4.7 | 80.9 ± 5.0 | 71.8 ± 4.7 | 82.9 ± 6.6 | 73.4 ± 4.7 |
| MAP-SDS | 0.0 ± 0.8 | -0.0 ± 0.8 | -0.2 ± 0.8 | -0.1 ± 0.8 | 0.1 ± 1.1 | 0.1 ± 0.8 |
| SBP load (%) | 6.8 ± 8.9 * | 7.8 ± 16.4 | 4.6 ± 6.4* | 7.9 ± 14.5 | 8.7 ± 10.1 | 10.5 ± 15.5 |
| DBP load (%) | 5.2 ± 11.8 | 9.8 ± 9.8 | 6.5 ± 8.4 | 10.4 ± 9.4 | 6.7 ± 9.0 | 12.7 ± 12.6 |
| SBP dipping (%) | 10.2 ± 3.4 | | 9.4 ± 4.1 | | 9.6 ± 4.9 | |
| DBP dipping (%) | 15.7 ± 4.9 | | 13.9 ± 6.1 | | 14.1 ± 7.3 | |

Data are presented as mean ± standard deviation.
DBP: diastolic blood pressure, MAP: mean arterial pressure, SBP: systolic blood pressure, SDS: Standard derivation score
*p <0.05; daytime systolic blood pressure load was significantly higher in enuretic children with polyuria compared to those enuretic children without polyuria

(p<0.05). When all three groups are compared, the highest MAP pressure was found in the control group, but it did not reach to statistical significance (p >0.0.5, Table V).

No significant correlation was determined between the ABPM parameters and urinary excretion of electrolytes day and nighttime. Similarly, serum renin and aldosterone levels were not correlated with ABPM parameters (p>0.05).

## Discussion

Monosymptomatic nocturnal enuresis is a multifactorial health issue that affect approximately 10% of children between the ages of 5 -10 years.[7] Children with normal pediatric development, daytime bladder control is usually achieved between the age of 2–3 years and nighttime control is developed around 3–5 years of age. According to the criteria of International Children's Continence Society, children over 5 years of age are expected to have urinary control during sleep.[8] For this reason, our study included 83 enuretic children above 5 years of age with an average age of 9.2 ± 2.6 years.

It has been shown that enuresis is seen equally in both genders until the age of five, then the incidence increases gradually in boys and its incidence is twice higher in boys than girls around the age of 11.[9] In our study, 60.2% of the enuretic children were boys, which was compatible with the literature.[10] The frequent occurrence of enuresis in boys can be attributed to the lower rate of spontaneous recovery and the higher frequency of secondary enuresis in boys.[11,12]

In our study, the electrolyte excretion in daytime and nighttime urine samples of enuretic children and the children in the control group were calculated. No difference was determined on the daytime mean FENa and FEK values but the mean FENa and FEK values calculated in nighttime urine samples of enuretic children was found to be significantly higher than the control group. Review of the literature shows that FENa and FEK excretion were found to be higher in children with MNE compared to controls.[13] Another study including 30 enuretic children showed only increased potassium excretion.[5] Increased potassium excretion in those children suggests that there may be failure of some potassium -regulating mechanisms in distal tubules. Excessive K+ in the distal tubule together with the low ADH in enuretic may cause less tubular fluid reabsorption, and insufficiently reabsorbed K+ remains in the distal tubule concomitantly with water. Kir 4.1 is expressed in the kidney with high specificity only in the distal convoluted tubule (DCT) on the basolateral membrane, and speculated that it is critical for K+ recycling. KCNJ10 channel protein is a member of the Kir 4.1 family.[13] Balat et al.[13] studied relation between urinary electrolytes, especially K+ and KCNJ10 gene promoter polymorphism. They found that SNP3 in promoter of KCNJ10 gene was strongly associated with either distribution of genotype and allele frequency in enuretic, and TT genotype was associated with higher urinary K+ excretion. They also suggested that determination of the relationship between KCNJ10 gene polymorphism and polyuria in enuretic children could be more informative.

When polyuric–non polyuric children and control group were compared in terms of urinary excretion of electrolytes in our study, no difference was determined in daytime urine samples. In children with nocturnal polyuria, however, the mean nighttime FENa, the mean nighttime FEK urinary excretion, protein and calcium excretions were found to be significantly higher. These data, which are compatible with previous studies, suggest that FENa correlates with nocturnal polyuria and nocturnal enuresis, especially in enuretic patients with polyuria.[12]

When enuretic children and the control group were compared in terms of urinary calcium and protein excretion and TPR in daytime and nighttime urine samples, no significant difference was found between these two groups. When these parameters were evaluated

regarding enuretic children with vs. without nocturnal polyuria and the control group, however, the urinary calcium and protein excretion of children with nocturnal polyuria was determined to be higher. Raes et al.[14] determined hypercalciuria in 12% of children with nocturnal enuresis and stated a correlation between urinary calcium excretion, nocturnal urine volume and increased sodium excretion in urine. However, hypercalciuria is a symptom already present in the normal population with a ratio around 3 - 7%. Furthermore, enuresis is a symptom that can be seen in cases with primary hypercalciuria. Depending on these data, it can be stated that this correlation between enuresis and hypercalciuria should not be considered as a primary factor but as a co-morbid factor in the pathogenesis of enuresis.

Increased Na and K excretion accompanied by increased protein and calcium excretion in nighttime urine samples of enuretic children with nocturnal polyuria as shown by our study suggests that renal tubular defects may have a role in the etiopathogenesis in enuretic children with nocturnal polyuria. Yet, it should be also kept in mind that hormonal factors also have a role in the regulation of renal water and solute excretions. The literature contains limited data regarding the role of atrial natriuretic peptide, renin, aldosterone and angiotensin-2 in the etiology of enuresis.[3] In our study, enuretic children and control group were compared in terms of renin and aldosterone levels but no significant difference was found. Kamperis et al.[15] compared ANP, angiotensin-2, aldosterone and renin levels and, they determined no difference between enuretic children and the control group which is similar to the findings of our study. However, in the same study, a significant increase was determined in the prostaglandin E-2 (PGE2) level of patients with nocturnal polyuria and it was suggested that renal prostaglandins were the key molecule in the etiopathogenesis of enuresis. PGE2 prevents ADH's effects on tubular water reabsorption and at the same time potentiates its natriuretic effect. For this reason, even though the natriuresis in children with nocturnal polyuria cannot be directly explained with abnormalities in the circadian rhythm of ANP, renin, aldosterone and angiotensin-2, it is possible that increased PGE2 is the main factor responsible for this occurrence.[16]

When enuretic children and the control group was compared in terms of the findings of ABPM, no significant difference was determined between the two groups in terms of the MAP and the DBP values that were measured both during daytime and nighttime. The only difference determined between the two groups was the lower SBP in the enuretic group. However, it was considered that this reduction in daytime values was not involved in the pathogenesis of enuresis. Additionally, enuretic children with versus without nocturnal polyuria and the control group were also compared. No significant difference was found among all three groups in terms of the SBP, DBP and MAP as measured both daytime and nighttime.

Review of the literature shows that most of the studies on this subject revealed no significant difference between the nocturnal blood pressure values of enuretic children with polyuria versus without polyuria.[17,18] Normally, nocturnal decrease in urine production is accompanied by reduced blood pressure during sleep. On the basis of this information, one can expect to determine some variations in nocturnal blood pressure pattern in enuretic children with nocturnal polyuria. In order to explain why ABPM findings of children with polyuria versus without polyuria were found to be similar in our study and some other studies in the literature, one can speculate that sleep patterns of enuretic children included in the study may be different or disturbed. Another reason for this finding may be the presence of additional pathophysiological mechanisms, other than nocturia, affecting the blood pressure. Contrary to the data in our study, in a study conducted by Anne et al.[17] higher MAP values were found in enuretic children with nocturnal polyuria compared to enuretic children without nocturnal polyuria and to

the control group and it was reported that the enuresis might be caused by polyuria resulting from this increase in blood pressure. In another study evaluating the role of autonomic activity in the pathogenesis, selective nocturnal increase in the mean diastolic and mean arterial pressure was found in children with enuresis, and this increase was thought to be associated with sympathetic nervous system hyperactivation.[2]

Various conclusions in the literature related to nocturnal blood pressure changes in children with enuresis may be related to variability of nocturnal activity of the autonomic nervous system. Dundaroz et al.[19] analyzed the heart rate changes in children with nocturnal enuresis and increase in heart rate supporting sympathetic hyperactivity was determined.[20] Contrary to this, many studies in this field showed existence of parasympathetic neural system hyperactivity and consequent bladder hyperactivity.[20] Conflicting results in the literature, give rise to the thought that the autonomic nervous system can be more active in sympathetic or parasympathetic way in different enuretic patient groups.

According to the theory that anticipates a relationship between nocturnal blood pressure changes and enuresis; in enuretic children, it is expected that the nocturnal increase of blood pressure should increase glomerular filtration and consequently the urinary solute excretion. As a result, urinary volume should increase during the night. However, from the data obtained in our study, no correlations between ABPM findings and urinary electrolyte excretions and volumes were found.

In the literature, the nocturnal blood pressure increase was determined predominantly in enuretic children with polyuria.[17] In general, also in our study, approximately one out of every five enuretic children showed polyuria. However, results of separate evaluations of cases with polyuria provided similar nocturnal blood pressure levels with those without polyuria. These findings support the idea that the nocturnal blood pressure changes are not an invariable finding in enuretic children and that in addition to the blood pressure, different pathogenic mechanisms play a role in the etiology of enuresis.

In the literature, there are various studies on nighttime urinary electrolyte excretion in enuretic children. However, the number of studies on blood pressure changes in enuretic children is limited. Yet, our study included the highest number of patients compared to other studies. Additionally, the study bears a certain significance by evaluating both blood pressure and urinary volume as well as urinary electrolyte excretion. We can conclude that our study reflects the findings of enuretic children on a broader basis and provides more reliable data since it included a large number of patients than any other study in the literature and since it examined various parameters and their correlations. Our study compared the ABPM findings and urinary electrolyte excretions in detail rather than a specific pathogenetic mechanism, which may be considered a limitation. But still, this may provide a broader view to the subject.

In conclusion; it has been shown that nighttime urinary solute excretion is higher in enuretic children. This finding may play a role in the pathogenesis of nocturnal polyuria. Furthermore, it has been found that nocturnal blood pressure changes are not invariably present in all enuretic children. As a result, it has been concluded that pathogenesis of enuresis is multifactorial and different factors may play a role in each individual patient.

## Ethical approval

The study was approved by the Ethics Committee of Ministry of Health Ankara Training and Research Hospital (no: 0398/2010).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ZSE, PIA; data collection: ZSE, SE; analysis and interpretation of results: ZSE, PIA; draft manuscript preparation: ZSE, PIA, SE, YBD. 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. Kuwertz-Bröking E, Gontard A. Clinical management of nocturnal enuresis. Pediatr Nephrol 2018; 33: 1145-1154. https://doi.org/10.1007/s00467-017-3778-1
2. Yüce Ö, Bayrakçi US, Gülleroğlu K, Baskın E. Abnormal circadian blood pressure regulation in children with nocturnal enuresis. Ren Fail 2016;38:899-905. https://doi.org/10.3109/0886022X.2016.1164064
3. Bascom A, McMaster MA, Alexander RT, MacLean JE. Nocturnal enuresis in chil-dren is associated with differences in autonomic control. Sleep 2019; 42: zsy239. https://doi.org/10.1093/sleep/zsy239
4. Ritting S, Knudsen UB, Norgaard JP, et al. Diurnal variation of plasma atrial natriu-retic peptide in normals and patients with enuresis nocturna. Scand J Clin Lab Invest 1991; 51: 209-217. https://doi.org/10.1080/00365519109091109
5. Balat A, Cekmen M, Yürekli M, et al. Adrenomedullin and nitrite levels in children with primary nocturnal enuresis. Pediatr Nephrol 2002; 17: 620-624. https://doi.org/10.1007/s00467-002-0922-2
6. Wuhla E, Witteb K, Soergelc M, Mehlsa O, Schaefera F. Distribution of 24-h ambula-tory blood pressure in children: normalized reference values and role of body dimen-sions. J Hypertens 2002; 20: 1995-2007. https://doi.org/10.1097/00004872-200210000-00019
7. Lottmann HB, Alova I. Primary monosymptomatic nocturnal enuresis in children and adolescents. Int J Clin Pract Suppl 2007; 155: 8-16. https://doi.org/10.1111/j.1742-1241.2007.01464.x
8. Austin PF, Bauer SB, Bower W, et al. The standardization of terminology of lower urinary tract function in children and adolescents: Update report from the standardi-zation committee of the International Children's Continence Society. Neurourol Uro-dyn 2016; 35: 471-481. https://doi.org/10.1002/nau.22751
9. Mohammadi M, Vaisi Raiegani AA, Jalali R, Ghobadi A, Salari N. The prevalence of nocturnal enuresis among Iranian children: a systematic review and meta-analysis. Urol J 2019; 16: 427-432. https://doi.org/10.1186/s12978-019-0804-8
10. Ozden C, Ozdal OL, Altinova S, et al. Prevalence and associated factors of enuresis in Turkish children. Int Braz J Urol 2007; 33: 216-222. https://doi.org/10.1590/S1677-55382007000200013
11. Kajiwara M, Inoue K, Kato M, et al. Nocturnal enuresis and overactive bladder in children: an epidemiological study. Int J Urol 2006; 13: 36-41. https://doi.org/10.1111/j.1442-2042.2006.01217.x
12. Kahraman A, Dursun H, Hatipoglu S, et al. Non-dipping phenomenon in children with monosymptomatic nocturnal enuresis. Pediatr Nephrol 2013; 28: 1099-2103. https://doi.org/10.1007/s00467-013-2448-1
13. Balat A, Parlak M, Balcı SO, et al. Increased potassium excretion in children with monosymptomatic nocturnal enuresis: could it be related to Kir 4.1-KCNJ10 gene polymorphism?. Turk J Pediatr 2020; 62: 208-214. https://doi.org/10.24953/turkjped.2020.02.006
14. Raes A, Dossche L, Hertegonne N, et al. Hypercalciuria is related to osmolar excre-tion in children with nocturnal enüresis. J Urol 2010; 183: 297-301. https://doi.org/10.1016/j.juro.2009.08.175
15. Kamperis K, Rittig S, Jorgensen KA, Djurhuss JC. Nocturnal polyuria in monosymp-tomatic nocturnal enuresis refractory to desmopressin treatment. Am J Physiol Renal Phsiol 2006; 291: 1232-1240. https://doi.org/10.1152/ajprenal.00134.2006
16. Rittig S, Matthiesen TB, Pedersen EB, Djurhuss JC. Circadian variation of angioten-sin and aldosteronee in nocturnal enuresis: relationship to arterial blood pressure and urine output. J Urol 2006; 176: 774-780. https://doi.org/10.1016/S0022-5347(06)00594-5

17. Kruse A, Mahler B, Rittig S, Djurhuus JC. Increased nocturnal blood pressure in enu-retic children with polyuria. J Urol 2009; 182: 1954-1960. https://doi.org/10.1016/j.juro.2009.04.079

18. Kamperis K, Hagstroem S, Radvanska E, Rittig S, Djurhuus JC. Excess diuresis and natriuresis during acute sleep deprivation in healthy adults. Am J Physiol Renal Phys-iol 2010; 299: F404-F411. https://doi.org/10.1152/ajprenal.00126.2010

19. Dundaroz MR, Denli M, Uzun M, et al. Analysis of heart rate variability in children with primary nocturnal enuresis. Int Urol Nephrol 2001; 32: 393-397. https://doi.org/10.1023/A:1017539317009

20. Yakinci C, Müngen B, Durmaz Y, Balbay D, Karabiber H. Autonomic nervous sys-tem functions in children in children with nocturnal enuresis. Brain Dev 1997; 19: 485-487. https://doi.org/10.1016/S0387-7604(97)00069-7

The Turkish Journal of Pediatrics 2022; 64: 332-340
https://doi.org/10.24953/turkjped.2020.2372

**Original Article**

# Etiology-based strabismus classification scheme for pediatricians

Mehmet Cem Mocan[1], Aishwarya Pastapur[2], Lawrence Kaufman[1]

*[1]Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL; [2]College of Medicine, University of Illinois at Chicago, Chicago, IL.*

## ABSTRACT

**Background.** Pediatricians are regularly involved in the initial examination of children presenting with strabismus, a common ocular condition occurring in 3% of children. The objective of this review was to gain insight into pediatric residents, fellows and attendings' understanding of strabismus, and to propose an etiology-based strabismus classification scheme to aid this understanding.

**Methods.** A survey was conducted in a single Department of Pediatrics in a university academic institution in order to assess the degree of understanding of the classification, etiology and nomenclature of strabismus. A targeted literature review, pertinent to our classification scheme for strabismus in the pediatric age group, is provided to clarify the various underlying etiological conditions for pediatricians.

**Results.** The surveyed cohort (n=26) consisted of 10 (38.5%) attendings and 16 (61.5%) pediatricians-in-training. Although 69% of survey participants felt comfortable performing an ocular motility evaluation, only 19% had a clear understanding of the underlying etiology of strabismus, 8% had a clear understanding of strabismus nomenclature and none of the participants had clear knowledge of a classification scheme of strabismus. We propose an etiologic-based strabismus classification scheme with streamlined nomenclature geared towards Pediatricians to facilitate the management of pediatric patients with various ocular misalignments. Eight major categories of this classification scheme include (1) physiologic, (2) comitant, (3) paralytic, (4) sensory, (5) syndromic, (6) orbital, (7) supranuclear and (8) pseudostrabismus.

**Conclusions.** Pediatricians at all levels of professional experience have a limited command of strabismus. An etiology-based classification scheme of strabismus may assist in understanding the underlying causes and facilitate the management of strabismus in the pediatrician's office.

**Key words:** strabismus classification, strabismus etiology, pediatric strabismus.

Strabismus is a common ophthalmic condition in children, with a prevalence around 2-3%.[1-3] Pediatricians should promptly identify ocular motor disorders in children for two main reasons: strabismus may be the only sign of a critical ocular, neurologic or systemic disease with significant health implications[4], and an ocular misalignment may lead to irreversible amblyopia and loss of binocularity if treatment is delayed beyond the age of visual plasticity.[5-6]

✉ Mehmet Cem Mocan
cmocan@uic.edu

Received 4th August 2020, revised 3rd May 2021, accepted 4th July 2021.

An ocular motor disorder may be observed as a crossing in of an eye (esotropia), a drifting out of an eye (exotropia), a vertical misalignment (hypertropia or hypotropia), or an incomplete rotation of an eye. Pediatricians are often the first medical providers to encounter infants or children who have strabismus, and have a pivotal role in the identification and referral of these patients.

The term strabismus in medical texts is often narrowly defined to describe an ocular motor problem in which the eyes are not properly aligned. Ophthalmologists tend to use a broader usage to include any type of eye muscle disturbance that may not necessarily include

a misalignment of the eyes, such as in a gaze palsy or apraxia.

Various classification schemes exist for strabismus, most of which are penned by pediatric ophthalmologists for use within the ophthalmology community.[7] These same schemes show up in primary care texts, and classify strabismus in terms of direction of misalignment, whether constant or intermittent, as comitant versus incomitant, as patterns with over- or under-actions of oblique extraocular muscles, as a relationship to accommodation, as excesses or insufficiencies of convergence or divergence, and in terms of age of onset or laterality.[7] Confusing nomenclature such as high accommodative convergence/accommodation esotropia or divergence excess exotropia may show up in these types of schemes. Also, these classification schemes provide little insight as to why patients develop the strabismus, the necessity for early referral or the expected work-up.

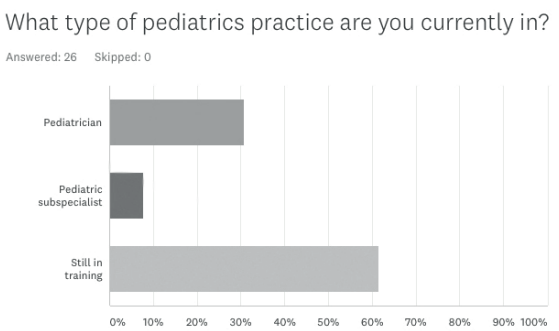
In the present study, we aimed to construct a clinically relevant classification of strabismus to simplify the differential diagnosis and work-up of pediatric patients for pediatricians and primary care physicians. In order to assess the current understanding of strabismus conditions by the pediatricians, we undertook a survey of the pediatrician faculty and pediatric residents/fellows at our academic institution to see if these individuals had a working knowledge or comfort level in understanding strabismus. We propose an alternative etiology-based classification scheme for strabismus with simplified nomenclature tailored towards the needs of pediatricians in dealing with this disorder.

## Material and Methods

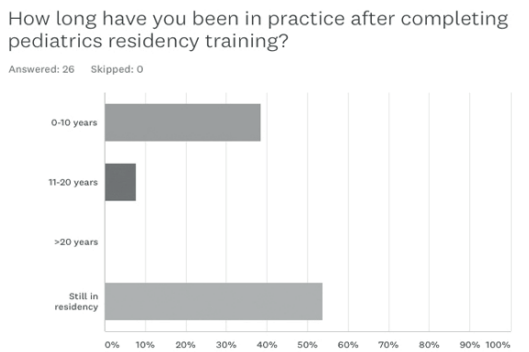
This cross-sectional survey was conducted on Pediatrician attendings and Pediatric trainees in a single university-based Department of Pediatrics. The demographics of the physicians surveyed are presented in Figure 1. A five-question survey was administered through a web-based portal (i.e. surveymonkey.com) in a blind fashion during a 4-week period (July-August 2019). The survey questions are presented in Table I, and the questions sought to identify the participant's level of comfort and knowledge regarding strabismus. No patient data was extracted and therefore an IRB approval was not required for this survey.

### *Data sources*

A PubMed data search was conducted between the years 1980 and 2020, utilizing the key words "strabismus", "strabismus classification",




| ANSWER CHOICES | RESPONSES | |
|---|---|---|
| Pediatrician | 30.77% | 8 |
| Pediatric subspecialist | 7.69% | 2 |
| Still in training | 61.54% | 16 |
| TOTAL | | 26 |




| ANSWER CHOICES | RESPONSES | |
|---|---|---|
| 0-10 years | 38.46% | 10 |
| 11-20 years | 7.69% | 2 |
| >20 years | 0.00% | 0 |
| Still in residency | 53.85% | 14 |
| TOTAL | | 26 |

**Fig. 1.** Demographics of physicians who participated in the survey according to current practice/training status.

and "strabismus etiology". Targeted searches based on the articles found through the initial PubMed query were additionally conducted. Publications written in a language other than English were not analyzed, apart from those that had abstracts provided in English.

## Results

The preliminary survey that served as the basis for the current review consisted of 26 physicians; 16 were pediatricians in training and 10 were attendings of the same department (Fig. 1). Of the surveyed physicians, 50% were comfortable with performing an eye screening exam on a pediatric patient and 69% were comfortable with performing an ocular motility examination. No physician surveyed had a clear understanding of a classification system of strabismus and only 19% had an understanding as to the etiology of strabismus in children. Furthermore, the majority (92%) of the surveyed population did not have a clear understanding of strabismus nomenclature. The response rates of pediatricians in training versus attending pediatricians are presented in Table I.

In the light of these results, a clinically relevant classification scheme was proposed to assist pediatricians with improving their understanding of the underlying causes of strabismus (see below and Table II). Eight major categories of this classification scheme include (1) physiologic, (2) comitant, (3) paralytic, (4) sensory, (5) syndromic, (6) orbital, (7) supranuclear and (8) pseudostrabismus, provided in more detail in the following section.

## Discussion

Our proposed classification scheme for strabismus is clear and concise, and provides a sense of the importance of identifying strabismus in the pediatric patient, the need for prompt referral and the possible work-up. It should be emphasized that the role of the pediatrician is not to assign an etiology to the strabismus, but to identify and refer, as the skills and knowledge for assigning an etiology in these cases require advanced training in ophthalmology.

Particular important clinical clues for *expedited* referral in suspected cases of strabismus include an acute onset, presence of diplopia, limited ocular rotations, visual impairment, leukocoria, pupillary abnormalities, proptosis, ptosis, signs of ocular inflammation or other neurologic findings.[8] Many forms of strabismus, including comitant strabismus, may present either as alternating strabismus where either eye will be used for fixing on objects of interest or non-alternating strabismus where there will be a dominant eye that will be used for fixation.

**Table I.** Responses to survey questions regarding the perception of pediatricians towards strabismus.

| Questions | All participants (n=26) | | Pediatricians in-training (n=16) | | Attendings (n=10) | |
|---|---|---|---|---|---|---|
| | Response, n (%) | | Response, n (%) | | Response, n (%) | |
| | Yes | No | Yes | No | Yes | No |
| 1. Do you feel comfortable performing a screening eye exam on a child? | 13 (50) | 13 (50) | 4 (25) | 12 (75) | 9 (90) | 1 (10) |
| 2. Do you feel comfortable performing the ocular motility portion of the exam? | 18 (69) | 8 (31) | 12 (75) | 4 (25) | 6 (60) | 4 (40) |
| 3. Do you have a clear understanding of the classification system of strabismus? | 0 (0) | 26 (100) | 0 (0) | 16 (100) | 0 (0) | 10 (100) |
| 4. Do you have a clear understanding as to the etiology of strabismus? | 5 (19) | 21 (81) | 1 (6) | 15 (94) | 4 (40) | 6 (60) |
| 5. Do you have a clear understanding as to the strabismus nomenclature? | 2 (8) | 24 (92) | 2 (13) | 14 (87) | 0 (0) | 10 (100) |

**Table II.** Proposed classification scheme for strabismus geared towards pediatricians.

| Type of strabismus | Feature | Work-up |
|---|---|---|
| 1. Physiologic misalignment of early infancy | Resolves by 6 months of age; mostly exodeviations | None |
| 2. Comitant (Essential) | Onset: 3 mo.-6 years; full ocular motility; often intermittent onset | Ophtho w/u required |
| 3. Paralytic | Limited ocular motility; diplopia may be reported in older children | Required (imaging and neurology w/u) |
| 4. Sensory | Visual loss in one or both eyes; may have absent red reflex | Ophtho w/u required |
| 5. Syndromic | Limited ocular motility; noted in early infancy | Ophtho w/u required |
| 6. Orbital | Associated with proptosis ± limited ocular motility | Ophtho w/u and imaging required |
| 7. Supranuclear (Neurologic) | Associated with neurologic findings; abnormal gaze findings may be present | Required (imaging and neurology w/u) |
| 8. Pseudostrabismus | Negative cover-uncover test result; epicanthal folds or flat nasal bridge noted on exam | None |

Ophtho: ophthalmology, w/u: work-up.

Non-alternating strabismus may be an ominous sign potentially indicative of an underlying amblyopia, structural pathology such as cataract, optic nerve hypoplasia or retinal pathology, or both in the non-fixing eye of the strabismic patient.

### *Etiology-based Strabismus Classification Scheme for Pediatricians*

**1. Physiologic strabismus:** Misalignment of the eyes in early infancy is a feature of normal development.[9,10] Normal ocular alignment is a learned process that should be complete by around 4-6 months of age. In one study, out of 2271 newborns, 67% showed some exotropia, 30% had straight eyes, 1% had esotropia and 2% had variable angle misalignments changing between exo- and esotropia.[9] All esotropes were noted to resolve by 2 months and almost all exotropes (97%) were resolved by 6 months of age.[9] Thus, esotropia that persists beyond 3 months of age and exotropia that persists beyond 6 months of age should be considered non-physiologic and then referred. A key feature of physiologic strabismus is full ocular rotations, and so if rotations are seen to be limited by voluntary movements or Doll's head maneuver, another etiology needs to be considered with an early referral.

**2. Comitant strabismus:** Term used by ophthalmologists to denote the commonly occurring childhood-type, benign strabismus. The word "comitant" was first introduced to describe an ocular horizontal misalignment that showed the same degree of misalignment in center, right and left positions of gaze, with no regard as to the etiology of the misalignment. The term has evolved with time to mean an etiologic grouping that includes the whole gamut of childhood-onset strabismus that does not require a neurologic or orbital work-up.[8] Key features include the same degree of misalignment in center, right and left positions of gaze, full ocular rotations, and no structural changes to the globe or orbit. Vertical misalignments and oblique extraocular muscle dysfunction often co-exist. Non-alternating comitant strabismus could indicate the presence of visual impairment.

The etiology is actually idiopathic, but probably represents a variety of dysfunctions of central nervous system (CNS) centers of ocular motor coordination and/or sensory centers of binocular vision, all below the resolution of current imaging techniques. Comitant strabismus is

the most common etiologic group of all ocular misalignments and occurs in approximately 3% of otherwise healthy, neurologically intact children.[11] This same type of strabismus also occurs in a much higher frequency in children with other neurologic impairments, such as Down syndrome and cerebral palsy.[12] Onset is usually between 3 months of age to 6 years.[13,14] The most common sub-types of strabismus falling into this etiologic group include accommodative esotropia, congenital/infantile esotropia, and intermittent exotropia.[13,14]

**3. Paralytic strabismus:** Ocular misalignments resulting from a lesion anywhere along the pathway of cranial nerves (CN) III, IV and VI from the lower motor nuclei in the midbrain to the neuromuscular junction with their corresponding extraocular muscles. Among a cohort of 627 pediatric patients with any type of strabismus, paralytic strabismus secondary to CN III palsy was observed in 11 (1.8%) of the patients, CN IV palsy in 13 (2.1%) and CN VI palsy in 25 (4.0%).[11] Paralytic strabismus can occur congenitally or develop secondarily due to intracranial neoplasms, trauma, inflammations, infarctions, demyelination, elevated intracranial pressure, aneurysms and myasthenia gravis.[15-18] The principal ophthalmic findings of paralytic strabismus are an ocular misalignment and impaired ocular rotation(s). Ptosis and pupillary dysfunction may occur with CN III palsy. An anomalous head tilt or a head turn in any direction may be present to compensate for the limited ocular motility.[19] These patients will often require pediatric neurology consultation, as well as magnetic resonance imaging (MRI) imaging of the brain and orbits with contrast.

**4. Sensory strabismus:** An ocular misalignment that develops secondary to poor vision in one or both eyes, due to any ocular sensory pathology or unilateral amblyopia.[20] Properly aligned eyes are not just a matter of anatomy or healthy extraocular muscles. Rather, there is a very active CNS process of checks and balances that maintains normal ocular alignment, referred to as fusional amplitudes. This process has a variety of requirements and inputs, one of which is a clear visual image from both eyes received by the CNS visual centers. With a loss of vision, the fusional amplitude process fails, and a secondary ocular misalignment may ensue, either in the form of an esotropia or an exotropia.[21] Sensory esotropia occurs in 6.7% of all strabismus cases in children younger than 19 years of age.[14] Underlying causes include retinal dystrophies, foveal hypoplasia, optic nerve disease, intraocular tumors, cataracts, glaucoma, and corneal opacification.[20] The presence of an abnormal red reflex, nystagmus and sluggish pupillary reflexes often point out the sensory nature of strabismus. This type of strabismus is also the second most common presentation of retinoblastoma.

When a young child presents with a unilateral loss of vision and strabismus, the ophthalmologist must distinguish if the loss of vision occurred first with a subsequent sensory strabismus, versus a child who develops a strabismus first and secondarily develops a strabismic amblyopia. These children may require ancillary testing for those cases in which a suspected primary loss of vision is not readily evident.

**5. Syndromic strabismus:** Specific forms of strabismus that have stereotypic and unique misalignment patterns, and are distinguishable from the other etiologic groups on an ophthalmologic exam. These strabismic "syndromes" are mostly isolated to the ocular motility system, but can also be a feature of systemic syndromes. Examples that fall within this group include Duane syndrome, Brown syndrome, Mobius syndrome, monocular elevation deficiency (double elevator palsy) congenital fibrosis of the extraocular muscles (CFEOM) and chronic progressive external ophthalmoplegia.[22] A key feature common to this group is a limitation of some ocular rotation. The specific entities within this group have a variety of etiologies, which include aplasia of cranial nerves, intra-uterine anomalous innervation and dysinnervation, tendon dysfunction, and CNS lesions, amongst others.[22] Some of these forms are strictly congenital, some

are strictly acquired, and some can be either. These patients may warrant limited, directed work-ups, such as an audiogram for patients with Duane syndrome, or genetics work-up for Duane syndrome and CFEOM.[23]

**6. Orbital strabismus:** Ocular misalignments that result from structural changes within one or both bony orbits. These changes can result from birth anomalies, trauma, inflammations, infections or masses.[24] Proptosis with or without limited ocular rotations often accompany this type of misalignment. Examples include the craniosynostoses, blow-out fractures, thyroid eye disease, orbital cellulitis and orbital neoplasms.[24-26] Additional testing, such as orbital computed tomography or MRI with ensuing surgical treatment of the underlying lesion is often required.[27]

**7. Supranuclear strabismus:** Ocular misalignments that develop secondary to lesions above the level of the midbrain lower motor nuclei.[28] The ocular motor system is complex and diffuse, with many diverse inputs such as from proprioceptors throughout the body and the vestibular system, and control centers such as those to maintain a steady gaze, to coordinate horizontal and vertical gaze, and for fast or slow eye movements.[29] The pathways from these control centers to the lower motor nuclei of CN III, IV and VI are also complex and indirect. Lesions anywhere along the various inputs, centers or pathways may result in ocular misalignments or eye movement abnormalities.[30] Sometimes, discrete lesions may produce localizing ocular motility abnormalities, such as an internuclear ophthalmoplegia from lesions of the medial longitudinal fasciculus.[31] Other times, lesions result in ocular motility abnormalities that are non-specific and non-localizing, such as those observed in the setting of fetal alcohol syndrome.[32] According to two previous US studies, 17.2% of childhood esotropias and 21.3% of childhood exotropias have been found to be associated with congenital or acquired abnormalities of the CNS.[13-14] Given the common association of childhood strabismus with CNS abnormalities, it is important to have a lower threshold for neurologic workup of childhood strabismus if the strabismus cannot be easily identified as "comitant" by a pediatric ophthalmologist. These supranuclear patients are often the most complex to diagnose, and may require hospitalization, imaging and various consultations in consideration of possible tumors, vascular and inflammatory processes, infections and neurodegenerations.

**8. Pseudostrabismus:** The false appearance of misaligned eyes in a patient with normal ocular alignment and no evidence of strabismus with the cover-uncover test.[19] Often, this appearance is due to normal morphological features of the child's face such as epicanthal folds, flat nasal bridge and wide-set or narrow-set eyes, creating an illusion of misalignment. Pseudostrabismus can also arise from pathological ocular or facial changes such as alterations of the visual axis from retinal dystopias (so called positive or negative angle kappas), blepharophimosis or facial dysmorphisms.[8,33] Pseudostrabismus can be a common diagnosis in a pediatrician's office but the true prevalence of the condition is unknown. As a caveat, studies suggest an increased incidence of true strabismus ranging between 10-12% in patients who had been diagnosed previously with pseudostrabismus.[33-35] True strabismus should be ruled out prior to diagnosing pseudostrabismus, and these patients may need to be followed at 4 to 6-month intervals to monitor for the development of true strabismus.[33,34]

It is not the intention of this paper to provide an in-depth literature survey of the individual types of strabismus conditions discussed here. Thus, the reader is encouraged to review relevant publications for a more comprehensive evaluation of these medical conditions. The preliminary survey was limited to one academic center; it is possible that pediatricians in other clinical settings may have different levels of competencies regarding strabismus evaluation. However, it is the authors' opinion that the results of the survey conducted are representative of academic centers where

Pediatrics residency programs are being implemented.

Strabismus is a fairly common ocular condition in children, and may be the presenting sign of a serious underlying medical condition. Pediatricians are often the first physicians to diagnose strabismus in their patients, and so, having a clear understanding of the underlying causes, the nomenclature and a classification scheme for strabismus is imperative. Certain ocular misalignments such as physiologic strabismus and comitant strabismus are benign in nature. However, other types of strabismus such as paralytic and supranuclear may be associated with life-threatening disorders and need a more expedited approach.

The classification scheme of strabismus presented in this paper is specifically developed for Pediatricians in light of an unmet need observed in our survey of our university's Department of Pediatrics. The classification scheme uses mostly familiar nomenclature, and emphasizes the underlying etiology of the strabismus in order to present a clearer understanding of strabismus to the Pediatrician. It is anticipated that this scheme can assist Pediatricians with improving their triaging skills when faced with various strabismic conditions in the outpatient setting. By providing non-ophthalmic medical terminology, this classification scheme can familiarize Pediatricians with various strabismic conditions and help identify which patients need to be promptly referred to the Ophthalmologist (i.e. non-alternating comitant, sensory, orbital) or urgently to the emergency room for urgent care (i.e. acute paralytic strabismus), thus expediting the work up and improving the overall care of children with strabismus.

## Ethical approval

No patient data was extracted and therefore an IRB approval was not required for this survey.

## Author contribution

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

## Source of funding

The study was carried out with funding from the core grant from NEI (P30 EY001792, Core Grant for Vision Research) and the RPB departmental grant (Unrestricted Grant from Research to Prevent Blindness).

