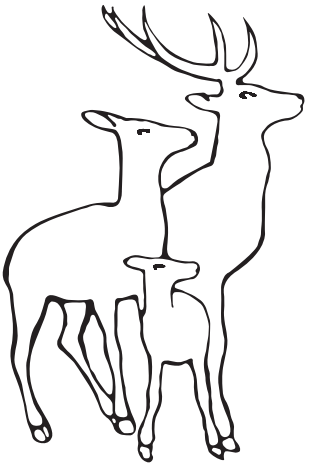
ISSN 0041-4301
www.turkishjournalpediatrics.org

# THE TURKISH JOURNAL OF PEDIATRICS

ORIGINAL ARTICLES



volume 63
number 3
May-June 2021



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www.turkishjournalpediatrics.org

**Volume 63 ▪ Number 3**
**May-June 2021**

**ISSN: 0041-4301**

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**ISSN 0041-4301**
www.turkishjournalpediatrics.org

Cilt: 63 Sayı: 3, Mayıs-Haziran 2021



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

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

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

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

**BASIM TARİHİ:** XX.XX.2021

**ISSN 0041-4301**
www.turkishjournalpediatrics.org

Vol: 63 Number: 3, May-June 2021



**PRODUCTION MANAGER**
**Owner on behalf of the Publishers**
Murat YURDAKÖK

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

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

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

**PUBLICATION TYPE**
International peer-reviewed journal

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

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

**PRINT DATE:** XX.XX.2021

# THE TURKISH JOURNAL OF PEDIATRICS



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.

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SHORT COMMUNICATION

The Turkish Journal of Pediatrics 2021; 63: 345-354
https://doi.org/10.24953/turkjped.2021.03.001

**Original Article**

# Enthesitis: an obscured extraintestinal manifestation in pediatric inflammatory bowel disease

Nelgin Gerenli[1], Betül Sözeri[2]

*Departments of [1]Pediatric Gastroenterology and [2]Pediatric Rheumatology, University of Health Sciences, Ümraniye Training and Research Hospital, İstanbul, Turkey.*

## ABSTRACT

**Background.** Enthesitis is an extra-intestinal manifestation of inflammatory bowel disease (IBD) which often remains underdiagnosed in children. We aimed to evaluate the frequency of enthesitis in pediatric IBD patients using physical examination and ultrasound (US) assessment as the reference standard.

**Methods.** 31 children, 7 -18 years of age, diagnosed with IBD were recruited for a cross-sectional study. All subjects completed a study questionnaire and underwent both physical and US examination for the presence of the enthesitis.

**Results.** Of 31 subjects (17girls; median age 14(6) years) enrolled, 17 (55%) had ulcerative colitis, 11 (35%) had Crohn's disease, and 3 (10%) had indeterminate colitis. The median time from IBD diagnosis was 1.2 years. At least one enthesitis (range 1–4) was identified in 14 (45%) patients of whom nine had more than one enthesitis with symmetric involvement in eight. The quadriceps femoris insertion at the superior portion of the patella was the frequently involved site (32%, 9 of 28 sites), followed by patellar ligament insertion at tibial tuberositas. The presence of enthesitis was associated with a higher intensity of the musculoskeletal pain (p=0.018), but physical activity remained unaffected (p=0.056).

**Conclusions.** Enthesitis is a common underestimated extra-intestinal manifestation of IBD that may impact the musculoskeletal health of children. Future studies with more extensive cohorts are needed to evaluate enthesial involvement both with physical examination and US in order to predict the long-term outcomes of the enthesitis on children with IBD.

**Key words:** pediatric inflammatory bowel disease, extraintestinal manifestations, enthesitis.

Inflammatory bowel disease (IBD) refers to a group of chronic inflammatory disorders affecting the gastrointestinal tract, which may be associated with extraintestinal manifestations involving the eyes, skin, hepatobiliary and musculoskeletal systems.[1] Musculoskeletal involvement, affecting especially the peripheral or axial joints, can precede, concur, or succeed IBD. Enthesitis which is an inflammation at the insertion site of the ligaments or tendons to the bone is a sign of musculoskeletal involvement.[2,3] It is a distinct clinical hallmark of spondyloarthropathies (SpA) in both children and adults, observed also in healthy children[4] as well as in children with juvenile idiopathic arthritis (JIA), particularly those with enthesitis-related arthritis (ERA).[5,6] Articular-peripheral complications occur in 23%, and axial involvement in 4% of adult patients with IBD.[7,8] Data are limited in children. One study that evaluated children with IBD-associated arthropathy found enthesitis and sacroiliitis (SI) in 7% and 25% of patients, respectively.[9] The pathophysiology of enthesitis includes the innate and adaptive immunity with overlap of the interleukin (IL)-23 and IL-17 axis. The IL-23 is a key driver of enthesitis in rats and acts via previously unidentified T cells.[10] Accordingly abnormal gut microbiome as seen in IBD

✉ Nelgin Gerenli
nelgingerenli@gmail.com

Received 17th July 2020, revised 18th November 2020, 26th December 2020, accepted 29th December 2020.

patients may play a role in the emerging of entheseal pathology.[11] Moreover, biomechanical factors such as obesity and physical activity may confound the pathogenesis of the enthesitis and might explain predominately lower limb distribution.[12] Enthesitis is generally independent of IBD activity index, and may be asymptomatic in the majority of patients.[13,14] Children with IBD are usually not examined for enthesitis and additionally there is no standard protocol for such examination. Yet, clinical findings including localized pain, tenderness, and swelling are suggestive of enthesitis.[15] If left untreated, enthesitis may cause osteopenia, erosions, soft tissue calcifications, and new bone formation. Osteopenia, which is present in many patients with IBD, may also aggravate enthesitis.[16] Ultrasonography (US) and power Doppler ultrasonography (PDUS) are used for the diagnosis of inflammatory enthesitis.[17] This study aimed to investigate the frequency of enthesitis in children with IBD.

## Material and Methods

The study included 31 children, 7 to 18 years of age, who had been diagnosed with Crohn's disease (CD), ulcerative colitis (UC), or indeterminate colitis (IC), at the Pediatric Gastroenterology Unit. The trial was approved by the University of Health Sciences Umraniye Research and Training Hospital Ethics Committee (approval number 19/12/2019-26670).

Inclusion was made regardless of the duration, phenotype, severity or current activity of the disease and all patients had been on 5 amino salicylic acid (5-ASA) and azathioprine treatment, with or without low dose of methylprednisolone. Disease activity indexes for CD and UC or IC were calculated using the Pediatric Cohn's Disease Activity Index (PCDAI) and the Pediatric Ulcerative Colitis Activity Index (PUCAI), respectively.[18,19] Disease phenotype at diagnosis was categorized according to the Paris classification.[20] All IBD patients were evaluated for coexistence of familial Mediterranean fever (FMF), using FMF diagnostic criteria.[21,22] Clinical information about previous diagnoses, anthropometric measurements and laboratory tests (albumin, C-reactive protein (CRP), complete blood count (CBC), and erythrocyte sedimentation rate (ESR)) were obtained from the hospital's medical record system. Informed consent was taken from the parents and children prior to inclusion. Patients who had been previously diagnosed with arthritis or SpA, had overuse tendinitis, were on non-steroidal anti-inflammatory drugs or were unable to describe pain accurately were excluded.

Initially, a questionnaire was given to the parents or the children themselves for demographic and historical characteristics and the presence or absence of musculoskeletal pain, joint symptoms and the degree of the daily physical activity. The level of the sportive activities done by the children was also detailed to exclude overuse tendinitis. Musculoskeletal pain intensity within the previous month was designed to range from 0 (no pain) to 10 (intense pain) using numerical pain rating scale (NPRS).[23,24] Physical activity score ranged from 0 (not active) to 5 (very active).[25]

***Musculoskeletal examination:*** The subjects were examined by the same pediatric rheumatologist who was blinded to the diagnosis of the disease phenotype and clinical history of the patients including musculoskeletal symptoms and medications. The patients were also examined for signs suggesting spondyloarthropathies as well as peripheral extraintestinal manifestations as dactylitis. Enthesitis was defined as the presence of tenderness on a vigorous tendon palpation.[26] A standardized bilateral examination for enthesitis was performed, including the humeral supraspinatus insertion at the greater tuberosity, common flexor tendon insertion at the medial epicondyle, common extensor tendon insertion at the lateral epicondyle, hip extensor insertion at the greater trochanter, quadriceps femoris insertion at the superior patella, patellar ligament insertion at the patella and tibial tuberosity, Achilles tendon

insertion at the calcaneus, and plantar fascial insertion at the calcaneus.[27]

***Ultrasound (US) examination:*** Patients with signs of enthesitis were further evaluated by the same pediatric rheumatologist who was also experienced in musculoskeletal US examination (certified by EULAR, the European League Against Rheumatism). Philips IU22 US device with a high-frequency linear array 12 MHz transducer was used.[26,27] Grey-scale and power Doppler evaluation of each enthesial site in both longitudinal and transverse planes- included assessment of the abnormalities in the tendon appearance, hypoechogenicity, lack of normal fibrillar aspect, enthesophytes, bony erosions, bursitis and power Doppler signal at enthesis. Power Doppler imaging was standardized with a pulse repetition frequency of 500–750 Hz and gain adjusted to the highest level without background noise artefact.[28,29] Enthesitis was graded according to its intensity: 0, absent; 1, minimal (1 spot); 2, moderate (2 spots); and 3, severe (≥3 spots).[5] Minimal power Doppler findings of enthesitis may be detected in normal children, thus findings of grade 2 or above were considered positive.[6,30] Patients with and without enthesitis were compared. Entheses thickness [expressed in millimeters and defined with Balint cut-off for quadriceps >6.1 mm, inferior pole of patella (proximal rotuleus) and tibial tuberosity (distal rotuleus) >4 mm, Achilles >5.29 mm, plantar fascia >4.4 mm] were recorded at each site and scored with US according to Glasgow Ultrasound Enthesitis Scoring System (GUESS), ranging from 0 to 36, which was validated by Balint et al.[31,32]

### *Statistical analysis*

The results were analyzed by the Statistical Package for the Social Sciences (SPSS) version 11 for Windows (SPSS Inc.; Chicago, IL, USA). Shapiro-Wilks test was used to determine the distribution of the variables. Independent samples t-test was used for comparison of normally distributed variables and the results were shown as mean and standard deviation (SD). Mann-Whitney U test was used for non-normally distributed variables and the results were shown as interquartile range (IQR). Pearson correlation test, Fisher's exact test, and chi-square test were used to compare the absence or presence of enthesitis and p values of less than 0.05 were accepted as significant.

## Results

The study included 31 children with a female to male ratio of 1.2 (F/M: 17/14). The median (IQR) age of the study population was 14 (6) years. Of 31 children, 17 (55%) had UC, 11 (35%) had CD, and 3 (10%) had indeterminate colitis (IC). The median (IQR) time from IBD diagnosis was 8 (6) months and all patients were receiving one or more medication (Table I). Pediatric disease activity index and bowel involvement are listed in Table I. All IBD patients had negative p-ANCA results and none had coexisting FMF disease. There was no relationship between enthesitis and clinical variables, including IBD phenotype, IBD activity index and levels of inflammatory markers. However, patients with enthesitis had younger age (p= 0.048), and higher body mass index SDS (BMI SDS) (p=0.041), compared to those with no entheseal involvement (Table II). Musculoskeletal pain intensity score within the previous month ranged from 1 (no pain) to 10 (severe intense pain) points. Children, having at least one enthesitis reported a greater intensity of pain than those without enthesitis (p=0.001). The level of physical activity was not found to be affected by the presence of pain or enthesitis (p=0.066). The results of blood tests obtained at the time of examination are summarized in Table II. A total of 28 inflamed entheseal sites were detected in 14 of 31 (45%) patients. Nine (64%) of the 14 patients with enthesitis had more than one tender enthesis (range 1–4), with symmetric involvement in eight (57%) of them. Only one patient with IC had entheseal involvement. The most common sites of enthesitis were the quadriceps femoris insertion at the superior portion of the patella (9 of 28 sites), patellar ligament insertion at tibial tuberositas (7 of 28 sites) and the Achilles tendon insertion at the calcaneus (7 of 28

**Table I.** Demographic and clinical characteristics of children with inflammatory bowel disease.

| Characteristics | All (N=31) | Ulcerative colitis (N=17) | Crohn's disease (N=11) | Indeterminate colitis (N=3) |
|---|---|---|---|---|
| Age (years), median (IQR) | 14 (6) | 15 (6) | 14 (6) | 9 (0) |
| Disease duration (months), median (IQR) | 8 (6) | 6 (6) | 11 (17) | 6 (15) |
| Disease location, n (%) | | | | |
| Rectosigmoid | 5(16%) | 3 (18%) | | 2 (67%) |
| Left sided disease | 5 (16%) | 4 (24%) | | 1 (33%) |
| Pancolitis | 10 (33%) | 10 (59%) | | |
| Large intestine only | 21 (68%) | 17 (100%) | 1 (9%) | 3 (100%) |
| Small intestine only | 1 (3%) | | 1 (9%) | |
| Small and large intestine | 9 (29%) | | 9 (82%) | |
| Medications, n (%) | | | | |
| 5-Acetylsalicylic acid | 31(100%) | 17 (100%) | 11 (100%) | 3 (100%) |
| Azathioprine | 28 (90%) | 17 (100%) | 11 (100%) | 0 (0%) |
| Methylprednisolone | 4 (13%) | 0 (0%) | 4 (36%) | 0 (0%) |

IQR: interquartile range

sites). The least affected ligament was the hip extensor insertion at greater trochanter (Table III). The most frequently observed entheseal pathology on ultrasonographic examination was thickening of tendon 12 (38%) followed by peritendenous edema 10 (31%), hypoecogenisity 6 (19%), loss of thickness 2 (6%) and increased Doppler signal (6%). No tendon tears, intra-tendinous calcifications, enthesophytes or bone erosions were observed (Table IV). Bursitis was detected in four patients, three with bilateral retrocalcaneal and one with bilateral infrapatellar involvement. The treatment for enthesitis was arranged as sulfasalazine or ibuprofen for mild cases (9 patients), methylprednisolone dosage adjustment for moderate (2 patients) and in three patients with severe disease the treatment was switched to anti-TNF agent (infliximab).

## Discussion

The present study reports the frequency of enthesitis in children with IBD, which to our knowledge is the first pediatric study investigating ultrasound-guided diagnosis of enthesitis. As far as we could find in the literature, only one study reported enthesitis in pediatric IBD patients with diagnosis based solely on physical examination findings,[33] another study reported enthesitis in a study investigating spondyloarthropathies in pediatric IBD.[9] The rate of enthesitis in pediatric IBD study was 21%, where 12% of the subjects had 3 or more tender enthuses.[33]

Compared with previous studies, the frequency of enthesitis in patients with IBD was higher in our study, with at least one ultrasonografically verified enthesitis in 45% of all patients. This was thought to be due to the diagnostic approach using both physical examination and US. This was similar to adult IBD studies in which both physical and US examinations had been performed and the frequencies had been reported to be between 44.3- 84.1%.[34,35] In a meta-analysis performed for the prevalence of the axial and peripheral manifestation in adults with CD and UC, the prevalence of the peripheral manifestations such as enthesitis was reported to range between 1- 54%.[36]

Jousse-Joulin et al.[5] found that entheseal tenderness does not always correspond to ultrasound abnormalities while radiographic enthesitis is also not associated with positive physical examination findings, suggesting subclinical enthesitis. In accordance, a

**Table II.** Comparison of clinical features, musculoskeletal pain and activity levels between patients with and without enthesitis.

| Features | No enthesitis (N=17) | Enthesitis (N=14) | p-value |
|---|---|---|---|
| Age (years), median (IQR) | 16 (6) | 11 (6) | 0.048 |
| Gender, n (%) | | | 0.015 |
| Male | 9 (53%) | 5 (36%) | |
| Female | 8 (47%) | 9 (64%) | |
| BMI SDS, mean ± SD | -0.57 ± 1.4 | 0.19 ± 0.79 | 0.041 |
| Disease duration (months), median (IQR) | 9 (17) | 6 (6) | 0.570 |
| Inflammatory bowel disease type, n (%) | | | |
| Crohn's disease | 5 (29%) | 6 (43%) | 0.715 |
| Ulcerative colitis | 10 (59%) | 7 (50%) | 0.480 |
| Indeterminate colitis | 2 (12%) | 1 (7%) | - |
| Pediatric Activity Index (PCAI or PUCAI), median (IQR) | | | |
| Crohn's disease (PCDAI) | 20.0 (25.0) | 30.0 (5.0) | 0.452 |
| Ulcerative colitis (PUCAI) | 20.0 (1.25) | 20.0 (10.0) | 0.229 |
| Inflammatory bowel disease activity, n (%) | | | 0.778 |
| Remission | 7 (33%) | 6 (38%) | |
| Mild to moderate disease | 10 (67%) | 8 (62%) | |
| Musculoskeletal pain score during last one month, median (IQR) | 3.0 (4.5) | 7.0 (3.25) | 0.001 |
| Physical activity level, n (%) | | | 0.066 |
| Not active | 0 (0%) | 0 (0%) | |
| A little active | 0 (0%) | 0 (0%) | |
| Regular activity | 0 (0%) | 1 (7%) | |
| Pretty active | 3 (17%) | 5 (36%) | |
| Very active | 14 (83%) | 8 (57%) | |
| Laboratory, median (IQR) | | | |
| WBC ( $x10^3/\mu l$) | 9.31 (2.9) | 8.6 (4.4) | 0.894 |
| Hemoglobin (gr/dl) | 12.6 (1.1) | 12.5 (1.85) | 0.116 |
| Platelets ($x\ 10^3/\mu l$) | 346 (103) | 352 (116) | 0.642 |
| Albumin (gr/dl) | 4.1 (0.4) | 3.9 (0.3) | 0.226 |
| ESR (mm/h) | 16 (10) | 28 (13) | 0.673 |
| CRP (mg/L) | 0.2 (0.1) | 0.4 (0.5) | 0.370 |

BMI SDS: body mass index standard deviation score, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, IQR: interquartile range, PCDAI: Pediatric Crohn's Disease Activity Index, PUCAI: Pediatric Ulcerative Colitis Activity Index, WBC: white blood cell

research in adult IBD patients without any musculoskeletal symptoms showed that a majority of the subjects had ultrasonographic findings suggestive of enthesitis, not observed in controls.[37] This trial supports the hypothesis that enthesitis is prevalent and asymptomatic in patients with IBD. Of note, compared with clinical examination US is a more sensitive method in detecting enthesitis. In a recent study, US detected enthesitis in 25 of 30 patients whereas clinical enthesitis was seen only in 15 of them.[6] Similar results had been reported in studies on concomitance of psoriasis and enthesitis, emphasizing that US is a valuable

**Table III.** Distribution of enthesitis among ligaments of lower limbs in children with inflammatory bowel disease (N=28 sites).

| Enthesitis | Left | Right | Bilateral |
|---|---|---|---|
| Hip extensor insertion at greater trochanter, n (%) | | 1 (4%) | |
| Quadriceps femoris insertion at superior portion of patella, n (%) | 1 (4%) | 2 (7%) | 6 (21%) |
| Patellar ligament insertion at tibial tuberositas, n(%) | 1 (4%) | 2 (7%) | 4 (14%) |
| Plantar fascial insertion at the calcaneus, n (%) | | 2 (7%) | 2 (7%) |
| Achilles tendon insertion at calcaneus, n (%) | 1 (4%) | 2 (7%) | 4 (14%) |

**Table IV.** Observed entheseal pathologies and involved sites.

| Entheseal pathology | Involved sites | | | | |
|---|---|---|---|---|---|
| | Hip extensor insertion at the greater trochanter | Quadriceps femoris insertion at the superior portion of patella | Patellar ligament insertion at the tibial tuberositas | Achilles tendon and fascia | Plantar fascia |
| Thickening of tendon (n=12) | 1 | 4 | 2 | 4 | 1 |
| Hypoechogenicity of tendon (n=6) | | 3 | 1 | 1 | 1 |
| Peritendinous edema (n=10) | | 3 | 2 | 5 | |
| Doppler signal (n=2) | | | 1 | 1 | |
| Loss of thickness (n=2) | | 1 | | | 1 |

examination tool.[38-41] The introduction of power Doppler increased the sensitivity, allowing visualization of abnormal vascularisation and hyperemia of the soft tissue.[41,42]

The current study also revealed that patients with increased BMI had statistically higher entheseal involvement, which had not been previously reported in pediatric patients with IBD. Similar data had been reported in several adult studies especially in patients with psoriasis related enthesitis where the increased BMI was found to be correlated with entheseal involvement.[36,37] The authors supposed that the increased BMI may have burdened ligaments of lower extremities.[38,44]

The frequency of enthesitis was found to be almost equal in both UC and CD, similar with recent trials which reported that concurrence of enthesitis was not different among patients with UC or CD.[33-36] Nevertheless patients with entheseal involvement had relatively younger age (p=0.048) with female predominance (p=0.015). Horton et al.[33] showed that there was no age and sex differences in patients with enthesitis, however Jose et al.[55] showed that extra-intestinal manifestations had been observed more frequently in girls with IBD. A female predominance of enthesial involvement was also found in studies performed on patients with psoriasis.[37,41]

Familial Mediterranean fever and inflammatory bowel disease association was reported in many studies. The prevalence of FMF in Turkish children with IBD was found to range between 15-21.2%.[42,43] Children with CD had increased FMF prevalence, whereas in UC the rate was found to be similar with Turkish healthy controls.[42,43] When patients with FMF were investigated for the presence of IBD a concomitant IBD was diagnosed in 15.4% of them.[44] Interestingly Yurtcu et al.,[45] did not find an association between FMF gene mutations and IBD phenotypic characteristics, but IBD patients without Mediterranean fever (MEFV) mutations had a statistically significant increase in extraintestinal disease frequencies. As enthesitis is also prevalent among patients with severe FMF[46] it is important to exclude FMF concurrence in children with IBD, however none

of our patients met the diagnostic criteria of FMF, hence we did not perform FMF mutation analysis.

Quadriceps femoris insertion at superior portion of patella was the most affected entheseal site, similar to the results of Weiss et al.[17] who showed that 30% of enthesitis-related arthritis in pediatric patients was at the quadriceps insertion of superior patella, followed by common extensor (12%) and Achilles (10%) tendons. Nevertheless, Cantini at al.[34] reported that the Achilles' tendon had been mostly affected, followed by proximal and distal insertions of the patellar tendons. According to other trials the patellar tendon insertion at the tibial tuberosity was affected mostly.[6,47-49] In our trial, increased tendon thickness was the most commonly detected finding which was consistent with most previous studies.[50,51] These results would help understand the differences in entheseal involvement between pediatric and adult patients, and as well as the differences between concurrent diseases.

The patients with enthesitis were more likely to have increased musculoskeletal pain than those without enthesitis.[33,51] In the current study the enthesitis was detected in patients who had more intensive pain within the previous month (p=0.001). Horton et al.[33] supposed that presence of enthesitis may reflect an inflammatory process which may cause pain or patients with enthesitis could have lower pain thresholds, or a combination of the two. The other hypothesis is that undertreated enthesitis could predispose affected individuals to experience more pain elsewhere.[33] The study of healthy school children showed that those with enthesitis reported tenderness at control sites at lower applied pressures.[52]

Even so, the presence of enthesitis did not seem to affect the level of activity in our patients. Horton et al.[33] also reported that the presence of enthesitis did not affect the level of activity in children with IBD. Palm et al.[53] investigated physical activity and quality of life in adult IBD patients with non-inflammatory joint pain (NIJP) and reported that NIJP could decrease physical activity and quality of life in these subjects. Accordingly a study on enthesitis and quality of life in patients with juvenile rheumatoid arthritis showed a marked decrease in both physical activity and quality of life.[54] The issue could be explained by the fact that these subjects had associated co-morbidities like arthritis. Further studies will provide a better understanding of these issues.

Enthesitis is responsive to treatment, but it is unclear, beyond pain relief, if the response translates to true resolution and reversal of the underlying morphological destruction.[56] There is no specified therapy for enthesitis in children with IBD, but treatment for JIA, psoriasis and SpA associated enthesitis is broadly discused.[56-58] The first line of choice for active enthesitis are non-steroidal anti-inflammatory drugs (NSAIDs), whereas in severe active enthesitis, American College of Rheumatology/Arthritis recommends using anti-TNF, anti-IL-17 agents and Janus kinase inhibitors. In children and adolescents with chronic active enthesitis unresponsive to NSAIDs, the bridging therapy with an oral glucocorticoids are recommended.[58] In our case, the treatment was tailored as sulfasalazine or ibuprofen for mild enthesitis, whereas for those with moderate disease the methylprednisolone was the choice and severe cases were switched to anti-TNF therapy.

The present study had a few limitations. First, the study was conducted with a limited number of patients. In addition, many of the drugs used to control IBD, including salicylate derivatives (used by all of our patients), cytotoxic medications such as azathioprine (used by 90%) and oral steroids (used by 12%), are also effective in the treatment of arthritis and enthesitis. This may interfere with the percentage of entheseal involvement. Moreover, we performed the study in a cohort of patients with a well-controlled disease. One could speculate that patients with more active intestinal disease and who are drug-free may have increased risk for other extraintestinal inflammatory conditions.

Two or more entheseal examinations, first at the diagnosis and second at the time of remission, would most likely give more extensive information about the entheseal involvement and response to treatment in children with IBD.

In conclusion, this trial supports the hypothesis that enthesitis is a prevalent extraintestinal manifestation of IBD regardless of the type of disease. The concurrence of enthesitis causing subclinical sign and symptoms may have impacts on musculoskeletal health of pediatric patients with IBD. A higher BMI could contribute to entheseal involvement, especially in lower extremities and aggravate the pain. Being different from adult IBD, children could have associated musculoskeletal pain which does not deteriorate their physical activity. To our knowledge, this is the first study using both physical examination and US to evaluate enthesitis in children with IBD. We supposed that powered Doppler evaluation is a more sensitive method to detect enthesitis, therefore, a combined clinical and US evaluation is probably the best approach to evaluate the presence of enthesitis in children with IBD. Further studies with more extensive cohorts are needed to evaluate the long-term impact of enthesitis in children with IBD.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Nelgin Gerenli and Betül Sözeri, data collection: Nelgin Gerenli, analysis and interpretation of results: Nelgin Gerenli and Betül Sözeri, draft manuscript preparation: Nelgin Gerenli.

All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

The trial was approved by the University of Health Sciences Umraniye Research and Training Hospital Ethics Committee (approval number 19/12/2019-26670).

## Source of funding

There has been no funding obtained for this work.

## Conflicts of interest

We declare no conflicts of interest associated with this publication.

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The Turkish Journal of Pediatrics 2021; 63: 355-362
https://doi.org/10.24953/turkjped.2021.03.002

**Original Article**

# Pediatric hospital healthcare workers and pertussis; a seroprevalence study

Selcen Özer, Vildan Avkan Oğuz

*Department of Infectious Diseases and Clinical Microbiology, Dokuz Eylul University Faculty of Medicine, İzmir, Turkey.*

**ABSTRACT**

**Background.** This study aimed to assess pertussis seroprevalence among healthcare workers (HCWs) of a university children's hospital and to determine their opinions on whether to get the pertussis vaccine booster dose in adulthood.

**Methods.** This cross-sectional study was carried out between January 2018 and March 2019. Data recording forms were filled by the face to face interview method. Anti-pertussis toxin IgG (Anti-PT IgG) antibody levels were determined quantitatively from the serum samples using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Anti-PT IgG results were interpreted according to World Health Organization (WHO) recommendations.

**Results.** Of the 169 HCWs included in the study, 67 (39.6%) were seronegative and susceptible to pertussis. Seropositivity was significantly higher among HCWs who worked 40–80 hours per week. Thirty-six (21.3%) HCWs had high anti-PT IgG levels, indicating recent infection in the past few years. High-level positivity was significantly more common among HCWs using macrolide antibiotics due to prolonged cough. Anti-PT IgG levels of three (1.8%) were compatible with acute infection and they were not followed up with suspicion of whooping cough and were not isolated. While 125 (74.0%) of the participants said they could get the pertussis vaccine booster dose, only three (1.8%) had done so.

**Conclusions.** The fact that 39.6% of HCWs were seronegative, emphasized the need for the pertussis booster dose. More than 20% of HCWs have had the acute infection without pertussis diagnosis in the past few years. Adult vaccination awareness in HCWs has not yet created a change in behavior. The high rate of seropositivity in macrolide users also shows that we cannot prevent its spread despite treatment. These findings highlight the fact that pertussis can occur in adult age groups and that eradication cannot be achieved without effective adult immunization and surveillance.

**Key words:** whooping cough, healthcare workers, seroprevalence, pertussis vaccine, attitudes.

Whooping cough is a highly contagious, vaccine-preventable disease that primarily affects infants. Primary pertussis vaccination programs have been implemented in most countries, and developed countries have high coverage rates for infants. Although the introduction of the pertussis vaccine dramatically reduced infection rates, it is known that neither infection nor immunization produces lifelong immunity to pertussis.[1] Protective immunity wanes 4-12 years after the vaccination or 4–20 years after natural infection, resulting in re-infections in adolescents and adults.[2] As a result, numerous studies have documented an increased incidence of pertussis among adolescents and adults.[3,4] In a review from our country, it was mentioned that the frequency of whooping cough increases in adolescents and adults as in many countries.[5] The main concern is that these groups may be a source of infection for young infants who have not yet completed their primary immunization.[6] In particular, symptomatic or asymptomatic pertussis infections

✉ Selcen Özer and Vildan Avkan Oğuz
selcen.es.ozer@gmail.com; vildan.oguz@gmail.com

Received 21st July 2020, revised 11th January 2021, accepted 18th January 2021.

among healthcare workers (HCWs) pose a greater threat to public health than in other adults.[7] Most infected HCWs remain undiagnosed, and treatment and isolation are not always possible, thus endangering susceptible populations in hospitals such as newborns or preterm infants. Nosocomial pertussis outbreaks show that the number of infected individuals may increase and affect the entire population.[8] The Advisory Committee on Immunization Practices (ACIP) recommends that HCWs who have direct patient contact should receive a single dose of tetanus toxoid/diphtheria toxoid/acellular pertussis vaccine (adult formulation, Tdap) if they have not been vaccinated previously as an adult, regardless of when they received their last dose of tetanus toxoid/diphtheria toxoid (Td).[9] However, the World Health Organization (WHO) emphasizes that a decision to introduce adolescent and/or adult boosters should only be taken after careful assessment of local epidemiology.[10] For this reason, several pertussis seroprevalence studies have been conducted in Turkey.[11-14] However, to the best of our knowledge, none of these studies have investigated the pertussis seroprevalence among HCWs employed in pediatric hospitals in Turkey. Therefore, the aim of this study was to evaluate the pertussis seroprevalence of HCWs working in a university children's hospital.

## Material and Methods

This cross-sectional study was approved by the Clinical Research Ethics Committee of Dokuz Eylul University hospital (decision number 13.07.2017, 2017 / 18-40).

### *Study population*

The study included all nurses, resident physicians, and specialists aged 18 and over employed in the pediatric hospital of Dokuz Eylul University between January 2018 and March 2019 who agreed to participate. Written informed consent was obtained from all participants before the study. The data registration form with questions about the participants' demographic characteristics, the units in which they worked, the patient profiles they worked with, and their immune status were completed in face-to-face interviews. The presence of cough persisting for longer than 3 weeks, the use of macrolide antibiotics due to prolonged cough, and vaccination history were evaluated based on the participants' verbal statements. They were also asked their opinions about the adult pertussis booster vaccination.

### *Sample collection and antibody detection*

Blood was collected from the study participants and the isolated serum samples were stored at - 20°C until analysis. Anti-pertussis toxin IgG (Anti-PT IgG) antibody levels were determined quantitatively from the serum samples using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cat No: El 2050-9601 G, Brand: EUROlMMUN AG, Germany) as per the manufacturer's instructions. This kit has a lower limit of detection of 0.2 IU/ml, sensitivity of 95.5–97.8%, and specificity of 100%, and conforms to the latest serological diagnostic guidelines for *Bordetella pertussis* infections.[15]

Anti-PT IgG results were interpreted according to WHO recommendations. HCWs with anti-PT IgG level <10.0 IU/ml were considered seronegative, while those with ≥10 IU/ml were regarded as seropositive. The seropositive HCWs were further classified into three groups: Low-level positivity indicating no recent exposure (anti-PT IgG 10–40 IU/mL) and high-level positivity indicating recent infection in the past few years (anti-PT IgG ≥40 IU/mL) or current/very recent (last 12 months) infection (anti-PT IgG ≥100 IU/mL). None of the HCWs had been vaccinated in the past 2 years.

### *Statistical analysis*

Kolmogorov-Smirnov test was used to assess the normality of data distributions. Descriptive statistics were presented as mean and standard deviation, median and minimum–maximum values, and percentage distribution.

Comparisons of means between two groups were performed using Student's t test for normally distributed data and Mann–Whitney U test for non-normally distributed data. Means of more than two groups were compared using analysis of variance (ANOVA) for normally distributed data and Kruskal–Wallis test for non-normally distributed data. Chi-square analysis (Fisher's exact test when necessary) was used to compare the percentage distributions between the groups. Significance level was accepted as $p < 0.05$.

## Results

Of all HCWs employed in the children's hospital (n=198), 169 (85.4%) were included in the study. The mean age of the study group was 30.4 ± 5.9 (23–52) years. Their demographic characteristics and occupational groups are presented in Table I.

The participants were divided into 3 groups according to hours worked per week: 50 (29.6%) worked ≤40 h/week, 88 (52.1%) worked 40–80 h/week, and 31 (18.3%) worked ≥80 h/week. Evaluation of these working hours by professional group is presented in Table II. All HCWs who worked ≥80 h/week were resident physicians and had been working in the children's hospital for 1 year or less (Table II).

Eighty-nine (52.7%) of the HCWs stated that they did not know their childhood pertussis vaccination status. While 125 (74.0%) of the participants said they could get the pertussis vaccine booster dose, only three (1.8%) had done so.

The mean anti-PT IgG antibody level of the entire study group was 25.1 ± 30.3 IU/ml (5–200 IU/ml). Sixty-seven (39.6%) HCWs were evaluated as seronegative (anti-PT IgG <10 IU/ml), while a total of 102 (60.4%) HCWs were seropositive (anti-PT IgG ≥10 IU/ml) and were grouped according to their antibody levels (Fig. 1). Of the 136 female HCWs of childbearing age included in the study, 104 (76.5%) had anti-PT IgG levels below 30 IU/ml.

There were no significant differences in seropositivity based on gender (p=0.35), age (p=0.21), other demographic characteristics (p>0.05), occupational group (p=0.72), or the unit in which they worked (p=0.34). Seropositivity was significantly higher among HCWs who worked 40–80 hours per week (p=0.03). The distribution of seronegative and seropositive participants according to their working hours is shown in Figure 2.

Thirty-six (21.3%) HCWs had high anti-PT IgG levels (≥40 IU/ml) indicative of recent infection in the past few years. There were no significant differences in high-level positivity based on gender (p=0.83), age (p=0.65), other demographic characteristics (p>0.05), occupational group (p=0.31), hospital department (p=0.68), working hours (p=0.68), or the presence of chronic cough in the past year (p=0.27). However, high-level positivity was significantly more common

**Table I.** Characteristics of health care workers included in the study (n=169).

| Characteristic | n (%) |
|---|---|
| Age group | |
| 19-29 years | 96 (56.8) |
| 30-39 years | 56 (33.1) |
| ≥40 years | 17 (10.1) |
| Sex | |
| Female | 138 (81.7) |
| Male | 31 (18.3) |
| Professional group | |
| Nurses | 89 (52.7) |
| Resident physicians | 56 (33.1) |
| Specialist physicians | 24 (14.2) |

**Table II.** Distribution of professional groups by hours worked per week.

| Professional group | Hours worked per week | | |
|---|---|---|---|
| | ≤40 h | 40-80 h | ≥80 h |
| | n: 50 (29.6%) | n: 88 (52.1%) | n: 31 (18.3%) |
| Nurses | 45 (90) | 44 (50) | 0 (0) |
| Resident physicians | 2 (4) | 23 (26.1) | 31 (100) |
| Specialist physicians | 3 (6) | 21 (23.9) | 0 (0) |

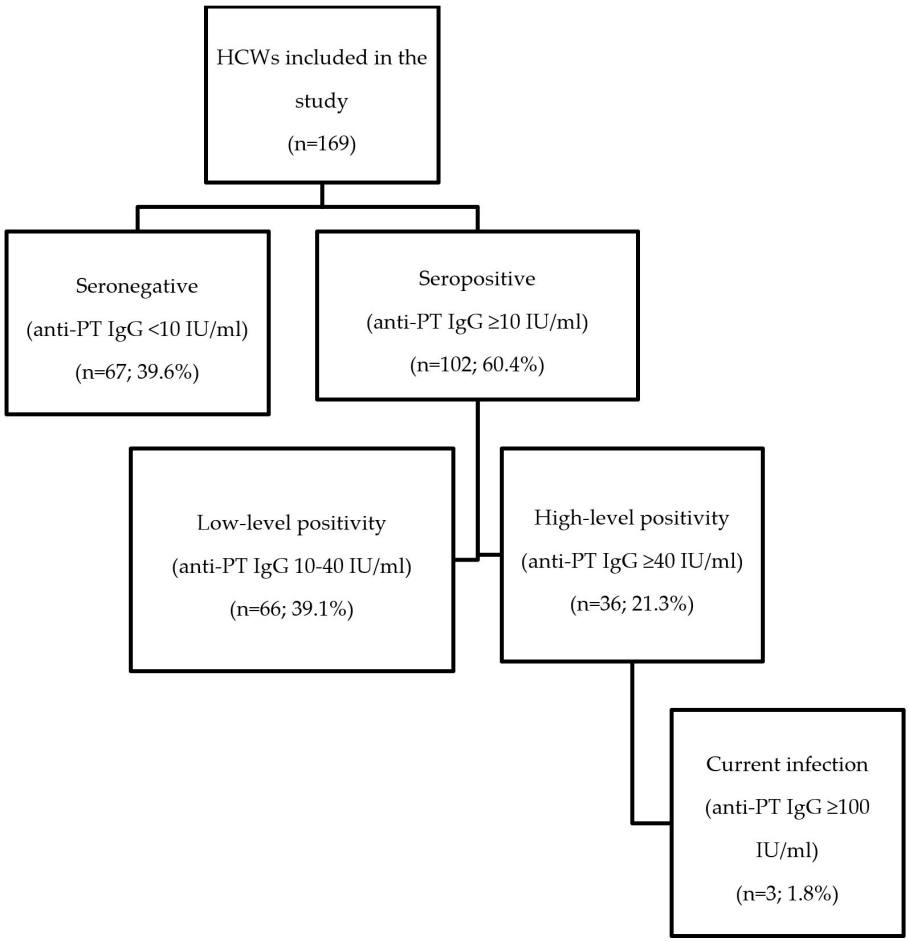


**Fig. 1.** Distribution of health care workers (HCWs) by anti-PT IgG level.

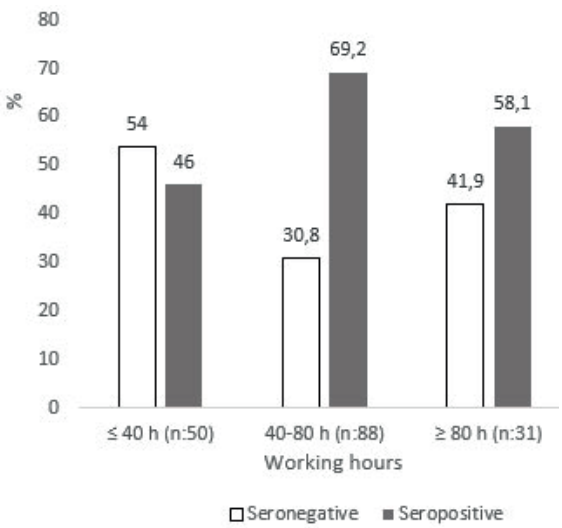


**Fig. 2.** Distribution of seronegative and seropositive health care workers (HCWs) according to hours worked per week.

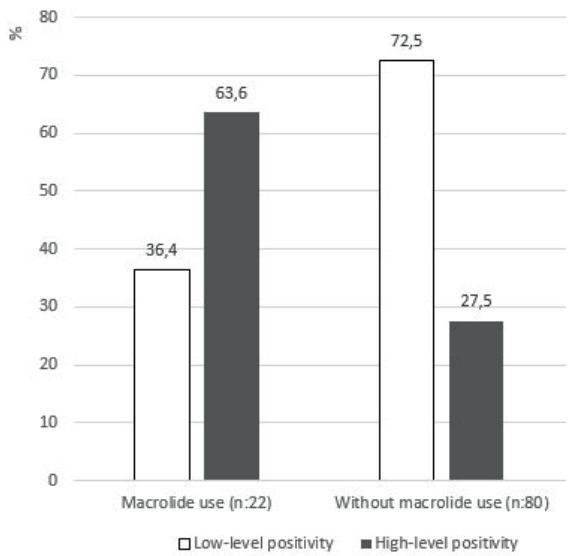


**Fig. 3.** Low-level and high-level seropositivity in health care workers with and without macrolide use due to persistent cough.

among HCWs using macrolide antibiotics due to prolonged cough than other HCWs (p=0.002) (Fig. 3).