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Williams C, Northstone K, Howard M, Harvey I, Harrad RA, Sparrow JM. Prevalence and risk factors for common vision problems in children: data from the ALSPAC study. Br J Ophthalmol 2008; 92: 959-964. https://doi.org/10.1136/bjo.2007.134700
2. Torp-Pedersen T, Boyd HA, Skotte L, et al. Strabismus incidence in a Danish population-based cohort of children. JAMA Ophthalmol 2017; 135: 1047-1053. https://doi.org/10.1001/jamaophthalmol.2017.3158
3. Friedman DS, Repka MX, Katz J, et al. Prevalence of amblyopia and strabismus in white and African American children aged 6 through 71 months the Baltimore Pediatric Eye Disease Study. Ophthalmology 2009; 116: 2128-2134. https://doi.org/10.1016/j.ophtha.2009.04.034
4. Hoyt CS, Good WV. Acute onset concomitant esotropia: when is it a sign of serious neurological disease?. Br J Ophthalmol 1995; 79: 498-501. https://doi.org/10.1136/bjo.79.5.498
5. Ing MR. Early surgical alignment for congenital esotropia. Trans Am Ophthalmol Soc 1981; 79: 625-663.

6. Wright KW, Edelman PM, McVey JH, Terry AP, Lin M. High-grade stereo acuity after early surgery for congenital esotropia. Arch Ophthalmol 1994; 112: 913-919. https://doi.org/10.1001/archopht.1994.01090190061022

7. Wright KW. Binocular fusion and introduction to strabismus. In: Wright KW, Strube YNJ (eds). Pediatric Ophthalmology and Strabismus. New York: Oxford University Press, 2012: 217-230.

8. Ticho BH. Strabismus. Pediatr Clin North Am 2003; 50: 173-188. https://doi.org/10.1016/S0031-3955(02)00108-6

9. Sondhi N, Archer SM, Helveston EM. Development of normal ocular alignment. J Pediatr Ophthalmol Strabismus 1988; 25: 210-211. https://doi.org/10.3928/0191-3913-19880901-03

10. Nixon RB, Helveston EM, Miller K, Archer SM, Ellis FD. Incidence of strabismus in neonates. Am J Ophthalmol 1985; 100: 798-801. https://doi.org/10.1016/S0002-9394(14)73370-7

11. Mohney BG. Common forms of childhood strabismus in an incidence cohort. Am J Ophthalmol 2007; 144: 465-467. https://doi.org/10.1016/j.ajo.2007.06.011

12. Aslan L, Aslankurt M, Aksoy A, Altun H. Preventable visual impairment in children with nonprofound intellectual disability. Eur J Ophthalmol 2013; 23: 870-875. https://doi.org/10.5301/ejo.5000304

13. Mohney BG, Huffaker RK. Common forms of childhood exotropia. Ophthalmology 2003; 110: 2093-2096. https://doi.org/10.1016/j.ophtha.2003.04.001

14. Mohney BG. Common forms of childhood esotropia. Ophthalmology 2001; 108: 805-809. https://doi.org/10.1016/S0161-6420(00)00639-4

15. Mudgil AV, Repka MX. Ophthalmologic outcome after third cranial nerve palsy or paresis in childhood. J AAPOS 1999; 3: 2-8. https://doi.org/10.1016/S1091-8531(99)70087-X

16. von Noorden GK, Murray E, Wong SY. Superior oblique paralysis. A review of 270 cases. Arch Ophthalmol 1986; 104: 1771-1776. https://doi.org/10.1001/archopht.1986.01050240045037

17. Lee MS, Galetta SL, Volpe NJ, Liu GT. Sixth nerve palsies in children. Pediatr Neurol 1999; 20: 49-52. https://doi.org/10.1016/S0887-8994(98)00090-3

18. Mullaney P, Vajsar J, Smith R, Buncic JR. The natural history and ophthalmic involvement in childhood myasthenia gravis at the hospital for sick children. Ophthalmology 2000; 107: 504-510. https://doi.org/10.1016/S0161-6420(99)00138-4

19. Curnyn KM, Kaufman LM. The eye examination in the pediatrician's office. Pediatr Clin North Am 2003; 50: 25-40. https://doi.org/10.1016/S0031-3955(02)00141-4

20. Havertape SA, Cruz OA, Chu FC. Sensory strabismus--eso or exo?. J Pediatr Ophthalmol Strabismus 2001; 38: 327-330. https://doi.org/10.3928/0191-3913-20011101-05

21. Wright KW, Mocan MC. Esotropia. In: Wright KW, Strube YNJ (eds). Pediatric Ophthalmology and Strabismus. New York: Oxford University Press, 2012: 281-305.

22. Wright KW. Complex strabismus: Restriction, paresis, dissociated strabismus and torticollis. In: Wright KW, Strube YNJ (eds). Pediatric Ophthalmology and Strabismus. New York: Oxford University Press, 2012: 338-367.

23. Wang SM, Zwaan J, Mullaney PB, et al. Congenital fibrosis of the extraocular muscles type 2, an inherited exotropic strabismus fixus, maps to distal 11q13. Am J Hum Genet 1998; 63: 517. https://doi.org/10.1086/301980

24. Castillo BV Jr, Kaufman L. Pediatric tumors of the eye and orbit. Pediatr Clin North Am 2003; 50: 149-172. https://doi.org/10.1016/S0031-3955(02)00115-3

25. Ganesh A, Edmond J, Forbes B, et al. An update of ophthalmic management in craniosynostosis. J AAPOS. 2019; 23: 66-76. https://doi.org/10.1016/j.jaapos.2018.10.016

26. Holt H, Hunter DG, Smith J, Dagi LR. Pediatric Graves' ophthalmopathy: the pre- and postpubertal experience. J AAPOS 2008; 12: 357-360. https://doi.org/10.1016/j.jaapos.2007.12.011

27. Burns NS, Iyer RS, Robinson AJ, Chapman T. Diagnostic imaging of fetal and pediatric orbital abnormalities. AJR Am J Roentgenol 2013; 201: W797-808. https://doi.org/10.2214/AJR.13.10949

28. Picard V. Vertical deviations secondary to supranuclear origins. Am Orthopt J 2011; 61: 23-27. https://doi.org/10.3368/aoj.61.1.23

29. Cassidy L, Taylor D, Harris C. Abnormal supranuclear eye movements in the child: a practical guide to examination and interpretation. Surv Ophthalmol 2000; 44: 479-506. https://doi.org/10.1016/S0039-6257(00)00114-4

30. Lemos J, Eggenberger E. Supranuclear eye movement disorders. Curr Opin Ophthalmol 2014; 25: 471-479. https://doi.org/10.1097/ICU.0000000000000104

31. Rizzo JL, Lloyd M, O'Hara MA. Pediatric internuclear ophthalmoplegia. J Neuroophthalmol 2013; 33: 134-136. https://doi.org/10.1097/WNO.0b013e3182881ad7

32. Miller M, Israel J, Cuttone J. Fetal alcohol syndrome. J Pediatr Ophthalmol Strabismus 1981; 18: 6-15. https://doi.org/10.3928/0191-3913-19810701-04

33. Sefi-Yurdakul N, Tuğcu B. Development of strabismus in children initially diagnosed with pseudostrabismus. Strabismus 2016; 24: 70-73. https://doi.org/10.3109/09273972.2016.1170046

34. Ryu WY, Lambert SR. Incidence of strabismus and amblyopia among children initially diagnosed with pseudostrabismus using the Optum data set. Am J Ophthalmol 2020; 211: 98-104. https://doi.org/10.1016/j.ajo.2019.10.036

35. Pritchard C, Ellis GS. Manifest strabismus following pseudostrabismus diagnosis. American Orthoptic Journal 2007; 57: 111-117. https://doi.org/10.3368/aoj.57.1.111

The Turkish Journal of Pediatrics 2022; 64: 341-349
https://doi.org/10.24953/turkjped.2021.204

Original Article

# Non-ocular risk factors in Turkish children with strabismus and amblyopia

Burçin Çakır, Nilgün Özkan Aksoy, Özlem Bursalı, Sedat Özmen

*Department of Ophthalmology, Sakarya University Medical Education and Research Hospital, Sakarya, Turkey.*

## ABSTRACT

**Background.** To evaluate non-ocular risk factors including family history, febrile seizure, history of trauma, neurological diseases, and prematurity in Turkish children with strabismus and amblyopia.

**Methods.** The records of patients diagnosed with strabismus and/or amblyopia below 18 years old, were recruited. The current mean age, sex, types and subtypes of strabismus and amblyopia, family history, history of trauma, and febrile seizure were investigated. The presence of neurological diseases and prematurity were noted. Family history was investigated whether the presence of strabismus or amblyopia was maternal or paternal. Blood relatives were divided into 3 groups including first, second, and third-degree relatives. The relationship between blood relative degrees and types of strabismus or amblyopia were assessed.

**Results.** There were 803 patients with a current median age of 8 years (1-29 years). Of these patients, 786 patients could be evaluated and 55% had esotropia (ET), 32.6% had exotropia (XT) and 12.5% had amblyopia as a primary diagnosis. Positive family history of strabismus or amblyopia was more common among all risk factors. There was a statistically significant rate of patients with a positive family history in the first-degree relatives, in the esotropic patient group (p= 0.002). Maternal positive family history was more common in patients with refractive ET (p= 0.024) and paternal positive family history was more common in patients with intermittent XT (p= 0.009).

**Conclusions.** The rates of positive family history of amblyopia and strabismus were not statistically different. Family history of strabismus in first-degree relatives of patients with esotropia was markedly high. The family history of strabismus on the maternal or paternal side might be different in patients with different subtypes of strabismus.

**Key words:** strabismus, amblyopia, non-ocular risk factors, family history.

Ocular misalignment and amblyopia are common and important ocular diseases that might cause visual impairment if left untreated in childhood. Ocular misalignment or in other words, strabismus might be caused by abnormalities in binocular vision or by anomalies of neuromuscular control of ocular motility. Amblyopia can be defined as a unilateral or bilateral reduction of best-corrected visual acuity (BCVA) that can not be attributed directly to the effect of any structural abnormality of the eye or visual pathways.[1]

The prevalence of strabismus was 1.93% worldwide, in a recent meta-analysis.[2] Ocular risk factors for strabismus development were found to be hyperopia, moderate anisometropia, and amblyopia, in a recent study.[3] Other risk factors, including positive family history, additional neurological diseases, history of trauma, Down Syndrome, prematurity, and low birth weight were assessed previously, in the literature.[4-8]

The prevalence of amblyopia was reported to be 2.8% in a recent study that investigated

✉ Burçin Çakır
b_koklu@yahoo.com

Received 15th January 2021, revised 28th April 2021, 29th July 2021, 31st August 2021, 17th October 2021, accepted 7th November 2021.

This paper was presented online at the Turkish Ophthalmology Association 2020 National and Compound Congress, December 2020, Antalya, Turkey.

amblyopia among children in an Eastern European Country.[9] Ocular risk factors for amblyopia were strabismus, anisometropia, hyperopia, astigmatism, and congenital nasolacrimal duct obstruction.[9,10] Non-ocular risk factors, including a family history of amblyopia, prematurity, low birth weight, maternal age, neurological diseases were found, in previous studies.[5,7,11,12]

To the best of our knowledge, there have been no studies focusing on these risk factors in Turkish children. In the present study, we aimed to assess non-ocular risk factors, including family history, previous trauma, febrile seizure history, prematurity, and neurological diseases in Turkish children with different types of strabismus or amblyopia. We focused on children with strabismus and/or amblyopia because these disorders are the most common disorders we have to deal with in our Pediatric Ophthalmology Department and they are related closely with each other. We also investigated possible differences in maternal or paternal family history and the differences in family blood relative degrees.

## Material and Methods

The present, retrospective study was conducted at Sakarya University hospital. Prior approval from the Institutional Review Board was taken (Sakarya University Ethical Committee, IRB number: 71522473/050.01.04/54). The study was performed in adherence to the Declaration of Helsinki.

A retrospective chart review of all records at the Pediatric Ophthalmology Department was performed. The chart reviews included medical records between January 2014 and December 2019. The records of patients with strabismus and amblyopia were enrolled. The number of patients with the primary diagnosis of esotropia (ET), exotropia (XT), hypertropia, and amblyopia was noted. When present, secondary and tertiary diagnoses were also noted. The inclusion criteria were the presence of ET, XT, hypertropia, and/or amblyopia in the patients who were diagnosed below the age of 18 years old. Patients who were diagnosed above the age of 18 years old and who had incomitant strabismus were the exclusion criteria of the study.

Strabismus types of patients were classified. Subtypes of ET were refractive accommodative ET (RAET), partially refractive ET (PAET), infantile ET (IET), basic acquired nonaccommodative ET (BANAET), and sensory ET (SET). Subtypes of XT were intermittent XT (IXT), constant XT (CXT), infantile XT, and sensory XT (SXT). Amblyopia might be defined as the reduction of best-corrected visual acuity of one or both eyes that cannot be attributed exclusively to a structural abnormality of the eye.[13] Amblyopia was classified as isoametropic, anisometropic, strabismic, and visual deprivation (VD) amblyopia.

The mean age, gender, mean BCVA which was measured by Snellen chart, spherical equivalent (SE), the value of astigmatism, presence of myopia or hyperopia, presence of inferior oblique overaction (IO OA), and strabismus surgery requirement were noted. Refractive error measurements were done under cycloplegic conditions by using an auto refractometer (Nidek ARK-30) or retinoscopy. An alternate cover test in near and distance was performed by using prism bars to measure ocular deviations.

Positive family history was defined as the presence of the same type of strabismus or amblyopia in blood relatives. The presence of strabismus or amblyopia in the first, second, and third-degree relatives of patients were investigated. Additionally, the information, whether these relatives were maternal or paternal, was also assessed. These data were obtained from the parents of children besides first and second degree relatives were also examined in our strabismus department. The characteristics of family history were evaluated in different subtypes of strabismus and amblyopia.

Previous febrile seizure history of patients was noted and the rates of febrile seizure history was investigated in subtypes of strabismus or amblyopia.

Previous head trauma which was linked to the beginning of strabismus, in the infancy period was asked to all patients or parents of patients and noted. The distribution of the number of patients with trauma history, among patients with different types of strabismus, was also investigated.

Neurological diseases including hydrocephalus, developmental delay, cerebral palsy and attention deficit and hyperactivity disorder (ADHD) were found out and evaluated in strabismic or amblyopic patients. Besides, patients who had Down Syndrome were also noted and evaluated in this group.

Prematurity was investigated among patients with strabismus or amblyopia. The effect of prematurity on different types of strabismus was also evaluated. Besides, the mean birth weight, gestational age, and neonatal intensive care unit (NICU) requirement were noted.

### *Statistical Analysis*

Statistical analysis was performed by using SPSS statistical software (IBM SPSS Statistics, Version 23.0. Armonk, NY: IBM Corp.) Descriptive analyses were performed to provide information on the general characteristics of the study population. Kolmogorov-Smirnov test was used to evaluate whether the distributions of numerical variables were normal. The numeric variables were presented as mean ± standard deviation. Categorical variables were compared by the Chi-Square test. A p-value <0.05 was considered significant.

## Results

Totally 803 patients were enrolled in this study. Table I reveals the characteristics of patients. The mean age given in the Table I was the current age of patients. All patients were diagnosed with strabismus and amblyopia below 18 years old. The number of patients with solely vertical deviation was only 9 and 8 patients had unclassifiable diagnoses such as Duane syndrome, restrictive strabismus. So 786 patients with the diagnosis of exo-, esodeviations, and amblyopia could be analyzed in comparisons (Of these patients, 432 had ET, 256 had XT, and 98 had amblyopia as a primary diagnosis). The number of patients who had vertical deviations as a secondary diagnosis was 16. In Table II, subtypes of eso- and exodeviations and amblyopia are seen. Patients

**Table I.** General characteristics of patients.

| | |
|---|---|
| The median age (minimum-maximum) | 8 years (1-29 years) |
| Female/male ratio | 412/391 |
| The mean BCVA of right eyes (decimal) (n:687) | 0.80±0.24 |
| The median SE of right eyes (n:797) (min-max) | 2.25D (-0.50-13.5D) |
| The median astigmatism of right eyes (n:797) (min-max) | 0.50D (0D-5.25D) |
| The median astigmatism axe of right eyes (n:797) | 65° (0°-180°) |
| The percentage of patients with ET | 55 % |
| The percentage of patients with XT | 32.6 % |
| The percentage of patients with amblyopia as a primary diagnosis | 12.5 % |
| Presence of IO OA (%) | 21% |
| Patients with surgery requirement (%) | 31,3% |
| The mean GA of preterms (n:134) | 32.0±3.6 week |
| The mean BW of preterms (n:134) | 1856.3±787.6 gram |

BCVA: best corrected visual acuity, SE: spherical equivalent, IO OA: inferior oblique overaction, GA: gestational age, BW: birth weight, ET: esotropia, XT: exotropia

**Table II.** Number of patients with subtypes of ET, XT, and amblyopia.

| | | N | % |
|---|---|---|---|
| Subtypes of ET | RAET | 207 | 26.3 |
| | PAET | 84 | 10.7 |
| | IET | 46 | 5.9 |
| | BANAET | 86 | 10.9 |
| | Sensory ET | 9 | 1.1 |
| | Total | 432 | 55 |
| Subtypes of XT | IXT | 189 | 24 |
| | CXT | 56 | 7.1 |
| | Infantile XT | 3 | 0.4 |
| | Sensory XT | 8 | 1 |
| | Total | 256 | 32,5 |
| Subtypes of Amblyopia* | Strabismic | 124 | 15.8 |
| | Anisometropic | 152 | 19.3 |
| | Isometropic | 39 | 5 |
| | VD | 12 | 1.5 |
| | Total | 327 | 41.6 |

ET: esotropia, RAET: refractive accommodative esotropia, PAET: partially refractive esotropia, IET: infantile esotropia, BANAET: basic nonaccommodative esotropia, XT: exotropia, IXT: intermittent exotropia, CXT: constant exotropia, VD: visual deprivation
*: patients with the diagnosis of amblyopia in all groups.

with RAET in the esodeviation group and IXT in exodeviation group were more common. In the amblyopia group, anisometropic amblyopia was more common. The number of patients who had amblyopia as a primary diagnosis was 98 (12,5%) but in total 327 amblyopic patients were evaluated (Fig. 1).

The percentage of patients with a non-ocular risk factor was 83.9%. Table III reveals these rates in detail. Positive family history was found to be more common among all risk

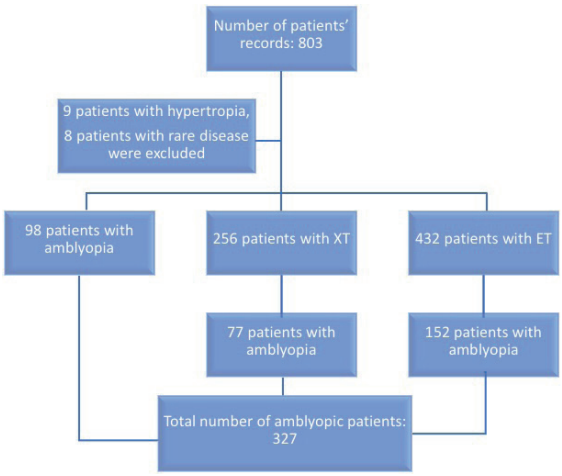



**Fig. 1.** The flow chart of the patients recruited in the study.

factors. Neurological diseases included global developmental delay, cerebral palsy, hydrocephalus, epilepsy, history of intracranial hemorrhage, attention deficit disorders, and 21 patients had Down Syndrome. The diagnosis of these neurological diseases were obtained from the records of patients in the hospital system and from the parents of the children.

Positive family history was more common in patients with esotropia but statistically, a significant difference was not seen between amblyopic patients and patients with eso- and exodeviatons (p= 0.088). Rates of other risk factors including febrile seizure, history of trauma, presence of neurological diseases, and prematurity were also not different between groups (p=0.16, p=0.50, p=0.20, p=0.42, respectively).

Table IV reveals positive family history rates of patients with subtypes of eso-, exodeviations,

**Table III.** Percentages of esotropic, exotropic, and amblyopic patients with nonocular risk factors.

| | ET n (%) | XT n (%) | Amblyopia n (%) | Total n (%) |
|---|---|---|---|---|
| Positive family history | 151(19.2) | 75 (9.5) | 42 (5.3) | 268 (34.1) |
| Positive febrile seizure history | 36 (4.6) | 33 (4.2) | 9 (1.1) | 78 (9.9) |
| Previous trauma history | 28 (3.6) | 18 (2.3) | 3 (0.4) | 49 (6.2) |
| Presence of neurological diseases | 80 (10.2) | 50 (6.4) | 12 (1.5) | 142 (18.1) |
| Prematurity | 81 (10.3) | 40 (5.1) | 12 (1.5) | 133 (16.9) |

ET: esotropia, XT: exotropia

**Table IV.** Positive family history in patients with different subtypes of ET, XT, and amblyopia.

| | | N | % within each group |
|---|---|---|---|
| Subtypes of ET | RAET | 78 | 51.7 |
| | PAET | 32 | 21.2 |
| | IET | 14 | 9.3 |
| | BANAET | 24 | 15.9 |
| | Sensory ET | 3 | 2 |
| Total ET | | 151 | 100 |
| Subtypes of XT | IXT | 58 | 77.3 |
| | CXT | 12 | 16 |
| | Sensory XT | 3 | 4 |
| | Infantile XT | 2 | 2.7 |
| Total XT | | 75 | 100 |
| Subtypes of amblyopia* | Strabismic | 28 | 27.5 |
| | Anisometropic | 54 | 52.9 |
| | Isometropic | 15 | 14.7 |
| | Visual deprivation | 5 | 4.9 |
| Total amblyopia | | 102 | 100 |

ET: esotropia, RAET: refractive accommodative esotropia, PAET: partially refractive esotropia, IET: infantile esotropia, BANAET: basic nonaccommodative esotropia, XT: exotropia, IXT: intermittent exotropia, CXT: constant exotropia, VD: visual deprivation
*: all amblyopic patients with positive family history.

and amblyopia. There were no statistically significant differences in patients with different subtypes of diseases (p=0.08). All amblyopic patients were assessed to find out the possible differences between subtypes.

Table V reveals information about the degrees of relatives in patients with positive family history. A positive family history of strabismus in the first-degree relatives was more common in patients with esotropia (43.3%). There was a significant difference between esotropia, exotropia and amblyopia groups in terms of degrees of relatives (p=0.002).

Table VI reveals whether the family history of strabismus was maternal or paternal. Of 268 patients with positive family history, we could receive 213 patients' information (79,5%) about this item. There was no statistically significant difference between patients with eso-, exodeviations, and amblyopia (p=0.08). When evaluating patients with subtypes of these diseases, there was a statistically significant difference among patients with subtypes of ET (p= 0.024) and positive maternal family history was more common in patients with RAET and PAET (38 %). A statistically significant difference was found among patients with XT

**Table V.** Degrees of relatives in esotropic, exotropic, and amblyopic patients with positive family history.

| | First degree (n/%) | Second degree (n/%) | Third degree (n/%) | Total |
|---|---|---|---|---|
| (n/%) | | | | |
| Esotropic patients | 116/43.3 | 31/11.6 | 4/1.5 | 151/56.3 |
| Exotropic patients | 47/17.5 | 23/8.6 | 5/1.9 | 75/28 |
| Amblyopic patients* | 39/14.6 | 2/0.7 | 1/0.4 | 42/15.7 |
| Total | 202/75.4 | 56/20.9 | 10/3.7 | 268/100 |

*: patients who had amblyopia as a primary diagnosis and had positive family history.
Chi-square test, p=0.002

**Table VI.** Differences between suptypes of ET, XT, and amblyopia in terms of positive maternal and paternal family history.

| | | Maternal family history (n/%) | Paternal family history (n/%) |
|---|---|---|---|
| Subtypes of ET | RAET | 38/25.3 | 25/16.7 |
| | PAET | 19/12.7 | 8/5.3 |
| | IET | 5/3.3 | 5/3.3 |
| | BANAET | 6/4 | 11/7.3 |
| | Sensory ET | 1/0.7 | 1/0.7 |
| ET Total | ET | 69/26.8 | 50/19.2 |
| Subtypes of XT | IXT | 22/29.3 | 30/40 |
| | CXT | 4/5.3 | 6/8 |
| | Sensory XT | 2/2.7 | 0/0 |
| XT Total | XT | 28/10.7 | 36/13.8 |
| Subtypes of amblyopia | Strabismic | 11/10.8 | 14/13.7 |
| | Anisometropic | 25/24.5 | 16/15.7 |
| | Isometropic | 5/4.9 | 3/2.9 |
| | Visual deprivation | 3/2.9 | 1/1 |
| Amblyopia Total | Amblyopia | 44/6.5 | 34/5 |

Chi-square test, p=0.024 in ET group, p=0.009 in XT group, p=0.32 in amblyopia group.
ET: esotropia, RAET: refractive accommodative esotropia, PAET: partially refractive esotropia, IET: infantile esotropia, BANAET: basic nonaccommodative esotropia, XT: exotropia, IXT: intermittent exotropia, CXT: constant exotropia, VD: visual deprivation.

(p=0.009) and a positive paternal family history was more common in patients with IXT (40 %).

## Discussion

In this current study, 33,7% of patients with strabismus or amblyopia had a positive family history of strabismus or amblyopia. Cotter et al.[14] reported that positive family history of strabismus was independently associated with a greater risk for exotropia. In another study, children with a family history of strabismus were observed and the development of constant or intermittent esotropia occurred in 17,6% of these children.[15] Positive family history of strabismus seems to be an important risk factor for the development of strabismus in a child thus this entity should be asked during the ophthalmic examination of a child.

In the literature, family history was found to be positive in only 2.6% of patients with IXT. The sample of this study was composed of 1228 patients from South China.[16] In our study, 30,7 % of patients with IXT had a positive family history of strabismus. Differences in race, ethnicity, and sample sizes could explain these results. Further evaluation should be performed on these patients to find out the possible genetic factors.

Matsuo et al.[17] studied risk factors for different types of comitant strabismus. They reported that when compared with patients with infantile esotropia, positive family history was found to be a marked risk factor in patients with IXT, constant XT, and accommodative esotropia. Eroğlu et al.[18] reported that the risk of strabismus development was high in esotropic patients with positive family history. The most common form was found to be the non-Mendelian type. In our study, there was no statistically significant difference between esotropic, exotropic, and amblyopic patients in terms of positive family history of strabismus or amblyopia.

Mocanu et al.[9] reported an association between amblyopia development and family history

of amblyopia. Guimaraes et al.[11] reported that positive family history was more common in patients with strabismic amblyopia. Not only amblyopia but also the presence of strabismus might have affected this result. When we investigated positive family history in patients with different subtypes of amblyopia, we found no statistically significant difference. Further studies are needed to clarify this.

In this current study, positive family history of strabismus was more common in the first-degree relatives of patients with esotropia. Ziakas et al.[19] reported that first-degree family history was remarkably common in patients with hyperopic accommodative esotropia.

Having a first-degree relative with amblyopia was also found to be a risk factor for amblyopia, in the literature.[20]

The origin of the family history of strabismus was also investigated in this current study. Statistically, a significant difference was not found between patients with eso-, exodeviations, and amblyopia. On the other hand, positive maternal family history of strabismus was more common in patients with RAET and PAET and positive paternal family history of strabismus was more common in patients with IXT. Although genetic-based studies were available, this item was not deeply discussed in previous studies.[19-21] The origin of the family history of strabismus might be important in the development of strabismus.

In this current study, 16,7% of patients had a history of prematurity. Prematurity was also found to be associated with a higher risk of having eso- and exotropia in a study.[7] Low BW and preterm delivery were also found to be risk factors for isolated strabismus. In a recent review; prematurity was found to be a risk factor for amblyopia, too.[22] Prematurity was also found to be associated with a high risk of esotropia.[23] Subtypes of strabismus or amblyopia did not differ in preterm born patients, in our study. The alterations in the results might be due to the characteristics and sizes of samples.

Both patients with esotropia (17,2%) and exotropia (21,3%) were found to be associated with neurological diseases such as cerebral palsy and developmental delay.[24] In a review, the incidence of strabismus was found to be higher in patients with cerebral palsy than neurologically normal patients. Esotropia was the most common ocular misalignment according to this review.[25] In our study, 17,7% of patients had neurological diseases and neurological diseases were not different between patients with different types of strabismus or amblyopia.

In our country, the prevalence of febrile seizures in children was also found to be 4,3%, compatible with the literature.[26] In this current study, the prevalence of the history of febrile seizure was 9,7% in patients with strabismus or amblyopia. This higher rate was remarkable and in our opinion, further studies just focused on the possible link between febrile seizures and decompansation of binocular fusion might give us more accurate results. In the literature, there was no information about this item.

The prevalence of patients with a history of head trauma possibly linked with strabismus was 6,2% in this current study. This rate was not high and did not enlighten us about the effect of trauma history on comitant strabismus and amblyopia. Further studies might explain the effect more precisely.

The major limitation of this current study might be the relatively small sample size and the retrospective manner. Because of the retrospective manner, the characteristics of febrile seizures, the severity of neurological diseases and head trauma were not avaliable. Besides, additional factors such as maternal smoking and factors associated with birth were not investigated. On the other hand, the investigation of head trauma linked with the beginning of strabismus or amblyopia, febrile seizure, and comparisons between subtypes of strabismus and amblyopia in terms of all risk factors were the positive sides of this current study. The evaluation of only Turkish children

with strabismus and amblyopia was also the unique side of the study.

In conclusion; the rates of positive family history of amblyopia and strabismus were not statistically different between esotropic, exotropic, and amblyopic patients. Family history of strabismus in first-degree relatives of patients with esotropia was markedly high. The positive maternal family history of strabismus was common in patients with RAET and PAET. Finally, the positive paternal family history of strabismus was more common in patients with IXT.

### Ethical approval

Prior approval from the Institutional Review Board was taken (IRB number: 71522473/050.01.04/54).

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BÇ; data collection: ÖB, SÖ; analysis and interpretation of results: BÇ, NÖA, ÖB; draft manuscript preparation: BÇ, NÖA. 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. Brodie SE, Gupta PC, Irsch K, et al. Pediatric ophthalmology and strabismus. In: 2018-2019 BCSC: Basic and clinical science course. San Francisco: American Academy of Ophthalmology, 2018: 53-63.
2. Hashemi H, Pakzad R, Heydarian S, et al. Global and regional prevalence of strabismus: a comprehensive systematic review and meta-analysis. Strabismus 2019; 27: 54-65. https://doi.org/10.1080/09273972.2019.1604773
3. Schaal LF, Schellini SA, Pesci LT, Galindo A, Padovani CR, Corrente JE. The prevalence of strabismus and associated risk factors in a Southeastern Region of Brazil. Semin Ophthalmol 2018; 33: 357-360. https://doi.org/10.1080/08820538.2016.1247176
4. Chia A, Lin X, Dirani M, et al. Risk factors for strabismus and amblyopia in young Singapore Chinese children. Ophthalmic Epidemiol 2013; 20: 138-47. https://doi.org/10.3109/09286586.2013.767354
5. Altıntas O, Etus V, Etus H, Ceylan S, Çağlar Y. Risk of strabismus and amblyopia in children with hydrocephalus. Graefes Arch Clin Exp Ophthalmol 2005; 243: 1213-1217. https://doi.org/10.1007/s00417-005-0001-z
6. Pineles SL, Repka MX, Yu F, Lum F, Coleman AL. Risk of musculoskeletal injuries, fractures, and falls in medicare beneficiaries with disorders of binocular vision. JAMA Ophthalmol 2015; 133: 60-65. https://doi.org/10.1001/jamaophthalmol.2014.3941
7. Lingham G, Mackey DA, Sanfilippo PG, et al. Influence of prenatal environment and birth parameters on amblyopia, strabismus, and anisometropia. J AAPOS 2020; 24: 74.e1-74.e7. https://doi.org/10.1016/j.jaapos.2019.12.013
8. Creavin AL, Brown RD. Ophthalmic abnormalities in children with down syndrome. J Pediatr Ophthalmol Strabismus 2009; 46: 76-82. https://doi.org/10.3928/01913913-20090301-06
9. Mocanu V, Horhat R. Prevalence and risk factors of amblyopia among refractive errors in an Eastern European Population. Medicina (Kaunas) 2018; 54: 6. https://doi.org/10.3390/medicina54010006
10. Koo EB, Gilbert AL, VanderVeen DK. Treatment of amblyopia and amblyopia risk factors based on current evidence. Semin Ophthalmol 2017; 32: 1-7. https://doi.org/10.1080/08820538.2016.1228408

11. Guimaraes S, Vieira M, Queiros T, Soares A, Costa P, Silva E. New pediatric risk factors for amblyopia: strabismic versus refractive. Eur J Ophthalmol 2018; 28: 229-233. https://doi.org/10.5301/ejo.5001036

12. Shah S, Torner J, Mehta A. Prevalence of amblyogenic risk factors in siblings of patients with accommodative esotropia. J AAPOS 2008; 12: 487-489. https://doi.org/10.1016/j.jaapos.2008.03.012

13. Zhao PF, Zhou YH, Wang NL, Zhang J. Study of the wavefront aberrations in children with amblyopia. Chin Med J (Engl) 2010; 123: 1431-1435. https://doi.org/10.3760/cma.j.issn.0366-6999.2010.11.015

14. Cotter SA, Varma R, Tarczy-Hornoch K, et al. Risk factors associated with childhood strabismus: the multi-ethnic pediatric eye disease and Baltimore pediatric eye disease studies. Ophthalmology 2011; 118: 2251-2261. https://doi.org/10.1016/j.ophtha.2011.06.032

15. Aurell E, Norrsell K. A longitudinal study of children with a family history of strabismus: factors determining the incidence of strabismus. Br J Ophthalmol 1990; 74: 589-594. https://doi.org/10.1136/bjo.74.10.589

16. Yang M, Chen J, Shen T, et al. Clinical characteristics and surgical outcomes in patients with intermittent exotropia: a large sample study in South China. Medicine (Baltimore) 2016; 95: e2590. https://doi.org/10.1097/MD.0000000000002590

17. Matsuo T, Yamane T, Ohtsuki H. Heredity versus abnormalities in pregnancy and delivery as risk factors for different types of comitant strabismus. J Pediatr Ophthalmic Strabismus 2001; 38: 78-82. https://doi.org/10.3928/0191-3913-20010301-08

18. Çorak Eroğlu F, Oto S, Şahin Fİ, Terzi Y, Özer Kaya Ö, Tekindal MA. The role of heredity and the prevalence of strabismus in families with accommodative, partial accommodative, and infantile esotropia. Turk J Ophthalmol. 2020; 27: 143-150. https://doi.org/10.4274/tjo.galenos.2019.49204

19. Ziakas NG, Woodruff G, Smith LK, Thompson JR. A study of heredity as a risk factor in strabismus. Eye (London) 2002; 16: 519-21. https://doi.org/10.1038/sj.eye.6700138

20. Gopal SKS, Kelkar J, Kelkar A, Pandit A. Simplified updates on the pathophysiology and recent developments in the treatment of amblyopia: a review. Indian J Ophthalmol 2019; 67: 1392-1399. https://doi.org/10.4103/ijo.IJO_11_19

21. Plotnikov D, Shah RL, Rodrigues JN, et al. A commonly occurring genetic variant within the NPLOC4-TSPAN10-PDE6G gene cluster is associated with the risk of strabismus. Hum Genet 2019; 138: 723-737. https://doi.org/10.1007/s00439-019-02022-8

22. Tinley C, Grotte R. Comitant horizontal strabismus in South African black and mixed race children--a clinic-based study. Ophthalmic Epidemiology 2012; 19: 89-94. https://doi.org/10.3109/09286586.2011.645107

23. Gulati S, Andrews CA, Apkarian AO, Musch DC, Lee PP, Stein JD. Effect of gestational age and birth weight on the risk of strabismus among premature infants. JAMA Pediatr 2014; 168: 850-856. https://doi.org/10.1001/jamapediatrics.2014.946

24. Elflein HM. Elflein HM. Amblyopie. Epidemiologie, Ursachen, Risikofaktoren [Amblyopia. Epidemiology, causes and risk factors]. Ophthalmologe 2016; 113: 283-288. https://doi.org/10.1007/s00347-016-0247-3

25. Collins ML. Strabismus in cerebral palsy: when and why to operate. Am Orthopt J 2014; 64: 17-20. https://doi.org/10.3368/aoj.64.1.17

26. Canpolat M, Per H, Gumus H, Elmali F, Kumandas S. Investigating the prevalence of febrile convulsion in Kayseri, Turkey: an assessment of the risk factors for recurrence of febrile convulsion and for development of epilepsy. Seizure 2018; 55: 36-47. https://doi.org/10.1016/j.seizure.2018.01.007

The Turkish Journal of Pediatrics 2022; 64: 350-356
https://doi.org/10.24953/turkjped.2020.2842

Case Report

# Autoimmune/autoinflammatory syndrome induced by adjuvants after multi-component meningococcal serogroup B vaccination in a 7-year-old girl: a case report

Özge Atay[1], Suna Asilsoy[1], Gizem Atakul[1], Serdar Al[1], Özge Kangallı Boyacıoğlu[1], Tayfun Çinleti[2], Nevin Uzuner[1], Özlem Giray Bozkaya[2], Özkan Karaman[1]

*Departments of [1]Pediatric Immunology and Allergy and [2]Pediatric Genetics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey.*

## ABSTRACT

**Background.** Vaccines, which make it possible to be protected from many life-threatening infectious diseases, have been used safely and effectively for years. Most vaccines used today contain a variety of adjuvants and exogenous proteins. Severe reactions, in addition to transient and self-limiting mild reactions, mostly caused by these components, have been reported. The effects of vaccine adjuvants on the pathogenesis of immune-mediated diseases are still under investigation. The syndrome called Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) has been defined in the literature.