Three female HCWs of reproductive age had antibody levels above 100 IU/ml (115.7 IU/ml, >200 IU/ml, >200 IU/ml). None of them was tested or treated for suspected whooping cough and were not isolated. One of these HCWs was a specialist in the nephrology department, one was a nurse in the pediatric intensive care unit, and the other was a nurse in the neonatal intensive care unit. The antibody levels of the three HCWs who had received the booster dose were 55.5 IU/ml, 41.9 IU/ml, and 10.8 IU/ml. Of these participants, the two women HCWs stated that they were vaccinated during pregnancy and one male HCW received the vaccine while his wife was pregnant.

## Discussion

National vaccination programs in Turkey have included pertussis vaccines since 1937. Although 92% of children under the age of 5 were vaccinated in a mass vaccination campaign conducted in Turkey in 1985, 39.6% of the HCWs in our study were seronegative and susceptible (Fig. 1). Similarly, Urbiztondo et al.[7] found that 48.3% of HCWs in their study were susceptible, while Hashemi et al.[16] and Higa et al.[17] reported seronegativity rates of 52.4% and 56.2% among HCWs respectively. These data indicate that the susceptible population is increasing in other countries as well. It is known that the protective immune response provided by primary immunization with the acellular pertussis (aP) vaccine diminishes faster than with the whole cell (wP) vaccine and the duration of protection is 5–6 years.[18] Because the aP vaccine was introduced to Turkey in 2007, it is impossible for HCWs, nearly half of whom are susceptible, not to be affected by pertussis. As such, the development of a nosocomial pertussis epidemic is inevitable. Therefore, adult vaccination programs should be revised and pediatric HCWs should be considered a priority group to receive the pertussis vaccine booster dose.

Our results showed that 60.4% of HCWs in this study were seropositive. In a community-based seroprevalence study, Esen et al.[11] reported 60.8% seropositivity among 2.085 volunteers aged 0– 60 years. In other studies from our country, pertussis seroprevalence rates between 8.5% and 90% were determined using different kits and thresholds in different age groups.[11-14,19,20] Only one study by Tanriover et al.[14] included HCWs, and even then they only represented 2.6% of the study group. Although no previous study conducted in Turkey focused exclusively on HCWs, pertussis seroprevalence among HCWs has been evaluated in many other countries, with results similar to ours.[7,16,17,21,22] Anti-PT antibody seropositivity in HCWs has been reported at rates of 51.7% by Urbiztondo et al.[7], 43.8% by Higa et al.[17] among medical staff at a Japanese university hospital, and 47.6% among Iranian medical students in a study by Hashemi et al.[16] The reported prevalence varies between 10.2% and 98.7% in other studies conducted among HCW in different countries.[7,16,17,21,29] This wide range may be due in part to the use of different antigens and cut-off values, but may also reflect different epidemiological conditions.[24] Although anti-PT IgG levels were determined using the ELISA method in all of those studies, it is difficult to compare their results due to the absence of an FDA-approved ELISA kit.[8] For this reason, multicenter studies using the same kit and reference ranges are needed.

In our study, 76.4% of female HCWs of childbearing age had anti-PT IgG levels below the protective maternal antibody level (≤30 IU/ml). Similarly, in another study from Turkey, Esen et al.[11] reported that 51.7% of women of childbearing age women had antibody levels of ≤30 IU/ml. The fact that most female HCWs of childbearing age do not have a protective maternal antibody level shows that they can be a source of infection not only for their patients but also for their own children.

We observed a significantly higher seropositivity rate among the HCWs in our study who worked 40–80 hours per week (p=0.03). We believe

the low rate of seropositivity among HCWs working >80 hours per week can be explained by the fact that all of these HCWs were resident physicians who had worked at the children's hospital for one year or less. Similarly, Cunegundes et al.[21] reported that the risk of pertussis was highest among HCWs working 40 hours per week or more. In the present study, 21.3% of the HCWs had high-level anti-PT IgG positivity, suggestive of recent infection. This finding may be attributed to the increased risk of acquiring pertussis in HCW or the burden of disease in the community. Esen et al.[11] reported that 37.2% of their study group had antibody levels consistent with infection within the past few years. Ben Fraj et al.[28] reported high-level positivity in 11.4%, Urbiztondo et al.[7] in 7.8%, and Cunegundes et al.[21] in 6.4% of HCWs in their respective studies. Compared to studies in other countries, high-level positivity was more common in our study. Considering the cyclic pattern of pertussis, this may be related to the varying disease burden in different years and different countries. However, the WHO emphasized that this variability may result from different vaccination strategies utilized by countries in the past and present, and each country should consider local epidemiology while developing vaccination strategies.[6] The high-level positivity rates in our study, which indicate the persistence of circulation among HCWs, highlight the need for new vaccine strategies.

In our study, 63.6% of HCWs who used macrolide antibiotics due to chronic cough had serology suggestive of recent infection. In addition, high-level positivity was significantly more common in this group. It has been suggested that the use of empirical macrolides leads to misdiagnosis by masking clinical findings and decreasing the sensitivity of diagnostic methods in these HCWs. Therefore, to reduce the burden of pertussis and prevent transmission, pertussis should be suspected and serological methods used for diagnosis of adult patients with prolonged cough.

Rates of acute pertussis infection range from 1.3 to 4.5% in seroprevalence studies conducted among HCWs.[7,17,27,28] In our study, 1.8% had anti-PT IgG levels ≥100 IU/ml, indicative of active or very recent infection (within the last 12 months). One of these HCWs worked in the neonatal intensive care unit, and should have been isolated according to infection control guidelines. However, none of these three HCWs was suspected of a possible infection or isolated. Similarly, Koivisto et al.[27] reported that two nurses who had serological evidence of recent pertussis infection were not isolated. This finding is alarming, as asymptomatic transmission is known to be a risk factor for the spread of pertussis.

Vaccination coverage among HCWs varies from country to country according to vaccine strategies and awareness level. Some countries, such as the United States, Canada, Australia, the Netherlands, Germany, and the United Kingdom adopted booster dosing for all HCWs, while others, such as Austria, Finland, Norway, and Brazil, introduced Tdap only for those in contact with newborns and infants.[30] Nevertheless, it is also recognized that vaccine coverage among HCWs is still very low in all countries.[31] Although 74.0% of HCWs in our study stated that they considered a booster dose necessary and reliable, only 1.8% had gotten the booster dose. Similarly, Top et al.[32] reported that 76% of HCWs working in a children's hospital expressed an intention to get immunized, yet only 15% presented to a clinic where the pertussis booster vaccine was being provided free of charge. Urbiztondo et al.[7] determined that none of the HWCs in their study had received a booster dose, while Faruque et al.[24] reported that 23% had a booster dose.

The fact that 39.6% of HCWs in our study showed inadequate immunity highlights the need for the pertussis booster dose in HCWs. HCWs with acute infection act as infection sources for other workers and patients in the hospital. Most of the HCWs expressed a belief that the pertussis booster vaccine is necessary and safe;

however, the fact that hardly any of them had received the booster dose demonstrates that this awareness did not affect a change in behavior. All of these findings highlight that pertussis can occur in all age groups and that eradication cannot be achieved without effective adult immunization and surveillance.

**Acknowledgement**

Thanks to all healthcare workers who participated in our study.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: Selcen Özer and Vildan Avkan Oğuz; data collection: Selcen Özer; analysis and interpretation of results: Selcen Özer and Vildan Avkan Oğuz; draft manuscript preparation: Selcen Özer and Vildan Avkan Oğuz. All authors reviewed the results and approved the final version of the manuscript.

**Ethical approval**

This cross-sectional study was approved by the Clinical Research Ethics Committee of Dokuz Eylul University hospital (decision number 13.07.2017, 2017 / 18-40).

**Source of funding**

The study (Project no: 2018.KB.SAG.013) received financial support by Dokuz Eylul University Department of Scientific Research Projects.

**Conflict of interest**

None of the authors have a conflict of interest to declare.

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The Turkish Journal of Pediatrics 2021; 63: 363-371
https://doi.org/10.24953/turkjped.2021.03.003

**Original Article**

# Children with cervical lymphadenopathy: reactive or not?

Hilal Susam Şen[1], Süheyla Ocak[1], Pınar Yılmazbaş[2]

*[1]Division of Pediatric Oncology, [2]Department of Pediatrics, Health Sciences University, Okmeydanı Training and Research Hospital, İstanbul, Turkey.*

## ABSTRACT

**Background.** This study aims to evaluate the etiology of cervical lymphadenopathies in children and to define the significance of demographic, clinical, and laboratory features in the prediction of malignancy.

**Methods.** Medical records of 527 patients were reviewed retrospectively between 2015 and 2019. The patients were examined in terms of demographics, clinical, radiologic, and serologic findings. A lymph node biopsy was performed in selected patients. The risk factors for malignancy were evaluated.

**Results.** Out of 527 children, 26 had neck masses mimicking lymphadenopathy; 501 had lymphadenopathy. The most common location was the anterior cervical region and the median age was 5.7 years. Thirty-nine patients had malignancy (lymphoma in 34, nasopharyngeal carcinoma in 3, leukemia in 1 and neuroblastoma in 1). The risk of malignancy was associated with older age, duration of >4 weeks, lymph node size >3 cm, supraclavicular location, presence of systemic symptoms, and hepatosplenomegaly ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$). On laboratory evaluation, anemia, leukocytosis, and increased erythrocyte sedimentation rate were found to be associated with malignancy ($p<0.001$, $p=0.003$, $p<0.001$).

**Conclusions.** Cervical lymphadenopathies in children are generally benign but patients with persisting cervical lymphadenopathy, adolescent age, accompanying systemic symptoms and abnormal laboratory findings should be considered for an early biopsy.

**Key words:** cervical lymphadenopathy, children, malignancy, reactive lymphoid hyperplasia, supraclavicular location.

Neck masses in children have a wide range of differential diagnosis including congenital anomalies, infectious or inflammatory diseases, and malignant lesions.[1] Cervical lymphadenopathy (LAP) is the most common cause of a neck mass in childhood with estimates of 38 to 45% of otherwise healthy children.[2] Up to 90% of children between the ages of 4-8 years have palpable cervical lymph nodes due to a physiologic increase in lymphoid tissue by age.[3] Acute upper respiratory tract infections and infectious lymphadenitis are the major causes of enlarged cervical lymph nodes which usually regress spontaneously or with medication. Also, reactive hyperplasia of lymphatic tissue mainly due to local inflammation is very common in children. However, in clinical practice accurate and prompt diagnosis of malignancy is of importance, even if it is relatively rare. The primary goal in dealing with cervical masses in children is to define the essential workup and intervention algorithms for the diagnosis of malignant lesions while avoiding unnecessary diagnostic tests and therapies.[4] Several studies have shown that progressive increase in size, unresponsiveness to therapy, presence of systemic signs and symptoms, and abnormal leukocyte counts and high erythrocyte sedimentation rate (ESR) may indicate malignancy.[2] Aside from the malignancy, the presence of endemic diseases or regional factors affect the distribution of non-malignant diagnosis admits with cervical LAP. In this study, we evaluated 527 children with cervical mass to determine common etiologies

✉ Hilal Susam Şen
hilalsusam@hotmail.com

Received 10th June 2020, revised 29th July 2020, 15th September 2020, accepted 28th September 2020.

for cervical LAP in children and to define any clinical, laboratory, and pathological findings alarming for malignancy.

## Material and Methods

In this retrospective single center study 527 consecutive patients under the age of 19 years admitted to the Pediatric Oncology Department from 2015 to 2019 were evaluated. From each record we collected information regarding age, gender, medical history (including dental problems), sore throat, fever, cough, history of upper respiratory tract infection, animal contact and travel, duration of symptoms, and clinical course. Physical examination was noted for the location, size, laterality, mobility, and tenderness of the lymph node; associated systemic symptoms including weight loss, night sweats, fever, and hepatosplenomegaly for each patient. The patients were categorized into age categories (0–23 months, ≥24 months to <12 years, and ≥12 to 19 years). Complete blood count, ESR, lactate dehydrogenase (LDH), and C-reactive protein (CRP) levels were noted. Additional studies (the tuberculin skin test, serological tests for Epstein-Barr virus (EBV), cytomegalovirus, rubella, toxoplasmosis, Brucella, Mycobacterium tuberculosis, and human immunodeficiency virus, bone marrow examination, two-dimensional chest X-ray and ultrasonography) were also performed based on the clinical features. An excisional biopsy was performed in the case of suspected malignancy.

The lymph nodes were classified according to the anatomical region, size (<1 cm, 1-3 cm, >3 cm), extension (unilateral and bilateral), the number of involved sites (localized / one anatomic region or generalized / 2 or more noncontiguous lymph node regions) and the duration (less than 2 weeks, 2-4 weeks and >4 weeks). Lymph node size, extension, and the number of involved sites were evaluated by physical examination.

The clinical and laboratory features of malignant and benign cases were evaluated separately. Also, the cases with the benign diagnosis were grouped in eight as follows: reactive lymphoid hyperplasia, reactive micro-lymphadenopathy (<1 cm), EBV infection, infectious lymphadenitis, cat-scratch disease, abscess, tuberculosis lymphadenitis, and others (such as Castleman disease). Reactive lymphoid hyperplasia was described as a normally shaped and structured, but enlarged lymph node with a history of the previous infection and the absence of clinical signs of inflammatory disease. Children with complaints of snoring, sleeping difficulty, breathing through the mouth, hypo nasal speech, and recurring respiratory infections were noted for adenoid vegetation.

The data were analyzed using the statistical package for social sciences (SPSS, version 18). Comparisons of groups were made by using Chi-square or Fisher's exact test. A p-value of less than 0.05 was considered significant.

This study was approved by the Non-interventional Clinical Research Ethics Board of Health Science University Okmeydani Training and Research Hospital (2019/1304). All parents gave written informed consent for the study. It was performed in compliance with the 2009 Declaration of Helsinki.

## Results

Between 2015 and 2019, a total of 527 patients referred to our hospital with complaints of neck masses. Out of 527 patients, 26 who had neck masses mimicking LAP are listed in Table I. Their ages ranged from 1 month to 16.2 years with a median of 5.7 years. Most patients were male (male/female:16/8).

Of the 527 patients, 501 had cervical lymphadenopathy. Of these, 333 (66.5%) were males and 168 (33.5%) were females with a median age of 7.3±4.2 years (0.4-18 years). The diagnosis of cases with cervical LAP is listed in Table II. The median ages of patients with benign and malignant LAP were 6.8 years (range 4.8 months-18 years) and 12.7 years (range, 2.1-17.1 years) respectively.

**Table I.** Neck masses mimicking lymphadenopathy.

| Diagnosis | Number |
|---|---|
| Torticollis | 4 |
| Thyroglossal duct | 4 |
| Lymphangioma | 4 |
| Branchial cleft cyst | 3 |
| Neurofibroma | 2 |
| Pilomatrixoma | 2 |
| Ectopic thymus | 1 |
| Hemangioma | 1 |
| Vascular malformation | 1 |
| Fibromatosis | 1 |
| Nodular fasciitis | 1 |
| Benign lymphoepithelial cyst | 1 |
| Spindle cell mesenchymal tumor | 1 |

**Table II.** Diagnosis of cases with cervical lymphadenopathy.

| Diagnosis | Number | % |
|---|---|---|
| Malignant | 39 | 100 |
| Hodgkin lymphoma | 29 | 74.3 |
| Non-Hodgkin lymphoma | 5 | 12.8 |
| Nasopharyngeal carcinoma | 3 | 7.6 |
| Leukemia | 1 | 2.5 |
| Neuroblastoma | 1 | 2.5 |
| Benign | 462 | 100 |
| Reactive lymphoid hyperplasia | 321 | 69.4 |
| Unknown | 199 | 43 |
| Viral upper respiratory infection | 67 | 14.5 |
| Adenoid vegetation | 23 | 4.9 |
| Tooth decay | 9 | 1.9 |
| Tonsillitis | 9 | 1.9 |
| Dermatitis on scalp | 5 | 1 |
| Other | 9 | 1.9 |
| Reactive micro-lymphadenopathy | 78 | 16.8 |
| Ebstein-Barr virus infection | 32 | 6.9 |
| Lymphadenitis | 11 | 2.3 |
| Cat scratch disease | 7 | 1.5 |
| Abscess | 5 | 1 |
| Tuberculosis lymphadenitis | 4 | 0.8 |
| Castleman disease | 2 | 0.4 |
| Chronic granulomatosis disease | 1 | 0.2 |
| Rosai-Dorfman disease | 1 | 0.2 |

Cervical LAP was unilateral in 120 children (23%) and bilateral in 381 (76%) cases. The most common etiology was reactive lymphoid hyperplasia (69%). A specific etiology such as Ebstein-Barr virus infection, acute infectious lymphadenitis, cat-scratch disease, abscess, and tuberculosis lymphadenitis was found in only 12% of patients in children with a benign diagnosis. Hodgkin lymphoma was the most common cause of the malignant LAP group (74%). Empirical antibiotic therapy was started for all infectious lymphadenitis and most of the reactive lymphoid hyperplasia with the LAP size larger than one cm. Lymph node biopsy was performed in 62 (12%) cases because of one of the following factors: fixed and hard lymph nodes, supraclavicular localization, progressive increase in size, unresponsiveness to empirical antibiotics, the presence of constitutional symptoms like a fever of unknown origin, night sweats or weight loss. Thirty-eight (61.3%) of 62 biopsies were reported to be malignant. All patients who had undergone biopsy were over the age of two years. Histopathological diagnosis according to the age groups is shown in Table III.