**Case.** We found a novel mutation of autoinflammatory diseases in the genetic analysis of our patient. The patient developed symptoms of prolonged fever, rash, arthritis and serositis after multicomponent serogroup B meningococcal (Bexsero®) vaccination, without a previously known rheumatic disease. In the presence of clinical findings in our patient, the diagnostic criteria of ASIA syndrome were met.

**Conclusion.** To the best of our knowledge, this is the first case report of a patient diagnosed with the autoinflammatory disease with a novel mutation after Bexsero® vaccination. We consider that genetic examinations will be useful in patients with a systemic vaccine reaction in the presence of ASIA when diagnostic criteria are met.

**Key words:** vaccines, cryopyrin-associated periodic syndromes, ASIA syndrome, adjuvants, autoimmune diseases.

Vaccines, an indispensable public health measure, are very effective in preventing infectious diseases.[1] The multicomponent meningococcal serogroup B vaccine (4CMenB), Bexsero® (GSK Vaccines Srl, Siena, Italy) is the first vaccine approved to prevent the invasive disease caused by Neisseria meningitides serogroup B. The vaccine contains aluminum hydroxide in addition to its active ingredients.[2]

In general, there is a fear of reactions to vaccine components and that vaccines may trigger the development of various diseases.[1] Most of the adverse effects that develop after 4CMenB vaccination have been reported to be general disorders and administration site conditions. Suspected immune-mediated and/or neurological diseases are less common, but Kawasaki disease, Henoch-Schoenlein purpura, Juvenile idiopathic arthritis, myositis, vasculitis, epilepsy, paralysis, Guillain-Barre syndrome have been reported.[3]

✉ Özge Atay
dr_ozge@hotmail.com

Received 16th November 2020, revised 24th July 2021, accepted 8th August 2021.

Our manuscript had been presented as a poster in 26th National Allergy and Clinical Immunology Congress in Antalya between 9-13 November 2019.

Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) was first reported in 2011. The presence of 2 major or 1 major and 2 minor criteria is sufficient for diagnosis. (Table I).[4]

In recent years, as a result of a better understanding of the natural immune system regulation, immune activation pathways and in consequence of genetic studies, the number of diseases included in the group of autoinflammatory diseases has increased.[5]

We found a novel mutation in the NLRP12 gene in the genetic analysis of our patient who had no known disease other than seasonal allergic rhinitis before, who developed symptoms of prolonged fever, rash, arthritis and serositis after Bexsero® vaccination, and who met ASIA diagnostic criteria. Here, we presented the difficulties in the differential diagnosis and treatment of our patient in the light of the literature.

## Case Report

A 7-year-old girl was admitted to our emergency service with the signs of a rash, redness in the eyes, pain and swelling in her ankles and the complaint of high fever for 3 days. The Bexsero® vaccine had been administered 3 days ago. The patient had no known diseases except for seasonal allergic rhinitis and occasionally used antihistamines for the rhinitis. No previous side effects were observed in the patient, who was vaccinated according to the national vaccination schedule. Her mother was followed up with the diagnosis of Hashimoto's thyroiditis, and there were no additional features in her family history. On physical examination, her body weight was 24 kg (SDS: 0.06, Percentile: 52.39) and her height was 132 cm (SDS: 1.8, Percentile: 96.41). Her axillary temperature was 38.3$^0$C, pulse 150 beats per min, respiratory rate was 28 breaths per min, arterial blood pressure was 90/65mmHg. Her general condition was moderate, and widespread skin lesions compatible with erythema multiforme, bilateral non-purulent conjunctivitis and periorbital heliotrope rash were observed. Breathing sounds decreased in the base of the lungs on auscultation. On palpation, the liver was 3 cm below the costal margin. Swelling and warmth in the ankles were present (Fig 1). Her laboratory examination results were as follows; Leukocytes (WBC) 17300 (4000-10000)/μL, Neutrophils (NEU) 16200 (2000-6000)/

**Table I.** Diagnostic criteria for Autoimmune/inflammatory syndrome induced by adjuvants.

| | |
|---|---|
| Major criteria | • Exposure to external stimuli (infection, vaccine, silicone, adjuvant) before clinical manifestations |
| | • The appearance of "typical" clinical manifestations |
| | Myalgia, myositis, or muscle weakness |
| | Arthralgia and/or arthritis |
| | Chronic fatigue, unrefreshing sleep, or sleep disturbance |
| | Neurological manifestations (especially associated with demyelination) |
| | Cognitive impairment, memory loss |
| | Pyrexia, dry mouth |
| | • Removal of inciting agent induces improvement |
| | • Typical biopsy of involved organs |
| Minor criteria | • The appearance of autoantibodies or antibodies directed at the suspected adjuvant |
| | • Other clinical manifestations (ie, IBS) |
| | • Specific HLA (ie, HLA DRB1, HLA DQB1) Progression of an autoimmune disease (ie, MS, SS) |

HLA: human leukocyte antigen, IBS: irritable bowel syndrome, MS: multiple sclerosis, SS: systemic sclerosis.

μL, Lymphocytes (LYM) 1000 (1500-3500)/ μL, Eosinophils (EOS) 200 (0-500)/μL. CRP 375.80 (0-5) mg/L, erythrocyte sedimentation rate 91 (0-20) mm/h and procalcitonin was 0,98 (0-0.5) ng/ml. She was lymphopenic because her lymphocyte count was below 1500/ μL, but lymphocyte subsets and Ig G,A,M,E values were within normal reference ranges for her age. House dust, cat, grass/cereal for specific IgE values were <0.10 kU/L. Other laboratory findings planned to assess systemic involvement were: Creatine phosphokinase (CPK): 30 (0-145) U/L, CK MB: 0.6 (0.6-6.3) ng/ml, HS-TROPONIN I: 106.6 (8.4-18.3) ng/L. C3:1.65 (0,9-1,8) g/L, C4: 0.372 (0,1-0,49) g/L. HS-TROPONIN was high and other parameters were within normal range. On abdominal ultrasound (USG) hepatosplenomegaly with 43mm and minimal abdominal fluid; on neck USG, reactive lymph nodes smaller than 1 cm were observed. Minimal pleural effusion on the right was seen on chest radiography. The transthoracic echocardiography revealed 1-2. 1st-degree tricuspid regurgitation, 1st-degree mitral insufficiency and a 5-6 mm mild pericardial effusion adjacent to the right ventricle. Coronary arteries were evaluated as normal. These findings were compatible with pancarditis. She was hospitalized with pre-diagnoses of atypical Kawasaki disease, vaccine reaction, sepsis, and connective tissue disease. Oral cetirizine dihydrochloride and empirical intravenous (iv) cefotaxime treatment was initiated for the patient. TORCH, Ebstein

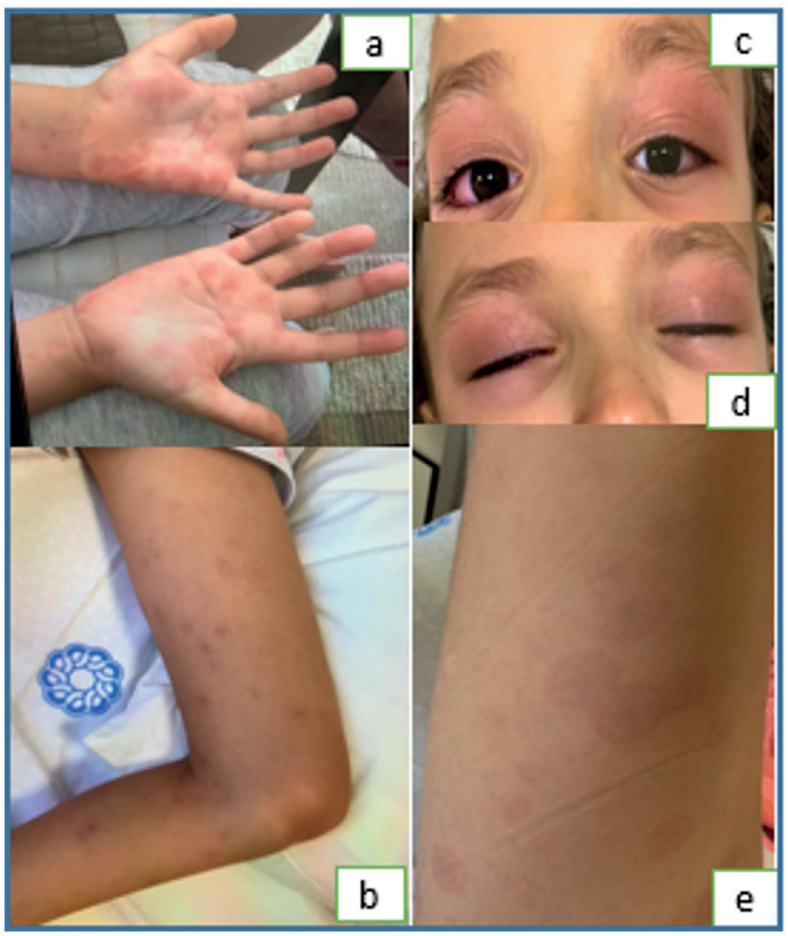



**Fig. 1.** Skin and occular findings of our patient.

**a, b.** Maculopapular erythema in the palm and lower extremities,

**c.** Nonpurulent conjunctivitis, **d.** Heliotrope rash, **e.** Erythema multiforme.

barr virüs (EBV), Human immunodeficiency virus (HIV) and Brucella serological test results of the patient were negative. Cefotaxime treatment was discontinued on the 4th day because no growth was found in blood, urine and throat cultures. In the follow-up, mild hypoalbuminemia (3.14 g / dL) developed along with microscopic hematuria and proteinuria. Antinuclear antibody (ANA) pattern was spotted and positive with a titer over 1/100. A genetic panel was run for autoinflammatory diseases due to clinical findings. Anti-extractible nuclear antigens (ENA) antibodies (Anti nRNP / Sm, Anti Sm, Anti SS-A, Anti SS-B, Anti Scl 70, Anti CENP-B, Anti Nucleosome, Anti Histone, Anti Jo1, Anti Ribosomal P, Anti PM-Scl) was negative. With a serum sickness-like reaction, atypical Kawasaki and autoinflammatory disease pre-diagnosis, the patient was started on intravenous (IV) methylprednisolone 2 mg/kg on the 5th day of hospitalization. Her fever dropped dramatically after the first dose of steroid therapy. Acute phase reactants and leukocytosis regressed over time. On the 4th day of the steroid treatment of the patient who clinically recovered, the CRP value decreased to 19.30 mg/L and the procalcitonin value was normal. Hypoalbuminemia and urine tests improved. Control laboratory test results were: WBC 24300 (4000-10000)/μL, NEU 13900(2000-6000)/μL, LYM 8600(1500-3500)/μL, EOS 100(0-500)/μL, HGB 11.7 (12-16) g/dL, HCT 34%, PLT 1212000(150000-450000) /μL. It was thought that neutrophilia and thrombocytosis developed secondary to steroid treatment. The steroid dose was reduced to 1mg/kg/day. Control echocardiography showed mild left ventricular hypertrophy and minimal mitral insufficiency. The antiaggregant dose of aspirin (5mg/kg/day) treatment was initiated for the patient. The patient, whose control laboratory findings, active complaints and skin findings improved, was discharged on the 8th day of admission to continue outpatient follow-up. Steroid (1mg/kg/day) and aspirin (100 mg/kg/day) treatment was discontinued within two weeks and the patient had no active complaints at her follow-up control. Genetic analysis revealed pathogenic heterozygous p Lys695Arg (c.2084A>G) mutation in the 10th exon of the MEFV gene; Possibly pathogenic, heterozygous novel p.Val151GlnfsTer41 (c.451_452delGT) mutation in exon 3 of NLRP12 gene and heterozygous, of unknown clinical significance p.Arg860Trp (c.2578C>T) mutation in exon 6 of NLRP12 gene. Informed consent was received from her family.

## Discussion

Diseases with a rash accompanying fever are common in children. The most common causes are infections, collagen tissue diseases and drug reactions.[6] Possible infectious causes were primarily excluded in our patient, who was accompanied by symptoms of rash, arthritis, serositis and fever that continued for three days after vaccination. We considered the pre-diagnosis of a serum sickness-like reaction (SSLR) in our patient, who had a clinic compatible with serum sickness, because complement levels were found to be normal.

SSLR is a systemic hypersensitivity reaction which generally occurs secondary to drugs. The characteristic symptoms, which begin within 1-2 weeks after exposure to the causative agent, are rash, fever, polyarthralgia and/or polyarthritis. It is differentiated from serum sickness with the absence of immune complexes in the circulation and normal complement levels.[7]

After vaccination, the onset of the findings in the patient was shorter than three days and her clinic did not improve with time. Due to these reasons, we ruled out the diagnosis of SSLR.

Juvenile dermatomyositis disease, which was mentioned in the preliminary diagnosis due to the finding of heliotrope rash and muscle weakness, was excluded because of the absence of additional dermatological findings, normal CPK values and the development of acute clinical findings after vaccination. In addition, although ANA positivity was detected in our patient, the diagnosis of other collagen tissue diseases was excluded because the ENA profile

was negative and the clinical findings were not sufficient for diagnosis.

Kawasaki disease (KD) is a systemic vasculitis that is most commonly seen in males between the ages of six months and five years, and mostly involves the middle and small arteries. The presence of fever lasting five days or longer and at least 4 of the following clinical findings: changes in the periphery of the extremities, polymorph exanthema, bilateral conjunctival congestion, changes in the oropharyngeal mucosa, and cervical lymphadenopathy are necessary for the diagnosis. In addition to the fever criterion, atypical KD is also mentioned in patients with lung and gastrointestinal system involvement that do not meet all the diagnostic criteria.[8]

In our patient, fever lasting more than five days, diffuse maculopapular rash, serositis, non-purulent conjunctivitis were findings compatible with KD. However, oropharynx involvement, pathological cervical lymphadenopathy, coronary artery involvement, were not observed in our patient. However, the diagnosis of atypical Kawasaki with the detection of pleural effusion and free fluid in the abdomen remained among the possible diagnoses.

Studies also support that vaccines induce autoinflammatory diseases, especially in genetically susceptible individuals. Severe exacerbations of chronic rheumatic diseases have frequently been reported in the literature following vaccination. Based on this, ASIA syndrome has been defined.[9-12] Sarcoidosis, Sjögren's syndrome, undifferentiated connective tissue disease, silicone implant incompatibility syndrome, and immune-related adverse events are defined as classic examples of ASIA. These disorders have been described after an adjuvant stimulus (silicone implantation, drugs, infections, metals, vaccines, etc.) among genetically predisposed individuals, which induce an hyperstimulation of the immune system resulting in the production of autoantibodies, eventually leading to the development of autoimmune diseases.[12]

However, the presence of ASIA syndrome is still debated in studies due to the lack of syndrome-specific diagnostic criteria and evidence for the triggering role of adjuvants in this syndrome. Also, the time between antigen exposure and the development of ASIA syndrome is unclear. A recent study reported that this time ranged from 2 days to 23 years.[4]

In the presence of clinical findings developing 3 days after vaccination in our patient, 2 major diagnostic criteria of ASIA syndrome were met. Although there was no family history and recurrent complaints, genetic analysis was planned for the patient with suspected autoinflammatory disease. In the treatment plan for possible diagnoses, we preferred IV methylprednisolone and oral cetirizine dihydrochloride treatments, since there were no contraindications for any disease and this is a common treatment method. At the end of two weeks of treatment, the patients clinical and laboratory findings improved. During her follow-up, a significant mutation in the NLRP12 gene was detected in her genetic analysis.

To date, only 62 patients with NLRP12 mutations were reported in a 2020 review. Therefore, a comprehensive description of the phenotypic variations associated with this mutation is lacking. Similar to our patient, the main symptom reported in the cases was fever (93%), while other findings were rash, urticaria, myalgia, polyarthralgia/arthritis, abdominal pain (41-62%).[13]

In a different study involving 246 children with periodic fever of unknown cause, NLRP12 mutation was identified in 15 patients as a result of next-generation sequencing analysis. The age of the first autoinflammatory disease-related fever attack has been reported to vary between 2 months and 13 years. While short-term corticosteroid therapy was found to be effective in the treatment of attacks in most patients, it has been reported that canakinumab (anti-interleukin-1p antibody) was successfully used in a severe case.[14]

Familial cold autoinflammatory syndrome-2 (FCAS2) is an autoinflammatory disease characterized by recurrent rash, fever, urticaria, arthralgia and myalgia in which autosomal dominant mutations are detected in the NLRP12 gene. In most patients, exposure to cold is a trigger for attacks. Although the age of onset varies from the first to middle ages of life, the clinical symptoms are also quite heterogeneous.[5]

In our case, there was no history of a periodic finding and cold exposure was not a triggering factor. A definitive diagnosis was made in our patient with the help of the genetic tests planned as a result of the presence of non-specific but multisystemic findings occurring after vaccination.

As a result, to the best of our knowledge, no autoinflammatory disease diagnosed with a novel mutation after Bexsero® vaccination in children has been reported in the literature. The differential diagnosis of rash and prolonged fever findings accompanied by systemic findings is not always easy, as in our patient. It is beneficial to use genetic tests for autoinflammatory diseases in suspected cases, even if it is the first attack. In this way, the genetic variations and phenotype relationships of rare autoinflammatory diseases will become apparent in time.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: OA, SA, OGB, NU, OK; data collection: OA, TÇ; analysis and interpretation of results: OA, SA, GA, ÖKB; draft manuscript preparation: OA, SA, TC, SA. All authors reviewed the results and approved the final version of the manuscript.

### Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Nilsson L, Brockow K, Alm J, et al. Vaccination and allergy: EAACI position paper, practical aspects. Pediatr Allergy Immunol 2017; 28: 628-640. https://doi.org/10.1111/pai.12762
2. Nompari L, Orlandini S, Pasquini B, et al. Quality by design approach in the development of an ultra-high-performance liquid chromatography method for Bexsero meningococcal group B vaccine. Talanta 2018; 178: 552-562. https://doi.org/10.1016/j.talanta.2017.09.077
3. Mentzer D, Oberle D, Keller-Stanislawski, B. Adverse events following immunisation with a meningococcal serogroup B vaccine: report from post-marketing surveillance, Germany, 2013 to 2016. Euro Surveill. 2018; 23: 17-00468. https://doi.org/10.2807/1560-7917.ES.2018.23.17.17-00468
4. Ameratunga R, Gillis D, Gold M, Linneberg A, Elwood JM. Evidence refuting the existence of autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA). J Allergy Clin Immunol Pract 2017; 5: 1551-1555. https://doi.org/10.1016/j.jaip.2017.06.033
5. Dilek N, Uksal Ü. Çocuklarda otoinflamatuvar hastalıkların deri bulguları. Turk J Dermato 2014; 8: 216-222. https://doi.org/10.4274/tdd.2157
6. McKinnon HD Jr, Howard T. Evaluating the febrile patient with a rash. Am Fam Physician 2000; 62: 804-816.
7. Misirlioglu ED, Duman H, Ozmen S, Bostanci I. Serum sickness-like reaction in children due to cefditoren. Pediatr Dermatol 2012; 29: 327-328. https://doi.org/10.1111/j.1525-1470.2011.01539.x
8. Yalaki Z, Atik F, Uslu Gökçeoğlu A. Septik şok gelişen atipik Kawasaki olgusu. J Pediatr Inf 2020; 14: 38-41. https://doi.org/10.5578/ced.202005
9. Watad A, Quaresma M, Brown S, et al. Autoimmune/inflammatory syndrome induced by adjuvants (Shoenfeld's syndrome)-An update. Lupus 2017; 26: 675-681. https://doi.org/10.1177/0961203316686406
10. Shoenfeld Y, Agmon-Levin N. 'ASIA' - autoimmune/inflammatory syndrome induced by adjuvants. J Autoimmun. 2011; 36: 4-8. https://doi.org/10.1016/j.jaut.2010.07.003
11. Guimarães LE, Baker B, Perricone C, Shoenfeld Y. Vaccines, adjuvants and autoimmunity. Pharmacol Res 2015; 100: 190-209. https://doi.org/10.1016/j.phrs.2015.08.003
12. Borba V, Malkova A, Basantsova N, et al. Classical examples of the concept of the ASIA syndrome. Biomolecules 2020; 10: 1436. https://doi.org/10.3390/biom10101436

13. Del Porto F, Cifani N, Proietta M, et al. NLRP12 gene mutations and auto-inflammatory diseases: ever-changing evidence. Rheumatology (Oxford) 2020; 59: 3129-3136. https://doi.org/10.1093/rheumatology/keaa304

14. Kostik MM, Suspitsin EN, Guseva MN, et al. Multigene sequencing reveals heterogeneity of NLRP12-related autoinflammatory disorders. Rheumatol Int 2018; 38: 887-893. https://doi.org/10.1007/s00296-018-4002-8

The Turkish Journal of Pediatrics 2022; 64: 357-363
https://doi.org/10.24953/turkjped.2020.30

Case Report

# Pregabalin abuse in adolescence: a case series

Burcu Ersöz Alan[1,*], Melis Pehlivantürk Kızılkan[2,*], Sinem Akgül[2]

*Departments of [1]Child and Adolescent Psychiatry and [2]Division of Adolescent Medicine, Deprtment of Pediatrics, Hacettepe University Faculty of Medicine, Ankara Turkey.*

**ABSTRACT**

**Background.** Pregabalin is an inhibitor of gamma-aminobutyric acid (GABA) and can be abused, especially by polydrug abuser adults. Drug abuse is one of the many risky behaviors that can be seen during adolescence. Here, three adolescents with pregabalin abuse were described.

**Case.** These adolescents abused pregabalin to cope with their depressive and anxiety symptoms, become tranquilized, boost other drug effects, and reduce withdrawal symptoms. Therefore, the risk factors should be assessed while pregabalin is prescribed to adolescents.

**Conclusions.** The cases were reported in order to increase awareness concerning the abuse risk of pregabalin among adolescents.

**Key words:** adolescent, pregabalin, substance use disorder, prescribed drug addiction.

According to a study from Turkey, the lifetime prevalence of tobacco and substance use among adolescents was 45.4% for hookah, 34.2% for alcohol, 24.4% for cigarettes, 4.9% for volatile substances, 3.8% for benzodiazepines, 2.9% for marijuana, 0.6% for cocaine, and 0.4% for heroin.[1] According to the Turkish National Monitoring Center for Drugs and Drugs Addiction (TUBIM, 2019), alcohol and cigarette smoking were the antecedents of using other drugs, and the marijuana trial was the most common one at the beginning.[2]

Gabapentinoids, (pregabalin, and gabapentin) are inhibitors of the presynaptic voltage-dependent calcium channels of gamma-aminobutyric acid (GABA). They are prescribed for partial-onset seizures, anxiety, restless legs syndrome, neuropathic and non-neuropathic pain.[3] However, they are also assumed to have the potential for abuse.[4] Here, three cases of pregabalin abuse with different clinical presentations will be discussed. The first case is an adolescent girl presenting pregabalin dependency. The second case is an adolescent boy who had been using pregabalin to overcome heroin withdrawal symptoms, and the last case, also an adolescent boy who had been using pregabalin to increase the hedonic effects of cannabis.

These cases aim to increase awareness concerning the use of pregabalin and its addictive potential. Informed consent was received from the cases and their families for this report.

## Case 1

Sixteen years-8 months girl applied to the child and adolescent outpatient clinic with her mother due to pregabalin abuse. She had been taking 900-1500 mg/day pregabalin, smoking cigarettes (1 package/day) for two years and occasionally drinking alcohol in social settings. Her family was of low socioeconomic status. Her parents divorced when she was seven, and she has been living with her father and two sisters (15 years and 13 years, respectively).

✉ Burcu Ersöz Alan
burcuersoz02@gmail.com
*The first and second author contributed equally and therefore share first authorship.

Received 6th January 2020, revised 19th June 2021, accepted 3rd July 2021.

The parents were not close with her. Instead, they blamed her 19-year-old boyfriend, who also suffered from substance abuse problems of synthetic cannabinoids and pregabalin.

She started using pregabalin two years ago, after her boyfriend was hospitalized because of a fight. At first, she was using pregabalin to tranquilize herself and help her sleep. Then she progressively increased pregabalin use when she was angry, unhappy and anxious, and described withdrawal symptoms of sweating, irritability, insomnia, and loss of appetite. Her longest period of abstinence from pregabalin was two weeks. No other substance use was mentioned.

She had repeated 9th grade two years ago due to truancy and academic failure, and she dropped out the year before. She did not have any plans or hobbies, was unemployed, and all her friends were using heroin and pregabalin. She reported being depressive and hopeless, feeling aimless, having concentration problems, and cutting herself, but she had no suicidal ideation. At her initial psychiatric assessment, although she described depressive symptoms, she was euthymic. She had no formal and content thinking problems. Her judgment, insight and motivation were poor. Physical and neurological examinations and laboratory investigations were normal. Besides truancy, her parents reported acting-outs, running away from home, lying, stealing money from home and self-harm behaviors. Family history was significant for substance abuse (her uncle), conduct disorder (her sister), and antisocial personality disorder (her uncle).

She was diagnosed with oppositional defiant disorder (ODD) with conduct problems, substance abuse disorder, and unspecified anxiety disorder. She was prescribed olanzapine and sertraline, which gradually increased to 7.5 mg/day and 100 mg/day respectively during weekly follow-ups. She had been spending her days doing nothing except being with her friends. During the follow-up, although she reported not using pregabalin, her parents stated otherwise. After separating from her boyfriend, she refused treatment, started cutting herself, and expressed suicidal ideation in her acting-outs. Her parents admitted her to social services. Approximately four months after the initial assessment, she dropped out of follow-up. Social services continued to follow her.

**Case 2**

Seventeen years-6 months boy applied voluntarily to the adolescent medicine clinic for poly-substance abuse. He had been smoking cigarettes since the age of 12 (currently, approximately 20 cigarettes/per day) and drinking alcohol occasionally for the last three years. He started using cannabis two years ago, and synthetic cannabinoids one year ago. He had only tried hallucinogens for once. Two months ago, he started inhaling heroin two or three times a week. When he could not find heroin, he used pregabalin 900-1200 mg/day to deal with depressive feelings, loss of joy, and exhaustion. He described his motives for substance use as self-medication, peer pressure, and impulsivity. He had not used any drugs for the last two days. He stayed at home under his father's supervision for these 2 days with some vegetative withdrawal symptoms such as sweating, tremor, and aggression and started feeling better at the current hospital visit. He had never undergone a detoxification trial before.

Two years ago, he first presented to the emergency department with visual hallucinations, drowsiness, and confusion after trying synthetic cannabinoids for the first time. His blood toxicology panel was negative at that time, and the urine toxicology panel was only positive for clomipramine (521 ng/ml). After the emergency room workup and treatment, he was consulted to the department of child and adolescent psychiatry and was discharged with a risperidone prescription. After a while he stopped using risperidone due to feeling drowsy all the time and dropped out of follow-up.

He had been living with his father and his eight years old brother in the family home. Family history was negative for substance use disorders. He had dropped out after finishing 8th grade due to academic failure. His academic performance was always poor. Due to difficulty focusing on lessons and impulsivity in elementary school, he was taken to a child and adolescent psychiatrist and diagnosed with attention deficit hyperactivity disorder (ADHD). He was prescribed methylphenidate but stopped taking this medication due to severe appetite loss, and he was never properly treated afterward. He had been working in a marketplace during night shifts as a porter for the last two years. According to his father, he had anger management issues where he harms himself and others during anger episodes. He was also engaged in several fights while attending school.

During the interview, he was slightly agitated. Physical examination and laboratory investigation were normal. Although he was consulted to the department of child and adolescent psychiatry, he refused to go. He was referred to a withdrawal center for further evaluation and detoxification.

**Case 3**

Fifteen years-9 months old boy applied voluntarily to the adolescent medicine clinic for his poly-substance abuse problem. He had been smoking cigarettes (6-7 cigarettes per day) and using cannabis regularly since he was 13. One year ago, after his cousin's death, who also had substance abuse problems, there was a 6-month long abstinence period for cannabis. However, after a while, he started using cannabis again, progressively increased the amount up to 3-4 times a week, and experimented with volatile substances such as paint thinner, lighter fluid, automotive fuel, and adhesives. He rarely consumed alcohol. For the last six months, he started taking pregabalin (900 mg/day) and phenprobamate (up to 4 grams) right after using cannabis to boost its euphoric effects. He reported that he had been using drugs to suppress his agitation, anger, depressive feelings and improve his mood. He had been using drugs mostly alone and sometimes with peers. He had been living with his parents and 18 years old brother. Other than his cousin, there was no family history of substance use disorders. His academic performance was good until last year. Then, he dropped out of regular school after 10th grade due to truancy and started attending an apprenticeship training school for the last three weeks.

On his first visit, he had been abstinent for one week. He had been evaluated at two child and adolescent psychiatry clinics during the past week, but was unsatisfied with the treatment. Other than these visits he had not received treatment for substance abuse before or had not been diagnosed with a psychiatric disorder. The physical and neurological examination and laboratory investigation were normal. The urine toxicology panel was negative. He was consulted to the child and adolescent psychiatry department.

In his psychiatric examination, he was dysphoric with blunt affect; his speech, formal and content of thinking was normal, but his motivation was poor. He was prescribed Quetiapine XR 150 mg/day and was referred to a withdrawal center for further evaluation and detoxification.

**Discussion**

In this article, three adolescents with pregabalin abuse are described: An adolescent girl with pregabalin use disorder who had many individual and environmental risk factors, an adolescent boy with multidrug abuse who had been using pregabalin to self-manage heroin withdrawal symptoms, and an adolescent boy using pregabalin to boost the euphoric effects of cannabis. Based on these cases, different aspects of pregabalin abuse in adolescence will be discussed and summarized in Table I.

Besides epilepsy, pregabalin can be used to treat anxiety disorders[5], alcohol, and

**Table I.** Different aspects of pregabalin abuse among the 3 adolescents.

| Risk factors | Case 1 | Case 2 | Case 3 |
|---|---|---|---|
| Sex/Age | Female/16 y 8 m | Male/17 y 6 m | Male/ 15 y 9 m |
| Smoking/Alcohol | One package/day for 2 years / occasionally in social settings | 20 cigarette/day for 5 years / occasionally for 3 years | 6-7 cigarette/day for 2 years /rarely |
| Academic performance | Dropped out of school because of truancy and academic failure | Dropped out of school due to academic failure | Good academic performance, but dropped out of school because of truancy |
| Family history | Low SES, divorced, antisocial uncle, drug-abuser uncle, sister with CD | Low SES, No history of drug abusers | Substance abuser cousin |
| Social setting | Drug-abuser friends and boyfriend | Drug-abuser friends | Drug-abuser friends |
| Pregabalin abuse duration | Two years | Two months | Six months |
| Pregabalin abuse doses | 900-1500 mg/day | 900-1200 mg/day | 900 mg/day |
| Purpose of pregabalin abuse | To tranquilize and sleep, continue to use in order to reduce withdrawal symptoms | To deal with depressive symptoms and heroin withdrawal symptoms | To boost cannabis euphoric effects |
| Withdrawal symptoms | Sweating, irritability, sleep disturbances, loss of appetite | Sweating, tremor, aggression | None |
| Drug abuse other than pregabalin | None | Cannabis, synthetic cannabinoids, heroin | Volatile substances, phenprobamate |
| Comorbid psychiatric disorder | ODD, Anxiety disorder, Symptoms of depression and CD | ADHD, Depression | Depressive symptoms |
| Individual factors | Poor judgment, having no insight and any goal settings, poor problem solving skills | Impulsivity | Hopelessness, prolong mourning |

ADHD: attention deficit hyperactivity disorder, CD: conduct disorder, ODD: oppositional defiant disorder, SES: socioeconomic status

benzodiazepine dependence.[6] The therapeutic doses of pregabalin are between 150-600 mg/day, and the risk of abuse increases at high doses as its dose-dependent anxiolytic, euphoric and dissociative effects increase along with the risk of withdrawal symptoms.[7] Pregabalin abuse can be seen especially in individuals with a history of polydrug misuse or addiction.[8,9] In a qualitative research from Jordan, some participants described combining pregabalin with other substances, mainly hashish, and caffeinated sweet beverages, to enhance the euphoric effects.[10] The use of pregabalin to induce the anxiolytic, sedative, or euphoric effects of other substances such as alcohol[11], opioids[12], benzodiazepines, cannabis, and amphetamines[7,13,14] was also reported previously. In literature, the treatment of pregabalin withdrawal symptoms has not been fully established yet.[15] Physicians should be careful about the withdrawal symptoms, including insomnia, headache, nausea, anxiety, depression, diarrhea, flu syndrome, and convulsion.[16] Benzodiazepine use for pregabalin withdrawal symptoms can be controversial in terms of abuse risk.

Self-medication is defined as using drugs to self-treat a medical problem without receiving healthcare providers' advice. The risks of self-medication have been well documented, leading

to a delay in seeking medical advice, excessive dosages or prolonged drug-use duration, and drug abuse.[17] Appropriate treatment of psychiatric conditions could have prevented the use of pregabalin in our cases. Substance abuse, especially opioid abuse, is associated with anxiety disorders.[18] Also many pregabalin abusers have comorbid anxiety problems.[19] Self-medication is highly prevalent among adolescents with psychiatric disorders[20], so screening for self-medication is recommended.

Pregabalin might potentially be used to treat opioid, benzodiazepine, nicotine, cannabinoid, and alcohol abuse withdrawal symptoms. However, there is limited evidence for the efficacy and safety of pregabalin.[6,21] Evidence for the use of pregabalin to treat opioid addiction is based on case reports.[22-24] On the other hand, the abuse potential of pregabalin makes prescription controversial.[8,25] In a study of 124 patients with opiate dependency syndrome, 12.1% of urine samples were tested positive for pregabalin, suggesting pregabalin has a potential of abuse among individuals with opiate addiction. It can act as a weak rewarding substance in patients with long-term opioid tolerance.[9] A systematic review of 59 studies estimated the prevalence of gabapentinoids abuse to be 1.6%. However, the prevalence increases from 2% to 68% among patients with opioid abuse.[26] A study investigating the postmortem toxicology results of medico-legal deaths demonstrated that 91.4% of cases with pregabalin abuse also were using opioids.[27] Additionally, a case study reported an opioid dependent patient using pregabalin to reduce the opiate withdrawal symptoms.[22] Instead of an intention to experience its hedonic effects, opioid-addicted patients might use pregabalin to self-treat symptoms of opiate withdrawal syndrome. In a qualitative study analyzing the online reports of pregabalin, gabapentin and clonazepam users through Google search revealed that some cases were using pregabalin to overcome heroin withdrawal symptoms and cravings.[7] In the presence of sedative drug abuse, clinicians should be careful about the possibility of additional pregabalin abuse, as the risk of overdose and death increases with their combination.[28]

Pregabalin has been perceived as an easily accessible drug that can be abused to induce euphoria.[29] Pregabalin rarely causes a mild, dose-dependent euphoria effect.[30] Patients increasingly had been using higher than the recommended doses of pregabalin to achieve these euphoric effects.[26] Other than taking orally, pregabalin might also be abused rectally, intravenously, and with inhalation.8 Pregabalin has been hypothesized to have euphoric effects due to its direct or indirect effect on the dopaminergic reward system.[31]

Being easily accessible can make pregabalin addiction is increasing among adolescents. Abuse of over the counter and prescription drugs is a global problem.[32] Previously, pregabalin could be obtained through a regular prescription written by any physician from pharmacies in Turkey. However, the Turkish Ministry of Health upgraded the prescription category of pregabalin as a psychotropic drug with a potential of abuse that can only be obtained through special prescription recently. Therefore, physicians should carefully assess the risk factors for drug abuse when they are prescribing pregabalin.

## Author contribution

The authors confirm contribution to the paper as follows: Evaluation and treatment of all 3 patients. BEA, MPK and SA; draft manuscript preparation: BEA, MPK. All authors reviewed and approved the final version of the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Evren C, Ogel K, Demirci AC, Evren B, Yavuz BG, Bozkurt M. Prevalence of lifetime tobacco, alcohol and drug use among 10th grade students in Istanbul. Bull Clin Psychopharmacol 2014; 24: 201-210. https://doi.org/10.5455/bcp.20140131023259
2. Turkish Drug and Addiction Report, TUBIM. 2019. Available at: http://www.narkotik.pol.tr/2019-uyusturucu-raporu-yayinlandi (Accessed on March 13, 2019).
3. Calandre EP, Rico-Villademoros F, Slim M. Alpha2delta ligands, gabapentin, pregabalin and mirogabalin: a review of their clinical pharmacology and therapeutic use. Expert Rev Neurother 2016; 16: 1263-1277. https://doi.org/10.1080/14737175.2016.1202764
4. Kapil V, Green JL, Le Lait MC, Wood DM, Dargan PI. Misuse of the γ-aminobutyric acid analogues baclofen, gabapentin and pregabalin in the UK. Br J Clin Pharmacol 2014; 78: 190-191. https://doi.org/10.1111/bcp.12277
5. Hadley SJ, Mandel FS, Schweizer E. Switching from long-term benzodiazepine therapy to pregabalin in patients with generalized anxiety disorder: a double-blind, placebo-controlled trial. J Psychopharmacol 2012; 26: 461-470. https://doi.org/10.1177/0269881111405360
6. Oulis P, Konstantakopoulos G. Efficacy and safety of pregabalin in the treatment of alcohol and benzodiazepine dependence. Expert Opin Investig Drugs 2012; 21: 1019-1029. https://doi.org/10.1517/13543784.2012.685651
7. Schifano F, D'Offizi S, Piccione M, et al. Is there a recreational misuse potential for pregabalin? Analysis of anecdotal online reports in comparison with related gabapentin and clonazepam data. Psychother Psychosom 2011; 80: 118-122. https://doi.org/10.1159/000321079
8. Schifano F. Misuse and abuse of pregabalin and gabapentin: cause for concern?. CNS Drugs 2014; 28: 491-496. https://doi.org/10.1007/s40263-014-0164-4
9. Grosshans M, Lemenager T, Vollmert C, et al. Pregabalin abuse among opiate addicted patients. Eur J Clin Pharmacol 2013; 69: 2021-2025. https://doi.org/10.1007/s00228-013-1578-5
10. Al-Husseini A, Wazaify M, Van Hout MC. Pregabalin misuse and abuse in Jordan: A qualitative study of user experiences. Int J Ment Health Addict 2018; 16: 642-654. https://doi.org/10.1007/s11469-017-9813-4
11. Grosshans M, Mutschler J, Hermann D, et al. Pregabalin abuse, dependence, and withdrawal: a case report. Am J Psychiatry 2010; 167: 869-869. https://doi.org/10.1176/appi.ajp.2010.09091269
12. Baird CR, Fox P, Colvin LA. Gabapentinoid abuse in order to potentiate the effect of methadone: a survey among substance misusers. Eur Addict Res 2014; 20: 115-118. https://doi.org/10.1159/000355268
13. Papazisis G, Garyfallos G, Sardeli C, Kouvelas D. Pregabalin abuse after past substance-seeking behavior. Int J Clin Pharmacol Ther 2013; 51: 441-442. https://doi.org/10.5414/CP201881
14. Yazdi K, Hemetsberger U, Baier C. Pregabalin abuse of benzodiazepine and alcohol addicted patient. Psychiatr Danub 2015; 27: 278-279.
15. Barrett JA, Kittler LM, Singarajah C. Acute pregabalin withdrawal: a case report and review of the literature. Southwest J Pulm Crit Care 2015; 10: 306-310. https://doi.org/10.13175/swjpcc059-15
16. Ponton R. Pregabalin misuse: preventing potential problems in New Zealand. N Z Med J 2018; 131: 50-54.
17. Lee CH, Chang FC, Hsu SD, Chi HY, Huang LJ, Yeh MK. Inappropriate self-medication among adolescents and its association with lower medication literacy and substance use. PloS One 2017; 12: e0189199. https://doi.org/10.1371/journal.pone.0189199
18. Welsh JW, Knight JR, Hou SSY, et al. Association between substance use diagnoses and psychiatric disorders in an adolescent and young adult clinic-based population. J Adolescent Health 2017; 60: 648-652. https://doi.org/10.1016/j.jadohealth.2016.12.018
19. Schjerning O, Rosenzweig M, Pottegård A, Damkier P, Nielsen J. Abuse potential of pregabalin. CNS Drugs 2016; 30: 9-25. https://doi.org/10.1007/s40263-015-0303-6
20. Robinson J, Sareen J, Cox BJ, Bolton JM. Role of self-medication in the development of comorbid anxiety and substance use disorders: a longitudinal investigation. Arch Gen Psychiat 2011; 68: 800-807. https://doi.org/10.1001/archgenpsychiatry.2011.75
21. Freynhagen R, Backonja M, Schug S, et al. Pregabalin for the treatment of drug and alcohol withdrawal symptoms: a comprehensive review. CNS Drugs 2016; 30: 1191-1200. https://doi.org/10.1007/s40263-016-0390-z
22. Kämmerer N, Lemenager T, Grosshans M, Kiefer F, Hermann D. Pregabalin for the reduction of opiate withdrawal symptoms. Psychiatr Prax 2012; 39: 351-352. https://doi.org/10.1055/s-0032-1305042
23. Kontoangelos KA, Kouzoupis AV, Ferentinos PP, Xynos ID, Sipsas NV, Papadimitriou, GN. Pregabalin for opioid-refractory pain in a patient with ankylosing spondylitis. Case Rep Psychiatry 2013; 2013: 912409. https://doi.org/10.1155/2013/912409

24. Scanlon A. Pregabalin for detoxification from opioids: a single case study. Mental Health and Substance Use 2014; 7: 263-285. https://doi.org/10.1080/17523281.2014.924549

25. Bonnet U, Scherbaum N. How addictive are gabapentin and pregabalin? A systematic review. Eur Neuropsychopharmacol 2017; 27: 1185-1215. https://doi.org/10.1016/j.euroneuro.2017.08.430

26. Evoy KE, Morrison MD, Saklad SR. Abuse and misuse of pregabalin and gabapentin. Drugs 2017; 77: 403-426. https://doi.org/10.1007/s40265-017-0700-x

27. Häkkinen M, Vuori E, Kalso E, Gergov M, Ojanperä I. Profiles of pregabalin and gabapentin abuse by postmortem toxicology. Forensic Sci Int 2014; 241: 1-6. https://doi.org/10.1016/j.forsciint.2014.04.028

28. Carrus D, Schifano F. Pregabalin misuse-related issues; intake of large dosages, drug-smoking allegations, and possible association with myositis: two case reports. J Clin Psychopharm 2012; 32: 839-840. https://doi.org/10.1097/JCP.0b013e318272864d

29. Schifano F, Chiappini S. Pregabalin: A range of misuse-related unanswered questions. CNS Neurosci Ther 2019; 25: 659-660. https://doi.org/10.1111/cns.13115

30. Pfizer Inc. Prescribing information: Lyrica. 2011. Available at: http://www.pfizer.com/products/product-detail/lyrica (Accessed on March 12, 2019).

31. Bucur M, Jeczmien P. Pregabalin and libido-case reports. Open Neuropsychopharmacol J 2011; 4: 8-9. https://doi.org/10.2174/1876523801104010008

32. Casati A, Sedefov R, Pfeiffer-Gerschel T. Misuse of medicines in the European Union: a systematic review of the literature. Eur Addict Res 2012; 18: 228-245. https://doi.org/10.1159/000337028

The Turkish Journal of Pediatrics 2022; 64: 364-374
https://doi.org/10.24953/turkjped.2020.2012

Case Report

# Spinal muscular atrophy with respiratory distress type 1 (SMARD1): a rare cause of hypotonia, diaphragmatic weakness, and respiratory failure in infants

Serdar Pekuz[1], Yiğithan Güzin[1], Serdar Sarıtaş[1], Özgür Kırbıyık[2], Aycan Ünalp[1], Ünsal Yılmaz[1]

[1]*Department of Pediatric Neurology, University of Health Sciences, Dr. Behçet Uz Children's Education and Research Hospital, İzmir;*
[2]*Genetic Diagnostic Center, University of Health Sciences, Tepecik Training and Research Hospital, İzmir, Turkey.*

## ABSTRACT

**Background.** Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is a very rare autosomal recessive disorder caused by mutations in the immunoglobulin μ-binding protein-2 (IGHMBP2) gene on chromosome 11q13.2-q13.4. The initial symptoms of patients with SMARD1 are respiratory distress and distal muscle weakness manifesting in the infantile period due to progressive degeneration of $\alpha$-motor neurons. Preterm birth, intrauterine growth retardation, feet deformities, sensory and autonomic neuropathy are other main features.

**Case.** Herein, we report the characteristics of a 6-year-old Turkish girl with a diagnosis of SMARD1 confirmed by homozygous c.1738G>A (p.Val580Ile) missense *IGHMBP2* variant. She had unusual features such as vocal cord paralysis, nystagmus, and lack of congenital foot deformities besides typical findings including hypotonia, respiratory distress, and diaphragmatic weakness in the early infantile period. Epileptic seizures, cognitive impairment, and brain magnetic resonance imaging (MRI) abnormalities were other, unexpected, features which developed during the course of the disorder possibly due to several hypoxic episodes.

**Conclusions.** SMARD1 should be kept in mind in hypotonic infants with diaphragmatic weakness and respiratory failure during the early infantile period, even in the presence of unexpected findings including vocal cord paralysis, nystagmus, epileptic seizures, and brain MRI abnormalities.

**Key words:** spinal muscular atrophy with respiratory distress type 1, hypotonia, diaphragmatic weakness, vocal cord paralysis.

Spinal muscular atrophy with respiratory distress type 1 (SMARD1, OMIM #604320) is a very rare autosomal recessive disorder caused by mutations in the immunoglobulin μ-binding protein 2 (IGHMBP2, OMIM #600502) gene on chromosome 11q13.2-q13.4.[1] Only slightly more than 100 SMARD1 patients have been reported in the literature thus far. Although SMARD1 and spinal muscular atrophy (SMA) share some similar pathological characteristics, such as anterior horn motor neuron degeneration, they occur as a result of mutations in distinct genes and have diverse clinical phenotypes. The initial symptoms of patients with SMARD1 are respiratory distress and distal muscle weakness, manifesting during the infantile period due to progressive degeneration of $\alpha$-motor neurons.[2] Progressive respiratory distress is thought to be related with unilateral or bilateral diaphragmatic weakness.[3] Although lower limbs and distal muscle involvement are more prominent, muscle weakness becomes generalized during the course of the disease. Preterm birth, intrauterine growth retardation, feet deformities, sensory

✉ Serdar Pekuz
pekuzserdar@hotmail.com

Received 12th July 2020, revised 9th January 2021, accepted 14th February 2021.

and autonomic neuropathy are other main features of SMARD1.[4,5] In 2003, Pitt et al.[4] developed a set of clinical, histopathological and electrophysiological criteria for SMARD1 diagnosis (Table I). A few years later, a cluster analysis showed that the onset of respiratory distress between the ages of 6 weeks and 6 months, in combination with preterm birth or diaphragmatic weakness, had a 98% sensitivity and 92% specificity for predicting *IGHMBP2* mutation in 47 out of 141 patients presenting with respiratory distress and a spinal muscular atrophy phenotype.[5]

IGHMBP2 consists of 993 amino acids and has ATP-dependent 5→3 helicase activity. It is composed of three domains: DNA/RNA helicase, a R3H and a zinc-finger. Inactivation of the helicase domain is likely to be the cause of clinical symptoms and the majority of the pathological variants are found in this domain. R3H domain is thought to play a regulatory role on helicase activity. Although the precise function of IGHMBP2 is still uncertain, it has been demonstrated that it has a role in transcription, pre-mRNA processing, and translation.[6,7]

Herein, we report the characteristics of a 6-year-old Turkish girl with homozygous c.1738G>A (p.Val580Ile) missense *IGHMBP2* variant.

## Case Report

The subject was the only child of healthy nonconsanguineous Turkish parents. She was born by a caesarian section weighing 2,180 g at 39 weeks of gestation due to fetal distress. There was no abnormality at antenatal follow up visits. Among her relatives, there were individuals who died during the infantile period (Fig. 1). She was admitted to the neonatal intensive care unit on the first day of life due to respiratory distress during postnatal adaptation. Following clinical improvement with non-invasive respiratory support, she was discharged from the hospital in a week.

At about 6 weeks of age, the parents noticed less than usual spontaneous movement in her legs. Physical and neurological examination revealed laryngomalacia, prominent generalized hypotonia, lack of head control and deep tendon reflexes at the age of 3 months. There were no fasciculations on the tongue. Complete blood count, serum biochemistry tests, creatine phosphokinase level, thyroid function tests, ferritin, vitamin B12, folate, uric acid, and metabolic tests (lactate, pyruvate, ammonia, homocysteine, biotinidase activity, long chain fatty acids, serum free carnitine / acyl carnitine profile, serum and urine amino acids, and urine organic acid) were all within normal limits.

**Table I.** Diagnostic criteria of spinal muscular atrophy with respiratory distress type 1 (SMARD1) from *Pitt et al.*[4]

Clinical criteria
- Low birthweight below the 3rd centile
- Onset of symptoms within the first 3 months
- Diaphragmatic weakness either unilaterally or bilaterally
- Ventilator dependence within less than one month of onset with an inability to wean
- Absence of other dysmorphology or other conditions

Histopathological criteria
- Reduction of myelinated fiber size in sural nerve biopsies
- Minimal evidence of ongoing myelinated fiber degeneration in biopsies taken up to 3 ± 4 months
- No evidence of regeneration or of demyelination that might account for the change in fiber size.

Electromyography criteria
- Evidence of acute or chronic distal denervation
- Evidence of severe slowing [<70% of lower limit of normality in one or more nerves (motor or sensory)]

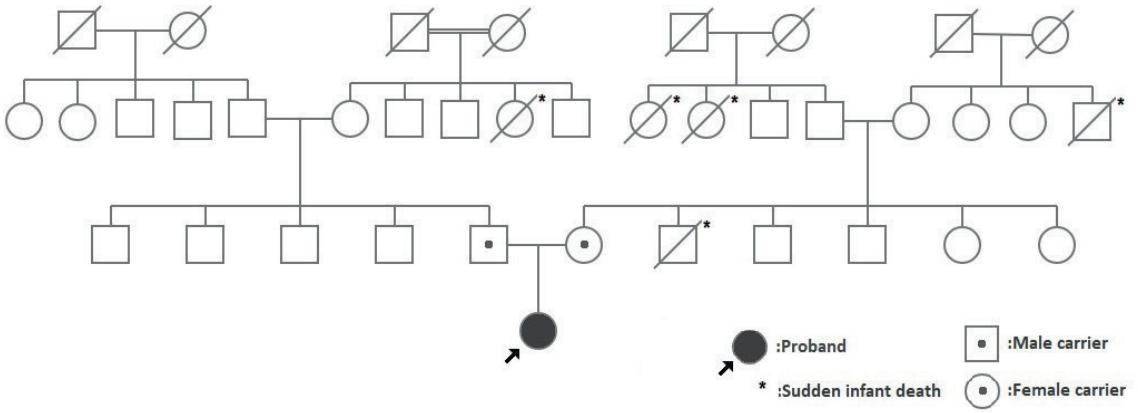



**Fig. 1.** Family pedigree of the SMARD1 case. The asterix (*) indicates sudden infant death.

Multiplex ligation probe amplification (MLPA) methodology and sequence analysis of the SMN gene revealed non-diagnostic heterozygous deletion in exon 8. At 4 months of age, she was admitted to the emergency department with respiratory distress and intubated during follow-up. Elevation of the right hemidiaphragm was noted on a chest X-ray (Fig. 2), and paradoxical movement on ultrasonography. Laryngoscopic examination of the vocal cord revealed bilateral mild impairment in abductor motion. The duration of intubation was longer than six months and repeated extubation attempts failed. Hemidiaphragm elevation has ameliorated only at moderate to high peak inspiratory pressures on the ventilator, and it was obvious following extubations. Tracheostomy and percutaneous endoscopic gastrostomy (PEG) were performed at the age of 14 months and 20 months, respectively. She had to be admitted to hospital several times with respiratory symptoms. At 17 months of age, she experienced clonic and myoclonic seizures during hospitalization for pneumonia in the intensive care unit. Interictal electroencephalography (EEG) revealed left temporal, frontotemporal and generalised sharp waves with normal background activity. Complete seizure cessation was achieved with levetiracetam treatment. Dilatation of lateral ventricles and decreased frontal white-matter volume were detected on brain magnetic resonance imaging (MRI) at 18 months of age. Nerve conduction studies demonstrated undetectable motor action potentials of peroneal, posterior tibial, median and ulnar nerves. Muscle biopsy of gastrocnemius muscle indicated neurogenic changes with atrophic myofibers. She had episodes of sweating, constipation, hypertension, sinus bradycardia, and tachycardia attributed to autonomic dysfunction. There was no abnormality in echocardiography. A custom target capture – based on next generation sequencing (NGS) panel (Celemics, Inc., Seoul, Korea) containing 579 genes associated with hereditary diseases indicated a homozygous c.1738G>A (p.Val580Ile) variant in *IGHMBP2* gene which was likely pathogenic according to ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/9114/), confirming the

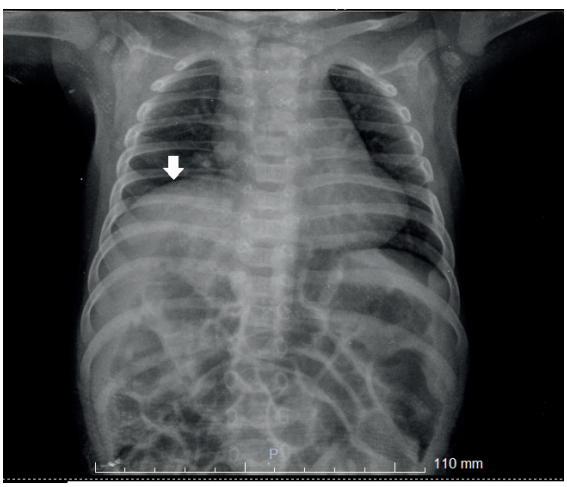



**Fig. 2.** Right hemidiaphragm elevation (white arrow) on chest X-ray at 5 months old.

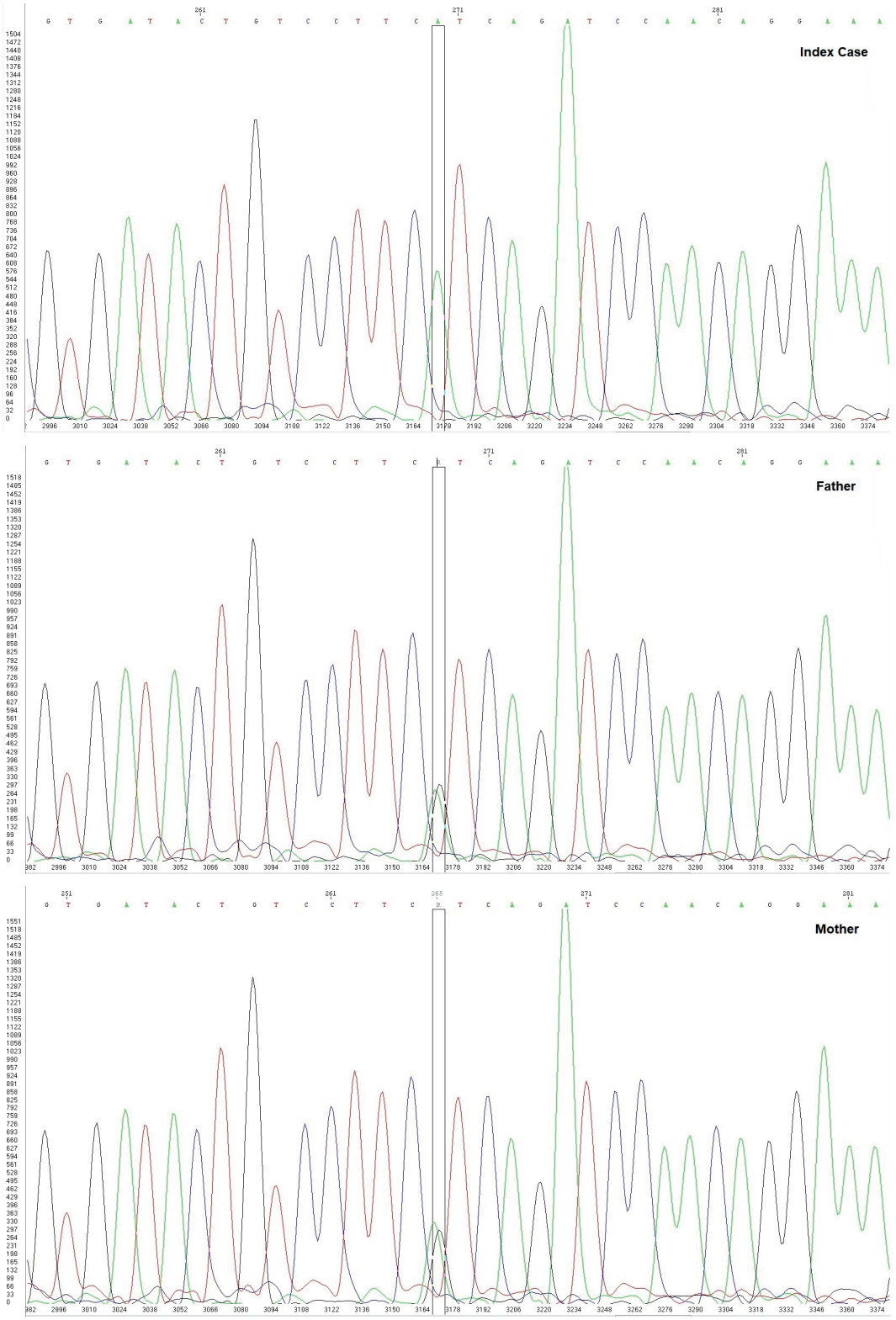



**Fig. 3.** Sanger sequencing electropherogram of IGHMP2 gene of the patient and family. Homozygous c.1738G>A (p.Val580Ile) variant in *IGHMBP2* gene of the patient, and maternal and paternal heterozygosity were confirmed by Sanger sequencing.

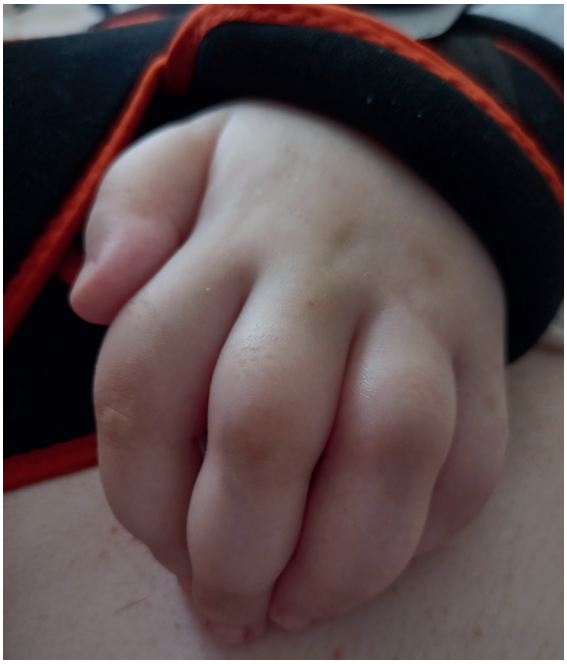


**Fig. 4.** Fatty pads on the paralyzed fingers of the patient with SMARD1.

diagnosis of SMARD1 at the age of 3.5 years. Sanger sequence analysis of parents revealed that both father and mother are heterozygous carriers of c.1738G>A (p.Val580Ile) variant (Fig. 3).

At the most recent examination, she had severe mentally retardation, unable to move both proximal and distal parts of her extremities against gravity, dependent on mechanical ventilation and gastrostomy tube feeding at the age of 6 years. Fatty pads at the fingers (Fig. 4), moderate contractures in the wrist, elbow, knee, and ankle were apparent. Facial weakness, absence of deep tendon reflexes, lack of eye tracking ability and bilateral horizontal nystagmus were the other features.

Informed consent was received from the family of the patient for publication.

## Discussion

SMARD1 is a very rare autosomal recessive neuromuscular disorder with high mortality. Low birth weight, early onset respiratory distress (requiring permanent artificial ventilation), diaphragmatic paralysis, and progressive wasting of the distal muscles are the main delineated features of reported patients, (Table II) although variations exist in clinical phenotypes. In this case report, we presented a 6-year-old Turkish girl with a homozygous c.1738G>A (p.Val580Ile) variant in *IGHMBP2* gene causing SMARD1. This rare variant has been previously reported in a few SMARD1 patients with compound heterozygosity and to the best of our knowledge in only one other Turkish girl with homozygosity.[1,8] Experimental studies have demonstrated that this variant impairs enzymatic activity in vitro conditions.[9]

The subject was born as a full term baby weighing 2,180 g, small for gestational age, indicating intrauterine growth retardation and she had respiratory distress shortly after delivery. SMARD1 patients have been characterized by intrauterine growth retardation, premature birth and decreased fetal movements, but respiratory distress is rare in the early neonatal period.[2,5] Onset of respiratory failure appears a key clinical diagnostic factor for SMARD1 patients with *IGHMBP2* mutation. While the single criterion scoring highest in favor of an *IGHMBP2* mutation with 87% sensitivity and 92% specificity was the ''manifestation of respiratory failure between 6 weeks and 6 months'', ''congenital manifestation of respiratory failure'' was found to be the single criterion shown scoring highest against the diagnosis of SMARD1 with a 66% sensitivity and 98% specificity in a cluster analysis.[5] Consistently, respiratory failure developed at about 4 months of age in our case, although transient respiratory distress was observed in the early neonatal period, where elevation of the diaphragm was not a chest radiograph finding. Among the expected findings of SMARD1, weakness manifested by congenital foot deformities was not found in our patient.[3,5]

Life threatening progressive respiratory distress in infancy, due to diaphragmatic paralysis which requires mechanical ventilation, is the most prominent presenting symptom

**Table II.** Characteristics of spinal muscular atrophy with respiratory distress type 1 (SMARD1) patients in previously published studies with detailed analysis of clinical, electrophysiological and histopathological findings.

| Characteristics | Studies | | |
|---|---|---|---|
| | Grohmann K. et al., 2003 | Viguier et.al., 2019 | Rudnik-Schöneborn S. et al., 2004 (Previously reported patient with homozygous c.1738G>A IGHMBP2 variant*) |
| No of patients (male:female) | 29 (15:14) | 22 (15:7) | 1 female |
| Consanguinity | 7 of 29 families (24%) | 38% of the families | + |
| IUGR | ¾ of the patients | 11 of 22 patients (50%) | - |
| Distal and/or foot deformities | 19 of 22 patients (86%) | 14 of 21 patients (67%) | Multiple distal contractures |
| Poor feeding | 15 of 26 patients (58%) | All 22 patients (100%) had tube feeding | + (tube feeding was initiated at 11 weeks of age) |
| Onset of respiratory distress | Median: 3 months (range: 0.1 -12) | Median: 2 months | 2 months |
| Respiratory support | All 29 patients (100%) had respiratory failure at a median age of 3.5 months | 15 of 22 patients required artificial ventilation at a median age of 10 months | Tracheostomy at 4 months of age |
| Inspiratory stridor | 7 of 14 patients (50%) | Not mentioned | + (soon after birth) |
| Diaphragm elevation | 23 of 25 patients (92%) | 20 of 20 patients (100%) | + (right hemidiaphragm) |
| Muscular hypotonia | 22 of 27 patients (86%) | 20 of 22 patients (91%) (median: 2 months) | + |
| Cognitive functions | Not mentioned | Normal cognition in 14 of 17 patients (82%) | Appropriate for age (able to communicate with an electronic communication system) |
| Seizures | Not mentioned | 5 of 20 patients (25%) | + |
| Cardiac arrhythmias | 5 of 7 patients (71%) | 7 of 22 patients (32%) | Not mentioned |
| Electromyography | 22 of 25 patients (88%) had neurogenic changes | 17 of 17 patients (100%) had motor and 14 of 17 patients (82%) had sensory neuropathy | Normal motor and sensory conduction velocities at 5 months, but no action potentials in 7 months |
| Muscle biopsy | Neurogenic muscle atrophy with fiber hypertrophy (21 of 22 patients) | Neurogenic muscle atrophy with a heterogeneous decrease in skeletal muscle fiber caliber and endoneural fibrosis | Neurogenic muscle atrophy |
| Sural nerve biopsy | Axonal degeneration (10 of 15 patients). | Hypomyelination or demyelination | Axonal atrophy, occasional hypermyelination in electron microscopy |

* Patient #3 in reference 15. IUGR: intrauterine growth retardation.

in SMARD1.[1,2,9] In a retrospective study of twenty-nine SMARD1 patients, all patients had respiratory distress between 1–13 months of life with a median age of 3.5 months.[2] Muscle weakness usually becomes obvious shortly after the onset of respiratory symptoms.[9] However, the initial parental concern of our patient was the less than usual spontaneous movement of the legs, which was regarded as distal muscle weakness by two months of age before the respiratory symptoms started. Respiratory distress was the chief complaint at 4 months of life and she was intubated at the age of 5 months. She had inspiratory stridor and a weak cry at 3 months of age, both of which are usually the first indicators of respiratory distress in SMARD1.[2] Inspiratory stridor due to laryngomalacia and impaired abduction of the vocal cords were remarkable and gave rise to the thought of vagus or its branch recurrent laryngeal nerve paralysis. Vocal cord paralysis is an unexpected finding for SMARD1, although it can be significant for other certain types of SMA-like motor neuron disorders.[10,11] Laryngomalacia and tracheomalacia without vocal cord paralysis were also reported in two infants with SMARD1, presenting with stridor.[12,13] Interestingly, they had the mutation c.1737C>A (p.Phe579Leu) which is located in exon 12 of *IGHMBP2* gene, the same affected exon as in our patient.

Diaphragm elevation leading to respiratory failure is the hallmark of SMARD1, in comparison with SMA, which is characterised by the involvement of the intercostal muscles causing a bell-shaped thorax deformity, whilst the diaphragm is relatively spared. It is more commonly seen in the right side, presumably secondary to the mass effect of the liver, but bilateral involvement is possible.[14] Interestingly, diaphragmatic elevation may not be apparent in chest X-rays at the onset of respiratory symptoms and occur with a delay.[14] A chest X-ray of our patient revealed eventration of the right hemidiaphragm which was confirmed by paradoxical movement on ultrasonography at the onset of respiratory symptoms. Thin and membranous appearance of diaphragm was observed during surgery of the previously published patient with the same homozygous variant who underwent plication.[4] Plication of the diaphragm has not been shown to be beneficial to the clinical courses of reported SMARD1 patients and therefore is not a recommended intervention.[12,13,15] The main cause of death in the infantile period is respiratory failure. Most of the patients need invasive or non-invasive respiratory support and once mechanical ventilation is initiated, it is highly unlikely that the patient will be able to be weaned off ventilation.[15] However, a case was reported in which a girl had regained independent breathing for twelve hours per day by 4 years of age after a dramatic loss of independent breathing in the first year of life, despite persisting diaphragmatic paralysis.[9] A multicentric retrospective study has shown that tracheostomy had a positive impact on life expectancy of children with SMARD1, and only 1 of 22 patients survived without artificial ventilation beyond 2 years of age.[14] Therefore, families should be encouraged in favor of this procedure.

After the initial distal muscle weakness, predominantly in lower limbs, progressive generalized hypotonia, and absent deep tendon reflexes are inevitable features in SMARD1 patients. The natural course of the disease leads to complete paralysis of both limbs and trunk, which were noted on the follow-up examinations of our patient.[9,16] Marked distal weakness and atrophy caused adipose tissue replacement as fatty pads on the fingers, another characteristic finding of SMARD1 patients. This was a late manifestation in our patient, appearing after 2 years of age.[3,9,16]

Facial muscle weakness and tongue fasciculations can be observed due to the involvement of facial and hypoglossal nerves during the course of the disease.[2] Eckart et al.[9] have observed approximately one third of patients had facial weakness over an observational period of eight years. On the other hand, the oculomotor nerve is mostly spared and eye

movement disorders have not been previously reported in SMARD1 patients. A complete lack of eye tracking ability and bilateral horizontal pendular nystagmus of our patient was noted at 18 months of age although she had appropriate eye coordination and ability to follow brightly colored objects at 3 months of age prior to the onset of respiratory distress. Besides recurrent severe pneumonia episodes and autonomic dysfunction, she developed chronic filamentary keratitis with prominent epithelial erosion and mucopurulent discharge involving both eyes. Repeated slit lamp examinations of eyes revealed mucoepithelial strands attached to the corneal surface and epithelial defects after a long-term intubation period at 1-year old. Facial weakness and sedatives, to avoid patient-ventilator asynchrony, caused lagophthalmos and aqueous tear deficiency, which were thought to be main risk factors for filamentary keratitis. Fundoscopic examination and visual evoked potential responses were normal. In addition, hypoxic episodes may have contributed to eye movement disorders and visual impairment. Thereby, nystagmus and visual tracking disorder of our patient do not seem to be etiologically related to the disease itself, but most likely occurred as a secondary complication.

Epileptic seizure was another manifestation in our patient, uncommon in SMARD1 and generally thought to be related to secondary phenomena such as hypoxic episodes.[3,17] Electroencephalography revealed left temporal, frontotemporal and generalized sharp waves. The seizures were well controlled by levetiracetam treatment. No abnormalities were detected in brain ultrasonography during the infantile period. Brain MRI revealed mild dilatation of lateral ventricles and decreased frontal white-matter volume at 18 months of age, after the onset of epileptic seizures. Imaging abnormalities of the central nervous system are not expected in SMARD1. In a multicentric study, brain MRIs were normal in 71.4% of SMARD1 patients and most of the rest had non-specific imaging features.[13] However, an atypical SMARD1 patient was previously reported with microcephaly, cerebral atrophy and thin corpus callosum.[18] Cognitive skills and social interactions of SMARD1 patients were usually found appropriate for age in the literature.[15,19] Viguier et al.[14] in 2018, reported normal cognition in 14 of 17 children with SMARD1, and 7 of 9 survivors beyond 2 years of age were also able to talk and had normal facial expressions. Moreover, Eckart et al.[9] noted that most SMARD1 patients were well integrated into their home environment and two thirds of them were able to attend kindergarten or school, although severe disabilities in a long term follow-up were observed in 11 SMARD1 patients. In contrast, our patient had obvious impairment in cognitive functions although she wasn't assessed with objective psychometric tests. Both MRI findings and cognitive impairment may be attributed to the hypoxic episodes but not the natural course of the disease.

Dysfunction of the autonomic nervous system in SMARD1 is not rare and symptoms of autonomic neuropathy should not be overlooked or misinterpreted. Sudden changes in vital signs due to dysautonomia may lead to deterioration in the clinical condition of critically ill patients. Cardiac arrhythmia, variability of blood pressure, excessive sweating, urinary retention and constipation may be observed in the course the of the disease.[9,14] The prevalence of constipation, excessive sweating and cardiac arrhythmia were found in 53%, 58%, and 71% of 29 patients SMARD1 patients respectively.[2] Moreover, in a longitudinal study with a mean observational period of 7.8 years, all of 11 SMARD1 patients developed signs of autonomic neuropathy during the course of the disease, the latest was beyond 9 years of age.[9] Our patient had multiple periods of constipation, sweating, blood pressure fluctuations and arrhythmia which were prominent features consistent with autonomic neuropathy.

Reduction of the compound muscle action potential and abnormal nerve conduction velocities are commonly seen in patients with SMARD1. Electromyography (EMG) studies of SMARD1 patients revealed motor neuropathy, frequently accompanied by sensory neuropathy, and muscle biopsies indicated distally pronounced neurogenic muscular atrophy.[14] Nerve conduction studies of our patient failed to reveal any electrophysiological response in peroneal, posterior tibial, median and ulnar nerves, and muscle biopsy revealed neurogenic changes with atrophic myofibers at 1-year old. Although SMARD1 is also currently named as distal spinal muscular atrophy 1 (DSMA1, MIM#604320) and distal hereditary motor neuropathy type VI (dHMN6), both of which are confusing because these terms do not take into account sensory neuropathy and demyelination. This confusion of terminology may have occurred as a result of a broad spectrum of manifestations and electrophysiological results of defined SMARD1 patients initally.[20] Grohmann et al.[2] described clinical features of 29 SMARD1 patients who had neurogenic changes in EMG studies (22 of 25 patients), decrease in motor nerve conduction velocity (16 of 20 patients), and absent motor response after maximum stimulation (11 of 12 patients). Needle EMG results indicated chronic and active denervation in more than two thirds of patients.[14] Axonal degeneration was observed in sural nerve biopsies (10 of 15 patients) and histopathological examinations of muscle biopsy specimens revealed neurogenic changes with fiber hypertrophy and atrophy (21 of 22 patients).[2] Abnormal EMG results of the diaphragm compatible with denervation have previously been mentioned in patients with SMARD1.[4]

Homozygous c.1738G>A (p.Val580Ile) missense *IGHMBP2* variant in our patient was previously reported in only one other Turkish girl.[1,15] These two patients with the same homozygous variant, had similar clinical characteristics in the infantile period, although the onset of respiratory distress was earlier in the previously reported patient. Inspiratory stridor, facial weakness, absent deep tendon reflexes, distally marked weakness and progressive muscular hypotonia were common features. They were completely paralysed at 2 years of age but a slight improvement in the motor functions of the previous patient, mentioned above, was noted. Although she was not able to speak, her cognitive and social skills were appropriate for her age in contrast to our patient.[15]

SMARD1 is a severe motor neuron disease and the prognosis is usually poor.[9,14] The majority of patients die due to respiratory problems unless ventilatory support is initiated and few will survive into adulthood.[9,19] Viguier et al.[14] have shown that patients with diaphragmatic paralysis or areflexia before 3 months of age had significantly lower survival rates. In a multicentric retrospective study, tracheostomized patients had evidently higher survival rates and all survivors beyond 32 months of life were tracheostomized, and weaning from ventilation was unlikely once initiated.[14,15] Although most of the patients develop respiratory distress in the first few months of life and become non-ambulant and ventilator dependent during the infantile period, a plateau phase or even slight improvement of clinical symptoms may occur in survivors beyond 2 years of age.[9] Milder clinical courses with a late onset respiratory distress and distal muscle weakness have also been reported.[3,9,21] In 2015 Hamilton et al.[19] reported one of the oldest SMARD1 patients, a 21-year-old tracheostomized woman who had presented with respiratory distress at 16 months of age and remained stable for several years with only nocturnal mechanical ventilation. She was working full time in an office using her self-propelled electric wheelchair after completing her education. Previous studies showed high residual levels of IGHMBP2 enzymatic activity in fibroblasts and lymphoblastoid cells of the patients which correlated with a late onset form of the disease and/or better prognosis.[5,7,9,21] On the other hand, there is no evidence for a

genotype-phenotype correlation of the disease.[14] Treatment is primarily supportive with no known cure. Studies are ongoing for new treatment modalities and therapies.[10,22] Genetic counseling is crucial for the families with history of a child with SMARD1 and preimplantation genetic diagnosis should be considered to prevent this rare and lethal disease. Recent clinical advances in the treatment of another similar neuromuscular disease, SMA, give hope to development of new possible therapeutic approaches for this disease as well.