The most common localizations were upper anterior cervical (43%) and submandibular region (27%). The local features, clinical characteristics, and laboratory results of benign and malignant LAP groups are compared in Table IV. Patients between the ages of 12 and 19 years were mainly presented with chronic and

**Table III.** Histopathological diagnoses of the biopsies according to age groups.

| Diagnosis | 2-12 years (n=30) | 12-18 years (n=32) |
|---|---|---|
| Reactive lymphoid hyperplasia | 11 | 4 |
| Hodgkin lymphoma | 8 | 21 |
| Non-Hodgkin lymphoma | 3 | 2 |
| Nasopharyngeal carcinoma | 2 | 1 |
| Neuroblastoma | 1 | 0 |
| Other* | 5 | 4 |

*: Tuberculosis lymphadenitis, cat scratch disease, castleman disease

**Table IV.** Clinical characteristics and laboratory results of lymphadenopathy in the benign and malignant groups.

| | Benign LAP (n=461) | | | Malignant LAP (n=39) | |
|---|---|---|---|---|---|
| | n | % | | n | % |
| Age group | | | | | |
| 0-2 years | 43 | (9.3) | | 0 | 0 |
| 2-12 years | 363 | (78.7) | | 15 | (38.5) |
| 12-18 years | 55 | (11.9) | | 24 | (61.5) |
| | | | *p < 0.001* | | |
| Location | | | | | |
| Supraclavicular | 1 | (0.2) | | 22 | (56.4) |
| Other | 460 | (99.8) | | 17 | (43.6) |
| | | | *p < 0.001*[x] | | |
| Extension | | | | | |
| Local | 439 | (95) | | 34 | (87) |
| Generalized | 22 | (5) | | 5 | (13) |
| | | | *p= 0.033* | | |
| Size | | | | | |
| <1 cm | 101 | (21.9) | | 0 | 0 |
| 1-3 cm | 327 | (70.9) | | 7 | (17.9) |
| >3 cm | 33 | (7.2) | | 32 | (82.1) |
| | | | *p < 0.001* | | |
| Duration | | | | | |
| <2weeks | 36 | (7.8) | | 1 | (2.6) |
| 2-4 weeks | 274 | (59.4) | | 8 | (20.5) |
| >4 weeks | 151 | (32.8) | | 30 | (76.9) |
| | | | *p < 0.001* | | |
| Laterality | | | | | |
| Bilateral | 366 | (79.4) | | 15 | (38.5) |
| Unilateral | 95 | (20.6) | | 24 | (61.5) |
| | | | *p < 0.001* | | |
| Hepatosplenomegaly | | | | | |
| Positive | 13 | (2.8) | | 7 | (17.9) |
| Negative | 448 | (97.2) | | 32 | (82.1) |
| | | | *p < 0.001*[x] | | |
| Associated symptoms [xx] | | | | | |
| Positive | 36 | (7.8) | | 15 | (38.5) |
| Negative | 425 | (92.2) | | 24 | (61.5) |
| | | | *p < 0.001*[x] | | |
| Pathological LAP [xxx] | | | | | |
| Yes | 63 | (13.7) | | 39 | (100) |
| No | 398 | (86.3) | | 0 | 0 |
| | | | *p < 0.001* | | |

[x]: Fisher's Exact test; [xx]: fever, night sweats, weight loss; [xxx]: hard, fixed, conglomerate

**Table IV.** Continued.

| | Benign LAP (n=461) | | | Malignant LAP (n=39) | |
|---|---|---|---|---|---|
| | n | % | | n | % |
| Course of LAP | | | | | |
| Same size/increase in size | 123 | (26.7) | | 39 | (100) |
| Decrease in size | 338 | (73.3) | | 0 | 0 |
| | | | *p < 0.001* | | |
| Anemia | (n=450) | | | | |
| Yes | 72 | (16) | | 23 | (59) |
| No | 378 | (84) | | 16 | (41) |
| | | | *p < 0.001* | | |
| Leukocytosis | (n=450) | | | | |
| Yes | 109 | (24.2) | | 18 | (46.2) |
| No | 341 | (75.8) | | 21 | (53.8) |
| | | | *p = 0.003* | | |
| Platelet count | (n=450) | | | | |
| Elevated | 30 | (7) | | 12 | (30) |
| Normal/low | 420 | (93) | | 27 | (70) |
| | | | *p < 0.001* | | |
| Erythrocyte sedimentation rate | (n=247) | | | (n=36) | |
| Normal | 207 | (83.8) | | 15 | (41.7) |
| Elevated | 40 | (16.2) | | 21 | (58.3) |
| | | | *p < 0.001* | | |
| Lactate dehydrogenase | (n=252) | | | (n=38) | |
| Normal | 241 | (95.6) | | 36 | (94.7) |
| Elevated | 11 | (4.4) | | 2 | (5.3) |
| | | | *p = 0.682* | | |
| C- reactive protein | (n=198) | | | (n=32) | |
| Normal | 140 | (70.7) | | 19 | (59.4) |
| Elevated | 58 | (29.3) | | 13 | (40.6) |
| | | | *p = 0.198* | | |

[x]: Fisher's Exact test; [xx]: fever, night sweats, weight loss; [xxx]: hard, fixed, conglomerate

localized LAP and a biopsy was performed in 40% of the age group. Clinical characteristics of LAP according to the age groups are shown in Table V.

## Discussion

Cervical LAP in children is one of the most common reasons for admission to pediatric departments.[1-3] As seen in our series, neck masses including congenital cystic lesions, vascular malformations, and benign tumors may also mimic LAP and be referred to a pediatrician or a pediatric oncologist.[3,5]

The various diseases may cause cervical LAP; the most common etiology in children is reactive lymphoid hyperplasia following a viral or bacterial infection that resolves without any sequela within a limited period.[2,6] In our study,

**Table V.** Clinical characteristics of lymphadenopathy according to the age groups.

| | 0-2 years (n=43) | | 2-12 years (n=378) | | 12-18 years (n=80) | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| Gender | | | | | | |
| Male | 33 | (76.7) | 242 | (64) | 58 | (72.5) |
| Female | 10 | (23.3) | 136 | (36) | 22 | (27.5) |
| Extension | | | | | | |
| Local | 43 | (100) | 360 | (95.2) | 71 | (88.8) |
| Generalized | 0 | 0 | 18 | (4.8) | 9 | (11.2) |
| Duration | | | | | | |
| <2weeks | 8 | (18.6) | 25 | (6.6) | 4 | (5) |
| 2-4 weeks | 23 | (53.5) | 225 | (59.5) | 34 | (42.5) |
| >4 weeks | 12 | (27.9) | 128 | (33.9) | 42 | (52.5) |
| Laterality | | | | | | |
| Bilateral | 32 | (74.4) | 303 | (80.2) | 46 | (57.5) |
| Unilateral | 11 | (25.6) | 75 | (19.8) | 34 | (42.5) |
| Size | | | | | | |
| <1 cm | 27 | (62.8) | 65 | (17.2) | 9 | (11.3) |
| 1-3 cm | 14 | (32.6) | 279 | (73.8) | 42 | (52.5) |
| >3 cm | 2 | (4.7) | 34 | (9) | 29 | (36.3) |
| pathological LAP[x] | | | | | | |
| Positive | 4 | (9.3) | 58 | (15.3) | 41 | (51.3) |
| Negative | 39 | (90.7) | 320 | (84.7) | 39 | (48.8) |
| Hepatosplenomegaly | | | | | | |
| Positive | 1 | (2.3) | 14 | (3.7) | 5 | (6.3) |
| Negative | 42 | (97.7) | 364 | (96.3) | 75 | (93.8) |
| Associated symptoms [xx] | | | | | | |
| Positive | 2 | (4.7) | 36 | (9.5) | 13 | (16.3) |
| Negative | 41 | (95.3) | 342 | (90.5) | 67 | (83.8) |
| Course of LAP | | | | | | |
| Decrease in size | 23 | (53.5) | 274 | (72.5) | 41 | (51.3) |
| Same size | 20 | (46.5) | 99 | (26.2) | 30 | (37.5) |
| Increase in size | 0 | 0 | 5 | (1.3) | 9 | (11.3) |
| Biopsy | | | | | | |
| Yes | 0 | 0 | 30 | (7.9) | 32 | (40) |
| No | 43 | (100) | 348 | (92.1) | 48 | (60) |

[x]:hard, fixed, conglomerate; [xx]:fever, night sweats, weight loss

nearly 80% of patients had reactive lymphoid hyperplasia and this percentage is higher than the percentage in other studies.[2,3] The reason for this high percentage can be explained by the inclusion of LAP smaller than one cm in our study.

In cervical LAP, clinical history, physical examination, and laboratory findings provide valuable signs for the diagnosis of the majority of cases.[5] However, excisional biopsy is required in some cases for the definitive diagnosis. The rate of reported incidences of

malignancy in pediatric cervical LAPs varies from 1% to 72%.[6-11] In our study, the pathologic examination was required in 12% of the cases and malignant neoplasms were determined in 62% of the biopsy specimens (7% of all cases). In a prospective study, malignancy was reported in 2.7% of patients.[12] In another study by Yaris et al.[13] a biopsy was performed in 38.7% of the cases and malignant neoplasms were determined in 60% of biopsy specimens. In our study, the biopsy rate was lower than other studies because the children with cervical LAP were more frequently referred from the primary care to the pediatric oncology in fear of malignancy and as a result, the indication for biopsy was decided by a pediatric oncologist. However, the percentage of malignancy in biopsy specimens was similar to other studies reported by Pediatric Oncology- Departments.[13,14]

The studies demonstrated that lymphomas were the leading causes of cervical LAP in older children.[6,13,14] Our results were compatible with previous studies as Hodgkin lymphoma was the leading cause of the malignant LAP group in our study. Similar results were reported by Celenk et al.[15] who introduced Hodgkin lymphoma as the most common malignant diagnosis. On the other hand, non-Hodgkin lymphoma was reported to be the most common malignant cause of LAP in another study in which regions other than the cervical region were also included.[16]

The risk of malignancy in peripheral LAP increases with age.[13] In our study, 30% of patients in the 12-19 age group represented with malignant LAP. The size and duration of the LAP and local and systemic symptoms were suggested to predict malignancy and to help the decision of performing an excisional biopsy.[12,16] Supraclavicular LAP has a particularly high prevalence of malignancy compared to other sites.[4,13] Almost all patients (22/23) with supraclavicular localization had malignancy in our series and it is suggested that the supraclavicular LAP should be examined with a high suspicion of malignancy. Oguz and colleagues reported that the risk of malignancy in cervical LAP increases with the size of the lymph node, duration of the LAP, associated symptoms, and hepatosplenomegaly, which is compatible with our data.[17] In our study, lesions smaller than one cm on palpation were always associated with a benign etiology, whereas half of the lesions bigger than three cm were due to malignancies. Besides, most of the LAP of 1-3 cm in size were in the benign group. In a manner consistent with other studies,[12,14,17,18] our results showed that LAP larger than three cm in diameter had increased risk of malignancy. Based on these data, it is possible to say that the upper limit of LAP size can be three cm or more to be considered as a malignant process. Kumral et al.[14] demonstrated the elevated risk for malignancy with symptoms that last more than 4 weeks. The duration of LAP reported in some studies was not associated with the presence of serious pathology.[9,18] Besides, in a prospective study, the onset time of symptoms was similar between malignant and benign LAP groups.[12] Malignancy was more common in the chronic LAP group in our study. However, this might be associated with delayed admission to healthcare as awareness of pediatric cancer is low in our population.

Unlike other studies, systemic symptoms like fever, night sweating, and weight loss were more frequent in malignant cases which can be explained by the predominance of Hodgkin lymphomas in our study.[15,18] It has been shown that bilateral cervical LAP is an indicator of a reactive disease and malignancy is not associated with bilaterality.[18,19] Similar to those, 61.5 % of malignant LAP group was presented with unilateral LAP in our study. Besides, the frequency of unilateral LAP in the malignant LAP group was statistically higher than those in the benign LAP group and was associated with malignant disease (p=0.033).

Local features of cervical LAP may be predictive of malignancy. The lack of mobility seems to be highly associated with malignancy, which was also confirmed by Oguz et al.[17] We found that hard, fixed, and conglomerated LAP was associated with malignancy. Soldes

et al.[18] reported similar results with increased malignancy in hard, fixed lymph nodes. Conversely, the consistency of the LAP was reported as being unhelpful for differential diagnosis in another study.[6] Therefore, localized or generalized LAP, and bilaterality or unilaterality may be misleading in determining whether the cause of cervical LAP is benign or malignant.

Previous studies showed that LAP in children regressed either spontaneously or with medication in most cases.[12] And it is recommended that if the LAP does not regress in four weeks despite antibiotics, a biopsy should be performed.[18] In our study, 67% of our patients' LAP regressed without further intervention. We consider that the enlargement or stable size of LAP in the follow-up can be a predictive factor for malignancy.

Simple hematological tests are usually required in children with cervical LAP. In our study, the risk of malignancy increased in the presence of anemia, leukocytosis, thrombocytosis, and elevated ESR. However, LDH and CRP levels were not associated with malignancy. Oguz et al. reported that anemia, leukocytosis, elevated ESR were associated with malignancy, which is compatible with our data.[17] Reactive thrombocytosis due to malignancies and in particular those related to lymphomas have been well-defined.[20] Conversely, children with thrombocytopenia were found to have a greater frequency of malignant LAP in some studies.[12,17] The predominance of thrombocytosis in the malignant LAP group can be explained by the fact that most of the malignant cases were lymphoma in our study.

In conclusion, we found a low incidence of malignancy in cervical LAP in contrast to the other Pediatric Oncology-Hematology Departments. Children older than the age of 12 years, presented with LAP bigger than three cm, a history of more than four weeks, and supraclavicular localization in combination with abnormal complete blood count and LDH levels should alert the pediatrician for malignancy. But as studies are inconclusive on the predictive role of laterality, consistency, or conglomeration of lymph nodes, the differential diagnosis should not be used for decision making in cervical lymphadenopathy in children.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Hilal Susam Şen, Süheyla Ocak; data collection: Hilal Susam Şen, Pınar Yılmazbaş; analysis and interpretation of results: Hilal Susam Şen; draft manuscript preparation: Hilal Susam Şen, Pınar Yılmazbaş, Süheyla Ocak.

All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

This study was approved by the Non-interventional Clinical Research Ethics Board of Health Science University Okmeydani Training and Research Hospital (2019/1304).

## Source of funding

No funding or other financial support for each author.

## Conflict of interest

None declared.

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The Turkish Journal of Pediatrics 2021; 63: 372-383
https://doi.org/10.24953/turkjped.2021.03.004

**Original Article**

# Healthcare workers' knowledge level regarding anaphylaxis and usage of epinephrine auto-injectors

Mustafa Arga[1], Erdem Topal[2], Sıla Yılmaz[3], Pınar Canızcı Erdemli[3], Kübra Bıçakcı[3], Arzu Bakırtaş[4]

*Departments of [1]Pediatric Allergy and Immunology, [3]Pediatrics, İstanbul Medeniyet University Faculty of Medicine, İstanbul; [2]Department of Pediatric Allergy and Immunology, İnonu University Faculty of Medicine, Malatya; [4]Department of Pediatric Allergy and Asthma, Gazi University Faculty of Medicine, Ankara, Turkey.*

## ABSTRACT

**Background.** Inadequate practices in diagnosis and management of anaphylaxis in parallel with an increase in its prevalence may cause serious public health problems today. This is the first study aiming to assess the theoretical knowledge of professional and non-professional healthcare workers from different lines of the healthcare service chain about anaphylaxis management, and their practice approaches for epinephrine auto-injectors (EAIs) together.

**Methods.** The study included 697 participants comprising physicians, dentists, pharmacists, and school staff. In face-to-face interviews, each participant was asked to fill out the questionnaire forms prepared for assessing their demographic characteristics, experience with a case of anaphylaxis and EAI and theoretical knowledge about the diagnosis and treatment of anaphylaxis, and to demonstrate how to use EAI in practice with trainer device.

**Results.** The rates of 391 physicians, 98 dentists, 102 pharmacists and 105 school staff of knowing the diagnosis criteria of anaphylaxis were 47.6%, 31.6%, 31.1%, 19%, and knowing the first and life-saving treatment of anaphylaxis were 87.2%, 79.6%, 47.6%, 15.2%, respectively. Predictors that affected physicians in knowing the first and life-saving treatment of anaphylaxis were having experience with EAIs [OR:5.5, (%95CI:1.330-23.351, p=0.015)] and a case of anaphylaxis [OR:2.4, (%95CI:1.442-4.020, p=0.001)], and knowing the administration route of epinephrine correctly [OR:1.9, (%95CI:1.191-3.314, p=0.008)]. 31.1% of the participants demonstrated the EAI usage correctly. The EAI usage steps with the most errors were "Place the appropriate injection tip into outer thigh/Press the trigger so it 'clicks'" and "Turn the trigger to arrow direction" (60.3% and 34.9%, respectively).

**Conclusions.** Healthcare workers' knowledge level regarding anaphylaxis management and ability to use EAIs correctly are not adequate. That most errors were made in the same steps of EAI usage indicates that the industry should continue to strive for developing the ideal life-saving device.

**Key words:** anaphylaxis, epinephrine auto-injectors, healthcare workers, knowledge, management.

Anaphylaxis is a systemic hypersensitivity reaction that develops suddenly and may threaten life.[1] Therefore, early diagnosis and correct treatment of anaphylaxis is vital.[1] Its first and life-saving treatment is epinephrine.[2] It may recur despite all preventive measures.[1] Therefore, it is required to prepare an individual emergency written action plan for each patient[2,3] Epinephrine auto-injector (EAI) is one of the most important components of emergency action plans.[2] Physicians should provide patients and/or parents with a theoretical and practical training on when and how to use EAIs.[2] However, physicians worldwide may still perform critically inadequate practices in anaphylaxis management.[4] In most countries, pharmacies are the places where patients obtain

✉ Mustafa Arga
mustafarga@gmail.com

Received 16th September 2020, revised 24th November 2020, accepted 6th December 2020.

EAIs prescribed by physicians. The fact that pharmacists have adequate level of knowledge regarding anaphylaxis management and EAI usage and give patients and/or parents training on EAI usage may provide an important opportunity for rectifying potential errors in anaphylaxis management.

Most anaphylaxis episodes develop in non-hospital settings such as homes and public spaces. Considering the increase in food allergy prevalence in childhood in recent years, prevalence of anaphylaxis increases day by day in kindergartens and schools.[5] International and national allergy associations propose suggestions and provide support for reducing this risk and manage anaphylaxis optimally in case it develops in school settings.[6-9] It is a requirement that school managers prepare an emergency action plan regarding how to act in cases of anaphylaxis and how to treat it in their institutions, determine professional (e.g. physician, nurse) or non-professional [non-nursing staff (e.g. teacher, school staff)] healthcare workers and ensure they receive related training.[6-9] However, studies have shown that schools have considerable inadequacies regarding anaphylaxis management.[10,11] These inadequacies in the management of anaphylaxis, acknowledged today as a non-rare disease with ever increasing prevalence, can lead to morbidity and mortality for patients, and to serious psychosocial stress for the society and healthcare system.[12]

In this study, our primary aim is to determine the correct EAI usage rates of professional and non-professional healthcare workers from different lines of the healthcare service chain, and our secondary aim is to assess the theoretical knowledge of participants about anaphylaxis management.

## Material and Methods

The study included professional and non-professional healthcare workers from two different cities of our country (Istanbul and Malatya), who participated voluntarily. By statistical analysis, the minimum sample size required to detect a significant difference was determined to be at least 508, considering 6% deviance, type I error (alpha) of 0.05, power (1-beta) of 0.8, effect size of 0.12 and two-tailed alternative hypothesis (H1). The study included emergency medicine specialists, family physicians, pediatricians, internal medicine specialists and dentists who frequently encounter cases of anaphylaxis in health centers; pharmacists; and school staff (nursing and non-nursing staff) responsible for healthcare practices in primary schools who encounter them in public spaces. All participants provided written informed consent for voluntary participation before the study, which was approved by the institutional ethics committee of İnönü University (2020/8-630). Allergists, and healthcare workers who rejected to participate were excluded.