In conclusion; physicians should be alert for the possible diagnosis of SMARD1 in hypotonic infants presenting with respiratory distress and/or distal muscle weakness, and assess diaphragmatic weakness, congenital foot deformities, pre- and peri-natal medical history, siblings of sudden infant death syndrome, even in the presence of unexpected findings such as vocal cord paralysis, nystagmus, epileptic seizures, cognitive impairment, and brain MRI abnormalities. Concomitant motor and sensory neuropathy revealed by electrophysiological studies and dysautonomia are also noteworthy characteristics of the disease. Despite rapid progressive life threatening clinical symptoms in the first years of life, the clinical course of some survivors may remain stable without deterioration over many years and they may have normal social interactions with other people. Appropriate management, with supportive care, during the progressive phase of the disease and prevention of devastating complications may have a positive impact on quality of life and survival in these patients with an incurable disease.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SP, ÜY; data collection: YG; analysis and interpretation of results: SS, ÖK. SP; draft manuscript preparation: SP, AÜ. All authors reviewed the results and approved the final version of the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Grohmann K, Schuelke M, Diers A, et al. Mutations in the gene encoding immunoglobulin mu-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. Nat Genet 2001; 29: 75-77. https://doi.org/10.1038/ng703
2. Grohmann K, Varon R, Stolz P, et al. Infantile spinal muscular atrophy with respiratory distress type 1 (SMARD1). Ann Neurol 2003; 54: 719-724. https://doi.org/10.1002/ana.10755
3. Kaindl AM, Guenther UP, Rudnik-Schöneborn S, et al. Spinal muscular atrophy with respiratory distress type 1 (SMARD1). J Child Neurol 2008; 23: 199-204. https://doi.org/10.1177/0883073807310989
4. Pitt M, Houlden H, Jacobs J, et al. Severe infantile neuropathy with diaphragmatic weakness and its relationship to SMARD1. Brain 2003; 126(Pt 12): 2682-2692. https://doi.org/10.1093/brain/awg278
5. Guenther UP, Varon R, Schlicke M, et al. Clinical and mutational profile in spinal muscular atrophy with respiratory distress (SMARD): defining novel phenotypes through hierarchical cluster analysis. Hum Mutat 2007; 28: 808-815. https://doi.org/10.1002/humu.20525
6. Lim SC, Bowler MW, Lai TF, Song H. The Ighmbp2 helicase structure reveals the molecular basis for disease-causing mutations in DMSA1. Nucleic Acids Res 2012; 40: 11009-11022. https://doi.org/10.1093/nar/gks792
7. Guenther UP, Handoko L, Laggerbauer B, et al. IGHMBP2 is a ribosome-associated helicase inactive in the neuromuscular disorder distal SMA type 1 (DSMA1). Hum Mol Genet 2009; 18: 1288-1300. https://doi.org/10.1093/hmg/ddp028
8. Wong VC, Chung BH, Li S, Goh W, Lee SL. Mutation of gene in spinal muscular atrophy respiratory distress type I. Pediatr Neurol 2006; 34: 474-477. https://doi.org/10.1016/j.pediatrneurol.2005.10.022
9. Eckart M, Guenther UP, Idkowiak J, et al. The natural course of infantile spinal muscular atrophy with respiratory distress type 1 (SMARD1). Pediatrics 2012; 129: e148-e156. https://doi.org/10.1542/peds.2011-0544
10. McEntagart M, Spurlock G, Jackson C, Harper P, Rahman N. Distal spinal muscular atrophy with vocal cord paralysis (dSMA-VII) is not linked to the MPD2 locus on chromosome 5q31. J Med Genet 2000; 37: E14. https://doi.org/10.1136/jmg.37.8.e14

11. Velilla J, Marchetti MM, Toth-Petroczy A, et al. Homozygous TRPV4 mutation causes congenital distal spinal muscular atrophy and arthrogryposis. Neurol Genet 2019; 5: e312. https://doi.org/10.1212/NXG.0000000000000312

12. Baughn J, Gershan W, Rao A. Noisy breathing and hemidiaphragm paralysis progressing to respiratory failure in an infant. Pediatr Pulmonol 2011; 46: 817-819. https://doi.org/10.1002/ppul.21419

13. Wu S, Chen T, Li Y, et al. An atypical phenotype of a patient with infantile spinal muscular atrophy with respiratory distress type 1 (SMARD 1). Eur J Med Genet 2018; 61: 602-606. https://doi.org/10.1016/j.ejmg.2018.04.001

14. Viguier A, Lauwers-Cances V, Cintas P, et al. Spinal muscular atrophy with respiratory distress type 1: a multicenter retrospective study. Neuromuscul Disord 2019; 29: 114-126. https://doi.org/10.1016/j.nmd.2018.10.002

15. Rudnik-Schöneborn S, Stolz P, Varon R, et al. Long-term observations of patients with infantile spinal muscular atrophy with respiratory distress type 1 (SMARD1). Neuropediatrics 2004; 35: 174-182. https://doi.org/10.1055/s-2004-820994

16. van der Pol WL, Talim B, Pitt M, von Au K. 190 th ENMC international workshop: Spinal muscular atrophy with respiratory distress/distal spinal muscular atrophy type 1: 11-13 May 2012, Naarden, The Netherlands. Neuromuscul Disord 2013; 23: 602-609. https://doi.org/10.1016/j.nmd.2013.04.004

17. Litvinenko I, Kirov AV, Georgieva R, et al. One novel and one recurrent mutation in IGHMBP2 gene, causing severe spinal muscular atrophy respiratory distress 1 with onset soon after birth. J Child Neurol 2014; 29: 799-802. https://doi.org/10.1177/0883073813477203

18. Chiu ATG, Chan SHS, Wu SP, et al. Spinal muscular atrophy with respiratory distress type 1-a child with atypical presentation. Child Neurol Open 2018; 5: 2329048X18769811. https://doi.org/10.1177/2329048X18769811

19. Hamilton MJ, Longman C, O'Hara A, Kirkpatrick M, McWilliam R. Growing up with spinal muscular atrophy with respiratory distress (SMARD1). Neuromuscul Disord 2015; 25: 169-171. https://doi.org/10.1016/j.nmd.2014.10.005

20. Porro F, Rinchetti P, Magri F, et al. The wide spectrum of clinical phenotypes of spinal muscular atrophy with respiratory distress type 1: a systematic review. J Neurol Sci 2014; 346: 35-42. https://doi.org/10.1016/j.jns.2014.09.010

21. Guenther UP, Handoko L, Varon R, et al. Clinical variability in distal spinal muscular atrophy type 1 (DSMA1): determination of steady-state IGHMBP2 protein levels in five patients with infantile and juvenile disease. J Mol Med (Berl) 2009; 87: 31-41. https://doi.org/10.1007/s00109-008-0402-7

22. Saladini M, Nizzardo M, Govoni A, et al. Spinal muscular atrophy with respiratory distress type 1: clinical phenotypes, molecular pathogenesis and therapeutic insights. J Cell Mol Med 2020; 24: 1169-1178. https://doi.org/10.1111/jcmm.14874

The Turkish Journal of Pediatrics 2022; 64: 375-380
https://doi.org/10.24953/turkjped.2020.3601

Case Report

# A rare endocrinological complication of chronic kidney disease

Seçil Arslansoyu Çamlar[1], Berna Filibeli[2], Eren Soyaltın[1], Hayrullah Manyas[2], Gönül Çatlı[2,3], Demet Alaygut[1], Fatma Mutlubaş[1], Bumin Nuri Dündar[2,3], Belde Kasap Demir[1,4]

*Divisions of [1]Nephrology and [2]Endocrinology, Department of Pediatrics, University of Health Sciences, İzmir Tepecik Training and Research Hospital, İzmir; Divisions of [3]Endocrinology and [4]Nephrology and Rheumatology, Department of Pediatrics, İzmir Katip Çelebi University, İzmir, Turkey.*

**ABSTRACT**

**Background.** Chronic kidney disease (CKD) may lead to increase in serum levels of peptide hormones as a result of changes in peripheral metabolism. The pathogenesis of uremic hyperprolactinemia in CKD is not fully understood. Plasma prolactin levels are elevated in women, pubertal girls, and also in men with chronic kidney disease. But this is not comon in prepubertal boys. Also in prepubertal children and postmenopausal women, hyperprolactinemia rarely results in galactorrhea. We aimed to discuss hyperprolactinemia and galactorrhea in a 12-year-old male with CKD.

**Case.** A twelve-year-old boy with chronic kidney disease (CKD) suffered from bilateral galactorrhea. He was on follow-up at Pediatric Nephrology Department from the age of two due to bilateral dysplastic kidney. On physical examination, his weight was - 0.59 SDS, height was -2.82 SDS, Blood pressure was 115 / 72 (75p), stretched penis length was 6 cm, testicular volume was 3mL / 3mL, pubic hair was Tanner Stage 1, breast examination did not reveal plaque on bilateral breast. He was receiving recombinant erythropoietin, sodium bicarbonate, polystyrene sulfonate, calcium acetate, and calcitriol treatments. Glomerular filtration rate was 23ml/min/1.73 m2 (CKD stage IV). Serum prolactin (PRL) was >200 μg/L (N, 2.64-13.13). The pituitary adenoma was excluded with pituitary and cranial magnetic resonance imaging (gadolinium). Cabergoline (0.5 mg/ twice weekly) was initiated to decrease PRL levels and reduce galactorrhea. In the second week of treatment, serum PRL level was suppressed (0.4 μg/L) and galactorrhea was completely resolved.

**Conclusions.** Although uremic hyperprolactinemia is very rarely seen in childhood, it is important to evaluate, and initiate an appropriate treatment since it is associated with delayed puberty and infertility in adulthood in many cases.

**Key words:** children, chronic kidney disease, galactorrhea, hyperprolactinemia.

Many metabolic and endocrinological abnormalities are encountered in chronic kidney disease (CKD). Studies showed that decrease in glomerular filtration rate, and change in the metabolic environment due to uremia can cause impaired extrarenal metabolism and integrity of feedback controls regulating secretion or synthesis of the peptide hormones.[1]

✉ Seçil Arslansoyu Çamlar
secilars@yahoo.com

Received 6th December 2020, revised 6th January 2021, accepted 10th March 2021.

Prolactin (PRL) is responsible for the hormonal regulation of lactation and regulation of gonadal luteinizing hormone receptors in both genders. PRL also has many physiological functions such as osmoregulation, immune response and angiogenesis.[1] The pathogenesis of uremic hyperprolactinemia in CKD is not fully understood. In prepubertal children and postmenopausal women, hyperprolactinemia rarely results in galactorrhea.[2] Galactorrhea is not an expected finding in prepubertal cases. We aimed to discuss hyperprolactinemia and galactorrhea in a 12-year-old male with CKD.

## Case Report

A twelve-year-old boy with chronic kidney disease (CKD) suffered from bilateral galactorrhea. He was on follow-up at Pediatric Nephrology Department from the age of two due to congenital anomalies of kidney and urinary tract (congenital bilateral dysplastic kidney). In his medical history; he was born at 40 weeks' gestation with a birth weight of 3100 g and there was a hospitalization in the neonatal intensive care unit because of asphyxia. He received antibiotic therapy many times due to recurrent urinary tract infection. He had no vesico ureteral reflux. 99mTc dimercaptosuccinic acid (DMSA) scan show only bilateral small kidneys. On physical examination, his weight was 37.2 kg (- 0.59 SDS), height was 128.6 cm (-2.82 SDS), z-score of body mass index (BMIz score) +1.01 SDS, target height 172.1 cm (-0.61 SDS), blood pressure was 115/72 mmHg (75/75p), stretched penis length was 6 cm, testicular volume was 3mL/3mL, and pubic hair was at Tanner stage I. Breast examination did not reveal plaque or gynecomastia but galactorrhea was noted. Other systems and neurologic examinations were normal. Bone age according to Greulich- Pyle was 10 years and predicted adult height was calculated as 160 cm (-2.27 SDS) by the Bayley-Pinneau method. He was receiving recombinant erythropoietin, sodium bicarbonate, polystyrene sulfonate, calciumacetate, and calcitriol treatments. Glomerular filtration rate was 23 ml/min/1.73 m2 (CKD stage IV). Labaratory investigations revealed; Hb 10.2 g/dL, blood urea 152 mg/dL, creatinine 3.9 mg/dL, calcium 10.5 mg/dL, phosphate 6.1 mg/dL, alkaline phosphatase 264 mg/dL (160-500), 25-hydroxy vitamin D 32 μg/L (N, 20-100), parathyroid hormone 182 ng/L (N, 10-69), free T4 0.82 ng/dL (N,0.54-1.24), thyroid-stimulating hormone 3.2 mU/L (N, 0.34-5.6), luteinizing hormone(LH) 1 mIU/mL (N, <0.1), follicle stimulating hormone 2.8 IU/L (N, 0.1-4.3), total testosterone 10.3 ng/dL (N, <20), PRL>200 μg/L (N,2.64-13.13), and somatomedin-C (IGF-1) 168 g/L (N, 68-316). Pituitary and cranial magnetic resonance imaging with gadolinium showed no intracranial pathology. Pre-emptive renal transplantation was planned in our patient. However, peritoneal dialysis was started since no suitable donor could be found. Cabergoline (0.5 mg/ twice weekly) was initiated to decrease PRL levels and reduce galactorrhea. In the second week of treatment, serum PRL level was suppressed (0.4 μg/L) and galactorrhea was completely resolved. Informed consent was received from the parents.

## Discussion

Decrease in renal function causes changes in the synthesis, secretion, metabolism, and elimination of peptide hormones.[3] Deranged metabolic environment of uremia may contribute to impaired metabolism which regulates the integrity of feedback controls regulate the synthesis of secretion.[2] Elimination of PRL occurs both through glomerular filtration and tubular breakdown.[2] PRL is reabsorbed by tubular cells by blood flowing in peritubularcapillaries from the anti-luminal pole of proximal tubular cells.[4] The pathogenesis of uremic hyperprolactinemia in CKD is not fully understood. Chronic renal failure stimulates dysregulation of PRL secretion by a resistance of lactotrophs to dopaminergic inhibition.[5]

Plasma PRL correlates with serum creatinine levels; as the renal functions deteriorate, PRL level increases. Therefore, hyperprolactinemia is seen in patients with CKD with advanced or even end-stage renal failure. Peces et al.[1] reported that patients diagnosed with CKD who have hemodialysis had higher PRL levels compared to healthy controls and patients who had undergone kidney transplantation. The serum PRL levels in women were higher than in men in this study. In a study conducted by Ijaiya et al.[5] it was reported that serum PRL levels in children with acute renal insufficiency and renal transplantation were similar to healthy children, while those with chronic renal failure were 2.5 times higher. Moreover elevated basal PRL could not be stimulated by TRH stimulation.[5]

Dysfunction due to vitamin D deficiency, anemia, zinc depletion and pituitary regulation of PRL release can be the other etiological factors resulting in hyperprolactinemia in CKD patients.[6,7] The presence of inflammatory cytokines and chronic metabolic acidosis may contribute to dysregulation of the hypothalamic-pituitary-thyroid axis in CKD.[6] Pathological thyroid profile, including clinical or subclinical hypothyroidism, can also cause alterations in the hypothalamo-hypophyseal-gonadal axis and can manifest with hyperprolactinemia.[6] Thyroid function tests should be evaluated in cases with hyperprolactinemia. Thyroid function tests, vitamin D and hemoglobulin were normal in our patient. Many antipsychotic drugs increase PRL levels by affecting the dopaminergic system. Methyldopa and verapamil used in the treatment of hypertension are other causes of drug-related hyperprolactinemia.[8-10] Gulleroğlu et al.[9] reported an eleven-year-old boy on peritoneal dialysis with galactorrhea. He was using methyldopa for hypertension so they thought that galactorrhea was related to methyldopa instead of uremia as he was already on renal replacement therapy for long-term. Tacrolimus and amlodipine-induced hyperprolactinemia has been reported in a 19-year-old woman with kidney transplantation who presented with galactorrhea and mastalgia.[10] The medications of our patient were questioned in detail, but there were no agents causing drug-related hyperprolactinemia. Cases with chronic kidney disease and hyperprolactinemia reported in the literature are summarized in Table I .

Hyperprolactinemia is defined as an increase in serum prolactin levels. Normal prolactin levels are < 25 μ g/L in girls, and < 20 μ g/L in boys.[11] Plasma prolactin levels are elevated in women, pubertal girls, and also in men with chronic kidney disease.[12]

This situation is not usual in prepubertal boys. Our case was prepubertal according to testis volume, however, high levels of basal LH was incompatible with the prepubertal period. Hypothalamic and hormonal causes were excluded, so hyperprolactinemia in our patient was attributed to uremia. Our patient was clinically prepubertal, therefore, we focused primarily on a possible PRL-secreting adenoma. PRL levels > 200 μg/L are highly related to secretion PRL by prolactinoma.[13] Pituitary imaging is recommended for symptomatic patients.[14] Therefore, the patient was evaluated for pituitary adenomas by the pituitary and cranial magnetic resonance imaging. However, there were no findings related to pituitary compression, and no adenoma or tumor was found on MRI. Hyperprolactinemia can cause gonadotropin suppression, anovulation, irregular menstrual cycles, infertility, hypoestrogenism, gynecomastia, sexual dysfunction and galactorrhea in adults.[3,14] Male patients usually show intracranial pressure symptoms such as headache and visual loss due to tumor growth.[14]

Disturbances in the control of hypothalamic-pituitary-gonadal axis in men and women with CKD and end-stage renal disease is well-known; however, it is not fully understood. Delayed puberty and reduced pubertal growth are very pronounced in children with CKD due to long-term dialysis treatment and high glucocorticoid exposure.[15] Hyperprolactinemia also contributes to this situation by decreasing pulsatile gonadotropin-releasing hormone secretion and

**Table I.** Cases reported in the literature with hyperprolactinemia and chronic renal diseases.

| Cases | Age | Gender | Renal status | Suspected etiology |
|---|---|---|---|---|
| Bry-Gauillard[17] | 34 | male | Hemodialysis | Macroadenoma of the hypophisis |
| Pratap[7] | 42 | female | Hemodialysis | Methlydopa |
| Khira[9] | 19 | female | Renal tx | Tacrolimus, amlodipine |
| Rondeau[2] | 4 | female | Hemodialysis | Chronic kidney disease |
| Gulleroglu[8] | 11 | male | Periton dialysis | Methlydopa |

thus inhibiting luteinizing hormone (LH) and follicle-stimulating hormone secretion as well as gonadal steroidogenesis(hypogonadotropic hypogonadism).[11] In uremic patients, despite decreased LH secretion from the pituitary gland, serum LH concentration may be elevated due to impaired renal clearance, but bioactivity of LH is decreased.[16] Interestingly, central precocious puberty was also reported in girls and boys with CKD.[16-18] Furthermore, a case of precocious puberty associated with hyperprolactinemia was also reported.[19] The authors hypothesized that high prolactin levels might have both stimulated and inhibited the hypothalamic-pituitary-gonadal axis.[19] In brief, the pathophysiology of neuroendocrine dysregulation in uremic patients remains unclear. Despite the increased LH level, our patient's testicular volumes, serum total testosterone level and bone age were compatible with the prepubertal period. Besides, following normalization of PRL levels with cabergoline treatment, LH levels decreased to 0.5 mIU/mL and no progression in pubertal findings was observed at clinical follow-up.

Hyperprolactinemia treatment varies according to the underlying etiology. The main objectives of treatment are normalizing prolactin level, reducing the diameter of the adenoma and reducing clinical signs related to hyperprolactinemia.[11,14,15] The reduction of the drug dose or transition to another drug is enough for the treatment of drug-induced hyperprolactinemia.[14] Dopamine agonists are a successful therapeutic option in prolactinomas. Cabergoline has been shown to be more effective in normalizing prolactin levels and reducing tumor size with fewer adverse effects than bromocriptine.[13] A significant tumor shrinkage can be achieved with cabergoline in microadenomas (<10 mm), whereas surgery may be needed in addition to cabergoline treatment in macroprolactinomas (> 10 mm). In uremic hyperprolactinemia, it may be beneficial to start renal replacement therapy due to the contribution of uremia. Renal replacement therapies should be considered to eliminate the uremic state in hyperprolactinemia due to CKD. However, in the literature, hyperprolactinemia has been reported in cases receiving peritoneal dialysis. Frequent hemodialysis sessions did not decrease the prolactin levels.[20] Bilateral nephrectomy was performed in a 4-year-old girl with an end-stage renal failure due to uncontrolled severe hypertension. PRL levels rose following bilateral nephrectomy. After kidney transplantation, PRL levels decreased dramatically in 8 hours in this patient. Authors suggest that even uremic kidneys can eliminate PRL through tubular breakdown since PRL levels increase after bilateral nephrectomy.[2] After renal transplantation, hyperprolactinemia can usually be corrected or significantly improved.[21] It has been reported that PRL levels decrease or return to normal levels with the increase of glomerular filtration rate after kidney transplantation.[5] It has been demonstrated repeatedly that serum PRL levels rapidly return to normal after kidney transplantation, as in the cases reported in the literature.[8,22,23]

Early renal transplantation in the pediatric population has demonstrated adequate pubertal developmental outcomes. Therefore, the most appropriate approach is to find a suitable donor for the patient and perform renal transplantation. Although uremic hyperprolactinemia is very rarely seen in childhood, it is important to evaluate, and initiate an appropriate treatment since it is associated with delayed puberty and infertility in adulthood in many cases.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: SAÇ; data collection: BF, ES,DA; analysis and interpretation of results: HM, GÇ, FM; draft manuscript preparation: SAÇ, BND, BKD. All authors reviewed the results and approved the final version of the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Peces R, Horcajada C, López-Novoa JM, Frutos MA, Casado S, Hernando L. Hyperprolactinemia in chronic renal failure: impaired responsiveness to stimulation and suppression. Normalization after transplantation. Nephron 1981; 28: 11-16. https://doi.org/10.1159/000182087
2. Rondeau G, Merouani A, Phan V, Deal C, Robitaille P. Serum prolactin levels in uremic child: effects of bilateral nephrectomy and kidney transplantation. NDT Plus 2011; 4: 303-306. https://doi.org/10.1093/ndtplus/sfr092
3. Holley J. The hypothalamic-pituitary axis inmen and women with chronic kidney disease. Adv Chronic Kidney Dis 2004; 11: 331-337. https://doi.org/10.1053/j.ackd.2004.07.004
4. Emmanouel DS, Fang VS, Katz AI. Prolactin metabolism in the rat: role of the kidney in degradation of the hormone. Am J Physiol 1981; 240: F437-F445. https://doi.org/10.1152/ajprenal.1981.240.5.F437
5. Ijaiya K, Roth B, Schwenk A. Serum prolactin levels in renal insufficiency in children. Acta Paediatr Scand 1980; 69: 299-304 . https://doi.org/10.1111/j.1651-2227.1980.tb07082.x
6. Schaefer F. Endocrine and growth disorders in chronic kidney disease. In: Avner ED, Harmon WE, Niaudet P, Yoshikawa N (eds). Pediatric Nephrology (7th ed). Berlin: Springer, 2016; 2299-2300.
7. Caticha O, Norato DJY, Tambascia MA, Santana A, Stephanou A, Sarlis NJ. Total body zinc depletion and its relationship to the development of hyperprolactinemia in chronic renal insufficiency. J Endocrinol Invest 1996; 19: 441-448. https://doi.org/10.1007/BF03349889
8. Pratap B, Sundaram V, Abraham G, Matthew M, Bhaskar S. Cessation of galactorrhea in a chronic kidney disease patient with non-tumoral hyperprolactinemia after renal transplantation. J Assoc Physicians India 2008; 56: 54-55.
9. Gulleroglu K, Olgac A, Bayrakci U, Erdogan O, Kinik SB, Baskin E. Hyperprolactinemia as a rare cause of hypertension in chronic renal failure. Ren Fail 2012; 34: 792-794 . https://doi.org/10.3109/0886022X.2012.672313
10. Khaira A, Rathi OP, Gupta A, et al. Galactorrhoea and mastalgia in a renal transplant recipient on tacrolimus and amlodipine. Nephrology (Carlton) 2009; 14: 700-701. https://doi.org/10.1111/j.1440-1797.2009.01077.x
11. Catli G, Abaci A, Bober E, Büyükgebiz A. Clinical and diagnostic characteristics of hyperprolactinemia in childhood and adolescence. J Pediatr Endocrinol Metab 2013; 26: 1-11. https://doi.org/10.1515/jpem-2012-0327
12. Serret-Montaya J, Zurita-Cruz JN, Villasís-Keever MA, et al. Hyperprolactinemia as a prognostic factor for menstrual disorders in female adolescents with advanced chronic kidney disease. Pediatr Nephrol 2020; 35: 1041-1049. https://doi.org/10.1007/s00467-020-04494-7
13. Molitch ME. Diagnosis and treatment of pituitary adenomas: a review. JAMA 2017; 317: 516-524. https://doi.org/10.1001/jama.2016.19699
14. Eren E, Törel Ergür A, İşgüven ŞP, et al. Clinical and laboratory characteristics of hyperprolactinemia in children and adolescents: national survey. J Clin Res Pediatr Endocrinol 2019; 11: 149-156. https://doi.org/10.4274/jcrpe.galenos.2018.2018.0206
15. Halperin Rabinovich I, Cámara Gómez R, García Mouriz M, Ollero García-Agulló D; Grupo de Trabajo de Neuroendocrinología de la SEEN. Clinical guidelines for diagnosis and treatment of prolactinoma and hyperprolactinemia. Endocrinol Nutr 2013; 60: 308-319. https://doi.org/10.1016/j.endoen.2012.11.009
16. Avnon Ziv C, Tzvi-Behr S, Ben-Shalom E, et al. Early puberty in end stage renal failure and renal transplant recipients. J Pediatr Endocrinol Metab 2019; 32: 577-583. https://doi.org/10.1515/jpem-2019-0050
17. Loh KC, Salisbury SR, Accott P, Gillis R, Crocker JF. Central precocious puberty and chronic renal failure: a reversible condition post renal transplantation. J Pediatr Endocrinol Metab 1997; 10: 539-545. https://doi.org/10.1515/JPEM.1997.10.5.539
18. Winter S, Durand A, Brauner R. Precocious and early central puberty in children with pre-existing medical conditions: a single center study. Front Pediatr 2019; 7: 35. https://doi.org/10.3389/fped.2019.00035
19. Hardy M, O'Connell J, Gilbertson N, Barltrop D. Precocious puberty associated with hyperprolactinemia in a male patient. J Pediatr 1988; 113: 508-511. https://doi.org/10.1016/S0022-3476(88)80641-3

20. Hou SH, Grossman S, Molitch ME. Hyperprolactinemia in patients with renal insufficiency and chronic renal failure requiring hemodialysis or chronic ambulatory peritoneal dialysis. Am J Kidney Dis 1985; 6: 245-249. https://doi.org/10.1016/S0272-6386(85)80181-5

21. Huang W, Molitch ME. Evaluation and management of galactorrhea. Am Fam Physician 2012; 85: 1073-1080.

22. Bry-Gauillard H, Touraine P, Mamzer-Bruneel MF, Simoes-Vaz A, Kuttenn F, Legendre C. Complete regression of a major hyperprolactinaemia after renal transplantation. Nephrol Dial Transplant 1999; 14: 466-468. https://doi.org/10.1093/ndt/14.2.466

23. Saha MT, Saha HH, Niskanen LK, et al. Time course of serum prolactin and sex hormones following successful renal transplantation. Nephron 2002; 92: 735-737. https://doi.org/10.1159/000064079

The Turkish Journal of Pediatrics 2022; 64: 381-384
https://doi.org/10.24953/turkjped.2020.3609

Case Report

# Cardiac failure in a child with tuberculous meningitis as a complication of Paroxysmal sympathetic hyperactivity

Pınar Yazıcı Özkaya[1], Eşe Eda Turanlı[1], Hatice Feray Arı[1], Serap Kurt[2], Bülent Karapınar[1]

[1]*Division of Intensive Care Unit,* [2]*Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey.*

## ABSTRACT

**Background.** Paroxysmal sympathetic hyperactivity (PSH) is a disorder due to the loss of regulation of autonomic activity. The most common condition predisposing to the development of PSH is traumatic brain injury (TBI), followed by anoxic brain injury, stroke, tumors, and infections. Awareness about the condition and early recognition is important to avoid life threatening complications.

**Case.** We report a 4-year-old child with tuberculous meningitis with symptoms of PSH who developed cardiac failure. PSH episodes were treated with beta blocker, benzodiazepine, morphine, dexmedetomidine, baclofen, and tizanidine. Three weeks after readmission PSH episodes decreased and the patient was transferred to the general ward.

**Conclusions.** PSH assessment tool has benefits such as monitoring the patient, evaluating response to treatment and early diagnosing PSH patients.

**Key words:** paroxysmal sympathetic hyperactivity, tuberculous meningitis, child.

Paroxysmal sympathetic hyperactivity (PSH) is a disorder due to the loss of regulation of autonomic activity. PSH has previously been described as autonomic storms, hypothalamic dysregulation syndrome, dysautonomia, paroxysmal autonomic instability with dystonia, and diencephalic autonomic epilepsy. The most common condition predisposing to the development of PSH is traumatic brain injury (TBI), followed by anoxic brain injury, stroke, tumors, and infections. In 2014, an international panel was convened and published a consensus statement to define symptoms and diagnostic criteria of PSH.

Hereby we report a 4-year-old child with tuberculous meningitis with symptoms of PSH who developed cardiac failure.

✉ Pınar Yazıcı Özkaya
pinar.ozkaya@ege.edu.tr

Received 4th December 2020, revised 7th March 2021, accepted 10th March 2021.

## Case Report

A four-year old boy was admitted with fever, headache for three days, intermittent vomiting, and loss of consciousness. On his examination, neck stiffness has been determined, Glasgow coma scale (GCS) was 11. Vital signs were within the normal range. The diagnosis of tuberculous meningitis was made after head magnetic resonance imaging (MRI) and cerebrospinal fluid examination. Therapy of isoniazid, pyrazinamide, rifampin, and ethambutol has been initiated. On the 2nd day, he had a seizure, his computed tomography showed hydrocephalus. He was referred to our hospital after a ventriculoperitoneal (V/P) shunt placement and because of the need for tertiary intensive care follow up.

On admission to the pediatric intensive care unit (PICU), his heart rate was 110 beats per minute (bpm), blood pressure 105/60 mmHg, temperature 37.8 °C, GCS was 8. Physical examination revealed no abnormalities except

increased muscle tone. On the 2nd day in the PICU the patient deteriorated with a GCS of 5. Cerebral MRI showed progression of hydrocephalus and angio-MRI showed left anterior carotid artery (segment $A_2$) and posterior carotid artery (segment $P_1$, $P_2$) occlusion with embolic infarcts in the left cerebral hemisphere.

Tracheostomy was placed for long term ventilation and the patient has transferred to the general ward in a stable condition with home mechanical ventilatory support on the 24th day of admission. Cerebrospinal fluid culture detected *M.tuberculosis* with multiple drug resistance. His treatment was changed to linesolide, moxifloxacin, streptomycin, and ethambutol. Baclofen and tizanidine were continued for dystonia.

After 2 months of transfer to the general ward, he was readmitted to the PICU with symptoms of congestive heart failure. The heart rate was 190 bpm, blood pressure 190/110 mmHg, temperature 38 °C, and oxygen saturation 85% with fractional inspired oxygen of 50%. On his physical examination, his liver was palpable 4 cm under the right costal margin. On neurological examination, he had extensor posturing, mydriasis, and GCS of 4 points. Intravenous antihypertensive therapy (esmolol and glyceryl trinitrate) was immediately initiated. Transthoracic echocardiography revealed decreased systolic ventricular function with an ejection fraction (EF) of 30%.

On the 2nd day of readmission, antihypertensive treatment was weaned off gradually. During his observation in the PICU, he developed intermittent bradycardia and tachycardia episodes with high blood pressure, piloerection, mydriasis, dystonia, and fever. These episodes lasted approximately 30 minutes and improved after benzodiazepines. The electroencephalogram showed no epileptic discharge. During his stay in the PICU, sudden hypotension and hypertension periods with dystonia were observed. Serum cortisol level was within the normal range. Cardiac MRI revealed no abnormalities. Cultures of blood, urine, and tracheal aspirate were negative. A subsequent MRI showed progression of tuberculomas in the bilateral cerebral hemisphere and basal cisterns, and infarction in the basal ganglia.

The patient was considered as probable PSH according to the PSH assessment measure (PSH-AM). PSH episodes were treated with beta blocker, benzodiazepine, morphine, dexmedetomidine, baclofen, and tizanidine. Three weeks after readmission PSH episodes decreased and the patient was transferred to the general ward. Transthoracic echocardiography revealed improved systolic ventricular function with EF of 50%. Written informed consent was obtained from the parents for publication of the case.

## Discussion

PSH is a disorder in the regulation of the sympathetic nervous systems which is mostly caused by severe traumatic brain injury (TBI). In 2014, an international panel was convened and published a consensus statement to define symptoms and diagnostic criteria.[1] The reported incidence of PSH ranges from 8% to 33% in adults and %13 in children following acquired brain injury.[2,3] In adult studies, most PSH cases have been reported to occur after TBI. Anoxic brain injury, stroke, tumor, infections, subarachnoid hemorrhage, hydrocephalus, and suprasellar cyst have been reported as causes of PSH.[4-6]

PSH shows a wide spectrum of clinical symptoms. Main clinical symptoms include tachycardia, hypertension, tachypnea, fever, sweating, and/or increased muscle tone with possible dystonic posturing. Patients may present with various combinations of these symptoms. These clinical features can manifest spontaneously or in response to slight-noxious stimuli such as aspiration of secretions, change of position, or physiotherapy. In a previous study, tachycardia has been reported as the

most common symptom.[7] The combination of hypertension, diaphoresis, and dystonia best predicted a diagnosis of pediatric PSH.[3] In acute settings patient treatment generally includes deep sedation and analgesia which may hide the symptoms of PSH. It is important to recognize PSH because, if untreated, PSH can persist and potentially result in serious complications such as dehydration, muscle loss, and contractures.

Clinical Features Scale and Diagnosis Likelihood Score has been determined when dominant symptoms are observed, and appropriate interventions were made according to the clinical situation. Kirk et al.[3] diagnosed 10% PSH in TBI in pediatric rehabilitation settings by clinical diagnosis of dysautonomia. However, recently Alofisan et al.[8] showed a prevalence of 20% PSH in severe TBI children by using the diagnostic tool. The tool has benefits of monitoring the patient, evaluating response to treatment and diagnosing PSH patients early. In this case, the PSH-AM score was 22 and the score dropped to 9 before the patient was transfer to the general ward.

In the hyperacute phase, most brain injury patients require deep sedation and analgesia during their follow up in intensive care units which makes it difficult to recognize PSH. In this case, the patient underwent invasive procedures and needed a longer duration of sedoanalgesia. During his stay in the general ward, noninvasive monitorization resulted in underdiagnosed hypertension-hypotension periods and medical treatment was continued with baclofen and tizanidine only. The patient was admitted to the intensive care unit with a life-threatening complication due to a delay in diagnosis.

Recent studies reported that older age in children and early tracheostomy was associated with an increased risk for developing PSH.[8,9] It is important to develop tools to define risk factors that may help recognize PSH patients.

## Author contribution

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

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Baguley IJ, Perkes IE, Fernandez-Ortega JF, et al. Paroxysmal sympathetic hyperactivity after acquired brain injury: consensus on conceptual definition, nomenclature, and diagnostic criteria. J Neurotrauma 2014; 31: 1515-1520. https://doi.org/10.1089/neu.2013.3301

2. Baguley IJ, Slewa-Younan S, Heriseanu RE, et al. The incidence of dysautonomia and its relationship with autonomic arousal following traumatic brain injury. Brain Inj 2007; 21: 1175-1181. https://doi.org/10.1080/02699050701687375

3. Kirk KA, Shoykhet M, Jeong JH, et al. Dysautonomia after severe traumatic brain injury. Dev Med Child Neurol 2012; 54: 759-764. https://doi.org/10.1111/j.1469-8749.2012.04322.x

4. Perkes I, Baguley IJ, Nott M, Menon D. A review of paroxysmal sympathetic hyperactivity after acquired brain injury. Ann Neurol 2010; 68: 126-135. https://doi.org/10.1002/ana.22066

5. Choi HA, Jeon SB, Samuel S, Allison T, Lee K. Paroxysmal sympathetic hyperactivity after acute brain injury. Curr Neurol Neurosci Rep 2013; 370: 1-10. https://doi.org/10.1007/s11910-013-0370-3

6. Verma R, Giri P, Rizvi I. Paroxysmal sympathetic hyperactivity in neurological critical care. Indian J Crit Care Med 2015; 19: 34-37. https://doi.org/10.4103/0972-5229.148638

7. Hughes JD, Rabinstein AA. Early diagnosis of praoxysmal sympathetic hyperactivity in the ICU. Neurocrit Care 2014; 20: 454-459. https://doi.org/10.1007/s12028-013-9877-3

8. Alofisan TO, Algarni YA, Alharfi IM, et al. Paroxysmal sympathetic hyperactivity after severe traumatic brain injury in children: prevalence, risk factors, and outcome. Pediatr Crit Care Med 2019; 20: 252-258. https://doi.org/10.1097/PCC.0000000000001811

9. Li Z, Chen W, Zhu Y, et al. Risk factors and clinical features of paroxysmal sympathetic hyperactivity after spontaneous intracerebral hemorrhage. Auton Neurosci 2020; 225: 102643. https://doi.org/10.1016/j.autneu.2020.102643

The Turkish Journal of Pediatrics 2022; 64: 385-388
https://doi.org/10.24953/turkjped.2021.264

Case Report

# Primitive neuroectodermal tumor in a child with Currarino syndrome

Memnune Nur Çebi[1], Gizem Yılmaz[1], Gökçe Çelikdemir[2], Rahşan Özcan[2], Süheyla Ocak[1], Tülin Tiraje Celkan[1], Nil Çomunoğlu[3]

*Departments of [1]Pediatric Hematology and Oncology, [2]Pediatric Surgery and [3]Pathology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey.*

## ABSTRACT

**Background.** Curarino syndrome is a rare and complex anomaly with the triad of anorectal malformation, presacral mass and sacral bone deformation. The most common cause of the presacral mass is meningioma, but teratoma is the diagnosis in about one-third of the cases. Malignant transformation of teratoma in the form of carcinoma, rhabdomyosarcoma and leukemia have previously been reported on rare occasions.

**Case.** A 19 month-old-girl was referred with a presacral mass of 29mm x 23mm x 24mm. She was diagnosed as Currarino syndrome. The presacral mass was surgically resected and pathological examination revealed a foci of primitive neurectodermal tumor.

**Conclusions.** This is the first case of Currarino syndrome with a primitive neuroectodermal tumor (PNET) foci in the presacral mass. Considering the risk of malignant transformation, the accurate pathological examination is important for complete systemic evaluation and treatment plan in these children.

**Key words:** currarino syndrome, presacral teratoma, malignant transformation, PNET.

Currarino is a rare syndrome consisting of anorectal malformation, presacral mass and sacral bone deformation and may have different presentations. The type of the presacral mass, which is one of the components of the triad, is frequently meningocele, but may be teratoma in 20-40% of cases.[1] Malignant transformation of teratoma was reported in 6 children with Currarino syndrome in the literature as far as we know.[2] This transformation was in the form of carcinoma, rhabdomyosarcoma and leukemia. However, development of a primitive neuroectodermal tumor (PNET) has been observed in only two cases in the literature.[3,4] Here we report a girl with Currarino syndrome and a sacrococcygeal teratoma with a PNET foci inside.

✉ Süheyla Ocak
suheylaocak@gmail.com

Received 6th February 2021, revised 24th April 2021, 5th May 2021, accepted 13th May 2021.

## Case Report

Our case is a 19 month-old-girl born with ceasarian section weighing 2980gr at 37 weeks of gestation from consanguineous parents. It was learned that there were no similar cases in the family history of the patient. Informed consent was received from the family for this case report. Colostomy was performed on the second postnatal day due to vomiting and delayed stool discharge. In the lumbosacral MRI, a presacral mass lesion of 29mm x 23mm x 24mm with lobulated contours consisting of fat, dense content and cystic areas was detected, and interpreted as teratoma. In the preoperative examinations of the patient serum AFP level was 57 ng/ml (reference range for 6-12 months is 0-80 ng/ml) and serum NSE level was 21.7 ng/ml (reference range is <18 ng/ml). On examination under general anesthesia, it was observed that the rectum opened into the perineum in the form of a fistula, and presacral mass excision and anorectoplasty were

performed with a posterior sagittal incision due to anorectal malformation with perineal fistula. During the operation, dysmorphic coccyx and sacrum were observed and the coccyx was excised. The patient, in whom all 3 components of the syndrome observed, was diagnosed with Currarino syndrome. Pathological examination was revealed such that grade 2 ependymal components constituted approximately 30% of the tumor in a nodular infiltrative pattern. In addition, the primitive neuroectodermal tumor (PNET) area was seen in a focal area of approximately 1x1mm as a part of ependymal component. (Fig. 1)

The surgical margins were negative with a 0.1 mm to normal tissue. Given the presence of a PNET foci inside the tumor, the thorax CT and PET-MRI were performed, and no metastatic lesion was observed. Genetic testing was not done at this stage. The patient was discussed at the local tumor board. Considering the PNET foci is small and excised totally with no metastatic lesion, adjuvant therapy was not scheduled. She is under close follow-up with monthly AFP and MRI every three months, and 6 months since the diagnosis, with no evidence of disease.[3,4]

## Discussion

Currarino syndrome is a rare syndrome that includes one or more of the components of anorectal malformation, presacral mass and sacral bone deformation. While no pathology has been found in the cytogenetic examination of most cases, an autosomal dominant inherited mutation in the HLXB9 (MNX1) gene on chromosome 7q36 was observed in some cases.[5,6] There may be asymptomatic cases, as well as cases with compression symptoms such as intestinal obstruction or chronic constipation as observed in our case, and malignant transformation.

Sacrococcygeal teratoma, which may accompany the syndrome, is generally observed more frequently in sporadic cases and in females. It also carries a 1% risk of malignant transformation.[3] In a comparative study by Dirix

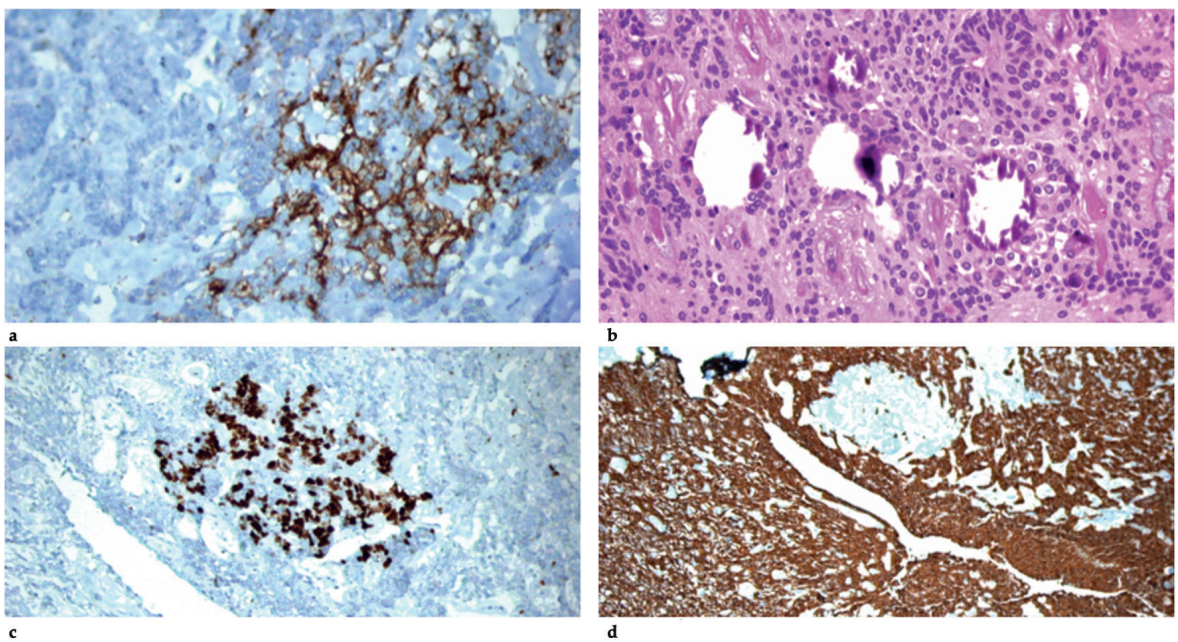



**Fig. 1. a.** Synaptophysin immunopositivity in PNET areas (Synaptophysin X200), **b.** Focal primitive neuroectodermal component in ependymal areas (H&EX200), **c.** High Ki-67 proliferative index in PNET areas (Ki-67X100), **d.** GFAP immunopositivity in ependymal component and immunonegative PNET areas (GFAPX100).

et al, the risk of malignant transformation was found to be higher in sporadic sacrococcygeal teratomas than components of Currarino syndrome. Also, malignant transformation of teratomas occurred at an older age in patients with the syndrome.[7,8] The authors proposed that the teratomas in Currarino syndrome might have a different biology and have a higher chance for complete resection. It was suggested that the treatment should be personalized for those patients.

As far as we know, malignant transformation of teratoma has been detected in 6 pediatric cases with Currarino syndrome in the literature.[2] This transformation is often in the form of carcinoma, rhabdomyosarcoma and leukemia. Peripheral PNET is an aggressive tumor with a high metastatic potential and systemic treatment is usually indicated irrespective of the size. Despite local and systemic treatment, the 5-year estimated survival rates are 75% in nonmetastatic cases and worse outcomes are reported in the presence of metastasis.[8] There are only two other cases of Currarino syndrome with malignant transformation of teratoma to PNET in the literature. A 3-year-old patient with Currarino syndrome having sacral teratoma component of PNET presenting with long-term constipation was mentioned in the case report of Sen et al.[4].The patient was treated according to the Euro Ewing 99 protocol, followed by local radiotherapy after resection. She remained in complete remission after 8 months from end of treatment. The second case with Currarino syndrome having malignant transformation to PNET within a sacral mass component had presented with a painless abdominal swelling at the age of 19.[9] After surgical excision of the tumor, considering that the pelvic washings were negative with no evidence of lymphovascular invasion, it was decided to closely monitor the patient without any additional intervention. The patient was monitored with abdomen and pelvis ultrasound and tumor markers (Carcinoma antigen 125, lactate dehydrogenase, beta human chorionic gonadotropin and a-fetoprotein) every 6 months. She remained in complete remission after 8 months of surgery. Our case is the third and the youngest one reported with a PNET foci inside the teratoma component of the Currarino syndrome.

The prognosis of Currarino Syndrome parallels the diversity in clinical involvement and death has been reported due to malignancy and sepsis in approximately 30% of cases.[10] As the malignant transformation to PNET was diagnosed in the first years of two cases, we think timely and margin-safe resection of the teratoma should be performed as soon as detected in these patients.

Since the clinical presentation of Currarino syndrome varies, it should be kept in mind that there may be asymptomatic cases that do not include all components of the triad, and Currarino syndrome should be included in the differential diagnosis in patients with at least one of the components of the syndrome. Considering the risk of malignant transformation, evaluation should be made in terms of systemic involvement and the treatment plan should be shaped specifically for sacrococcygeal teratoma in these patients.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MC, GC, SO; data collection: MC, GC, SO; analysis and interpretation of results: GC, SO, RO; draft manuscript preparation: MC, GC, SO, RO, NC, TTJ. All authors reviewed the results and approved the final version of the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Lynch SA, Wang Y, Strachan T, et al. Autosomal dominant sacral agenesis: Currarino syndrome. J Med Genet 2000; 37: 561-566. https://doi.org/10.1136/jmg.37.8.561
2. Mahmoud MA, Seddek AH. Incomplete Currarino syndrome: case report and a brief review of literature. Arch Clin Gastroenterol 2000; 6: 013-016.
3. Jaramillo-Huff A, Bakkar R, McKee JQ, Sokkary N. Primary primitive neuroectodermal tumor arising from an ovarian mature cystic teratoma in a 12-year-old girl: a case report. J Pediatr Adolesc Gynecol 2017; 30: 511-512. https://doi.org/10.1016/j.jpag.2016.11.007
4. Sen G, Sebire NJ, Olsen O, et al. Familial Currarino syndrome presenting with peripheral primitive neuroectodermal tumour arising with a sacral teratoma. Pediatr Blood Cancer 2008; 50: 172-175. https://doi.org/10.1002/pbc.20885
5. Pini Prato A, Cama A, Belloni E, et al. Currarino syndrome: proposal of a diagnostic and therapeutic. J Pediatr Surg 2004; 39: 1305-1311. https://doi.org/10.1016/j.jpedsurg.2004.05.003
6. Belloni E, Martucciello G, Verderio D, et al. Involvement of the HLXB9 homeobox gene in Currarino syndrome. Am J Hum Genet 2000; 66: 312-319. https://doi.org/10.1086/302723
7. Dirix M, van Becelaere T, Berkenbosch L, et al. Malignant transformation in sacrococcygeal teratoma and in presacral teratoma associated with Currarino syndrome: a comparative study. J Pediatr Surg 2015; 50: 462-464. https://doi.org/10.1016/j.jpedsurg.2014.07.014
8. Cash T, McIlvaine E, Krailo MD, et al. Comparison of clinical features and outcomes in patients with extraskeletal versus skeletal localized Ewing sarcoma: a report from the Children's Oncology Group. Pediatr Blood Cancer 2016; 63: 1771-1779. https://doi.org/10.1002/pbc.26096
9. Urioste M, Garcia-Andrade Mdel C, Valle L, et al. Malignant degeneration of presacral teratoma in the Currarino anomaly. Am J Med Genet A 2004; 128A: 299-304. https://doi.org/10.1002/ajmg.a.30028
10. Gardner PA, Albright AL. "Like mother, like son:" hereditary anterior sacral meningocele. Case report and review of the literature. J Neurosurg 2006; 104: 138-142. https://doi.org/10.3171/ped.2006.104.2.11

The Turkish Journal of Pediatrics 2022; 64: 389-393
https://doi.org/10.24953/turkjped.2021.385

Case Report

# Cutaneous Allergic reactions to pine processionary caterpillar (Thaumetopoea Pityocampa): a complicated cutaneous reaction in an infant and review of the literature

Nilüfer Galip[1], Burçin Şanlıdağ[2], Arzu Babayiğit[1,3], Nerin Nadir Bahçeciler[3]

*[1]Division of Pediatric Allergy & Immunology, Universıty of Kyrenia, Faculty of Medicine, Kyrenia, North Cyprus; [3]Division of Pediatric Allergy & Immunology, [2]Department of Pediatrics, Near East University, Faculty of Medicine, Nicosia, North Cyprus.*

## ABSTRACT

**Background.** Thaumetopoea Pityocampa (TP) are frequent in the Mediterranean region especially affecting forest workers in pinewood areas. The common symptoms include swelling, rash or burns like any form of dermatitis. The reactions can be triggered by mechanical, chemical or allergic factors and the "allergic" reaction is caused by sensitization to a hair protein named "thaumetopoein". This protein triggers the IgE mediated reaction resulting in the mast cell degranulation causing urticaria. Different kinds of allergic reactions like urticaria or anaphylaxis have been reported previously commonly in adults, especially in forest workers while severe reactions without direct contact are rare in pediatric population.

**Case.** A 28 month old healthy boy was admitted to Near East University Pediatric Allergy and Immunology Outpatient Clinic in March with complaints of pain, hyperemia and swelling on the left hand. His complaints had started the day before his admission just after walking around in their garden which is surrounded by pine trees. On admission, his physical examination revealed serious edema and hyperemia on his left hand limiting his finger movements with a few bullae on the skin. His temperature was 38 C and the other vital parameters were normal. Based on hyperemia, swelling and high acute phase reactants he was hospitalized with the differential diagnosis of soft tissue inflammation and cellulitis. The case was treated with iv antihistamines, systemic steroids and antibiotics.

**Conclusions.** Pine processionary (PP) is an important irritant and allergen especially in endemic areas like Cyprus which is a Mediterranean Country. It must be kept in mind in case of local or generalized urticaria, dermatitis, bullae and other allergic reactions even if there had been no direct contact with PP. Systemic involvement with fever and elevated acute phase reactants in infancy may necessitate hospitalization and intravenous treatment. Hereby, we reported an infant who presented with fever in addition to severe cutaneous lesions following the exposure to TP without direct contact. This is the first case reported from North Cyprus.

**Key words:** allergy, cutaneous reaction, pine caterpillar.

Processionary moths include many different species in Europe, the Middle East and African countries and their mature larvae are urticating for humans and many other mammalians. Several forms of cutaneous or less frequent ocular lesions caused by pine processionary caterpillar, named as Thaumetopoea Pityocampa (TP) are frequent in the Mediterranean region especially affecting forest workers in pinewood areas. The common symptoms include swelling, rash or burns like any forms of dermatitis. The first descriptions were made by Reaumur[1] in 1736 and since then, many studies have been performed to identify the pathogenesis of the reactions caused by TP. Nowadays, knowledge on the reactions is that they can be triggered by mechanical, chemical or allergic factors.[2-4] Mechanical way of the

✉ Nilufer Galip
dr_ngalip@yahoo.com

Received 18th February 2021, revised 22nd April 2021, accepted 25th April 2021.

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

damage occurs when the hairs of the caterpillar penetrate the skin. In the chemical reaction, there is a discharge of a toxic substance coming from the caterpillar and causing the irritation on the skin. The third and the "allergic" reaction is caused by sensitization to a hair protein named "thaumetopoein". This protein triggers the IgE mediated reaction resulting in the mast cell degranulation causing urticaria.[5] Different kinds of allergic reactions like urticaria or anaphylaxis have been reported previously commonly in adults, especially in forest workers.[6,7] In 2006, Aparicio et al.[8] from Spain, documented the largest group of pediatric patients having several types of allergic reactions to TP. This report demonstrated IgE mediated allergic reactions proven by either skin prick test (SPT) or specific IgE measurements including urticaria, angioedema, anaphylaxis, rhinitis, asthma and conjunctivitis in children between 6-14 years of age. Hereby, we reported an infant who presented with fever in addition to severe cutaneous lesions following the exposure to TP without direct contact. This is the first case reported from North Cyprus.

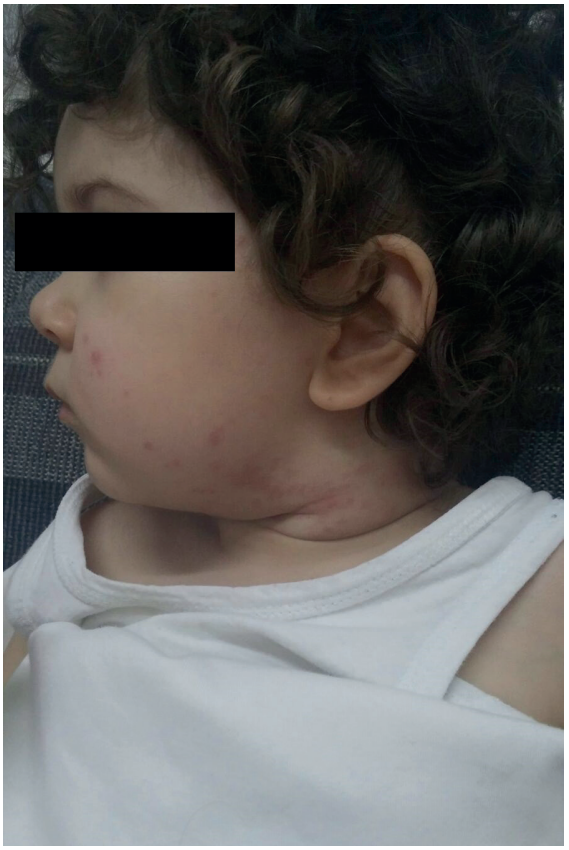


**Fig. 1.** Bullea, vesicles and edema on his left hand.

## Case Report

A twenty-eight- month-old healthy boy was admitted to Near East University Pediatric Allergy and Immunology Outpatient Clinic with complaints of pain, hyperemia and swelling on the left hand. His complaints had started the day before his admission just after walking around in their garden which was surrounded by pine trees. Hyperemia and urticarial rash with intense itching were the first signs on his first and the second fingers of the left hand, then, swelling spread through his whole hand. He also had some urticarial rash on his trunk. The father also developed urticarial lesions located at the back of the neck. (Fig. 1 and Fig. 2) The next morning, he had a fever of 38.2 C and he was unable to use his hand because of intense pain. On admission, his physical examination revealed serious edema and hyperemia on his left hand limiting his finger movements with a few bullae on the skin.

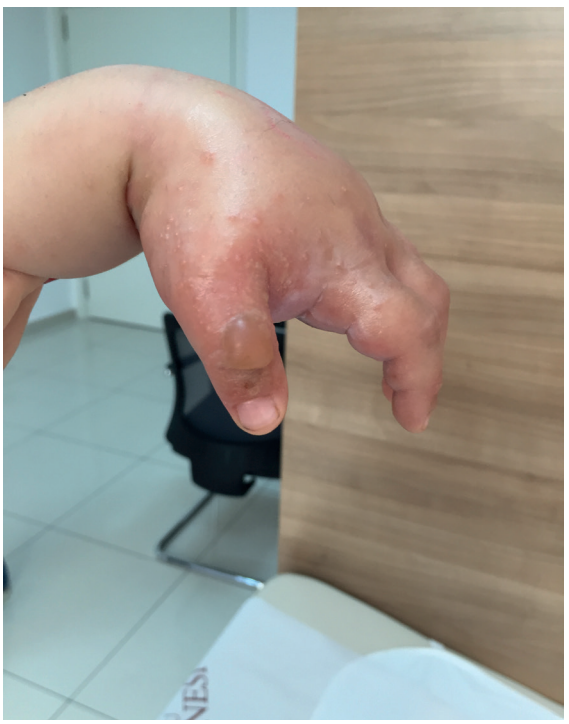


**Fig. 2.** Urticarial rashes on his neck.

His temperature was 38 C and the other vital parameters were normal. On his laboratory tests, white blood cell count was 19600/µl, neutrophils: 9380/µl, hemoglobin level: 12.3 gr/dl, platelet count: 332000/µl and C-reactive protein: 6.04 mg/dl. Based on hyperemia, swelling and high acute phase reactants he was hospitalized with the differential diagnosis of soft tissue inflammation and cellulitis. The patient was treated with intravenous 150mg/kg/day amoxicillin sulbactam, 1mg/kg/day methyl prednisolone and feniramin maleat. In addition, his left hand was elevated and cold compress was performed in order to prevent further swelling. On the 48th hour of his hospitalization the fever subsided, edema regressed, the pain was resolved and the patient was able to move his fingers. He was discharged from the hospital with oral antibiotics and antihistamine treatment. Informed consent was received from the family of the patient for publishing the patient data and pictures.

## Discussion

Processionary caterpillar is one of Lepidoptera species that can cause damage to human skin.[9] Pine processionary (PP) is commonly seen in the Mediterranean coast and European countries.[10] Contaminations are usually in pine forests and rarely in urban areas.[11,12]

Urticant hairs appear in the third stage of the caterpillars' development (L3) around September and increase up to the last stage (L5) which can be seen from January to May according to climate conditions.[13] There are two pathogenic mechanisms that PP can cause harmful effects, the first being direct contact with nests or caterpillars which can result in dermatitis. The second is aero-mediated contact that can result in skin, ocular and respiratory effects.[14]

Thaumetopoein is the protein that is isolated from processionary hairs. This protein acts directly on mast cells triggering degranulation which results in nonspecific urticarial lesions.[5] Beside toxic-irritant mechanisms; an Ig E mediated mechanism of hypersensitivity has been demonstrated in studies especially in adults.[3,6,7] Moneo et al.[15] described an IgE binding protein as the major allergen of PP that is named as Tha p 1. Less commonly delayed cutaneous reactions lasting several days, that is presented as small papules, papulo-vesicles and pustules are observed.[1,3,9,13] The responsible mechanism is thought to be toxic- irritant.[1,2,5,13,16] In the presented case, skin reaction was severe with bullae formation, severe edema and hyperemia with loss of function of the hand. In addition, the patient had systemic involvement signs such as fever and elevated acute phase reactants. This reaction was probably airborne, as the patient did not touch the caterpillars. PP setae can be released into the air and can penetrate the skin causing symptoms without touching like in the presented case.[5,17] On the other hand, as we did not test the patient for Tha p1 specific IgE, the pathogenesis of our case cannot be defined clearly.

In a study of the pediatric population; IgE mediated cutaneous reactions due to PP caterpillar were only 6.7%.[9] On the other hand, studies on adults revealed a rate of 50% of Ig E mediated cases.[3,4]

In the pediatric population, the most common symptomatology is contact urticaria.[9] Processionary dermatitis can be seen in every age, especially in children who tend to play with larvae. Involved body areas are forearms, digits, hand dorsum, face and neck.[3,9,18,19] In the pediatric age group, mostly extremities are found to be affected, followed by trunk, neck and head.[9] If direct contact is present the lesions may be limited to the contact region. If the exposure is aero-mediated multiple lesions may be seen, as in our case.[14]

In general, lesions cause intense and continuous itching, with pink to bright red macules and papules overlapping the urticarial base. Papules can be surrounded by vesicles or may sometimes accompany by bullous lesions.[12] Dermatitis resolves in 3-4 days and leaves a

brownish macule resolving in 1-2 weeks.[14] To date, no cases with fever and elevated acute phase reactants have been reported. Systemic involvement of our case may be due to secondary infection of the lesions.

For the diagnosis, direct contact with caterpillars or history of residing, passing through or nearby pine forest are important clues. Similar lesions can be detected in the accompanying family members or friends. In the presented case, the father also had lesions at the same time. Caterpillar hairs can be seen in microscopic examination like in our case.[14,20] Both our patient and his father were examined by magnifying glass but nothing was seen as they came after showering. We found irritant hairs on the father's clothes on microscopic examination. Diagnosis of IgE mediated reactions can be made by means of skin prick testing or detection of specific IgE in sensitized cases. Unfortunately, due to the lack of the material specific for the major allergen in our hospital, we could not perform those tests.

Approximately 10% of cases develop early or late ocular involvement and rarely respiratory involvement and anaphylaxis.[1,2,6,14,21]

Treatment includes systemic antihistamines, antipruritic lotions and topical steroids for persistent cutaneous reactions. For severe cases, systemic steroids are used.[21] Immediate epinephrine treatment is essential in case of anaphylaxis.[21] Our case was treated with IV antihistamines, systemic steroids and antibiotics.

PP is an important irritant and allergen especially in endemic areas like Cyprus which is a Mediterranean Country. It must be kept in mind in case of local or generalized urticaria, dermatitis, bullae and other allergic reactions even if there had been no direct contact with PP. Systemic involvement with fever and elevated acute phase reactants in infancy may necessitate hospitalization and intravenous treatment.

## Author contribution

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

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Ducombs G, Lamy M, Mollard S, Guillard JM, Maleville J. Contact dermatitis from processional pine caterpillar (Thaumetopoe Pityocampa Schiff Lepidoptera). Contact Dermatitis 1981; 7: 287-288. https://doi.org/10.1111/j.1600-0536.1981.tb04081.x
2. Ducombs G, Lamy M, Bergaud JJ, Tamisier JM, Gervais C, Texier L. La chenille processionnaire (Thaumetopoea pityocampa Schiff. lépidoptères) et l'homme. Etude morphologique de l'appareil urticant. Enquête épidémiologique [Thaumetopoea pityocampa caterpillar and man. Morphology of its venom apparatus. Epidemiological investigation]. Ann Dermatol Venereol 1979; 106: 769-778.
3. Vega JM, Moneo I, Armentia A, et al. Allergy to the pine processionary caterpillar (Thaumetopoea pityocampa). Clin Exp Allergy 1999; 29: 1418-1423. https://doi.org/10.1046/j.1365-2222.1999.00626.x
4. Vega JM, Moneo I, Armentia A, Vega J, De La Fuente R, Fernandez A. Pine processionary caterpillar as a new cause of immunologic contact urticaria. Contact Dermatitis 2000; 43: 129-132. https://doi.org/10.1034/j.1600-0536.2000.043003129.x
5. Lamy M, Pastureaud MH, Novak F, et al. Thaumetopoein: an urticating protein from the hairs and integument of the pine processionary caterpillar (Thaumetopoea pityocampa Schiff., Lepidoptera, Thaumetopoeidae). Toxicon 1986; 24: 347-356. https://doi.org/10.1016/0041-0101(86)90194-7
6. Vega JM, Moneo I, Armentia A, et al. Anaphlaxis to a pine caterpillar. Allergy 1997: 52: 1244-1245. https://doi.org/10.1111/j.1398-9995.1997.tb02532.x

7. Werno J, Lamy M, Vincendeau P. Caterpillar hairs as allergens. Lancet 1993; 342: 936-937. https://doi.org/10.1016/0140-6736(93)91989-Y

8. Fuentes Aparicio V, Zapatero Remón L, Martínez Molero MI, Alonso Lebreros E, Beitia Mazuecos JM, Bartolomé Zavala B. Allergy to pine processionary caterpillar (Thaumetopoea pityocampa) in children. Allergol Immunopathol (Madr) 2006; 34: 59-63. https://doi.org/10.1157/13086748

9. Vega ML, Vega J, Vega JM, Moneo I, Sanchez E, Miranda A. Cutaneous reactions to pine processionary caterpillar (Thaumetopoea pityocampa) in pediatric population. Pediatr Allergy Immunol 2003; 14: 482-486. https://doi.org/10.1046/j.0905-6157.2003.00066.x

10. de Boer JG, Harvey JA. Range-Expansion in Processionary Moths and Biological Control. Insects 2020; 11: 267. https://doi.org/10.3390/insects11050267

11. Leigheb G. Entomodermatosi. In: Giannetti A (ed). Trattato di Dermatologia. 2, cap. 40. Piccin: 2001: 1-57.

12. Bonamonte D, Profeta G, Foti C, et al. Humans and pine caterpillar (Thaumetopoea pityocampa Schiff. Ann Ital Dermatol Sifilogr 2007; 61: 91-98.

13. Werno J, Lamy M. Pollution atmosphérique d'origine animale: les poils urticants de la chenille processionnaire du pin (Thaumetopoea pityocampa Schiff.) (Insectes, Lépidoptères) [Atmospheric pollution of animal origin: the urticating hairs of the processionary caterpillar (Thaumetopoea pityocampa Schiff.) (Insects, Lepidoptera)]. C R Acad Sci III 1990; 310: 325-331.

14. Bonamonte D, Foti C, Vestita M, Angelini G. Skin reactions to pine processionary caterpillar Thaumetopoea pityocampa Schiff. ScientificWorldJournal 2013; 27: 867431. https://doi.org/10.1155/2013/867431

15. Moneo I, Vega JM, Caballero ML, Vega J, Alday E. Isolation and characterization of Tha p 1, a major allergen from the pine processionary caterpillar Thaumetopoea pityocampa. Allergy 2003; 58: 34-37. https://doi.org/10.1034/j.1398-9995.2003.23724.x

16. Garty BZ, Danon YL. Processionary caterpillar dermatitis. Pediatr Dermatol 1985; 2: 194-196. https://doi.org/10.1111/j.1525-1470.1985.tb01051.x

17. Rodriguez-Mahillo AI, Gonzalez-Muñoz M, Vega JM, et al. Setae from the pine processionary moth (Thaumetopoea pityocampa) contain several relevant allergens. Contact Dermatitis 2012; 67: 367-374. https://doi.org/10.1111/j.1600-0536.2012.02107.x

18. Hosler EW. Hossler EW. Caterpillars and moths: Part I. Dermatologic manifestations of encounters with Lepidoptera. J Am Acad Dermatol 2010; 62: 1-10. https://doi.org/10.1016/j.jaad.2009.08.060

19. Hossler EW. Caterpillars and moths: Part II. Dermatologic manifestations of encounters with Lepidoptera. J Am Acad Dermatol 2010: 62: 13-28. https://doi.org/10.1016/j.jaad.2009.08.061

20. Carruthers R. Caterpillar dermatitis. Br Med J (Clin Res Ed) 1967; 2: 765. https://doi.org/10.1136/bmj.2.5554.765

21. Vega JM, Moneo I, Ortiz JC, et al. Prevalence of cutaneous reactions to the pine processionary moth (Thaumetopoea pityocampa) in an adult population. Contact Dermatitis 2011; 64: 220-228. https://doi.org/10.1111/j.1600-0536.2011.01884.x

The Turkish Journal of Pediatrics 2022; 64: 394-399
https://doi.org/10.24953/turkjped.2020.2997

Case Report

# A case of juvenile systemic sclerosis and congenital pulmonary airway malformation related mucinous adenocarcinoma of the lung: paraneoplastic syndrome or just a coincidence?

Ayten Aliyeva[1], Amra Adrovic[1], Süheyla Ocak[2], Şebnem Batur[3], Mehmet Yıldız[1], Fatih Haşlak[1], Oya Köker[1], Sezgin Şahin[1], Kenan Barut[1], Özgür Kasapçopur[1]

*Departments of [1]Pediatric Rheumatology, [2]Pediatric Hematology and Oncology and [3]Pathology, İstanbul Cerrahpaşa University Faculty of Medicine, İstanbul, Turkey.*

## ABSTRACT

**Background.** Juvenile systemic sclerosis (JSS) is an extremely rarely seen auto-immune disease characterized by the increased fibrosis of skin and internal organs. Congenital pulmonary airway malformation (CPAM) is a developmental disorder of the lung, characterized by atypical cell hyperplasia which creates the ground for lung adenocarcinoma. In general, CPAM is diagnosed in early childhood, due to recurrent respiratory symptoms including cough, hemoptysis and respiratory infections. Although rare, there are some sporadic asymptomatic cases of CPAM that have been reported. We present a case with a coincidental presence of two rare diseases: JSS and CPAM.

**Case.** An adolescent female patient was admitted to hospital due to clinical signs of JSS. During the follow-up, the patient had been diagnosed with cystic adenoid malformation of the lung complicated by mucinous adenocarcinoma. The patient was previously healthy with an unremarkable history, including lack of respiratory symptoms. Left inferior lobectomy was performed. Considering the small size of malignant loci, the total resection of the tumor and absence of any sign for metastasis disease, adjuvant therapy was not scheduled.

We haven't found a pediatric case of CPAM associated adenocarcinoma of the lung presented by signs of JSS in the literature. In this case, the clinical signs of JSS possibly represent part of the paraneoplastic syndrome related to adenocarcinoma of the lung.

**Conclusions.** Internal organ involvement, including respiratory system, should not be omitted even in asymptomatic patients with JSS. Auto-antibody negativity represents a clue for the possible underlying condition. Further studies with a higher number of patients would reveal more relevant data.

**Key words:** adenocarcinoma, congenital pulmonary airway malformation, juvenile systemic sclerosis, KRAS.

Juvenile systemic sclerosis (JSS) is an extremely rare multisystemic disease characterized by skin stiffness, vasculopathy and internal organ involvement.[1-4] The precise worldwide incidence of the disease is unknown. The disease pathophysiology is not completely explained. Possible triggers (infections, trauma, chemicals etc.) initiate an auto-immune response which is characterized by the production of auto-antibodies and increased skin and internal organ fibrosis.[1,3]

Studies among adults reported an increased frequency of malignancies (namely lung cancer) in patients with systemic sclerosis (SS).[5-8] However, it is still controversial weather the

✉ Amra Adrovic
amra.adrovic@istanbul.edu.tr

Received 15th October 2020, revised 27th December 2020, accepted 6th April 2021.

signs of systemic sclerosis appear as a part of paraneoplastic syndrome or whether systemic sclerosis itself carries an increased risk for malignancies (lung cancer, gastric cancer, etc.). The possible association between systemic sclerosis and malignancy has been explained by a variety of theories, majority of them requiring further investigation and confirmation.[6-10]

Previously known as congenital cystic adenomatoid malformations (CCAM), the congenital pulmonary airway malformation (CPAM) represents localized developmental defects of airway formation at different times of lung development with an estimated incidence at 1:25,000 –1:35,000 births.[11] Previous studies showed that atypical goblet cell hyperplasia (AGCH) in CPAM represents a precursor lesion for pulmonary adenocarcinomas.[12,13]

In this report, we present a case with coincidental presence of two rare diseases: JSS and CPAM, an adolescent female patient admitted to hospital due to clinical signs of JSS. During the follow-up, the patient was diagnosed with cystic adenoid malformation of the lung. The pathophysiologic evaluation revealed mucinous adenocarcinoma. We sought to emphasize the importance of further evaluation of patients with JSS, especially those with negative autoantibodies.