All physicians completed an eleven-item questionnaire, which we had used in our previous study and consisted of questions regarding demographic characteristics (age, gender, and duration as a physician), experience with a case of anaphylaxis and EAIs, theoretical knowledge about the diagnosis and management of anaphylaxis and the indications of EAIs.[13] The questions related to the diagnosis of anaphylaxis and the indications of EAIs were prepared according to the recommendations of the Second National Institute of Allergy and Infectious Disease and Food Allergy and Anaphylaxis Network Symposium, and of the European Academy of Allergy and Clinical Immunology.[14,15]

Pharmacists completed an eight-item questionnaire consisting of questions regarding demographic characteristics (age, gender, duration as a pharmacist), experience with a case of anaphylaxis and EAIs, theoretical knowledge about the diagnosis and treatment of anaphylaxis and about the first and life-saving treatment of anaphylaxis. School staff completed a ten-item questionnaire consisting of questions, in addition to those asked to

pharmacists, regarding their professions and whether they had previously received emergency aid training.

Following the questionnaires, each participant was asked to demonstrate how to use EAIs in one-to-one practice. Participants who responded to the questionnaire that they had previously seen an EAI were given an EAI trainer (Penepin® trainer; Vem Pharmaceuticals, Ankara, Turkey) and asked to demonstrate its usage. Participants who stated that they had never seen an EAI or had seen one but did not know how to use it were given written and visual instruction sheets that showed the steps of EAI usage, which we had used in our previous study, and asked to demonstrate how to use it on an EAI trainer (Penepin® trainer) (Fig. 1).[16] Those who applied the six steps of EAI usage in the right order were accepted to use the EAI correctly (Fig. 1). Participants' demonstrations and errors in EAI usage steps were recorded on forms prepared beforehand. Meanwhile, participants who made mistakes in usage steps were given training by the responsible researcher on the correct usage of the EAI, which continued until participants demonstrated all steps correctly.

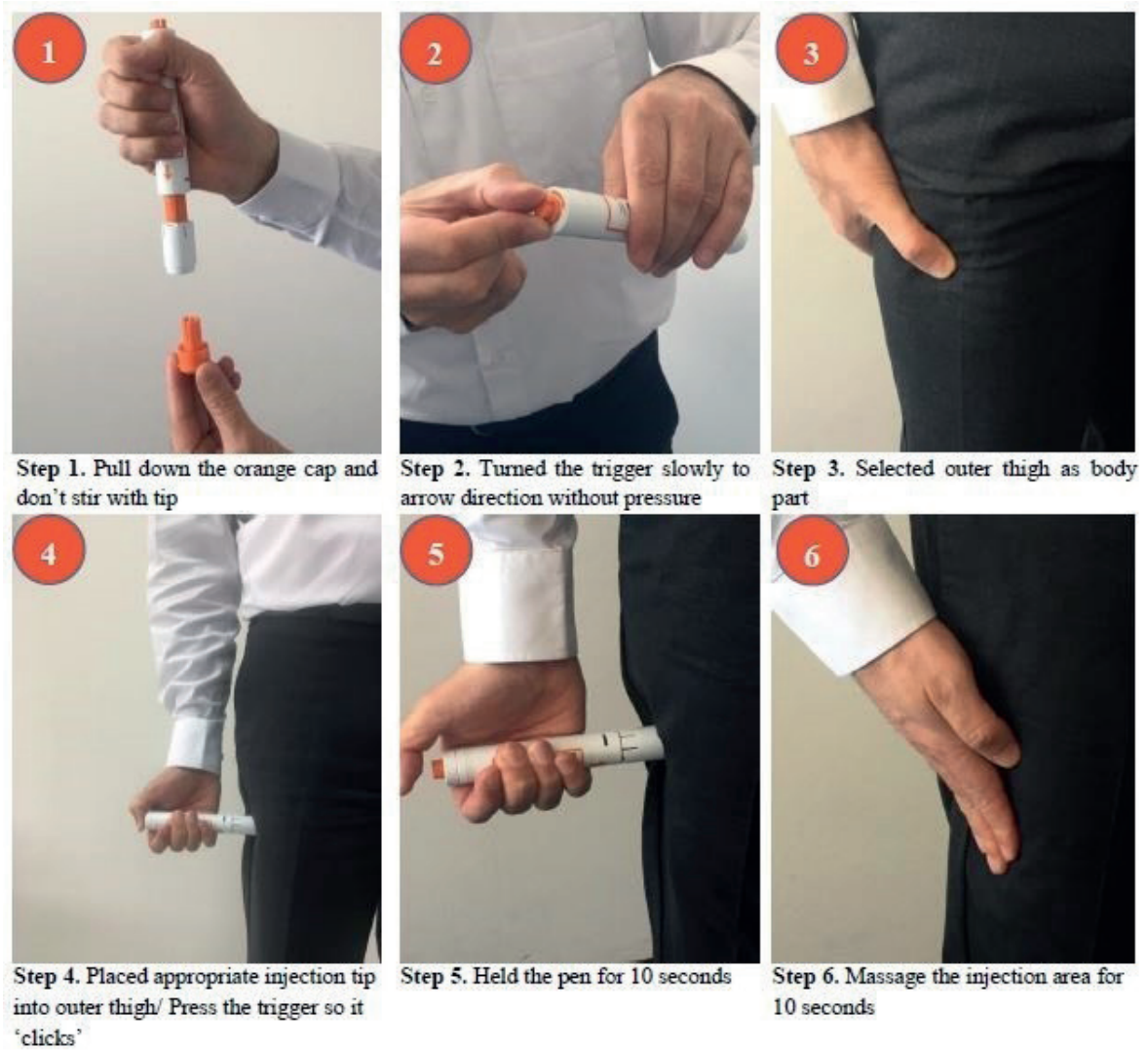


**Fig. 1.** Instruction sheet for epinephrine auto-injector (Penepin®) usage.

### *Statistical Analysis*

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 20.0 software (SPSS Inc., Chicago, IL). Normality was evaluated by the Kolmogorov-Smirnov test. Descriptive statistics were expressed as the frequency and percentage for categorical variables, whereas quantitative data were expressed as the median (min-max) for non-normally distributed data. The categorical and quantitative variables were compared using the chi-square test and/or the Mann-Whitney U test. A 2-sided p<0.05 was considered statistically significant.

## Results

The study included a total of 697 participants, comprising 391 physicians, 98 dentists, 102 pharmacists and 105 school staff (nurse or non-nursing staff). Demographic characteristics of the participants are shown in Tables I and II.

Of the physicians, 294 (75.2%) had experience with at least one case of anaphylaxis, 186 (47.6%) knew the diagnosis of anaphylaxis, and 341 (87.2%) knew that the first and life-saving treatment of anaphylaxis was epinephrine. 167 (42.7%) and 281 (71.9%) of the physicians correctly knew the epinephrine dose in anaphylaxis treatment and the administration route of epinephrine, respectively (Table I). Family physicians' rates of having experience with a case of anaphylaxis and knowing the first and life-saving treatment of anaphylaxis were lower compared to other physician groups (p<0.001 and p<0.001, respectively) (Table I). Dentists had less experience with a case of anaphylaxis than physician groups (p<0.001). While dentists' rate of knowing the first and life-saving treatment of anaphylaxis was similar to that of physicians, their rates of knowing the epinephrine dose in anaphylaxis treatment and the administration route of epinephrine were found to be significantly low (p=0.078, p<0.001 and p<0.001, respectively) (Table I).

Furthermore, 27 (26.2%) of pharmacists and 16 (15.2%) of school staff had experience with

**Table I.** Demographic characteristics of physicians and dentists, and their level of knowledge regarding the diagnosis and management of anaphylaxis.

| Questionnaire Responses | Family Physician (n:96) | Pediatrician (n:102) | Internal Medicine Specialist (n:95) | Emergency Medicine Specialist (n:98) | ¶p | All Physicians (n:391) | Dentist (n:98) | ¶¶p |
|---|---|---|---|---|---|---|---|---|
| Female† | 50 (52.1) | 43 (42.2) | 47 (49.5) | 43 (43.9) | 0.463 | 183 (46.8) | 49 (50) | 0.571 |
| Age, year [median (min-max)] | 46 (26-55)* | 37 (25-59) | 39 (26-56) | 34 (25-54) | <0.001 | 38 (25-59) | 32 (25-54) | <0.001 |
| Duration as a physician or dentist, year [median (min-max)] | 21 (3-31)* | 14 (2-36) | 14 (1-33) | 10 (2-30) | <0.001 | 14 (1-36) | 9 (2-31) | <0.001 |
| Have experience with a case of anaphylaxis† | 50 (52.1)* | 80 (78.4) | 66 (69.5) | 98 (100)* | <0.001 | 294 (75.2) | 31 (31.6) | <0.001 |
| Know the diagnostic criteria of anaphylaxis† | 39 (40.6) | 47 (46.1) | 34 (35.8) | 66 (67.3)* | <0.001 | 186 (47.6) | 31 (31.6) | 0.005 |
| Know the first and life-saving treatment of anaphylaxis† | 64 (66.7)* | 94 (92.2) | 89 (93.7) | 94 (95.9) | <0.001 | 341 (87.2) | 78 (79.6) | 0.078 |
| Know the epinephrine dose in anaphylaxis treatment correctly† | 30 (31.3) | 40 (39.2) | 40 (42.1) | 57 (58.2)* | 0.002 | 167 (42.7) | 14 (14.3) | <0.001 |
| Know the administration route of epinephrine correctly† | 55 (57.3) | 81 (79.4)* | 61 (64.2) | 84 (85.7)* | <0.001 | 281 (71.9) | 39 (39.8) | <0.001 |
| Know the indications of EAI† | 38 (39.6) | 46(45.1) | 38 (40.0) | 53 (54.1) | 0.147 | 175 (44.8) | 26 (26.5) | <0.001 |
| Have experience with EAI† | 7 (7.3) | 9 (8.8) | 17 (17.9) | 26 (26.5)* | <0.001 | 59 (15.1) | 4 (4.1) | <0.001 |
| Know the epinephrine dose in EAI† | - | - | 2 (2.1) | 4 (4.1) | NC | 6 (1.5) | - | NC |

†, n (%); ¶, p value in comparisons between physician groups; ¶¶, p value in comparisons between physicians and dentists; *, statistically significant group; EAI: epinephrine auto-injector, NC: non-calculated

**Table II.** Level of knowledge of pharmacists and school staff regarding management of anaphylaxis and epinephrine auto-injectors.

| Questionnaire Responses | Pharmacist | School Staff |
|---|---|---|
| Female† | 60 (58.3) | 82 (78.1) |
| Age, year [median (min-max)] | 37 (25-55) | 38 (25-60) |
| Duration as a pharmacist or school staff, year [median (min-max)] | 14 (1-28) | 10 (1-34) |
| *Career position† | | |
| Nursing staff | | 18 (17.1) |
| Non-nursing staff (teacher) | | 87 (82.9) |
| Have experience with a case of anaphylaxis† | 27 (26.2) | 16 (15.2) |
| Know the diagnostic criteria of anaphylaxis† | 32 (31.1) | 20 (19.0) |
| Know the first and life-saving treatment of anaphylaxis† | 81 (78.6) | 30 (28.6) |
| What should be used as the first and life-saving treatment of anaphylaxis?† | | |
| Corticosteroid (e.g. prednol®, decort®) | 28 (27.2) | 2 (1.9) |
| Antihistamine (e.g. atarax®, avil®, zyrtec®, deloday®) | 4 (3.9) | 10 (9.5) |
| Epinephrine | 49 (47.6) | 16 (15.2) |
| Antibiotics (e.g. penicillin) | - | 2 (1.9) |
| Have experience with EAI† | 31 (30.1) | 5 (4.8) |
| *Have received emergency aid training† | | 42 (38) |

†, n (%); EAI: epinephrine auto-injector; *This question was used only for school staff.

a case of anaphylaxis. Even though 81 (78.6%) of pharmacists and 30 (28.2%) of school staff responded "yes" to the question of "Do you know the first and life-saving treatment in anaphylaxis treatment?", only 49 (47.6%) of pharmacists and 16 (15.2%) of school staff preferred epinephrine as the first and life-saving treatment (Table II).

Predictor factors that affected knowing the first and life-saving treatment of anaphylaxis were having experience with EAIs [OR:5.5, (%95CI:1.330-23.351, p=0.015)], having experience with a case of anaphylaxis [OR:2.4, (%95CI:1.442-4.020, p=0.001)] and knowing the administration route of epinephrine correctly [OR:1.9, (%95CI:1.191-3.314, p=0.008)] for physicians; whereas having experience with EAIs [OR:6.1, (%95 CI:1.887-20.322, p=0.003)] for pharmacists. There were no significant factors that affected dentists and school staff in knowing the first and life-saving treatment of anaphylaxis.

217 (31.1%) of the participants demonstrated EAI usage correctly in the first attempt, only 32 (4.6%) of whom demonstrated EAI usage correctly without needing the instruction sheet (Table III). Rates of correct demonstration of EAI usage were found similar across physician groups (p=0.584) (Table III). On the other hand, pharmacists' rate of correct demonstration of EAI usage was higher than physicians, dentists and school staff (for each parameter, p<0.001) (Table III). The EAI usage steps with the most frequent errors in all participant groups were "Place the appropriate injection tip into outer thigh/Press the trigger so it 'clicks'" and "Turn the trigger to arrow direction". While the error rates in these steps were not significantly different across physician groups (p=0.938 and p=0.977, respectively), pharmacists were found to make fewer errors in these steps compared to physicians, dentists and school staff (for each parameter, p<0.001) (Table III). 480 (68.8%) participants could not demonstrate the EAI usage steps correctly. However, 273 (56.8%), 165 (34.3%), and 46 (9.5%) of them managed to demonstrate all usage steps correctly after they were demonstrated by the responsible researcher once, twice, and thrice, respectively.

**Table III.** Participants' rates of correctly demonstrating the usage steps of the epinephrine auto-injector (Penepin®).

| Usage steps of the EAI | Physician Groups | | | | ¶p | All Participant Groups | | | | ¶¶p |
|---|---|---|---|---|---|---|---|---|---|---|
| | Family Physician (n:96) | Pediatrician (n:102) | Internal Medicin (n:95) | Emergency Medicine (n:98) | | Physician (n:391) | Dentist (n:98) | Pharmacis (n:103) | School Staff (n:105) | |
| Remove the safety cap† | 81 (84.4) | 92 (90.2) | 79 (83.2) | 88 (89.8) | 0.331 | 340 (87) | 82 (83.7) | *98 (95.1) | 87 (82.9) | <0.001 |
| Turn the trigger to arrow direction† | 59 (61.5) | 63 (61.8) | 59 (62.1) | 57 (58.2) | 0.938 | 238 (60.9) | 66 (67.3) | *85 (82.6) | 65 (61.9) | <0.001 |
| Select outer thigh as body part† | 96 (100) | 102 (100) | 95 (100) | 98 (100) | NC | 391 (100) | 95 (96.9) | 103 (100) | 101 (96.2) | NC |
| Place the appropriate injection tip into outer thigh/Press the trigger so it 'clicks'† | 31 (32.3) | 35 (34.3) | 32 (33.7) | 31 (31.6) | 0.977 | 129 (33) | 40 (40.8) | *68 (66.1) | 40 (38.1) | <0.001 |
| Hold the pen for 10 seconds† | 76 (79.2) | 84 (82.4) | 70 (73.7) | 80 (81.6) | 0.435 | 310 (79.3) | 76 (77.6) | *90 (87.4) | 80 (76.2) | <0.001 |
| Massage the injection area for 10 seconds† | 79 (82.3) | 90 (88.2) | 80 (84.2) | 88 (89.8) | 0.397 | 337 (86.2) | 86 (87.8) | *99 (96.1) | 87 (82.9) | <0.001 |
| Correct demonstration of the EAI in all assessment steps† | 22 (22.9) | 29 (28.4) | 20 (21.1) | 21 (21.4) | 0.584 | 92 (23.5) | 32 (32.7) | *60 (58.3) | 33 (31.4) | <0.001 |
| Correct demonstration of the EAI in all assessment steps without needing the instruction sheet† | (1) | 3 (2.9) | 5 (5.2) | 7 (7.1) | NC | 16 (4.1) | 4 (4) | 12 (11.6) | 0 | NC |

†, n (%); ¶, p value for comparisons between physician groups; ¶¶, p value for comparisons between physicians, dentists, pharmacists and school staff; *, statistically significant group; EAI: epinephrine auto-injectors, NC: non-calculated

## Discussion

Our study showed that healthcare workers from different lines of the healthcare service chain had critical inadequacies in the management of anaphylaxis. This inadequacy is more obvious in pharmacists and school staff. Only one-seventh of the participants stated to have experience with EAIs, only one-third of whom could demonstrate the EAI usage correctly even though instruction sheets were given. The fact that the two most frequent errors were made in the same steps during the demonstration of EAI usage in all participant groups support the view that EAI design can have an important effect on correct usage.

87.2% of our physicians knew that the first and life-saving treatment of anaphylaxis is epinephrine. Altmanet al.'s[17] national follow-up study, which included 266 physicians from different specialities similarly to our study, found that this rate was between 81-98%, the lowest rate being in family physicians. In our study, this rate was lowest in family physicians and highest in emergency medicine specialists, as well. Two different studies ascertained that frequent experience with cases of anaphylaxis and knowledge of the diagnosis criteria of anaphylaxis were predictive factors in determining the first treatment correctly.[19,20] Similarly, our study found that having experience with a case of anaphylaxis (OR:2.4) and additionally having experience with EAIs (OR:5.5) and knowing the administration route of epinephrine correctly (OR:1.9) were significant factors in knowing the first and life-saving treatment of anaphylaxis correctly.

The epinephrine dose in anaphylaxis treatment and the administration route of epinephrine were determined correctly by 42.7% and 71.9% of our physicians, respectively. Studies showed that 23.8-92.6% of physicians chose the administration route of epinephrine and 26.8-81.6% chose the epinephrine dose correctly, depending on their branches.[20,21] In our country, various studies carried out in the last decade showed that 46% of pediatricians, 43.3% of family physicians and 20% of internal medicine specialists chose the administration route of epinephrine correctly, which may support the increase of physicians' knowledge levels with regard to correctly determining the administration route of epinephrine.[22-24] Nevertheless, the rate of pediatricians in our country choosing the epinephrine dose correctly has not changed significantly in the last seven years, in fact it has even decreased.[13,25] However, it is absolutely necessary to apply all steps in the management of a case of anaphylaxis immediately and correctly for preventing irrecoverable outcomes for the patient.[1,2] In this regard, in another study in which we assessed pediatricians' competence in anaphylaxis management through case scenarios, we ascertained that only 11.3% of the physicians were able to correctly apply all management steps from diagnosis to discharge recommendations.[26]

It is required that physicians prepare an individual emergency action plan for each patient before discharge, prescribe EAI to them and train them about when and how to use it.[9,15] In our study, less than half of the physicians knew the indications of EAIs correctly; and again, the highest rate belonged to emergency medicine specialists. In addition, 15% of our physicians had experience with EAIs, while only one-fourth of them could demonstrate the use of EAI correctly without needing the instruction sheet. Previous studies revealed that more than half of the trainer physicians did not have adequate knowledge about EAI usage.[13,15] Even though it was shown that giving theoretical and practical trainings to physicians increased their knowledge level about EAI usage, these studies suggest barriers to EAI usage that are not solely practical but incorporate complex psychological features.[27] On the other hand, Mahoney et al.[28] found in their recent study that "training physicians in psychologically informed strategies produce sustained improvements in their confidence and knowledge around patient auto-injector education, and their likelihood of using strategies in clinical practice". According

to all these results, it is necessary that theoretical and practical training to be given to physicians contain all steps of anaphylaxis management, provide psychological information and be repeated regularly in order to achieve improvement in anaphylaxis management at the required level. These comprehensive training programs to be given to physicians will also ensure correct self-management of patients and/or parents during anaphylaxis.