## Case Report

A 14- year-old female patient was admitted to our outpatient department due to swelling and stiffness of her fingers continuing for 2.5 years (Fig. 1). The patient was previously healthy with an unremarkable medical history. All complaints started 6 months prior to admission. At the physical examination, she had marked Raynaud's phenomenon on her fingers. The skin of the face looked "shiny and stiff". She had unremarkable initial laboratory test results: white blood cells count 4.900/mm$^3$, hemoglobin 13.1 gr/dl, thrombocytes count 244.000/ mm$^3$. Acute phase markers were in normal ranges: erythrocyte sedimentation rate 11 mm/h, CRP 0.04 mg/dl. The values of urea, creatinine, alanine-aminotransferase, aspartate-aminotransferase were in referent values. Evaluation of autoantibodies revealed the positivity of anti-nuclear antibody (ANA, >1/100), while anti-double strand DNA (ds-DNA), anti-Smith (Sm) and anti-Scl 70 antibodies were negative.

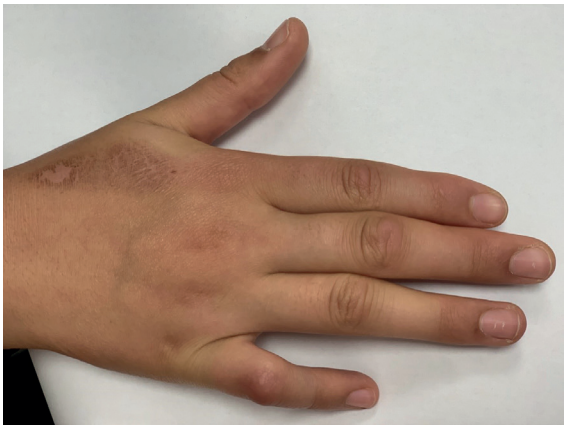


**Fig. 1.** Swelling of fingers, Gottron papules, Raynaud's phenomenon. Contracture in the proximal interphalangeal joint of the 5th finger of the right hand.

The patient was diagnosed with JSS according to Pediatric Rheumatology European Society (PReS)/American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for JSS[10] and prednisolone (10 mg/day, per oral) and methotrexate (15 mg/m$^2$/ week, subcutaneous) were started.

### *Lung involvement*

Although the patient had no respiratory symptoms and her previous medical history was unremarkable, the evaluation of the respiratory system was performed as a part of the routine workup for JSS patients. During the regular screening for internal organ involvement, the chest X-ray and the spirometry were performed and found to be in the normal range with a forced vital capacity (FVC) of 80%, forced expiratory volume in 1 second (FEV1) 76%,

FEV1/FVC 92% and carbon-monoxide diffusion capacity (DLCO) 92%. The X-ray revealed a round shaped cystic lesion in the basal part of the left lung. The irregularity of the patient's X-ray was a reason to perform the thoracic high-resolution computerized tomography (HRCT) which revealed the cystic lesion (57 x 44 mm) in the basal part of the left lung lobe (Fig. 2). The left inferior lobectomy was performed.

### *Histopathological evaluation*

Pathologic specimen demonstrated two focal mucinous adenocarcinoma (0.4 cm in diameter) with lepidic and focal acinar growth pattern without pleural invasion (Fig. 3A). Mucinous adenocarcinoma exhibited diffuse cytoplasmic expression for CK7 (Fig. 3B), CK20 staining was negative (Fig. 3C). The genetic analysis of the Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation revealed KRAS 12 (612C, 612S) positivity. The pathology report suggested congenital pulmonary airway malformation (CPAM type-2) as a basis for development of mucinous adenocarcinoma.

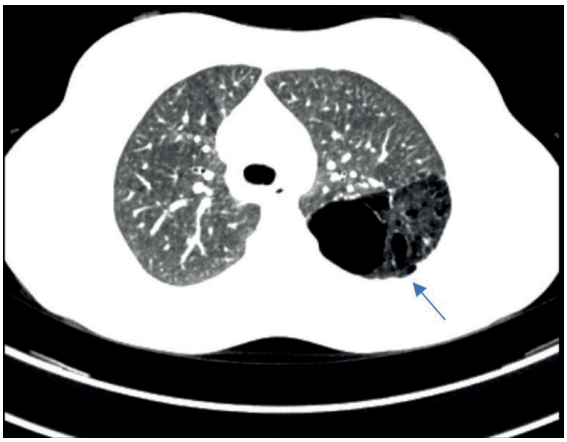


**Fig. 2.** Thoracic high-resolution computerized tomography (HRCT) of the patient showing cystic lesion (57 x 44 mm) in the basal part of the left lung lobe (arrow).

### *Follow-up*

Considering the small size of malignant loci, the total resection of the tumor and absence of any sign for metastasis disease, adjuvant therapy was not scheduled and clinical surveillance with periodical thorax HRCT was planned. After 1.5 years of follow-up, at the last clinic visit, the patient had no complaints. The stiffness of the skin remarkably decreased modified Rodnan skin score (mRSS; 38 vs. 16). She had no signs of Raynaud phenomenon but still had puffy fingers in the physical examination with normal thoracic HRCT findings. The immunosuppressive treatment was continued during the 12 months after the surgical treatment. Due to regression of clinical findings (except for limited range of motion in the proximal interphalangeal joints in 4th and 5th fingers of the right arm, which are considered as disease sequela), the immunosuppressive treatment was canceled. Physiotherapy for her fingers continued.

Written consent to publish was obtained from the study participant.

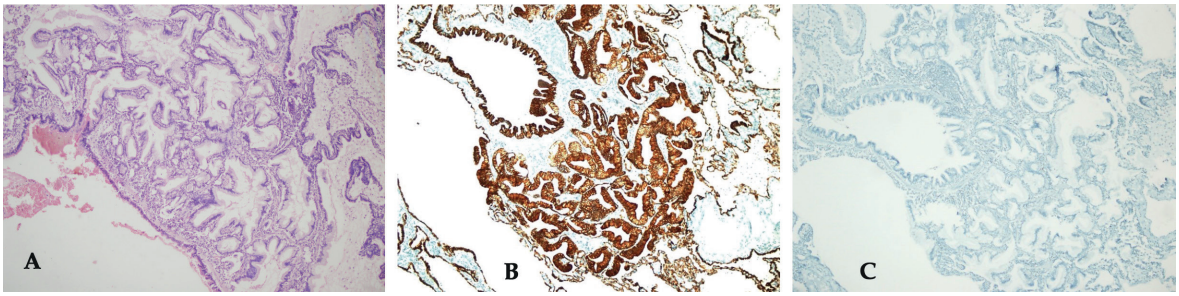



**Fig. 3.** Histopathological evaluation of the surgical material obtained by the left basal lobectomy. **A)** Mucinous proliferation composed of back-to-back glands, consistent with mucinous adenocarcinoma (arrows, H&E x100). **B)** Mucinous adenocarcinoma exhibiting diffuse cytoplasmic expression for CK7. **C)** CK20 staining was negative.

## Discussion

This is the first report of a patient with JSS and congenital pulmonary airway malformation complicated by the mucinous adenocarcinoma of the lung.

The increased frequency of malignancy among patients with systemic sclerosis has been reported among adults.[6-9] Studies including pediatric patients are scarce due to rarity of the disease and the increased risk for cancers among children with JSS has not been reported yet.

The main dilemma regarding our patient was whether the lung mucinous adenocarcinoma as the complication of CPAM 2 represented the basis for clinical signs of JSS or the two conditions appeared coincidentally.

CPAM is a developmental disorder of the lung, characterized by the atypical cell hyperplasia which creates the ground for lung adenocarcinoma.[11-13] Fakler et al.[13] reported that 5 out of 33 CPAM patients developed lung adenocarcinoma. All of them had positive KRAS oncogenic mutation, which is considered to be an oncogenic driver in this patients' group.[13,14] Similarly, our patient had positive KRAS mutation which was confirmed in the cancer tissue.

In general, CPAM is a condition recognized early in childhood due to prominent respiratory symptoms including recurrent pulmonary infections, productive cough, and hemoptysis.[15-26] However, a certain number of patients remain asymptomatic until adulthood.[15,20,23,27] Frick et al. reported a 68-year-old male patient with invasive lung mucinous adenocarcinoma on the basis of CPAM.[17] The reported patient presented with chest pain but no systemic symptoms including signs of vasculopathy and systemic sclerosis were reported. Abecasis et al.[23] described an asymptomatic 14-year-old male patient who was coincidentally diagnosed with a mucinous bronchioloalveolar carcinoma associated with a CPAM type-1. The patient underwent right inferior lobectomy. After two years of follow-up he is completely asymptomatic and free of complications.

In a study by Pogoriler et al.[14], over a 2.5-year period 184 surgical specimens from 174 infants with congenital lung lesions were identified. No malignancy was identified. There were no cases of pleuropulmonary blastoma or acinar dysplasia.

Papagiannopoulos et al.[20] reported their 19-years' experience with cystic lesions of the lung among pediatric and adult population. A total of 46 operations were performed on 44 patients (24 children and 20 adults). Cystic adenomatoid malformation was the most common pathological finding. Malignancy was confirmed in three pediatric patients (12.5%): bronchoalveolar carcinoma in 2 and pleuropulmonary blastoma in 1 patient. In the adult population cystic adenomatoid malformation was the second most common lesion, seen in 5 (25%) patients. In half of the adults 11 (55%) were asymptomatic while the rest had signs and symptoms related to expansion or infection of the cysts. However, none of the adults had malignancy at the pathophysiological evaluation of the surgical specimen. Authors suggest resections of the congenital cysts in childhood, in order to avoid later complications, which could make operating more difficult.[20]

As we mentioned previously in the manuscript, our patients fulfilled PReS)/ACR/EULAR Classification Criteria for Juvenile Systemic Sclerosis.[10] She had proximal sclerosis and induration of the skin (initial mRSS 38), sclerodactyly, Raynaud's phenomenon and ANA positivity. The scleroderma specific anti Scl-70 was negative, but we should keep on mind the low percentage of its positivity in JSS patients. The acute phase markers and routine laboratory work-up was non-significant as it is in the majority of JSS patients.[1,3-5]

Bearing in mind the rarity of both conditions (JSS and mucinous adenocarcinoma associated

with CPAM) makes it inappropriate to consider both of them separately. Consequently, in our patient we prefer to consider JSS findings as a possible paraneoplastic sign of mucinous adenocarcinoma of the lung. Improvement of skin stiffness and disappearance of Raynaud's phenomenon after the resection of the tumor support our hypothesis.

Still, this is the first reported case which requires further confirmation by extending patient numbers. The other limitation is that the serologic evaluation of our patients has been limited since we were not able to perform analysis of tests which are associated to malignancy in systemic sclerosis.

As far as we know, this is the first report of patients with JSS with co-existing mucinous adenocarcinoma of the lung associated with cystic adenoid malformation. The underlying condition should be evaluated in patients with JSS, especially those with negative autoantibodies. Further studies with higher number of patients would reveal more relevant data.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AA, AAd, MY, ÖK; data collection: FH, OK, SŞ, KB; analysis and interpretation of results: AA, ŞB; draft manuscript preparation: AA, AAd, ÖK. All authors reviewed the results and approved the final version of the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Zulian F, Cuffaro G, Sperotto F. Scleroderma in children: an update. Curr Opin Rheumatol 2013; 25: 643-50. https://doi.org/10.1097/BOR.0b013e3283641f61
2. Foeldvari I. Update on juvenile systemic sclerosis. Curr Rheumatol Rep 2015; 17: 18. https://doi.org/10.1007/s11926-014-0491-y
3. Adrovic A, Şahin S, Barut K, Kasapçopur Ö. Juvenile scleroderma: a referral center experience. Arch Rheumatol 2018; 33: 344-351. https://doi.org/10.5606/ArchRheumatol.2018.6578
4. Adrovic A, Sahin S, Barut K, Kasapcopur O. Juvenile scleroderma-what has changed in the meantime?. Curr Rheumatol Rev 2018; 14: 219-225. https://doi.org/10.2174/1573397114666180423105056
5. Foeldvari I, Klotsche J, Torok KS, et al. Are diffuse and limited juvenile systemic sclerosis different in clinical presentation? Clinical characteristics of a juvenile systemic sclerosis cohort. J Scleroderma Relat Disord 2018; 4: 49-61. https://doi.org/10.1177/2397198318790494
6. Shah AA, Rosen A. Cancer and systemic sclerosis: novel insights into pathogenesis and clinical implications. Curr Opin Rheumatol 2011; 23: 530-535. https://doi.org/10.1097/BOR.0b013e32834a5081
7. Olesen AB, Svaerke C, Farkas DK, Sorensen HT. Systemic sclerosis and the risk of cancer: a nationwide population-based cohort study. Br J Dermatol 2010; 163: 800-806. https://doi.org/10.1111/j.1365-2133.2010.09861.x
8. Bonifazi M, Tramacere I, Pomponio G, et al. Systemic sclerosis (scleroderma) and cancer risk: systematic review and meta-analysis of observational studies. Rheumatology 2012; 52: 143-154. https://doi.org/10.1093/rheumatology/kes303
9. Maria ATJ, Partouche L, Goulabchand R, et al. Intriguing relationships between cancer and systemic sclerosis: role of the immune system and other contributors. Front Immunol 2019; 9: 3112. https://doi.org/10.3389/fimmu.2018.03112
10. Zulian F, Woo P, Athreya BH, et al. The Pediatric Rheumatology European Society/American College of Rheumatology/European League against Rheumatism provisional classification criteria for juvenile systemic sclerosis. Arthritis Rheum 2007; 57: 203-212. https://doi.org/10.1002/art.22551
11. Stocker JT. Congenital pulmonary airway malformation: a new name and an expanded classification of congenital cystic adenomatoid malformation of the lung. Histopathol 2002; 41: 424-431.
12. Chang WC, Zhang YZ, Wolf JL, et al. Mucinous adenocarcinoma arising in congenital pulmonary airway malformation: clinicopathological analysis of 37 cases. Histopathology 2021; 78: 434-444. https://doi.org/10.1111/his.14239

13. Fakler F, Aykutlu U, Brcic L, et al. Atypical goblet cell hyperplasia occurs in CPAM 1, 2, and 3, and is a probable precursor lesion for childhood adenocarcinoma. Virchows Arch 2020; 476: 843-854. https://doi.org/10.1007/s00428-019-02732-4

14. Pogoriler J, Swarr D, Kreiger P, Adzick NS, Peranteau W. Congenital cystic lung lesions: redefining the natural distribution of subtypes and assessing the risk of malignancy. Am J Surg Pathol 2019; 43: 47-55. https://doi.org/10.1097/PAS.0000000000000992

15. Frick AE, Decaluwé H, Weynand B, Proesmans M, Van Raemdonck D. Invasive mucinous adenocarcinoma of the lung arising in a type 1 congenital pulmonary airway malformation in a 68-year-old patient: a case report. Acta Chir Belg 2021; 121: 55-60. https://doi.org/10.1080/00015458.2019.1634926

16. Soler-Perromat A, Vollmer I, Ramírez J, Sánchez M. Invasive mucinous adenocarcinoma in congenital pulmonary airway malformation: a case report. Arch Bronconeumol 2019; 55: 384-385. https://doi.org/10.1016/j.arbres.2018.12.001

17. Koh J, Jung E, Jang SJ, et al. Case of mucinous adenocarcinoma of the lung associated with congenital pulmonary airway malformation in a neonate. Korean J Pediatr 2018; 61: 30-34. https://doi.org/10.3345/kjp.2018.61.1.30

18. Ohye RG, Cohen DM, Caldwell S, Qualman SJ. Pediatric bronchioloalveolar carcinoma: a favorable pediatric malignancy?. J Pediatr Surg 1998; 33: 730-732. https://doi.org/10.1016/S0022-3468(98)90200-7

19. Granata C, Gambini C, Balducci T, et al. Bronchioloalveolar carcinoma arising in congenital cystic adenomatoid malformation in a child: a case report and review on malignancies originating in congenital cystic adenomatoid malformation. Pediatr Pulmonol 1998; 25: 62-66. https://doi.org/10.1002/(SICI)1099-0496(199801)25:1<62::AID-PPUL8>3.0.CO;2-Q

20. Papagiannopoulos K, Hughes S, Nicholson AG, Goldstraw P. Cystic lung lesions in the pediatric and adult population: surgical experience at the Brompton Hospital. Ann Thorac Surg 2002; 73: 1594-1598. https://doi.org/10.1016/S0003-4975(02)03469-0

21. Stacher E, Ullmann R, Halbwedl I, et al. Atypical goblet cell hyperplasia in congenital cystic adenomatoid malformation as a possible preneoplasia for pulmonary adenocarcinoma in childhood: a genetic analysis. Hum Pathol 2004; 35: 565-570. https://doi.org/10.1016/j.humpath.2004.01.008

22. Ioachimescu OC, Mehta AC. From cystic pulmonary airway malformation, to bronchioloalveolar carcinoma and adenocarcinoma of the lung. Eur Respir J 2005; 26: 1181-1187. https://doi.org/10.1183/09031936.05.00011705

23. Abecasis F, Gomes Ferreira M, Oliveira A, Vaz Velho H. Bronchioloalveolar carcinoma associated with congenital pulmonary airway malformation in an asymptomatic adolescent. Rev Port Pneumol 2008; 14: 285-290. https://doi.org/10.1016/S0873-2159(15)30236-1

24. Giubergia V, Barrenechea M, Siminovich M, Pena HG, Murtagh P. Congenital cystic adenomatoid malformation: clinical features, pathological concepts and management in 172 cases. J Pediatr (Rio J) 2012; 88: 143-148. https://doi.org/10.2223/JPED.2177

25. Li J, Chen GS, Zhang X, Moore L, Cheng H. Congenital cystic adenomatoid malformation with associated mucinous bronchioloalveolar carcinoma in a neonate. Fetal Pediatr Pathol 2014; 33: 29-34. https://doi.org/10.3109/15513815.2013.842272

26. Balkanlı S, Özturk MA, Köse M, et al. A report of adenocarcinoma in situ and congenital pulmonary airway malformation in a three-day-old infant with a review of the literature. Turk J Pediatr 2014; 56: 299-302.

27. Ribet ME, Copin MC, Soots JG, Gosselin BH. Bronchioloalveolar carcinoma and congenital cystic adenomatoid malformation. Ann Thorac Surg 1995; 60: 1126-1128. https://doi.org/10.1016/0003-4975(95)00494-6

The Turkish Journal of Pediatrics 2022; 64: 400-407
https://doi.org/10.24953/turkjped.2021.611

Case Report

# Severe acute reentry high altitude pulmonary edema in pediatric patients: report of three cases and literature review

Ali Alsuheel Asseri[1], İbrahim Ali Asiri[1], Haifa' Hisham Alwabel[1], Ameerah Mohammed Asiri[1], Walaa Ibrahim Asiri[2]

*[1]Department of Child Health, College of Medicine, King Khalid University, Abha, Saudi Arabia; [2]Department of Pediatric, Abha Maternity and Children Hospital, Abha, Saudi Arabia.*

## ABSTRACT

**Background.** High Altitude Pulmonary Edema (HAPE) is a fatal form of severe high-altitude illness. It is a form of noncardiogenic, noninfectious pulmonary edema secondary to alveolar hypoxia. The exact incidence of HAPE in children is unknown; however, most literature reports an incidence between 0.5-15%. There are three proposed HAPE types including classic HAPE, reentry HAPE, and high-altitude resident pulmonary edema (HARPE).

**Case.** We present three pediatric patients who were diagnosed with re-entry high altitude pulmonary edema and did not have any underlying cardiac abnormalities. All patients reside in areas of high altitude with a history of travelling to places of lower altitude. They had respiratory infections prior to the manifestation of HAPE.

**Conclusions.** These are the first reported cases of children with reentry HAPE in Saudi Arabia. Reentry HAPE can occur in otherwise healthy children. Rapid ascent to high altitude and recent respiratory infections are the most commonly reported triggers. Prognosis is very favorable with a very rapid response to oxygen therapy. Education about HAPE is mandatory for families and health care workers working in high altitude areas.

**Key words:** high altitude pulmonary edema, children, re-entry HAPE.

High Altitude Pulmonary Edema (HAPE) is a fatal form of severe high-altitude illness. It is a form of noncardiogenic, noninfectious pulmonary edema secondary to alveolar hypoxia. Dyspnea on exertion, dry cough, followed by dyspnea at rest, and chest crackles that happen after a rapid ascent for more than 2500 meters are the common clinical manifestations of HAPE.[1-4] However, HAPE has been reported in patients with underlying cardiac diseases at an altitude as low as 1400 meters.[5] There are three proposed HAPE types including classic HAPE, reentry HAPE, and high-altitude resident pulmonary edema (HARPE). Classic HAPE occurs in people who live in low altitude areas and travel to high altitude, while reentry HAPE happens in people living in high altitude, returning from travels near sea level.[4] HARPE happens without changing altitude, and respiratory infections are the usually obvious trigger.[2] In addition, genetic polymorphisms have been reported as a critical factor of HAPE predisposition.[6,7] The exact incidence of HAPE in children is unknown; however, most literature reports an incidence between 0.5-15%.[3]

The risk of HAPE increases with rapid ascent above 2500 meters, recent respiratory tract infection, previous HAPE episode, and male sex. The underlying pathophysiology is an increase in pulmonary artery pressure, secondary to hypoxic pulmonary vasoconstriction, which results in leakage of fluids into the alveolar spaces.[3,8] As per the Lake Louise diagnostic

✉ Ali Alsuheel Asseri
alsoheel11@kku.edu.sa

Received 22nd February 2021, revised 21st March 2021, accepted 30th March 2021.

criteria for HAPE, a recent gain in altitude associated with at least two of the four typical symptoms (dyspnea at rest, cough, weakness/ decreased exercise performance, and chest tightness/congestion) and at least two of the four typical signs (crackles/ wheezes, central cyanosis, tachypnea, and tachycardia) are suggestive the diagnosis of HAPE.[1] In addition, the chest radiograph is mandatory to confirm the diagnosis, which mainly shows bilateral opacities.[4] If HAPE is left untreated, it can progress to severe respiratory failure and a mortality rate of up to 50%. Unfortunately, HAPE is commonly misdiagnosed as pneumonia or asthma.[9] Herein, we report three cases of severe reentry HAPE, which are the first reported cases from Saudi Arabia to the best of our knowledge.

## Case Reports

### *Patient 1*

A 9-year-old female presented to the emergency room with acute onset of shortness of breath and cough for a 12-hour duration that happened after arriving at Abha city (her residential area 2200 Meters above sea level) from sea level area (Tehama area). There was a history of mild upper respiratory infection symptoms, fever, mild cough, and sore throat. She had a history of two similar episodes requiring hospitalization, with the average hospital stay being 2-3 days. She was in third grade and had good school performance. The patient was fully immunized. She also reported intermittent snoring and morning headaches for almost a year. She scored 15 out of 22 in the on The Sleep Related Breathing Disorder (SRBD) scale. SRBD scale is a validated pediatric sleep questionnaire that was described by a group of experts in pediatric sleep medicine with good sensitivity and specificity for obstructive sleep apnea diagnosis.[10] According to the publishing group, a score of 7 out of 22 has most frequently been used as diagnostic of OSA.[10] Physical examination showed she was in acute distress and severe hypoxia with SpO2 of 35% at room air; heart rate was 175 beats per minute, blood pressure was 120/ 72 mmHg (normal for age). Her weight was 51 kg above the 95th percentile. Her lung exam revealed bilateral diminished air entry with diffuse inspiratory crackles. Chest x-ray on admission (Fig. 1a) showed bilateral patchy opacities. Echocardiography showed normal heart function and structure without pulmonary hypertension which was done on day 2 of admission. Laboratory tests are summarized in Table I. She was admitted to the pediatric intensive care unit (PICU), started on High Flow Nasal Cannula (HFNC) running at 15 liter per minute (LPM) due to persistent hypoxic respiratory failure, and treated for presumed severe asthma exacerbation. She was started on continuous Albuterol nebulizer and intravenous (IV) methylprednisolone.

The pediatric pulmonary team was consulted. The patient was diagnosed with re-entry high-altitude pulmonary edema (reentry HAPE) given her past medical history, traveling to high altitude areas, and good response to oxygen therapy. On the second day of admission, the patient's symptoms improved, and was able to tolerate oxygen support at 2 LPM via nasal cannula. On the third day, she was discharged home on RA with normal heart rate, and significant improvement of patchy opacities on chest x-ray (Fig. 1b). Parents were instructed to monitor SpO2 when returning to high altitude from low altitude and, if SpO2 was less than 92% on RA, they were advised to start oxygen therapy immediately. After 18 months, she has been doing well without any episodes of HAPE. Informed consent was taken from patient and patient's parents.

### *Patient 2*

An 11-year-old boy, twin A, from Abha (specifically AlSouda 3,015 m above sea level) presented with a one-day history of progressive dyspnea and productive cough, two days after returning from the city of Jizan (sea level) to Abba. The family spent their winter vacation (three weeks) in the Jizan area. Before traveling back to Abha, the mother reported

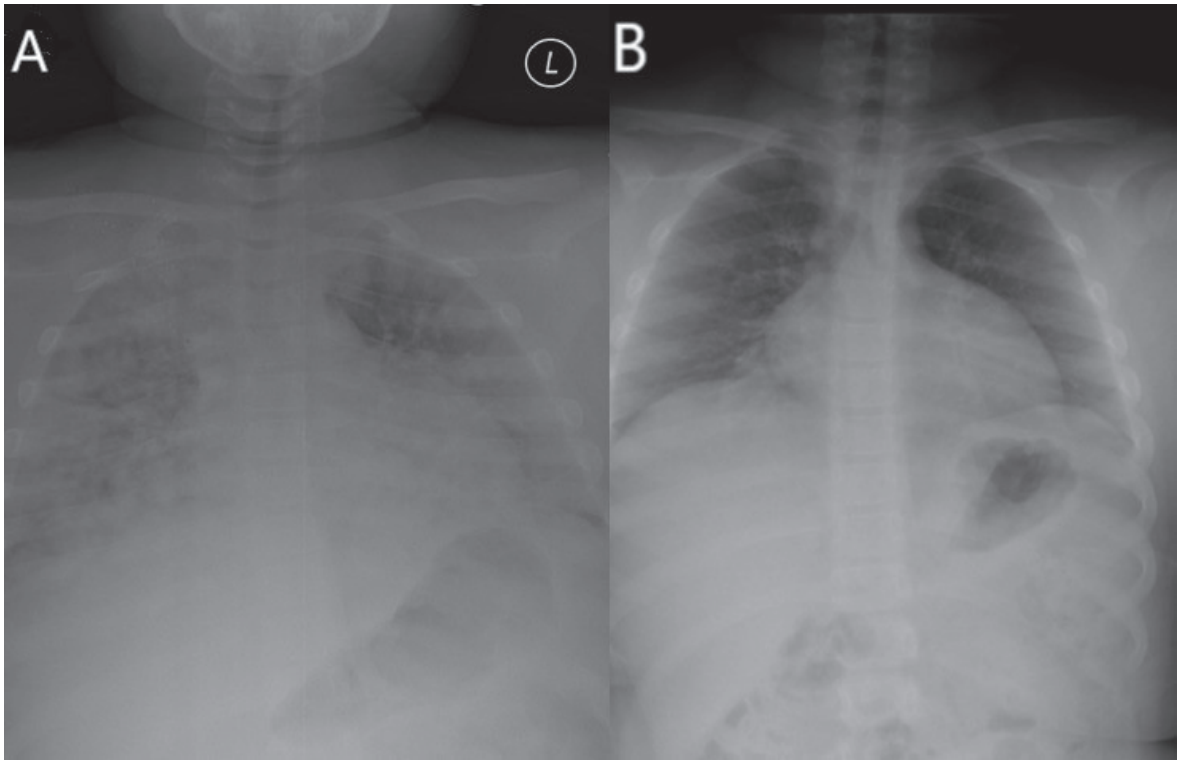



**Fig. 1.** Chest x-ray upon admission (A) and prior to discharge (B) for Patient 1.

**Table I.** Clinical characteristics, laboratory results and hospital outcome measures for the patients.

| Variables | Patient 1 | Patient 2 | Patient 3 |
|---|---|---|---|
| Age in years/sex | 9/F | 11/M (twin A) | 11/M (twin B) |
| Clinical characteristics | | | |
| - Previous similar illness | + | - | - |
| - Recent respiratory infection | + | + | + |
| - Dyspnea | + | + | + |
| - Chest pain | - | - | - |
| - Initial ER [a] SpO2 [b] | 37% | 79% | 63% |
| - Altered mental status | + | - | - |
| Laboratory results | | | |
| - WBC [c] (4-11x$10^3$/ul) | 21.21 | 6.71 | 8.99 |
| - Hb [d] (12-16 g/dl) | 12.4 | 15.4 | 15.6 |
| - HCT [e] (%) | 37.1 | 46.4 | 47.2 |
| - Echocardiography | Normal | Normal | Normal |
| Therapies and Hospital outcome | | | |
| - Asthma therapies | + | + | + |
| - Broad spectrum antibiotics | + | + | + |
| - Duration of oxygen therapy | 24 hours | 36 hours | 36 hours |
| - Length of stay (days) | 3 | 3 | 3 |

[a] ER: emergency room; [b] SpO2: oxygen saturation; [c] white blood cell counts; [d] hemoglobin level; [e] hematocrit

a history of respiratory infection in all family members, but there was no history of contact with confirmed or suspected Coronavirus Disease 2019 (COVID-19) patients. His vital signs on admission to the emergency unit were a heart rate of 145 bpm, respiratory rate of 28 breaths/min, blood pressure of 110/ 65 mmHg, temperature of 36.5°C, and SpO2 of 79% on RA. The lung examination revealed mild respiratory distress with crackles most commonly heard at lung bases. The throat examination revealed oropharyngeal erythema without tonsillar inflammation. Neurologically, the patient was fully awake with a Glasgow coma scale of 15/15. Laboratory tests are summarized in Table I. Chest x-ray revealed bilateral heterogeneous opacities that involved both lung fields (Fig 2a). He was admitted to the general pediatric ward with suspected COVID-19 pneumonia. The patient was placed on 4 liters of oxygen through a nasal cannula and started on broad-spectrum antibiotics (ceftriaxone and vancomycin).

Nasopharyngeal swab for SARS-CoV-2 PCR was sent, and the patient was placed in an isolation room. On the second day of admission, the patient's condition improved with resolution of respiratory symptoms and normalization of oxygen saturation (97% oxygen saturation on ambient air). Cardiology was consulted on the second day of admission. The patient underwent echocardiography that showed normal cardiac structure and function without evidence of pulmonary hypertension or pulmonary artery anomalies. On the third day of admission, the SARS-CoV-2 PCR result returned negative, and the chest x-ray revealed complete resolution of opacities (Fig 2b). The patient was discharged home with diagnosis of re-entry HAPE given the clinical symptoms, history of travelling from low altitude areas to high altitude areas, improving respiratory symptoms, and chest x-ray abnormalities on oxygen support. Informed consent was taken from patient and patient's parents.

### *Patient 3*

An 11-year-old boy, twin B, (sibling of patient 2) from the city of Abha (specifically AlSouda 3,015 m above sea level) presented with a one-day history of progressive dyspnea and productive cough, two days after returning from the town of Jizan (sea level) to Abha. The family spent their winter vacation (three weeks) in the Jizan area. Before traveling back to Abha, the mother reported a history of respiratory infection in all family members, but there was no history of contact with confirmed or suspected COVID-19 patients. His vital signs on admission to the emergency unit were a heart rate of 145 bpm, respiratory rate of 28 breaths/min, blood pressure of 110/ 65 mmHg, temperature of 36.5°C, and SpO2 of 63% on RA. The lung examination revealed mild respiratory distress with crackles most commonly heard at

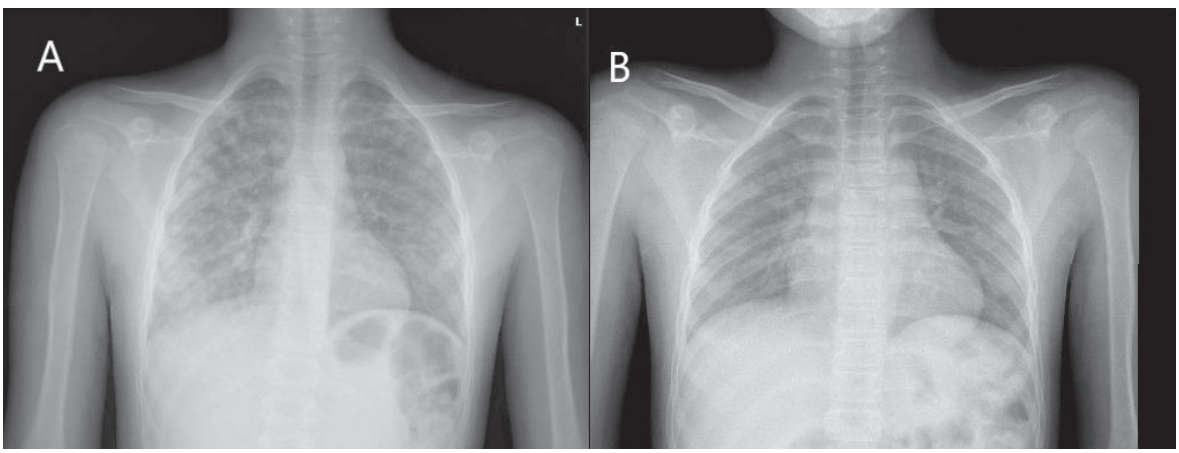



**Fig. 2.** Chest x-ray upon admission (A) and prior to discharge (B) for Patient 2.

lung bases. The throat examination revealed an oropharyngeal erythema without tonsillar inflammation. Neurologically, the patient was fully awake with a Glasgow coma scale of 15/15. Laboratory tests are summarized in Table I. Chest x-ray revealed bilateral heterogeneous opacities that involved both lung fields (Fig 3a). He was admitted to the general pediatric ward with suspected COVID-19 pneumonia. The patient was placed on 4 liters of oxygen through a nasal cannula and started on broad-spectrum antibiotics (ceftriaxone and vancomycin).

Nasopharyngeal swab for SARS-CoV-2 PCR was sent, and the patient was placed in the isolation room. On the second day of admission, the patient's condition improved with resolution of respiratory symptoms and normalization of oxygen saturation (97% oxygen saturation on ambient air). Cardiology was consulted on the second day of admission. The patient underwent echocardiography that showed normal cardiac structure and function without evidence of pulmonary hypertension or pulmonary artery anomalies. On the third day of admission, the SARS-CoV-2 PCR result returned negative, and the chest x-ray revealed complete resolution of opacities (Fig 3b). The patient was discharged home with diagnosis of re-entry HAPE given the clinical symptoms, history of traveling from low altitude area to high altitude area, improving respiratory symptoms, and chest x-ray abnormalities on oxygen support. Informed consent was taken from patient and patient's parents.