Concerning anaphylaxis, which is increasingly becoming a public health problem today, it is crucial that pharmacists take part as professional healthcare workers in the preparation of anaphylaxis emergency action plan and provision of trainings on EAI usage for patients. In our study, however, even though nearly three-fourths of the pharmacists stated that they knew the life-saving treatment of anaphylaxis, less than half of them preferred epinephrine as the first treatment. In the questionnaire study carried out by Wormet al.[29] in Germany, in which the knowledge levels of 213 pharmacists regarding anaphylaxis management were assessed, 53.9% preferred epinephrine as the first treatment. Although EAIs are sold only in pharmacies in our country, only one-third of the pharmacists in our study stated that they had experience with EAIs, only one-third of whom could demonstrate the practical usage of the EAI correctly without needing the instruction sheet. Nevertheless, other studies on this topic also ascertained that 24.4% and 17% of pharmacists could demonstrate EAI usage correctly.[29,30] While Salter et al.[31] found that physicians' rate of correct EAI usage increased to 88% after reading the instruction sheet, this rate increased to 58% in our study. This may be primarily associated with study designs. Regular allergist follow-up, repetition of training at regular intervals and encouraging patients and/or parents about EAI usage enhance their frequency of use.[32] However, it may not be easy for patients to continue regular follow-ups or contact the allergist if they want to ask something on this topic during follow-up. Therefore, in-time training of patients on EAI usage is crucial for enhancing their awareness of and competence with EAIs. Hence, pharmacists are an important link between the patient and the physician. Indeed, Salter et al.[31] determined that pharmacists who asked patients if they had an anaphylaxis emergency action plan, told them to go to the emergency department after using the EAI and inform them about epinephrine's side effects demonstrated EAI usage 16, 4.5 and 4 times more correctly. These results indicate that when pharmacists have detailed and extensive knowledge about anaphylaxis, they can act willingly about and contribute greatly to this issue. A study from Australia revealed that giving pharmacists e-learning or lecture programs including the national anaphylaxis emergency action plan nearly doubled their related minimum standard knowledge levels (45% pretest, 87% posttest), and this could continue for seven months.[33] Therefore, giving pharmacists detailed trainings on anaphylaxis beginning from their education years will render them a crucial link of the anaphylaxis management chain within the healthcare system.

Schools are places where development of anaphylaxis is observed most frequently in non-domestic social life.[5-8] Schools have legal liabilities for the protection of students' health; therefore, it is important for schools to establish policies and give school staff training about anaphylaxis management in order to take preventive measures for children with anaphylaxis, make early diagnosis when its symptoms develop, and perform the correct treatment.[5] However, results of studies from around the world demonstrate the inadequacies in prevention and management of anaphylaxis in schools that might lead to serious outcomes. Mohammed Elhassanet al.[34] found that school managements prohibited performing injection in 16% of schools that had students with anaphylaxis history; while Korematsu et al.[35] ascertained that in 79% of students who developed anaphylaxis at school, school staff did not administer epinephrine with EAI but wait for parents to come. In our

study, although one-fourth of the school staff said that they knew the first and life-saving treatment of anaphylaxis, only 15% preferred epinephrine. Additionally, there were no professional healthcare workers (nursing staff) in 82.9% of schools, and teachers (non-nursing staff) were the responsible ones in case students need emergency medical aid. In two studies that assessed the applications of preschools and primary schools in our country regarding anaphylaxis management in the last eight years, availability rates of professional healthcare workers in schools were similar.[11,36] One of these studies found teachers' rate of preferring epinephrine as the first and life-saving treatment as 0%, and the other as 3%.[11,36] However, in order to successfully implement scientific suggestions in countries, it is crucial that legislators and official authorities become more aware of this issue and legal infrastructures be established. None of the healthcare practitioners in schools in our study knew how to use EAIs, while Ercan et al.[11] found this rate as 4%. Studies showed that training given to non-nursing staff about prevention and management of anaphylaxis and EAI usage were effective in anaphylaxis management and EAI usage.[37] Furthermore, Devetak et al.[38] demonstrated that specific training on this topic provided to future teachers in their first year at university considerably improved their attitudes and knowledge levels about anaphylaxis in their last year. In our country, the Ministries of National Education and Health manage the policies on healthcare practices. The fact that these institutions cooperatively develop policies for prevention and management of anaphylaxis in schools and trainings on anaphylaxis management are added to the college curriculum of future teachers considering they play a crucial role as healthcare practitioners in schools will be the main determinants in resolving these problems and improving anaphylaxis outcomes.

Only around one-third of the participants given the written and visual instruction sheet were able to demonstrate the EAI usage correctly in practice. The two EAI usage steps with the most frequent errors in all participant groups were "Place the appropriate injection tip into outer thigh/Press the trigger so it 'clicks'" and "Turn the trigger to arrow direction". Various studies carried out with Penepin® and other commercial EAIs showed that the steps in which errors were made could be the same for each EAI regardless of the applier's identity. This supports the fact that the errors made in EAI application might be associated with EAI design.[13,16] It was shown that the reduction of the number of steps in EAI usage in recent years, keeping the needle within a protective shield after application and adding audio instructions were effective in increasing the rates of correct usage and reducing problems related to erroneous applications.[39] However, despite the industry's intensive efforts in the improvement of EAI design, unfortunately current commercial auto-injectors do not possess all of the ideal features required for a life-saving treatment.[40] For Penepin®, making modifications that eliminate the need for the application step of "Turn the trigger to arrow direction" can be effective in enhancing correct usage rates.

Our study found that pharmacists' rate of correct EAI usage was significantly high compared to all other participant groups; while the rates of school staff and dentists were high compared to physicians, though not significantly. This may have two reasons. The first one may be that these groups were more willing to learn and considered the study process an educational opportunity; and the second one is that physicians may have felt highly stressed during one-to-one demonstrations due to the study design, which may have reduced correct usage rates. Though this was the case for each participant, physicians may have felt more stressed than other participants, as they knew they were being assessed by their colleagues. This is one of the restrictive aspects of our study. Secondly, investigators were not the same person for the two study centers located in different parts of Turkey; therefore, each investigator's judgment may have led to bias. Thirdly, although the total

number of participants was high, the number of participants in each individual group was low. Fourthly, our study did not include the responsible people in certain public spaces (e.g. shopping malls, playgrounds and camps) where anaphylaxis develops. And the fifth restrictive factor is that the primary design of the study did not include other professional healthcare practitioners (e.g. nurses and paramedics) in addition to physicians and dentists.

In conclusion, healthcare workers' knowledge about anaphylaxis management and capability to use EAIs correctly are not at the required level. Adequate improvement could not be achieved on this topic despite the effort spent in the last ten years. Therefore, in order for current guidelines to be implemented, there is a necessity for regular, sustainable and extensive training for all healthcare workers constituting the healthcare chain, which include all steps of anaphylaxis management, cover its psychological aspects, and are supported by healthcare authorities through legal policies. The fact that the most frequent errors in EAI usage were made in the same steps indicates that the industry should continue to strive to develop the ideal life-saving device.

## Authors contribution

The authors confirm contribution to the paper as follows: study conception and design: Mustafa Arga, Arzu Bakırtaş; data collection: Erdem Topal, Sıla Yılmaz, Pınar Canızcı Erdemli, Kübra Bıçakcı; analysis and interpretation of results: Mustafa Arga, Erdem Topal, Sıla Yılmaz, draft manuscript preparation: Mustafa Arga, Erdem Topal, Arzu Bakırtaş. All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

This study was approved by the institutional ethics committee of İnönü University (2020/8-630).

## Source of funding

The sponsor had no involvement in this clinical study's design, the collection of data, analysis, and interpretation of data, the writing of the report and the decision to submit the paper for publication. An honorarium, grant, or other form of payment was not given to anyone to produce the manuscript.

## Conflict of interest

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 384-392
https://doi.org/10.24953/turkjped.2021.03.005

**Original Article**

# Seroprevalence of Anti- N-methyl-D-aspartate receptor antibodies in children with seizures of unknown cause

Mohammed Abdulrasol Abdulamer[1], Nebal Waill Saadi[1,2], Batool Ali Ghalib Yassin[3], Imad Al-Jumaili[4]

*[1]Unit of Pediatric Neurology, Children Welfare Teaching Hospital, Medical City Complex, Baghdad , Iraq; Departments of [2]Pediatrics and [3]Community, College of Medicine, University of Baghdad, Baghdad , Iraq; [4]Clinical Chemistry, MSc. Al-Nadaer Clinical Lab. Lab Director, Baghdad, Iraq.*

## ABSTRACT

**Background.** Anti- N-methyl-D-aspartate receptor (NMDAR) antibodies were found most probably to be accompanied by seizures, particularly in children and sometimes it may be the sole presenting feature. Therefore, testing these antibodies in children with seizure of unexplained cause might be helpful to identify the spectrum of these antibody-mediated disorders. The objective of this study was to determine the frequency of anti-NMDAR antibodies in patients who presented with seizures of unknown cause.

**Methods.** A case-control study was conducted in two hospitals in Medical City Complex-Baghdad in 2019. Children aged 2-18 years who manifested seizures solely without identified causes were recruited over a period of ten months, with an additional sex- and age-matched control group (forty children in each group). Serum was tested in both groups for anti-NMDAR antibodies.

**Results.** In the study group, males predominated in ages younger than 5 years. The mean age was 6.6 years and the mean duration since their seizures' onset was 2 months. In contrast to male patients, female patients manifested more focal seizures. Only 5 patients (12.5%) were positive for Anti- NMDAR antibodies, in contrast to no one in the control group. Significantly, most of the seropositive patients were females (4, 80%) and showed focal types of seizures (4, 80%).

**Conclusions.** This is a preliminary epidemiological study about the prevalence of anti-NMDAR antibodies in a sample of pediatric patients with isolated seizures of unknown cause. Anti-NMDAR antibodies were found to be prevalent in a relatively small proportion of children who presented with seizures of unknown causes. Demographic characteristics of the patients with variable testing status were found to be nearly comparable to the results from other related studies.

**Key words:** autoimmune, seizures, anti-NMDAR, children.

Epilepsy type of unknown cause is presumed to be due to an underlying cause not diagnosed yet.[1,2] An important area that deserves to be researched is the presence of specific antibodies associated with seizures.[3] Those types of epilepsies are either associated with signs and symptoms of encephalitis or presented primarily as recurrent seizures without features of encephalitis.[4] Symptoms as isolated seizures occur more frequently in children, are commonly of focal type, with an insidious onset or maybe aggressive, very frequent and prolonged with status epilepticus modality.[5] N-methyl-D-aspartate receptor (NMDAR) is a neuronal surface antibody and one common type implicated in disorders like epilepsy.[6] In the human brain, the NMDAR is a ligand of glutamate, the primary excitatory neurotransmitter. Its major role is in synaptic plasticity. Therefore, testing anti-NMDAR antibodies in children with seizure

✉ Nebal Waill Saadi
nebalpedneu2013@gmail.com

Received 28th April 2020, revised 13th August 2020, 3rd October 2020, 31st October 2020, accepted 24th November 2020.

of unexplained cause might be a significant approach to identify the spectrum of this antibody-associated clinical condition.[7] It is more sensitive to test for anti-NMDAR antibodies in the cerebrospinal fluid (CSF) than the serum.[8] Nevertheless, diagnostic sensitivity can be improved by searching for those antibodies in both the serum and CSF.[9] It is recommended to treat the disease early and extensively because of the intimidating course of the disorder and the recurrent relapses.[10] The aim of this study was to determine the prevalence of anti-NMDAR antibodies in a group of patients presenting with seizures of unknown cause in comparison to corresponding healthy volunteers.

## Material and Methods

A case – control study was conducted in two hospitals of Medical City Complex (emergency department, outpatient neurology clinic and neurology ward in Children Welfare Teaching Hospital (CWTH), and epilepsy clinic in Baghdad Teaching Hospital) in the period from February to October, 2019. Eighty children were enrolled in the study and divided into two groups: study group and control group with forty children in each. The inclusion criteria were: 1) Age ranged 2 - 18 years; 2) History of seizures, first or recurrent attacks with or without anti-seizure drugs and within the preceding 6 months. The researchers aimed to evaluate the role of anti-NMDAR antibodies in the development of seizure as an isolated feature. Therefore, those children who had additional features like psychiatric or encephalopathic signs and symptoms like behavioural changes, disturbed level of consciousness, or movement disorders which are highly suggestive of autoimmune encephalitis were, particularly excluded. Other exclusion criteria included: 1) evidence of provoked seizures (structural, tumor, infection, metabolic or electrolyte disturbance, or fever (≥ 38 °C)), 2) well-established electro-clinical epileptic syndrome, 3) personal or family history of autoimmune disorders or epilepsy and 4) patients with pre-existing developmental, motor or psychiatric abnormalities. All children and their caregivers were interviewed to explain the objectives of the study and written consent for participation were obtained.

In the study group, a full history and complete physical and neurological examination were performed, and the necessary data were collected using a special form. The gathered data included; gender, age, age of onset of the first attack, semiology of seizures, duration of each attack, type and number of anti-seizure drugs (if present), and if the seizures were controlled or not. Every patient was sent for brain magnetic resonance imaging (MRI) (most had 1.5 Tesla and few had 3 Tesla) and those with either normal study or signal changes not otherwise specified were included in this study. An electroencephalography (EEG) study was also done (standard protocol in all patients except one who had level 2 EEG, and all were assessed by a specialized neurophysiologist), and those with epileptic or normal findings were included.

The classification of the patients' seizures was made by a pediatric neurologist who was blinded to the results of the antibody testing. Classification was made according to the latest ILAE report, so that seizure type at presentation was classified into focal, generalized and unknown onset. This has been made depending on the history taking and also the home video if available. Then the epilepsy type was classified according to the clinical background supported by EEG into focal, generalized, combined focal and generalized and unknown. After that, the patients were tested for antibodies directed against NMDAR with enzyme linked immunoassay (ELISA) by using commercial kits provided by MyBioSource, MBS705691 kits and Mindray MR 96A Elisa Reader used for sero-analysis. Serum samples (3 milliliters) were obtained from each patient, centrifuged, stored at -20 C° to be tested within one month. The cut-off value for this kit was 2.1. Results of values less than 2.1 were considered normal and those equal or more than 2.1 were considered positive. The control group consisted of hospitalized children

who had serum collected as part of their routine investigations for non-neurological disorders like respiratory, gastrointestinal, hematological and others during the same period of collection of samples from the epileptic patients. They were age- and sex- matched with the study group. Autoimmune disorders were assessed by analysing antinuclear antibodies (ANA). This study was approved by the ethical committee of the Children Welfare Teaching Hospital (IRB: 548, Date 15.12.2018).

Data were analysed using Microsoft excel version 10. Categorical variables were presented as frequency and relative frequency (%), and continues variables were presented as mean± standard deviation. Student T-test was used to test the significant differences between means and chi-square and/or Fischer exact test were used to test the significance of association between categorical variables. P-value of less than 0.05 was considered statistically significant.

## Results

Of the 40 patients enrolled in the study group, 26 (65%) were boys and 14 (35%) were girls. Their age ranged between 2-14 years with a mean age of 6.6 years ± 3.3. The mean duration since their seizures' onset was 2 ± 2.3 months and the mean age at diagnosis of seizure was 6.4±3.4 years. In the study group, the male gender predominated (13 boys versus 2 girls) in the age group younger than 5 years. While both genders presented equally above and equal to 5 years (12 girls versus 13 boys). The association between gender and age of onset of the first seizure in the study group was statistically significant ($\chi 2$= 4.9, df=1, P=0.026). Female patients manifested more focal seizures than males (8 (57%) versus 6 (23%) respectively), in contrast to male patients who presented with generalized seizures more than females (20 (77%) versus 6 (43%) respectively), a result that emphasized statistically significant association between gender and seizure's type ($\chi 2$= 4.6, df=1, P=0.0312). Comparison of anti-NMDAR antibodies results between the study and control groups showed that only five patients (12.5%) were positive for anti- NMDAR antibodies, in contrast to no one in the control group (corrected OR=12.5; 95% CI (0.6-216.7)). The majority of patients with positive anti-NMDAR antibodies were females (4 (80%) versus 1 (20%)), in contrast to male predominance in the patients with negative anti-NMDAR antibodies (25 (71.3%) versus 10 (28.7%)), with a statistically significant association ($\chi 2$= 5.08, df=1, P=0.024).

**Table I.** Variable characteristics of the study group stratified by anti-NMDAR antibody status.

| Variable | | Anti-NMDA +ve No. (%) | Anti-NMDA –ve No. (%) | p value |
|---|---|---|---|---|
| Age (years) | | | | |
| Range | | 3-12 | 2-14 | 0.76 |
| Mean ± SD | | 7.1 ± 3.7 | 6.6 ± 3.3 | |
| Age at diagnosis (years) | | | | |
| Range | | 3-12 | 1.9-13.9 | 0.7 |
| Mean ± SD | | 7.03 ± 3.8 | 6.4 ± 3.4 | |
| Duration since seizure onset (months) | | | | |
| Range | | 0.25-2 | 0.25-10 | 0.3 |
| Mean ± SD | | 0.95 ± 0.7 | 2.2 ± 2.5 | |
| Type of seizure | Focal | 4(80) | 10(29) | 0.024 |
| | Generalized | 1(20) | 25(71) | |
| Treatment with anti-seizure drugs | Not treated | 1(20) | 15(43) | 0.6 |
| | Treated | 4(80) | 20(57) | |

The distribution of the study group by age, age at onset of first seizure and duration since seizure onset according to the results of anti-NMDAR antibodies is shown in Table I. The mean of both patients' ages and age of first seizure were higher in those with antibody positive result, yet, it was not statistically significant (*P* value 0.76 and 0.7 respectively). Anti-NMDAR positive patients presented earlier (within one month) with no significant statistical result (*P* value 0.3). The percentage of focal seizures was significantly higher in patients with antibody positive results (80%) compared to those with negative results. This was statistically significant (P-value =0.024) as shown in Table I. Before testing the anti-NMDAR antibodies, the usual treatment of children in the study group were anti-seizure drugs. No immunosuppressive or immune modulators were administered before or after obtaining the results in anti-NMDAR positive patients. The association between anti-NMDAR status and the use of anti-seizure drugs is described in Table I, and was found to be non-significant (P = 0.6), yet a higher percentage of those with positive anti-NMDAR status were treated.

All patients in the study group had normal EEG and brain MRI results. Demographic and clinical characteristics of the five patients with positive anti-NMDAR status is presented in Table II. Only one patient presented at a relatively young age (3 years) with only one attack of generalized tonic-clonic seizure. His EEG was reported as normal and he was on no anti-seizure medications. The other four patients were 5 years and older, of female gender, had focal type of epilepsy with frequent focal seizures and fairly partial response to anti-seizure medications. On one-month follow up, three patients became seizure free, while contact was lost with the other two patients. Anti-NMDAR antibodies were not tested again.

## Discussion

In the human brain, NMDAR is a ligand of glutamate, the primary excitatory neurotransmitter. Its major function is in synaptic plasticity, substantial for memory function and excitotoxicity that is implicated in a number of diseases like epilepsy and Alzheimer's. It is detected all around the central nervous system (CNS), in approximately 80% of cortical neurons. Several subtypes of NMDAR are found, each one is made up of two N1 subunits and either two N2 or two N3

**Table II.** Demographic and clinical characteristics of patients with positive anti-NMDAR antibodies.

| Patient | Age (years) | Gender | Time of sampling since onset of seizure | Type of seizure | Type of epilepsy | MRI & EEG | ASD | Follow up |
|---|---|---|---|---|---|---|---|---|
| 1 | 5.5 | Female | 2 months | Focal to bilateral tonic-clonic | Focal | Normal | Levetiracetam 20mg/kg/day | Seizure free / On treatment |
| 2 | 12 | Female | 1 month | Focal | Focal | Normal | Oxcarbazepine 15mg/kg/day | Lost contact |
| 3 | 10 | Female | 2 weeks | Focal tonic | Focal | Normal | Carbamazepine 10mg/kg/day | Lost contact # |
| 4 | 3 | Male | 2 days | Attack of Generalized tonic seizure | Non applicable | Normal | None | Seizure free / No treatment |
| 5 | 5 | Female | 1 month | Focal | Focal | Normal | Levetiracetam 20mg/kg/day | Seizure free / No treatment |

MRI: magnetic resonance imaging, EEG: electroencephalography, ASD: anti-seizure drugs
# Last contact was before 4 months during that time she was free of seizure and discontinued her treatment

subunits.[11] The construction of the receptor is formed generally of an extracellular part for ligand binding and an ion channel which allows the entrance of cations in and out of the cells controlled by the force of the individual electrochemical gradients. Once the ligand is tied up to its sites on the receptor, modifications in configurations in the receptor protein allows the entry of Na and Ca in and the emergence of K out of the cell which causes depolarization and neuronal excitation.[12] The activity status of the NMDAR may determine the clinical profile in the way that over-activity is causing excitotoxicity with subsequent features like epilepsy, stroke, and dementia, while under-activity results in symptoms of schizophrenia.[13] Immunoglobulin G antibodies are found to cause epilepsy when it is directed toward GluN1 subunit of the NMDAR resulting in extended openings of that receptor and causing acquisition-of-function.[14]

The occurrence of antibodies against NMDAR in patients with encephalitis has been the focus of several studies, yet very little has been reported about their prevalence in seizures of unknown cause in children. A study taken place in the UK in 2010 reported 4 (9%) samples collected from patients (44) tested positive for anti-NMDAR antibodies, two belonged to males (both aged 23) with a 4-year history of drug-resistant temporal lobe epilepsy, and two females (aged 17 and 33 years) who presented with an acute-onset of complex partial status epilepticus, had had minimal or no cognitive involvement and did not develop any movement disorders or other features consistent with the later stages of the disease.[15] Positive result of anti-NMDAR antibodies were also reported in studies conducted in Sydney by Suleiman et al.[6] (6% of pediatric patients with seizures of unknown cause),[6] and in the UK in 2013 (1.7% of patients older than 16 years with established and newly diagnosed epilepsy).[16] In the current study, the prevalence of anti-NMDA antibodies in patients with seizures of unknown causes was found to be 12.5%, which showed significant statistical feature (OR=12.5), yet it cannot be applied to the general population as the CI was 0.6 – 216.7, which may be related to the small size of the sample and we might have obtained different percentage if both CSF and serum were tested for anti-NMDAR antibodies as it is known that serum testing has lower sensitivity than CSF.[17] This heterogeneity of anti-NMDAR antibodies positivity among studies might be due to different lab techniques used to assess the autoantibodies and different sampling time.

The current study showed that the male gender predominated in the study group, at ages below and above 5 years. Nevertheless, the number of female patients increased gradually and approached that of males after 5 years of age (male: female ratio was 6.5:1 versus 1.1:1 respectively). The latter may be attributed to the hormonal changes that may play a significant role in female epilepsy near adolescence. The same was reported by studies conducted in Western Nepal in 2013 by Adhikari et al.[18] and in China in 2016 by Mwipopo et al.,[19] where prospective and retrospective analyses were taken place for children admitted with acute seizures. Sex differences in epilepsy and seizure are not always clear. Particularly if considering all epilepsy types. Documented effects of sex on seizures have been reported, and numerous effects of gonadal steroids have been shown throughout the rodent brain. There is a broad agreement among studies that males have greater exposure to risk factors for lesional epilepsy and acute symptomatic seizures.[20,21] However, inconsistencies were reported regarding epilepsy prevalence in different genders. Some cohorts showed a higher prevalence of epilepsy in men while others showed an opposite result. This may be due to pooling subjects with different epilepsy syndromes or neuropathology.[22] The small sized sample of the study group, causes difficulty to explain males predominance in regard to seizure prevalence. Gender difference in children with anti NMDAR encephalitis were reported to be in favour of the female sex.[23-25]

The mean age of first seizure presentation was 6.4±3.4 SD years and this was relatively

higher than most of the studies that discuss the epidemiology of epilepsy,[18,19,26] which may be explained by the fact that the current study has included a wider range of ages (2-18 years) and also excluded patients with febrile seizures, CNS infection, congenital malformation, metabolic and electro-clinical syndromes which constitute the major causes of epilepsy in younger children.

Generalized seizures predominated in our cohort (63%), which agreed with that reported in Mwipopo et al.,[19] Chen et al.[26] and the meta-analysis in 2017 by Fiest et al.[27] , but disagreed with other studies like Berg et al.[28] and Camfield et al.[29] Hauser has suggested in Pellock's Pediatric Epilepsy that in children both single, unprovoked seizures and acute symptomatic seizures are predominantly generalized. This difference in distribution by seizure type may be related to the inclusion criteria used for each study.[30]

Most of our female patients presented with focal seizures compared to the males (P-value=0.0312). However, the few numbers of patients in the study group suggested that the results need to be interpreted with caution. Pituitary and gonadal hormones are known to affect cortical excitability. Intravenous infusion of estrogen directly applied in to the cerebral cortex can activate seizures and interictal discharges. This is partially caused by alteration of cell membrane permeability to calcium, reduction of chloride influx through the gamma-aminobutyric acid (GABA)-A receptor, and glutamate agonist action of estrogen in the hippocampus.[31] Variable results were reported about sex difference for seizure types, some showed that idiopathic generalized epilepsy or multiple seizure types are more common in females.[32] Other reported that atonic seizures were more common in males with generalized epilepsy, and that autonomic, visual, and psychic symptoms associated with non-acquired focal epilepsy, were more common in females.[33] The current study reported no one in the control group with positive anti-NMDAR antibodies, in contrast to other studies which showed positivity in the serum of healthy persons as in USA in 2014 (0.4%) and in Germany in 2013 (3%).[8,34]

Most of the patients with positive results were females (80%) with a female to male ratio of 4:1, and showed statistical significance. This was agreed by a study conducted in Spain in 2013 by Armangue et al.[16] and another one in the UK in 2010 by Vincent et al.[35] It may be related to the fact that the risk of autoimmune diseases increases in females. Male predominance was reported in Brenner et al.[16] in 2013 (male: female ratio 1.3:1) while both Suleiman et al.[6] and Irani et al.[15] found a 1:1 ratio. Female predominance was also reported in studies surveying children with anti-NMDAR encephalitis.[23-25]

The mean duration since the first seizure onset and timing of the samples was 28.5 days for the positive patients versus 66 days for the negative patients with no statistically significant difference. A similar result was found by Suleiman et al.[6] (17.5 days for positive tests versus 40 days for negative results with no statistical differences). Vincent et al.[35] has proposed that these antibodies usually present at their nadir early in the disease course and may gradually decrease thereafter even without treatment.[35] Based on this, those patients with negative results who presented later may have had positive anti-NMDA antibodies if they had been tested earlier.

The mean age at first seizure presentation in the positive patients was 7.1 years versus 6.4 years for those with negative results. This was higher than that in the study of Suleiman et al.[6] (4.4 years) which might be attributed to the larger age range collected in that study, and it was much lower than that found in the samples collected by Irani et al.[15] (23 years in two samples, 17 years and 33 years in another two samples) which could be explained by referring the samples of the patients from adult neurologists. Generally, studies reported older ages of patients presenting with anti-NMDAR antibodies encephalitis as in studies by Armangue et al.,[37] Zekeridou et al.,[5] Remy et

al.[23] and Florance et al.[36],

Eighty percent of patients with positive results had focal seizures. This may be related to the inflammatory nature of this disorder affecting certain areas in the brain more than others. This was similar to the results found by Brenner et al.[16] and Suleiman et al.[6] Other studies discussing anti-NMDA associated autoimmune encephalitis also reported the predominance of focal seizures in the patients.[5,36,37]

No difference was found between seropositive and seronegative groups regarding the use of anti-seizure drugs as shown in Table I. These medications have little or no effects on these antibody - associated disorders.[38] Additionally, most of our positive patients were newly diagnosed and the effects of these medications can't be evaluated appropriately. Epilepsy itself and antiepileptic drugs are reported to alter immune responses, and it is not clear which autoantibodies arise as a consequence and which are causative.[39]

This is a preliminary epidemiological study about the prevalence of anti-NMDAR antibodies in a sample of pediatric patients with isolated seizures of unknown cause. Anti-NMDAR antibodies were prevalent in a small proportion of children with isolated seizures, who lacked other familiar manifestations of autoimmune encephalitis. The demographic characteristics of children with positive anti-NMDAR antibodies was nearly similar to other studies. We could neither duplicate the testing in those who showed positive results, because of financial restraints, nor be able to treat them, as we were not sure of the cause - effect relationship, and the manifestations (seizures) of the anti-NMDAR positive patients were not persistent, progressive or reluctant to conventional anti-seizure medications, to encourage the start of immune suppressive medications. The small sample size, the shorter follow up duration and the case– control statistical design of this study are the main limitations, so we were unable to reach a strong conclusion, in regard of measuring a cause– effect relationship. The latter necessitates a cohort study that includes longer follow up. Instead, the prevalence of the anti – NMDAR antibodies were investigated in this group of patients.

Furthermore, there is a need for larger prospective analysis of paired serum and CSF anti-NMDAR antibody titer in children with isolated seizures of unknown cause to optimize the laboratory diagnostic sensitivity and characterize the true prevalence of these antibodies among those patients. In conclusion, it is recommended to consider and screen for autoimmune etiologies of epilepsy, particularly in epidemiologically typical circumstances.

**Acknowledgements**

We are grateful to the patients and their families for their informed consent and agreement to participate in this study.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: Nebal Waill Saadi, Mohammed Abdulrasol Abdulamer; data collection: Mohammed Abdulrasol Abdulamer; analysis and interpretation of results: Imad Al-Jumaili, Batool Ali Ghalib Yassin, Nebal Waill Saadi, Mohammed Abdulrasol Abdulamer; draft manuscript preparation: Nebal Waill Saadi, Mohammed Abdulrasol Abdulamer, Batool Ali Ghalib Yassin. All authors reviewed the results and approved the final version of the manuscript.

**Ethical approval**

This study was approved by the ethical committee of the Children Welfare Teaching Hospital (IRB: 548, Date 15.12.2018).

**Source of funding**

None.

## Conflict of interest

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 393-403
https://doi.org/10.24953/turkjped.2021.03.006

**Original Article**

# Using lactate dehydrogenase to predict the severity of respiratory distress in term newborn infants with no perinatal asphyxia

Miran Lee[1], Narae Lee[1], Mi Hye Bae[2], Young Mi Han[1], Kyung Hee Park[2], Shin Yun Byun[1], Choongrak Kim[3]

*[1]Department of Pediatrics, Pusan National University Children's Hospital, Pusan National University School of Medicine, Yangsan, South Korea; [2]Department of Pediatrics, Pusan National University Hospital, Pusan National University School of Medicine, Busan, South Korea; [3]Department of Statistics, Pusan National University, Busan, South Korea.*

## ABSTRACT

**Background.** We aimed to establish whether knowledge of lactate dehydrogenase (LDH) levels on day 1, as well as the change in these levels in the first three days, could be of clinical benefit in the diagnosis and/or prediction of severity of respiratory distress syndrome (RDS) and transient tachypnea of the newborn (TTN).

**Methods.** A retrospective study was conducted on 275 term infants (35 with RDS and 240 with TTN) admitted to the neonatal intensive care unit from January 2014 to June 2019. LDH levels were measured on admission and after three days.

**Results.** Both RDS and TTN groups had elevated LDH levels during admission. LDH levels were significantly higher in the RDS group than in the TTN group on both days. LDH levels in both groups significantly correlated with both the duration of respiratory support required, as well as the number of hospital days. We used these outcomes as a measure of severity of these conditions.

**Conclusions.** In patients with respiratory distress, it may not be clinically useful to use LDH levels on day 1 to differentiate between RDS and TTN, despite the statistically significant differences, because of the overlapping values. However, LDH levels on day 1 and day 3 may predict the degree and duration of the required respiratory support for both RDS and TTN groups.

**Key words:** lactate dehydrogenase, transient tachypnea of the newborn, neonate, hyaline membrane disease.

Respiratory distress in term neonates occurs relatively frequently, with the two most common differential diagnoses being transient tachypnea of the newborn (TTN) and respiratory distress syndrome (RDS). Although usually a mild and self-limited disease, TTN may require respiratory support.[1]

Lactate dehydrogenase (LDH) is an intracellular enzyme found in the cytoplasm of nearly all human tissues. Injured cells with loss of cell membrane integrity leak their LDH into the surrounding extracellular spaces. Cellular injury in newborns may be a result of different conditions, not only pre- and peri-partum, but also ongoing injury post-partum. Measurement of plasma LDH is routinely available in most hospitals. LDH has been extensively studied in neonates and has been used as a marker of the severity of various conditions. The suggested neonatal LDH cut-off values to identify "general illness" and the need for neonatal intensive care unit (NICU) support are 600 and 800 IU/L for different sensitivities and specificities.[2] In asphyxiated newborns, initial LDH levels may be useful in predicting the severity of their illness and the subsequent duration of mechanical ventilation.[3]

✉ Young Mi Han
skybluehym@gmail.com

Received 2nd September 2020,
accepted 31st December 2020.

One study of 54 newborns stated that "LDH is an excellent predictor to differentiate RDS from TTN soon after birth in full-term neonates with respiratory distress".[4] We wanted to look at LDH levels in a larger group of RDS and TTN patients to assess its diagnostic value, as well as its possible predictive value in terms of the degree and duration of respiratory support requirement.

## Material and Methods

### *Design*

This was a single-center, retrospective study of term infants admitted for respiratory distress without a history of fetal distress or perinatal asphyxia. A pediatric radiology specialist interpreted the chest X-ray. This study was approved by the Institutional Review Board of our hospital and the requirement for informed consent was waived (IRB No. 05-2019-126).

### *Study population*

This study included term infants (gestational age ≥37 weeks) who were admitted to a tertiary referral neonatal center for respiratory distress, within 24 hours of birth between January 2014 and June 2019, and diagnosed with either TTN or RDS. Both in-house births and transfers into the center were included. The exclusion criteria were: meconium aspiration syndrome, complex cardiac anomalies diagnosed by echocardiogram, lung anomalies diagnosed by chest X-ray, perinatal asphyxia (defined as resuscitation with more than 10 min of positive pressure ventilation before stable spontaneous respiration, pH <7.10 or base deficit ≥16 mmol/L, or Apgar score < 6 at 5 min), chromosomal anomalies, congenital infection including pneumonia and admission after 24 hours' age.

### *TTN diagnosis*

TTN was diagnosed based on initial clinical signs (onset of tachypnea [respiratory rate exceeding 60 breaths/min] within 6 hours postnatally, persistence of tachypnea for at least 12 hours) and one of the following radiologic findings: lung hyperinflation, prominent pulmonary vascular markings, flattening of the diaphragm, or fluid in the fissures.[5]

### *RDS diagnosis*

The diagnostic criteria of RDS included clinical features such as grunting, retractions, nasal flaring, cyanosis, tachypnea, an initial (pre-intubation) need for oxygen or pressure support, and radiographic findings such as a diffuse reticulogranular pattern, ground-glass appearance and superimposed air bronchograms.[6]

### *Respiratory stabilization*

Oxygen supplementation was provided to maintain peripheral oxygen saturation ($SpO_2$) above 95%. Additional respiratory support was administered via invasive positive pressure mechanical ventilation or non-invasive support, which included nasal continuous positive airway pressure (nCPAP), humidified high flow nasal cannula (HHFNC), or low flow nasal cannula, based on the degree of respiratory requirement.

Mechanical ventilation for the TTN group was determined by the attending physician based on one or more of the following criteria: sustained signs of respiratory distress such as chest retraction accompanied by grunting or nasal flaring and tachypnea (>60 breaths/min), desaturation of $SpO_2$ <93%, $PCO_2$ >60 mmHg, or repeated apnea of 20 seconds or longer accompanied by bradycardia <60 beats/min. Surfactant was administered to all of the RDS infants, but to none of the TTN infants given their assumed underlying pathophysiology. Extubation criteria were determined by the attending physician and were similar for both RDS and TTN groups.

### *Data collection and outcome measurement*

Basic information such as gestational age, birth weight, gender, multiple gestation, caesarean section, 1 and 5-minute Apgar scores, maternal

age, gestational diabetes status, pregnancy-induced hypertension, duration of invasive respiratory support, duration of overall respiratory support and duration of hospital stay were retrospectively collected from the medical records.

All patients underwent a complete blood count, blood chemistry including LDH, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP) and arterial blood gas analysis (ABGA) on admission (within 24 hours of birth); LDH and CRP were again measured on day 3 of life.

A Nova Critical Care Xpress and a Nova pHOx Ultra analyzers (Nova Biomedical, Waltham, MA, USA) were used to measure ABGA; the measurements included pH, $pCO_2$, $pO_2$, base excess (BE), and $HCO_3$-. A TBA-200FR NEO (Toshiba Medical Systems Corporation, Otawara, Japan) and an AU5800® Chemistry Analyzer (Beckman Coulter, Chaska, USA) were used to measure serum LDH, AST, and ALT levels. The receiver operating characteristic (ROC) curve was calculated to assess the usefulness of LDH in the diagnosis of RDS. Regression analysis was performed to assess the association between LDH levels (on admission and on day 3 after admission) and the total number of days on any respiratory support (invasive, non-invasive pressure support, or supplemental oxygen), and duration of hospital stay.

### *Statistical analysis*

Statistical analyses were performed using SPSS version 21.0 (IBM Co., Armonk, NY, USA). Continuous data are presented as mean and standard deviation and categorical data are presented as frequencies or percentages. The area under the curve (AUC) was obtained from the ROC curve to assess the predictive value of LDH on admission for diagnosis of RDS. The RDS group was compared with the TTN group using the independent Student's *t*-test for continuous data and the approximate z-test for comparing two population proportions. Logistic regression analysis was performed on the entire study population using LDH on admission and on day 3 after admission as dependent variables, and total duration of respiratory support requirement and duration of hospital stay as independent variables. Further, linear regression of the total duration of subsequent respiratory support and duration of hospital stay were done on the day 1 LDH level. Statistical significance was considered as a $p$-value <0.05.

## Results

Table I contains the demographic characteristics of the study participants. During the study period, 403 patients were admitted for respiratory symptoms. From those, we excluded 39 cases of meconium aspiration syndrome, 10 cases of complex cardiac anomalies, 17 cases of lung anomalies, 23 cases of perinatal asphyxia, and 29 cases of vertical infection including pneumonia. The 10 cases of tachypnea transferred/admitted 24 hours after birth were also excluded. Finally, 275 patients were eligible for the study, of which 173 patients (62.9%) were male; 237 (86.2%) were born elsewhere and subsequently transferred to our center. The mean gestational age and weight at birth were 38.6 ± 1.2 weeks and 3270.7 ± 473.2 g, respectively. The mean Apgar scores at 1 and 5 minutes were 7.4 ± 1.4 and 8.7 ± 1.1, respectively; no differences were found between both groups.

We diagnosed 35 patients with RDS and 240 with TTN. The mean gestational age (37.9 ± 0.7 weeks vs. 38.7 ± 1.2 weeks, respectively; $p$<0.001) was significantly lower in the RDS group than that of the TTN group. In contrast, the frequency of Caesarean sections (31/35 [88.6%] vs. 131/240 [54.6%]; $p$<0.001) and mean maternal age (34.8 ± 4.4 years vs. 32.4 ± 4.6 years; p=0.003) were both significantly higher in the RDS group.