## Discussion

The exact pathophysiological mechanisms of HAPE are unknown. However, several studies have proposed mechanisms such as dysfunctioning voltage-dependent potassium channel and calcium channel due to non-homogenous pulmonary circulation constrictions.[11] Further, decreased nitric oxide synthesis plays a crucial role in HAPE manifestations.[12] Exaggeration of hypoxic pulmonary vasoconstrictions from the mechanisms mentioned above lead to pulmonary hypertension and increased capillary permeability in genetically susceptible individuals.[3,4,6,13] An increase in inflammatory markers, interleukins, and tumor necrosis factors were also reported in several cases, which indicate possible role of viral-induced inflammation in capillary permeability.[12,14] All patients reported mild upper respiratory infection before their illnesses, which are the likely predisposing factor for HAPE diagnosis. Several studies have correlated HAPE prevalence with concurrent respiratory infections, specifically re-entry HAPE and HARPE.[2,9,15] The exact mechanism of upper respiratory tracts (URT) predisposing to HAPE is unknown; however, increased vascular permeability and priming the pulmonary

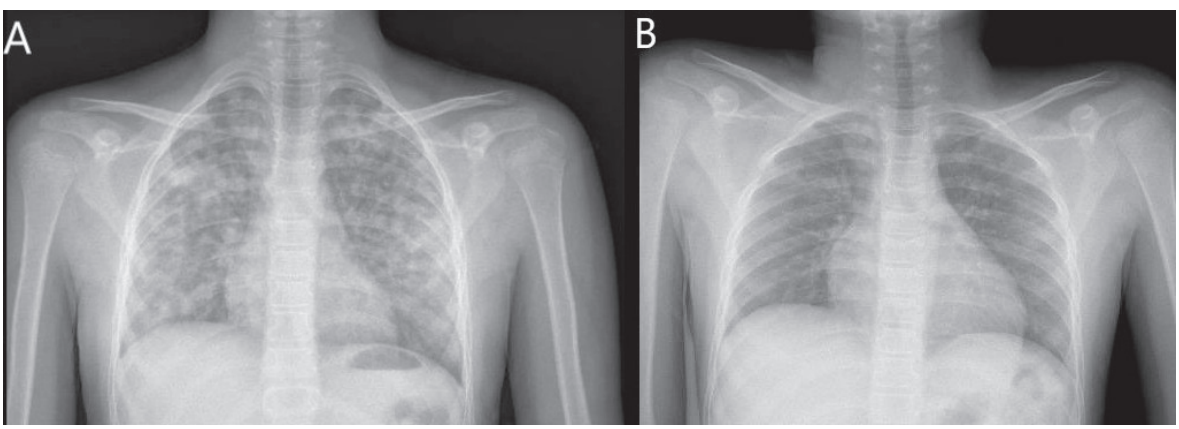



**Fig. 3.** Chest x-ray upon admission (A) and prior to discharge (B) for Patient 3.

capillaries for fluid leaking are the proposed pathological mechanisms.[2-4,16,17]

Our patients were misdiagnosed with severe asthma exacerbation vs pneumonia despite the absence of typical symptoms and signs of the two illnesses, which is likely due to the lack of awareness about the diagnosis of HAPE, especially re-entry type. Rapid improvement of our patients' clinical condition and resolution of radiographic changes support the diagnosis of reentry HAPE. Based on a published pediatric HAPE case series, more than two thirds of patients received antibiotics and around half of them were misdiagnosed with pneumonia.[9]

Most of the reported pediatric case series of reentry HAPE have male predominance (Table II). Patients 2 and 3 are males and have other three female siblings who have never had any HAPE episodes. The sex difference was observed in most of the published case series and reports, suggesting that sex hormones play a role in HAPE susceptibility. Male sex hormones could predispose patients to HAPE, or that female sex hormones are protective.[9,15,16] Further prospective and genetic studies with detailed phenotyping of HAPE patients are needed to investigate this finding.

Patient 1 reported chronic symptoms of obstructive sleep apnea with high scoring on the SRBD scale (15 out of 22) almost a year prior to her HAPE illnesses. Further, her body mass index was more than the normal age. These factors cause susceptibility to pulmonary hypertension and restrictive lung physiology with low functional residual capacity, respectively, and could explain HAPE development (patient 1) at moderate altitude (2200m).

Due to the low prevalence of this condition and the absence of randomized clinical trials to guide the treatment, management differs significantly between centers with overuse of antibiotics, steroids, and diuretics. HAPE is mostly misdiagnosed as asthma and pneumonia, which lead to overuse of the medications mentioned above.[9,15,17] Rapid descent and supplemental

**Table II.** Clinical characteristic of children reported with reentry high altitude pulmonary edema.

| References | Number of Patients | Age (Y)/ Gender (male-female) | History of URTs | Altitude | Treatment | Hospital stays | Outcome |
|---|---|---|---|---|---|---|---|
| Merino-Luna A et. al.[15] | 1 | 4/M | + | 3052 m | Oxygen- dexamethasone- IV antibiotics | Three days | Recovered |
| Douglas Lopez de Guimaraes.[16] | 1 | 17/M | - | 3100 m | Oxygen | Three days | Recovered |
| Saiki[17] | 1 | 10/M | + | 3400 m | Oxygen intravenous dexamethasone, oral acetazolamide | Four days | Recovered |
| Baniya et. al.[20] | 1 | 7/M | + | 3500 m | Oxygen intravenous dexamethasone | One day | Recovered |
| Viruez-Soto et. al.[21] | 1 | 14/M | - | 4090 m | Oxygen Mechanical ventilation Dexamethasone acetazolamide Sildenafil Vitamin C | Five days | Recovered |
| Giesenhagen et al.[9] | 19 (single center experience | Median and range 10.2 [0.6-19.2]/ male 70% (all HAPE) | Not specified | 2798 m [1840-3536] | Oxygen Dexamethasone Acetazolamide antibiotics | Median duration 2.2 days for all HAPE | Recovered |

oxygen are the primary treatment of HAPE. Targeting saturation level of 93% and above is recommended to relieve the pulmonary vasoconstrictions and improve hypoxemia. A nasal cannula/ face mask is the common oxygen delivering device, and rarely mechanical ventilation is necessary. Pharmacological interventions are also recommended if rapid descent is not possible, or the patients are severely distressed with more oxygen requirements. Direct pulmonary vasodilators are the main therapies used. Nifedipine has been recommended in children, especially for those who do not improve after oxygen therapy and descent.[1,4,8] Furosemide is not recommended since it decreases the pulmonary circulation and worsens the hypoxemia as most patients present with low intravascular volume.[4]

Patients 2 and 3 presented at the time of the COVID-19 pandemic, and they were suspected to having complicated COVID-19 pneumonia despite the absence of contact with COVID-19 patients. Nasopharyngeal swabs for SARS-CoV-2 were sent and returned negative on the third day. Response to therapies and pathophysiology of HAPE and COVID-19 differ significantly.[18] Development of Pulmonary edema in HAPE is due to exaggerated hypoxic pulmonary vasoconstriction while in COVID-19 the lung injury is due to an intense host cytokine-mediated inflammatory response that eventually leads to capillary permeability and surfactant dysfunction.[19]

In conclusion, these are the first reported cases of children with re-entry HAPE in Saudi Arabia. Re-entry HAPE can occur in otherwise healthy children, with the rapid ascent to high altitude and recent respiratory infections being the commonly reported triggers. Prognosis is favorable with a very rapid response to oxygen therapy. Education about HAPE is mandatory for families and health care workers working in high altitude areas.

## Author contribution

The authors confirm contribution to the paper as follows: designed the research and drafted the manuscript: AAA, İAA; interpreted the data: AAA, HHA, AMA; performed the literature search and scientific overview of our case: AAA, WİA. All authors critically read and reviewed the final manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Pollard AJ, Durmowicz A, Durrer B, et al. Children at high altitude: an international consensus statement by an ad hoc committee of the International Society for Mountain Medicine, March 12, 2001. High Alt Med Biol 2001; 2: 389-401. https://doi.org/10.1089/15270290152608561
2. Ebert-Santos C. High-altitude pulmonary edema in mountain community residents. High Alt Med Biol 2017; 18: 278-284. https://doi.org/10.1089/ham.2016.0100
3. Garlick V, O'Connor A, Shubkin CD. High-altitude illness in the pediatric population: a review of the literature on prevention and treatment. Curr Opin Pediatr 2017; 29: 503-509. https://doi.org/10.1097/MOP.0000000000000519
4. Liptzin DR, Abman SH, Giesenhagen A, Ivy DD. An approach to children with pulmonary edema at high altitude. High Alt Med Biol 2018; 19: 91-98. https://doi.org/10.1089/ham.2017.0096
5. Breitnauer N, Bush D, Stillwell PC, Carpenter T. A case of pulmonary edema at moderate altitude: extending the spectrum of HAPE?. Am J Respir Crit Care Med 2016; 193: A5627.
6. Yang Y, Du H, Li Y, et al. NR3C1 gene polymorphisms are associated with high-altitude pulmonary edema in Han Chinese. J Physiol Anthropol 2019; 38: 4. https://doi.org/10.1186/s40101-019-0194-1
7. Whitlow KS, Davis BW. High altitude pulmonary edema in an experienced mountaineer. possible genetic predisposition. West J Emerg Med 2014; 15: 849-851. https://doi.org/10.5811/westjem.2014.7.22773

8. Bhattarai A, Acharya S, Yadav JK, Wilkes M. Delayed-onset high altitude pulmonary edema: a case report. Wilderness Environ Med 2019; 30: 90-92. https://doi.org/10.1016/j.wem.2018.11.002

9. Giesenhagen AM, Ivy DD, Brinton JT, Meier MR, Weinman JP, Liptzin DR. High altitude pulmonary edema in children: a single referral center evaluation. J Pediatr 2019; 210: 106-111. https://doi.org/10.1016/j.jpeds.2019.02.028

10. Chervin RD, Hedger K, Dillon JE, Pituch KJ. Pediatric sleep questionnaire (PSQ): validity and reliability of scales for sleep-disordered breathing, snoring, sleepiness, and behavioral problems. Sleep Med 2000; 1: 21-32. https://doi.org/10.1016/S1389-9457(99)00009-X

11. Murray F, Insel PA, Yuan JX-J. Role of O(2)-sensitive K(+) and Ca(2+) channels in the regulation of the pulmonary circulation: potential role of caveolae and implications for high altitude pulmonary edema. Respir Physiol Neurobiol 2006; 151: 192-208. https://doi.org/10.1016/j.resp.2005.10.003

12. Paralikar SJ. High altitude pulmonary edema-clinical features, pathophysiology, prevention and treatment. Indian J Occup Environ Med 2012; 16: 59-62. https://doi.org/10.4103/0019-5278.107066

13. Hotta J, Hanaoka M, Droma Y, Katsuyama Y, Ota M, Kobayashi T. Polymorphisms of renin-angiotensin system genes with high-altitude pulmonary edema in Japanese subjects. Chest 2004; 126: 825-830. https://doi.org/10.1378/chest.126.3.825

14. Droma Y, Hayano T, Takabayashi Y, et al. Endothelin-1 and interleukin-8 in high altitude pulmonary oedema. Eur Respir J 1996; 9: 1947-1949. https://doi.org/10.1183/09031936.96.09091947

15. Merino-Luna A, Vizcarra-Anaya J. Acute lung edema as a presentation of severe acute reentry high-altitude illness in a pediatric patient. Case Rep Pediatr 2020; 2020: 8871098. https://doi.org/10.1155/2020/8871098

16. Lopez de Guimaraes D. Acute pulmonary edema returning to a High Altitude Environment: report of a case observed in Huaraz, Ancash, Peru. Acta Médica Peru 2008; 25: 92-95.

17. Saiki K. Reentry high altitude pulmonary edema in a Japanese boy: a case report. Nihon Kyukyu Igakukai Zasshi 2012; 23: 403-408. https://doi.org/10.3893/jjaam.23.403

18. Luks AM, Swenson ER. COVID-19 Lung injury and high-altitude pulmonary edema. A false equation with dangerous implications. Ann Am Thorac Soc 2020; 17: 918-921. https://doi.org/10.1513/AnnalsATS.202004-327CME

19. Brugger H, Basnyat B, Ellerton J, Hefti U, Strapazzon G, Zafren K. Letter to the editor: COVID-19 lung injury is different from high altitude pulmonary edema. High Alt Med Biol 2020; 21: 204-205. https://doi.org/10.1089/ham.2020.0061

20. Baniya S, Holden C, Basnyat B. Reentry high altitude pulmonary edema in the Himalayas. High Alt Med Biol 2017; 18: 425-427. https://doi.org/10.1089/ham.2017.0088

21. Viruez-Soto JA, Tinoco-Solórzano A. High-altitude acute reentrant lung edema. Medical Horizon (Lima) 2020; 20: e943. https://doi.org/10.24265/horizmed.2020.v20n3.14

The Turkish Journal of Pediatrics 2022; 64: 408-411
https://doi.org/10.24953/turkjped.2021.1617

Case Report

# A rare complication of pulmonary tuberculosis in childhood: Rasmussen's aneurysm in a 9-year-old child with Down syndrome

Elif Böncüoğlu[1], Celal Çınar[2], Elif Kıymet[1], İlknur Çağlar[1], Aybüke Akaslan Kara[1], Nuri Bayram[1], İlker Devrim[1]

*[1]Department of Pediatric Infectious Diseases, University of Health Sciences Dr. Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, İzmir; [2]Department of Interventional Radiology, Ege University, Faculty of Medicine, İzmir, Turkey.*

**ABSTRACT**

**Background.** As an extremely rare entity reported in children, Rasmussen's aneurysm is an inflammatory pseudo-aneurysmal dilatation of a branch of the pulmonary artery adjacent to or within a tuberculous cavity.

**Case.** Here, we reported a 9-year-old child with Down syndrome who presented with massive hemoptysis. Endovascular coil embolization was performed for Rasmussen's aneurysm. During the 2-year follow-up period, she had no further episodes of bleeding.

**Conclusions.** In case of the development of massive hemoptysis in the follow-up of a patient with pulmonary tuberculosis and Down syndrome, this lethal complication should be considered.

**Key words:** child, Down syndrome, rasmussen's aneurysm, tuberculosis.

Pulmonary artery aneurysms and pseudoaneurysms are rare and reportedly associated with various etiologies including pulmonary tuberculosis, trauma, pulmonary hypertension, and congenital conditions. Rasmussen's aneurysm was first described in 1868 by Danish physician Fritz Rasmussen. It is described as an inflammatory pseudo-aneurysmal dilatation of a branch of the pulmonary artery adjacent to or within a tuberculous cavity.[1,2-5] Here, we reported a case of Rasmussen's aneurysm in a 9-year-old child with Down syndrome.

## Case Report

A 9-year-old female with Down syndrome was presented to the outpatient clinic with fever and productive cough. She had a history of recurrent lower respiratory tract infection episodes and had undergone cardiac surgery for correction of ductus arteriosus in infancy. She had no known contact with a person with tuberculosis. She had a BCG vaccine scar. On physical examination, bilateral crepitant rales were heard during lung auscultation. Hematological examination revealed lymphocytosis (19950 $10^3$ /uL), anemia (hemoglobin: 9.6 g/dl), thrombocytosis (758000 $10^3$ /uL), and an elevated serum C-reactive protein level (9.78 mg/dl). Combined antibiotic therapy (teicoplanin and ertapenem) was initiated following the presumed diagnosis of bacterial pneumonia. Thorax computerized tomography showed bilateral widespread areas of consolidation in the upper lobes of the lungs associated with mediastinal lymphadenopathy and an increase in the diameter of the main pulmonary artery. Tuberculin skin test induration was 0 mm. The sputum sample was positive for acid-fast bacilli, later confirmed by the BACTEC culture system to be *Mycobacterium tuberculosis* and the patient

✉ Elif Böncüoğlu
dr_ebos@hotmail.com

Received 26th May 2021, revised 22nd June 2021, 2nd July 2021, accepted 8th July 2021.

was started on quadruple antituberculosis drug therapy (isoniazid, rifampicin, pyrazinamide, and ethambutol). An immunology consultation was carried out. Lymphocyte subset analysis revealed CD19+ B cell deficiency (<5 percentile). Functional T cell deficiency was also suspected due to Down syndrome and fluconazole prophylaxis was recommended by the immunologist. Testing for T-cell function was postponed until the end of the antituberculosis treatment.

One month after the initiation of antituberculosis treatment, the patient was admitted to the emergency room with complaints of massive hemoptysis. Serum hemoglobin level was dropped from 10.6 g/dl to 8.5 g/dl in the following hours. Her contrast-enhanced thorax CT with pulmonary angiography revealed cavitary lesions in both upper lobes and a peripheral pseudoaneurysm in a pulmonary artery branch (upper lobe posterior segment). (Fig. 1a, 1b,) The upper lobe posterior segment of the pulmonary artery was coaxially catheterized with a 5F multipurpose catheter (Boston Scientific Co., USA) and a 2.4 F microcatheter (Direction, Boston Scientific Co., USA). (Fig. 2a, 2b) Superselective embolization was done using a 2.4-F catheter, the aneurysmal cavity was completely obliterated with detachable coils (Concerto Detachable Coil, Medtronic) and a 5 mm- Amplatzer Vascular Plug 4 (AGA Medical Corporation, Golden Valley, MN, USA). (Fig. 2c). The patient was treated with antituberculosis therapy for 12 months.

In the X-ray taken 1 year later, the materials of embolization was observed without any complications (Fig. 3). During the 2-year follow-up period, she had no further episodes of bleeding and no sign of relapsing tuberculosis.

Informed consent was obtained from legal guardians of the patient.

## Discussion

Rasmussen's aneurysm is a critical entity that requires urgent recognition and treatment. It may result in rupture of the pulmonary artery wall and life-threatening massive hemoptysis with high mortality rates.[6] The incidence is reported to be around 5% in cavitary tuberculosis[7,8], however the data are limited to only a few case reports in childhood.[2-5] Timely implementation of angiographic embolization for massive hemoptysis was reported to be successful in up to 90% of the cases.[9]

Hemoptysis is an unusual manifestation of pediatric pulmonary tuberculosis[10,11] and it could be seen due to the extensive disease

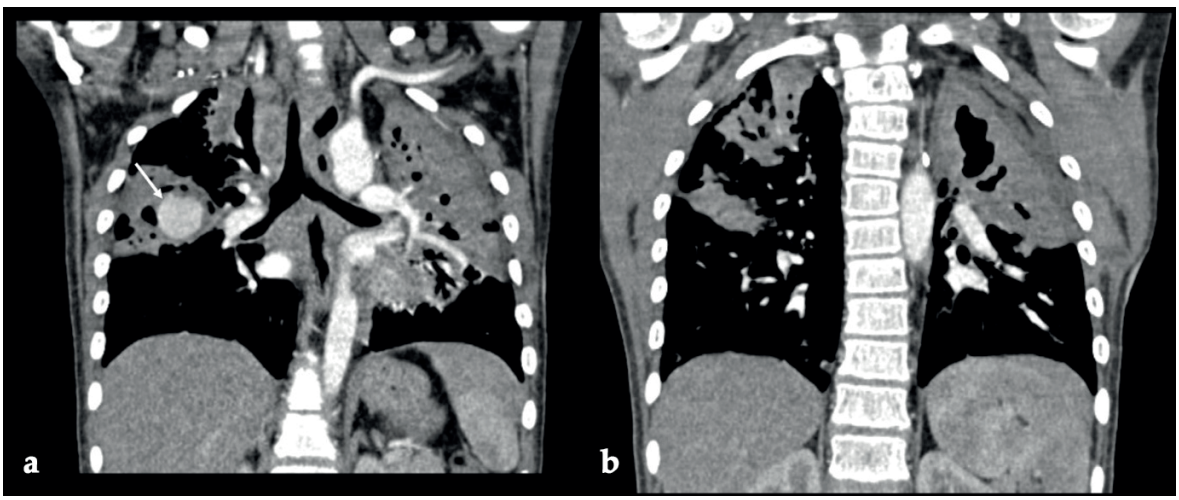



**Fig. 1. a.** Contrast-enhanced CT demonstrates a peripheral pseudoaneurysm in a pulmonary artery branch (upper lobe posterior segment) and cavitary lesions, **b.** Cavitary lesions in both upper lobes.

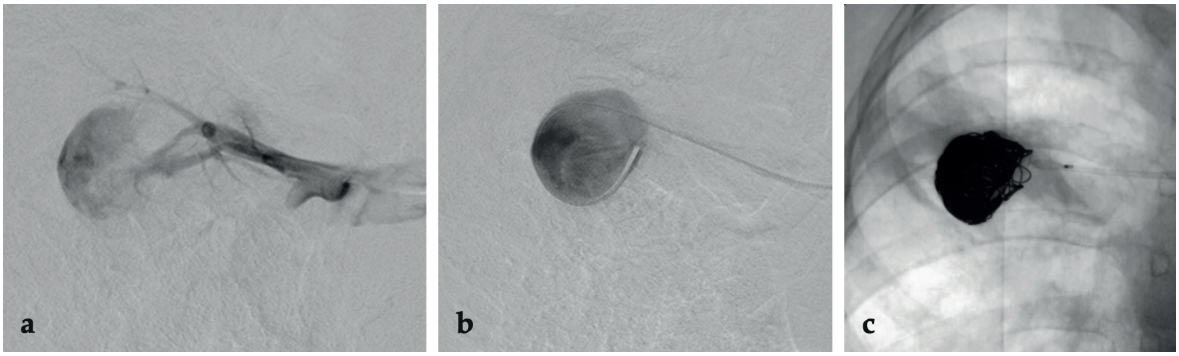



**Fig. 2. a.** A right selective upper lob pulmonary arteriogram demonstrates the aneurysm, **2b.** The upper lobe posterior segment pulmonary artery was coaxially catheterized with a 5F catheter (multipurpose, Boston scientific) and a 2.4 F microcatheter (Direction, Boston Scientific). A selective posterior segment pulmonary arteriogram demonstrated the presence of a Rasmussen's aneurysm, **2c.** Superselective embolization was done using a 2.4-F catheter, and the aneurysmal cavity was completely obliterated with detachable coils (Concerto Detachable Coil, Medtronic) and a 5 mm Amplatzer™ Vascular Plug 4(AGA Medical Corporation, Golden Valley, MN, USA).

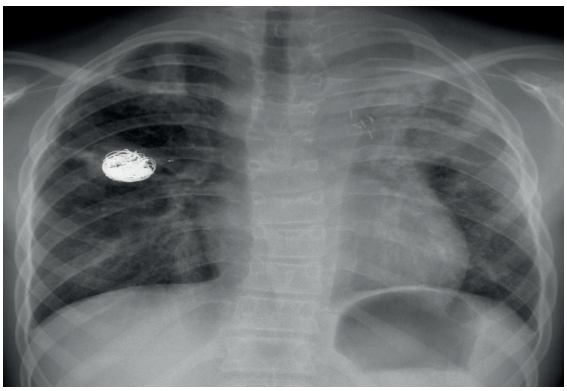


**Fig. 3.** One year later, chest X-ray showed materials of embolization of the Rasmussen's aneurysm.

and excavation and ulceration of blood vessels within the cavity wall. In our case, the patient had a history of recurrent episodes of lower respiratory tract infection which can be observed in patients with Down syndrome; and was probably misdiagnosed as bacterial necrotizing pneumonia instead of cavitary tuberculosis previously. Although the difference in the incidence of tuberculosis between the patients with Down syndrome and the general population has not been shown so far[12], we considered that T cell insufficiency associated with Down syndrome might have played a role in the spread of the disease and facilitated cavitation in the lung parenchyma in this patient. Furthermore, different types of aneurysms including infected (mycotic) and non-infected (such as sinus of Valsalva aneurysm) types are reported in Down syndrome so far.[13-15] However, the coexistence of Down syndrome with Rasmussen aneurysm has not been reported to date. We hypothesized that there may be an association between Down syndrome and structural abnormality of the connective tissue which may predispose the patient to the development of the aneurysm. Further data are needed to support the possible association.

In conclusion, in case of the development of massive hemoptysis during the follow-up of a patient with pulmonary tuberculosis and Down syndrome, this lethal complication should be considered.

## Author contribution

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

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Rasmussen V, Moore WD. On hæmoptysis, especially when fatal, in its anatomical and clinical aspects. Edinb Med J 1868; 14: 385-401.
2. Gandhi S, Jaiswal A, Joshi S, Shetty N, Shah I. Rasmussen's aneurysm in a child with multidrug-resistant pulmonary tuberculosis. Indian J Pediatr 2020; 87: 564. https://doi.org/10.1007/s12098-020-03211-4
3. Khera S, Simalti AK, Balasubramaniam D, Tiwari N. Rasmussen's aneurysm in a child with acute lymphoblastic leukemia. BMJ Case Rep 2020; 13: e235399. https://doi.org/10.1136/bcr-2020-235399
4. Gesuete V, Corzani A, Bronzetti G, Lovato L, Picchio FM. Rasmussen's aneurysm in childhood: a case report. Congenit Heart Dis 2013; 8: E41-E44. https://doi.org/10.1111/j.1747-0803.2011.00609.x
5. Horwitz M, Chaumoître K, Grimaldi C, et al. Spontaneous regression of multiple Rasmussen aneurysms in a child with Lemierre syndrome and pulmonary abscesses. Pediatr Infect Dis J 2013; 32: 1301-1302. https://doi.org/10.1097/INF.0b013e3182a638b7
6. Syed M, Irby J. Airway management of ruptured pulmonary artery "Rasmussen" aneurysm and massive hemoptysis. BMC Res Notes 2015; 8: 346. https://doi.org/10.1186/s13104-015-1313-7
7. Keeling AN, Costello R, Lee MJ. Rasmussen's aneurysm: a forgotten entity?. Cardiovasc Intervent Radiol 2008; 31: 196-200. https://doi.org/10.1007/s00270-007-9122-6
8. Shih SY, Tsai IC, Chang YT, Tsan YT, Hu SY. Fatal haemoptysis caused by a ruptured Rasmussen's aneurysm. Thorax 2011; 66: 553-554. https://doi.org/10.1136/thx.2010.135616
9. Woo S, Yoon CJ, Chung JW, et al. Bronchial artery embolization to control hemoptysis: comparison of N-butyl-2-cyanoacrylate and polyvinyl alcohol particles. Radiology 2013; 269: 594-602. https://doi.org/10.1148/radiol.13130046
10. Coss-Bu JA, Sachdeva RC, Bricker JT, Harrison GM, Jefferson LS. Hemoptysis: a 10-year retrospective study. Pediatrics 1997; 100: E7. https://doi.org/10.1542/peds.100.3.e7
11. Salazar GE, Schmitz TL, Cama R, et al. Pulmonary tuberculosis in children in a developing country. Pediatrics 2001; 108: 448-453. https://doi.org/10.1542/peds.108.2.448
12. Verma SK, Sodhi R. Down's syndrome and cardiac tamponade with pulmonary tuberculosis in adults. Indian J Hum Genet 2009; 15: 72-74. https://doi.org/10.4103/0971-6866.55219
13. Diab KA, Richani R, Al Kutoubi A, Mikati M, Dbaibo GS, Bitar FF. Cerebral mycotic aneurysm in a child with Down's syndrome: a unique association. J Child Neurol 2001; 16: 868-870. https://doi.org/10.1177/088307380101601114 05
14. Naughton PA, Wang TT, Keeling AN, Moneley D, Kelly CJ. Down syndrome: a risk factor for mycotic aneurysm?. Vascular 2010; 18: 297-298. https://doi.org/10.2310/6670.2010.00040
15. Benatar A, Decraene T, Feenstra A. Ruptured sinus of Valsalva aneurysm in a child with Down syndrome: a rare cardiac anomaly. Med Sci Monit 2010; 16: CS135-CS137.

# NOTICE TO AUTHORS

## Instruction for authors

## General Information:

The Turkish Journal of Pediatrics publishes the following peer-reviewed material oriented in the field of clinical pediatrics: original articles, case reports, review of the literature, short communications, clinicopathological exercises and letter to the editor. The Journal does not publish animal studies.

All papers are subject to editorial revision for the purpose of conformity to the style adopted by The Turkish Journal of Pediatrics, and authors may be requested to shorten or revise their papers. Statements and opinions expressed in the articles are those of the authors and not necessarily those of the Editor or publisher; the Editor and publisher disclaim any responsibility or liability for such material. Neither the Editor nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication; neither do they guarantee any claim made by the manufacturer of such product or service.

**Preparation of Manuscript:** Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts. Manuscript should be uploaded in a blinded manner; any affiliation must be covered.

**Format:** Manuscripts should be typed in 12pt (Times New Roman), double-spaced, with margins of 2.5 cm or 1 inch all round, and pages must be numbered on the right upper corner. Line numbers should be added to each page on the left margin.

**Covering Letter:** The covering letter is important to help the Editor in his/her preliminary evaluation, please indicate why you think the paper is suitable for publication. A statement that each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript must be provided. The covering letter will be uploaded as a separate file.

**Title Page:** A separate title page should include authors' names and academic degrees; departmental and institutional affiliations of each author, and sources of financial assistance, if any. Designate one author as the correspondent, and provide an address as well as business and home telephone numbers. Galley proofs and order forms for reprints will be sent to the corresponding author, at a later date. On the title page please add a short running title, word count (excluding abstract and references), number of tables and figures, ethical approval, funding, conflict of interest, authors contribution.

## Types of Articles:

**Original Article:** Original articles should report scientific findings within the pediatric field of interest. They must include a structured abstract of less than 300 words, with the following headings: background, methods, results and conclusions to appear after the title page. The manuscript should be structured with the following headings: introduction, materials and methods, results, and conclusions. The introduction should put the study in context with the current literature and reflect the purpose of the study. The materials and methods should include the study methodology, the setting for the study (although affiliation must be covered), the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The results section should include the outcome of the study and statistical significance, if appropriate. The conclusion(s) states the significance of the results and limitations of the study. The manuscript should be no longer than 3,500 words.

**Case Reports:** Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Case reports should consist of a structured abstract with the following headings: background, case and conclusions that summarizes the case(s), a brief introduction, a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process.

**Review Articles:** Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission.

Structured manuscript for systematic reviews are recommended. Headings should include: Structured abstract with the following headings: background and objectives, material and methods results and conclusions, Introduction, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions. The general instructions regarding submission (including cover letter, title page requirements, journal style guidance, and conflict of interest statements) also apply to Review Articles.

**Short communication:** Short communications are opinion pieces consisting of a main point and supporting discussion. They may address general issues or controversies in the field of pediatrics. No abstract is needed but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to short communications.

**Clinicopathological exercises:** Authors can submit for consideration an clinicopathological exercises that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references. No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to clinicopathological exercises.

**Letters to the Editor:** Letters to the Editor should pertain to papers published in The Turkish Journal of Pediatrics within the past year. Provide a unique title for the Letter on the title page with complete contact information for the author(s). References, including reference to the pertinent article(s) in The Journal, should conform to style for manuscripts. No abstract is needed for a letter to editor. Letters to the Editor may be sent to the authors of the original article to which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same issue as the Letter to the Editor.

**Summary / Abstract and key words:** An informative structured abstract not exceeding 300 words must accompany each manuscript, Unexplained abbreviations and references should not be given. The abstract should state the objective (the purpose of the study and hypothesis) and summarize the rationale for the study. It is critically important that all significant findings should be presented in the abstract. The abstracts should be structured with the following headings for an original article: Background, Methods, Results and Conclusions and Background, Case Report, Conclusion for a case report.

The authors should list three to five key words or phrases taken from Index Medicus

Medical Subject Headings (http://www.nlm.nih.gov/mesh/MBrowser.html).

**Tables:** Tables should be numbered with Roman numerals according to the order of mention in the text, each table must be typed double-spaced. A title for each table must be given. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, a footnote must give full credit to the original source. Tables should be added to the end of the main manuscript following the references.

**Figures:** Figures and illustrations should be of direct interest to the subject of the paper and numbered with Arabic numerals according to order of reference in the text. Art should be created/scanned and saved as either TIFF or JPEG format, submitted as a separate file, and not embedded in the text file. All lettering must be professional and in proportion to the drawing, graph, or photograph. A list of legends for the figures and illustrations must added to the end of the manuscript after the tables.

**Abbreviations:** Abbreviations must conform to accepted standards. Laboratory slang, clinical jargon and colloquialisms must be avoided. The full term for an abbreviation should precede its first use in the text. Generally, abbreviations should be limited to those defined in the AMA Manual of Style, current edition.

## Publication Ethics and Informed Consent:

The Turkish Journal of Pediatrics takes issues of publication ethics very seriously and subscribes to its principles on how to deal with acts of misconduct thereby committing to investigate allegations of misconduct in order to ensure the integrity of research. Scientific articles sent to journals should take into account the recommendations of the International Committee of Medical Journal Editors (ICMJE) and the International Standards for Editors and Authors of COPE (Committee on Publication Ethics).

The Materials and Methods section and the Title Page should include a statement that the research was reviewed and approved by an institutional review board, and that participation involved informed consent. Please give information concerning where and when the study was approved, with the report number.

Written consent from the family is required before a case report can be published. On submission, authors must attest that they have written consent from the family. Instances where there are extenuating circumstances in which family consent may be problematic will be handled on a case-by-case basis. A sentence stating that informed consent was received from the family should be added to the case section of the main document.

**Conflicts of Interest and Funding:** A conflict of interest is defined as any financial, personal, academically competitive or intellectual relationship between the author, referee or editor that involves article submission, referee evaluation, editorial decision and author-referee-editor correspondence and that could lead to a prejudiced and unfair action during the evaluation stage.

The authors must declare any financial relationship or conflict of interest areas that need to be known regarding the study. All financial contributions and sponsorships for the study and all financial relationships and conflict of interest areas should be included in the copyright transfer form that accompanies the article. The authors must explain the study sponsor's role in collecting, evaluating and analyzing the data, writing the article and submitting it for publication. Declaration of conflict of interest should be added to the title page. If there is no conflict of interest, this section must include a "The authors declare no conflict of interest" statement. All sources of funding should also be mentioned in the title page.

The evaluation process for the article is not started if a conflict of interest statement is not present at the time the article is sent. If a conflict of interest becomes apparent after the article is published, the journal may make an official correction or withdraw the entire article.

The referees also need to declare all their interests that they feel may represent a potential conflict of interest. The referees must declare to the editor any conflicts of interest that may prejudice their evaluation of the article and must avoid such evaluation of certain articles if a potential prejudice is present.

The editors who will make the final decision on the articles cannot have any personal, professional or financial relationship with any of the authors of the articles they will decide on. The editors will also try to avoid any referees who have a possible conflict of interest, for example those working in the same department or facility as any of the authors.

**Acknowledgement:** An "acknowledgement(s)" section may be added to thank those who helped the study or preparation of the article, if any. The acknowledgements should be added to the title page. This section contains statements of gratitude for personal, technical or material help, etc.

### Authors contribution:

We require that all authors take responsibility for the content of the work submitted for review. An author contribution section should be added to the title page

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

Please note that changes to the list of contributors are not permitted after the article has been accepted.

**References:** References to the literature should be limited to those cited by the author, arranged and numbered according to order they appear in the text; do not alphabetize. In text, tables, and legends, identify references with superscript Arabic numerals at the end of the sentence (after the full stop). List all authors up to 6; if more than 6, list the first 3 followed by "et al."

If reference to an author is made in the text the reference must be given after the name of the author rather than at the end of a sentence.

Example: In a study by Bier DM et al.[23] the prevalence of…….

Names of journals are to be abbreviated in accordance with the style of Index Medicus.

References to journal articles:

Example: Oral R, Coohey C, Zarei K, et al. Nationwide efforts for trauma-informed care implementation and workforce development in healthcare and related fields: a systematic review. Turk J Pediatr 2020; 62: 906-920.

References to books:

Example: Strasburger VC, Brown RT, Braverman PK, Rogers PD, Holland-Hall C, Coupey SM. Adolescent Medicine: A Handbook for Primary Care. Philadelphia: Lippincott Williams & Wilkins, 2006: 13-21.

References to chapters in books:

Example: Macumber IR, Flynn JT. Systemic hypertension. In: Kliegman RM, St Geme III JW, Blum NJ, Tasker RC, Shah SS, Wilson KM (eds). Nelson Textbook of Pediatrics (21st ed) Vol 2. Philadelphia: Elsevier, 2020: 2490-2499.

**Submission of article:** All manuscripts must be submitted on-line through the web site http://www.turkishjournalpediatrics.org. The corresponding author should upload the "Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form" with the main manuscript. The copyright transfer form can be downloaded during the online submission process.

**Footnote:** If the study has been presented at a scientific meeting or is part of a thesis this can be added to the end of the manuscript as a footnote.

**Copyright:** All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means without the prior written permission of the copyright owners. Permission is not required to copy summaries of papers or of articles on the condition that a full reference to the source is given. Multiple copying of the contents without permission is always illegal.

No manuscript will be considered for review until all authors have completed an uploaded the copyright form.

**Announcements:** Announcements of scheduled meetings, symposia, or postgraduate courses may be sent to the publisher for consideration at least 5 months in advance of the meeting date.

## Checklist

- Covering letter
- Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form.
- Title page
  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
  - Word count and number of tables and figures
  - Ethical approval
  - Funding
  - Conflict of interest
  - Acknowledgement
  - Authors contribution
- Article
  - Original articles: Title, Structured abstract, Key words, Introduction, Material and Methods, Results, Discussion, References, Tables, Figure legends
  - Case Reports: Title, Structured abstract, Key words, Introduction, Case Report, Discussion, References, Tables, Figure legends
- Manuscript should be uploaded in a blinded manner; any affiliation must be covered.
- Declaration of informed consent and ethical approval should be given in the material methods section and on the title page
- Tables should be added to the bottom of the main manuscript document
- Figures should be uploaded separately
- Figure legends should be added to the bottom of the main manuscript following the tables.

## Publication Process After Acceptance

Accepted papers will be passed on to the journal's English language editor for language editing. After this, the manuscript will be evaluated by the section editor/ associate editor before publication. If major changes have been made by the language editor the author identified as the formal corresponding author for the paper will receive an email asking them to evaluate the last edition and approve all changes.

The accepted paper will then be passed on to the Akdema Informatics and Publishing company for correct formatting. At this point, the manuscript is also checked for plagiarism.

### *Plagiarism Detection*

The journal employs a plagiarism detection system. By submitting your manuscript to our journal, you accept that your manuscript will be screened for plagiarism against previously published works after acceptance. If plagiarism is detected at this point the manuscript can be declined for publication even after previously being accepted.

## Proofing

Authors will receive an e-mail notification with their final pdf. Page proofs should be carefully proofread for any copyediting or typesetting errors. No special software is required, all common browsers are supported. The only acceptable changes at this stage are corrections to grammatical errors or data accuracy, or to provide higher resolution figure files. Any changes to scientific content will require editorial review and approval. Change in authors is not permitted at this point. Proofs must be returned within 72 hours of receipt of the email.

## CASE REPORTS



Printed by METEKSAN in Ankara, Turkey