Blood tests on admission revealed that both groups had elevated LDH levels (RDS group: 1337.2 ± 285.3 IU/L; TTN group: 1205.6 ± 399.0

**Table I.** Demographic characteristics of the study participants.

| | Total (n=275) | RDS (n=35) | TTN (n=240) | *p-value* |
|---|---|---|---|---|
| Gestational age (weeks) | 38.6 ± 1.2 | 37.9 ± 0.7 | 38.7 ± 1.2 | <0.001 |
| Birth weight (g) | 3270.7 ± 473.2 | 3151.2 ± 406.6 | 3288.1 ± 480.5 | 0.109 |
| Male (%) | 173 (62.9) | 20 (57.1) | 153 (63.7) | 0.569 |
| Twin (%) | 10 (3.6) | 1 (2.9) | 9 (3.8) | 1 |
| Caesarean section (%) | 162 (58.9) | 31 (88.6) | 131 (54.6) | <0.001 |
| Apgar score (1 min) | 7.4 ± 1.4 | 7.5 ± 1.8 | 7.4 ± 1.4 | 0.684 |
| Apgar score (5 min) | 8.7 ± 1.1 | 8.7 ± 1.5 | 8.7 ± 0.9 | 0.983 |
| Maternal age | 32.7 ± 4.6 | 34.8 ± 4.4 | 32.4 ± 4.6 | 0.003 |
| GDM (%) | 16 (5.8) | 1 (2.9) | 15 (6.2) | 0.678 |
| PIH (%) | 5 (1.8) | 0 (0) | 5 (2.1) | 0.853 |

Values are expressed as mean ± standard deviation or number (%).
GDM: gestational diabetes mellitus, PIH: pregnancy-induced hypertension, RDS: respiratory distress syndrome, TTN: transient tachypnea of the newborn.

**Table II.** Comparison of the laboratory test results on admission and on day 3 after admission between the RDS and TTN groups.

| | RDS (n=35) | TTN (n=240) | *p-value* |
|---|---|---|---|
| On admission | | | |
| AST (IU/L) | 67.0 ± 21.5 | 69.9 ± 42.7 | 0.524 |
| ALT (IU/L) | 11.3 ± 14.6 | 12.5 ± 7.8 | 0.438 |
| LDH (IU/L) | 1337.2 ± 285.3 | 1205.6 ± 399.0 | 0.019 |
| CRP (mg/dL) | 0.05 ± 0.06 | 0.31 ± 0.78 | <0.001 |
| pH | 7.35 ± 0.07 | 7.37 ± 0.08 | 0.165 |
| $PCO_2$ (mmHg) | 38.0 ± 8.8 | 33.6 ± 8.5 | <0.001 |
| $PO_2$ (mmHg) | 98.2 ± 51.8 | 94.4 ± 54.8 | 0.697 |
| BE | -4.86 ± 1.77 | -5.31 ± 3.16 | 0.242 |
| $HCO_3$- (mMol) | 20.4 ± 2.3 | 19.1 ± 3.3 | <0.001 |
| 3rd day | | | |
| LDH (IU/L) | 1560.7 ± 354.6 | 1234.2 ± 459.2 | <0.001 |
| CRP (mg/dL) | 0.86 ± 0.84 | 0.81 ± 1.08 | 0.775 |

Values are expressed as mean ± standard deviation.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, BE: base excess, CRP: C-reactive protein, LDH: lactate dehydrogenase, RDS: respiratory distress syndrome, TTN: transient tachypnea of the newborn.

**Table III.** Comparison of the respiratory support requirement and duration of hospital stay between RDS and TTN groups.

| | RDS (n=35) | TTN (n=240) | p-value |
|---|---|---|---|
| Need for oxygen support (%) | 34 (97.1) | 222 (92.5) | 0.512 |
| Total duration of any respiratory support (days) | 13.1 ± 6.4 | 5.5 ± 4.8 | <0.001 |
| Hospital day (days) | 15.6 ± 7.3 | 9.2 ± 5.4 | <0.001 |

Values are expressed as mean ± standard deviation or number (%).
RDS: respiratory distress syndrome, TTN: transient tachypnea of the newborn.

IU/L) when compared to reference values of normal LDH of 500–700 IU/L; there was a statistically significant difference between both groups.[7,8] C-reactive protein (CRP), $pCO_2$, and $HCO_3$- were significantly different between both groups, but the levels were within normal ranges, therefore, considered irrelevant for this study. On day 3, the LDH levels (Table II) were, again, significantly higher in the RDS group than the TTN group (RDS group: 1560.7 ± 354.6 IU/L; TTN group: 1234.2 ± 459.2 IU/L).

There was no significant difference in the need for oxygen supplementation between the two groups. However, the RDS group had a significantly greater total duration of any respiratory support (RDS: 13.1 ± 6.4 days vs. TTN: 5.5 ± 4.8 days, p<0.001). All 35 of the RDS infants were intubated for surfactant administration, as opposed to 49/240 infants with TTN requiring intubation as described above in "respiratory stabilization". One RDS infant received INSURE (intubation-surfactant-extubation). The mean duration of hospital stay (RDS: 15.6 ± 7.3 days vs. TTN: 9.2 ± 5.4 days, p<0.001) was significantly higher in the RDS group than the TTN group (Table III).

The area under the ROC curve of the LDH level on admission was 0.648 (Fig. 1). To assess the usefulness of LDH levels on admission and on day 3 after admission, the correlation with the following two variables was investigated: (1) duration of total respiratory support requirement, and (2) duration of hospital stay. Logistic regression analysis showed the LDH levels, both at admission and after 3 days, were significantly correlated with both variables, LDH levels on day 3 after admission showing a particularly strong correlation (Table IV). The univariate analysis of LDH level on admission and the two variables showed significant correlations (Fig. 2). Finally, to see the predicting ability of the fitted linear regression (i.e., the ability of the level of LDH at day 1 as a predictor of both variables), we used 200 observations as trainsets and 75 as test sets. As shown in Figure 3, the LDH level was significant in predicting both variables.

## Discussion

LDH levels are highest soon after birth, generally decreasing with age throughout childhood.[9] This may be related to the general vulnerability of tissues/organs in the newborn, temporary hypoxia during the birth process, and increased cell membrane permeability and

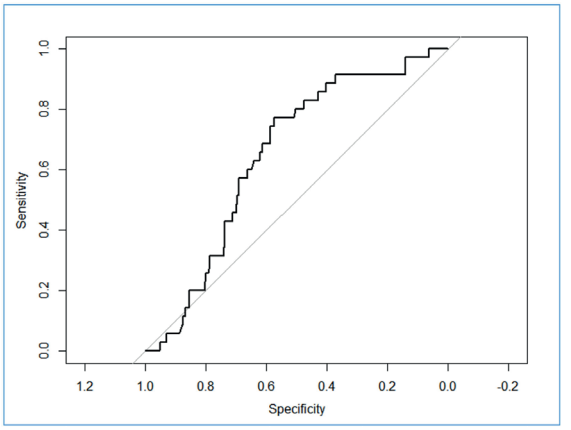


**Fig. 1.** ROC curve for the value of LDH as a predictor of RDS. The AUC is 0.648.

**Table IV.** Correlations between LDH levels and prognosis (respiratory support requirement and duration of hospital stay) in patients with respiratory distress: logistic regression between LDH and four prognosis variables.

| Response variable | On admission | | Day 3 | |
|---|---|---|---|---|
| | Increase of response variable with LDH 100 increase | *p*-value | Increase of response variable with LDH 100 increase | *p*-value |
| Total respiratory support (days) | 0.405 | <0.001 | 0.629 | <0.001 |
| Hospital stay (days) | 0.345 | <0.001 | 0.523 | <0.001 |

LDH: lactate dehydrogenase.

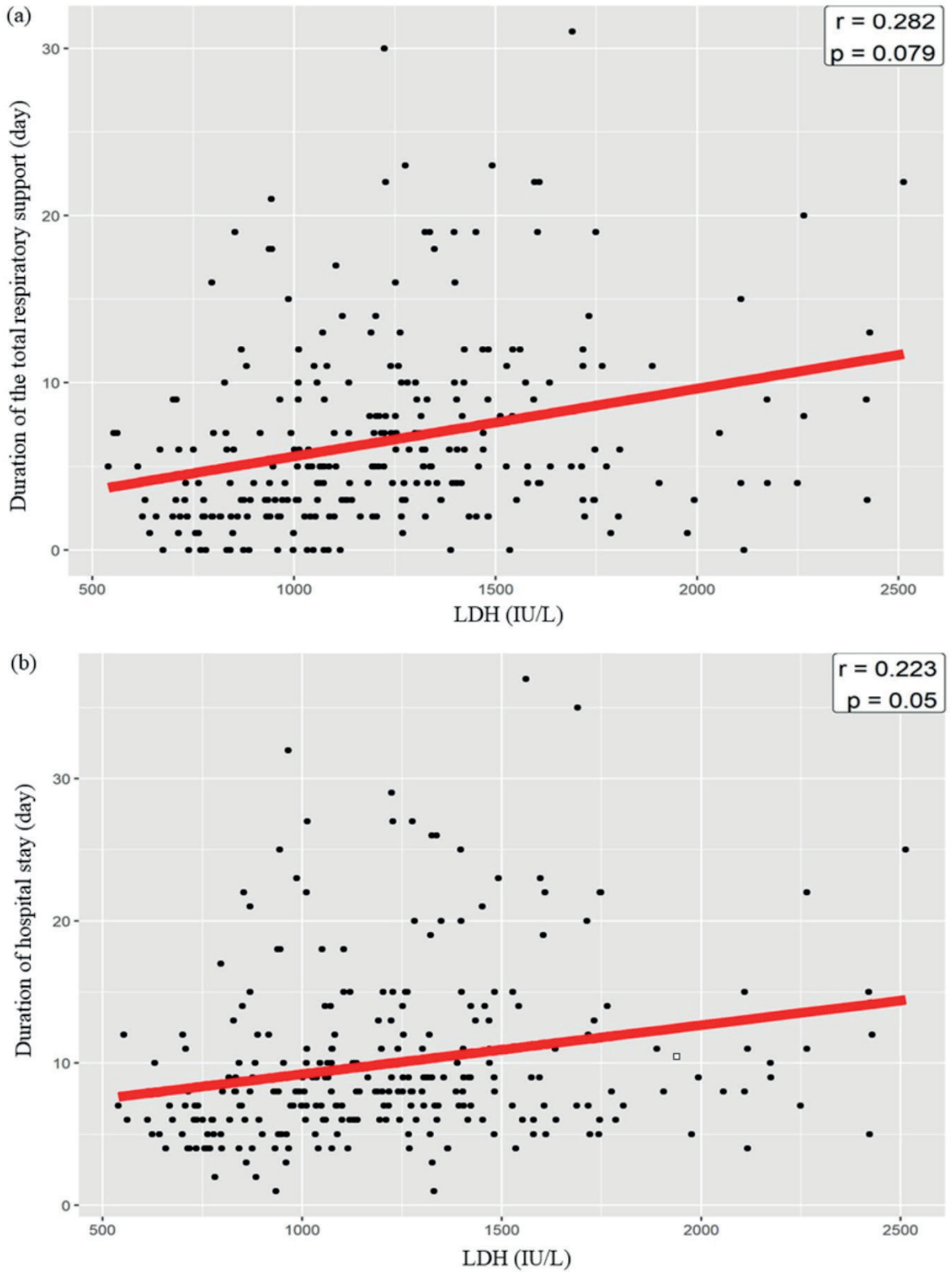


**Fig. 2.** In patients with respiratory distress, correlation between the LDH level on admission and the total respiratory support requirement (a), and the duration of hospital stay (b). A straight line is the fitted simple linear regression.

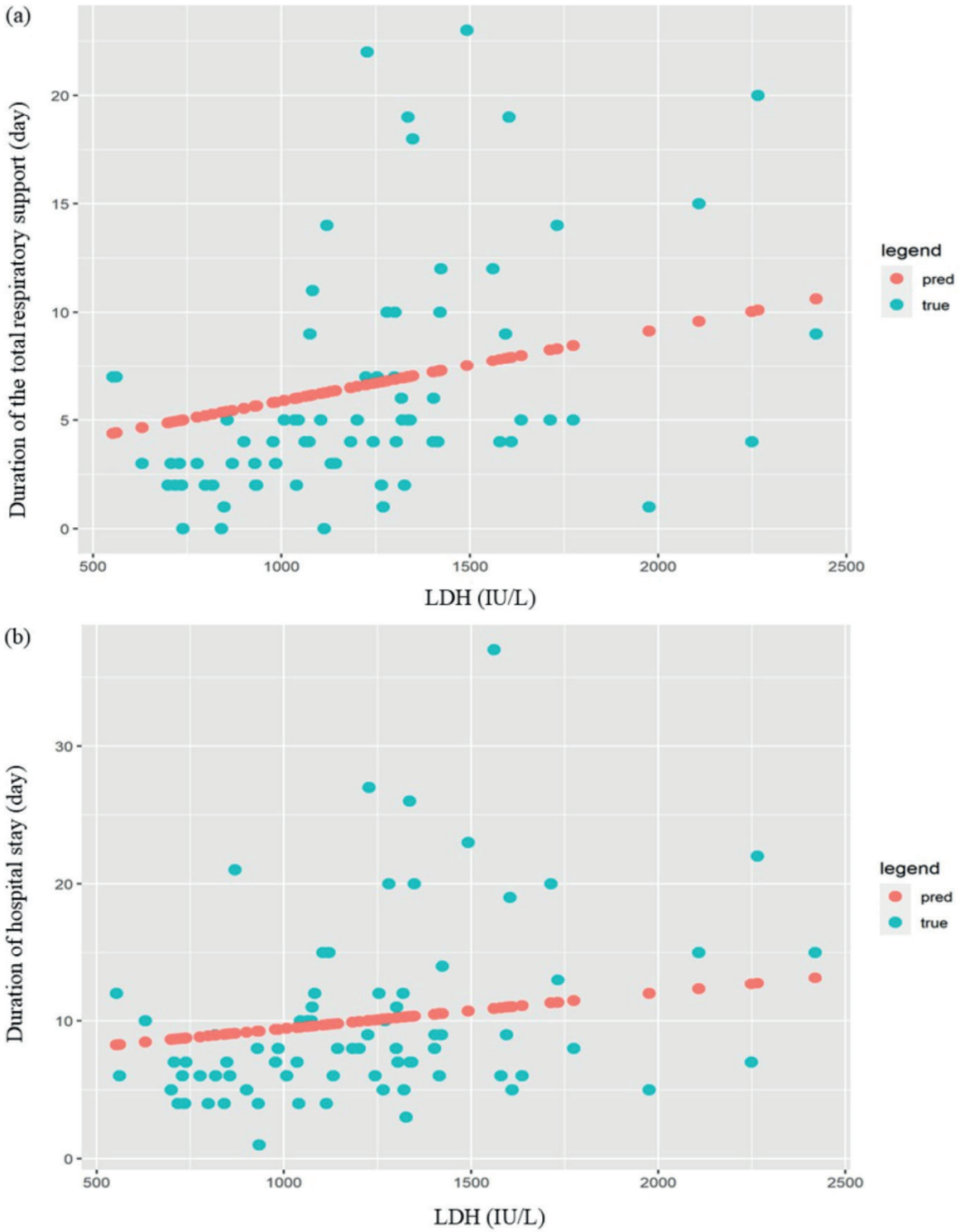


**Fig. 3.** Using the fitted model based on the 200 trainset, we compare the predicted value (orange) for the 75 test set with true value (green): the total respiratory support requirement (a), and the duration of hospital stay (b). A straight line is the fitted simple linear regression.

hemolysis after birth. In term healthy newborns, LDH peaks in the first 24 hours, then gradually decreases; with median LDH 358.5 IU/L at birth, 512.0 IU/L at 24 hours, and 431.0 IU/L at 72 hours.[8] For healthy non-asphyxiated newborns, normal LDH levels are between 330–700 IU/ L in the first 24 hours, and between 320–600 IU/L at 72 hours.[7,8]

Because perinatal hypoxia results in plasma LDH elevation postpartum, it is important to identify infants with some degree of perinatal hypoxia to appropriately interpret their postnatal LDH levels. We used the term "perinatal hypoxia" to denote some degree of oxygen deficiency, if insufficient to satisfy the "perinatal asphyxia" criteria defined earlier.

It is not always possible to obtain a reliable history, especially for newborns delivered at another institution. Historically, the Apgar score is an indicator of perinatal hypoxia. A low 5-minute Apgar score alone was correlated to elevated LDH.[10] The negative predictive value of the 5-minute Apgar score is the more reliable indicator; wherein a score of 7 or greater, "it is unlikely" that peripartum hypoxia would cause neonatal encephalopathy.[11] The mean 5-minute Apgar score for our cohort was 8.7.

Arterial pH and base excess (BE) at or soon after birth have been used as measures of prenatal hypoxia,[12] until later studies have shown lactate to be a more useful indicator.[13-15] In our study, cord arterial lactate would have been ideal to identify the presence and severity of perinatal hypoxia, but this is not routinely performed in many institutions, including ours. Postnatal arterial lactate, even within the first 24 hours, may normalize rapidly and thus no longer reflect preceding perinatal events.[15] Interestingly, there is a strong correlation between lactate and LDH levels on admission in sick neonates.[2]

"Physiological hypoxia" is a spectrum with no current accepted definition. Because perinatal hypoxia can affect the brain and all other body organs, an elevated LDH with some degree of perinatal hypoxia is a non-specific "marker" for hypoxic injury. The most commonly reported injuries secondary to asphyxia are those involving the kidneys and the brain; however, other organ systems, including the lungs, are also directly affected.[16] Once released from the cells, usually due to stresses of birth, plasma LDH reduction is dependent on the clearance capacity of the liver and possibly on ongoing cell injury, and thus, continuing LDH release.

Both LDH and AST in normal newborns were inversely related to pH and BE, which suggested that the more significant the hypoxia, the higher the LDH and AST levels.[7] Gunes showed increasing elevation of LDH with an increasing degree of perinatal asphyxia. Their mean AST on day 1 was higher in the mild (84 U/L), moderate (160 U/L), and severe asphyxia (227 U/L) groups compared to their control (70 U/L) group.[17] We showed a mean AST 67.0 and 69.9 U/L in our RDS and TTN groups respectively, suggesting that the elevated LDH in our study group was not secondary to significant perinatal hypoxia. In order to further eliminate the possibility of perinatal hypoxia accounting for our raised LDH levels on admission, we performed the same analysis on a subgroup of 138 patients whose AST was within the accepted laboratory reference range (< 60 U/L). The linear regression of the duration of total respiratory support requirement and duration of hospital stay, done on the day 1 LDH level, showed the same results; but an even more statistically significant correlation than what we found from our total patient pool.

Studies on perinatal asphyxia with or without HIE consistently show that the LDH in newborns with moderate and severe asphyxia peaks on day 3 after which it gradually decreases, normalizing within 10 days of life.[2,10,17] In mildly asphyxiated newborns, the elevated day 1 LDH decreased by day 3.[10,17] Thus, persistently raised and increasing LDH levels on day 3 in both our respiratory distress groups would be explained by perinatal asphyxia only if it was moderate or severe; however, we had no demonstrable perinatal asphyxia. Therefore, our increasing LDH levels would most likely be due to the respiratory conditions themselves: presumably

secondary to associated lung cellular injury, peripheral organ effects associated with the respiratory conditions, or possibly even by cellular injury caused by the administered respiratory support.

To tease out LDH elevation secondary to primarily respiratory disease, it is notably absent or immeasurable if there is a pre-existing raised LDH secondary to multi-organ hypoxia. Idiopathic respiratory distress syndrome (IRDS) superimposed on perinatal asphyxia does not further alter the already raised LDH and AST levels.[18] Thus, the cellular lung injury during global asphyxia may already have resulted in an early release of their LDH, which may be greater than any subsequent LDH leak associated with any coexistent respiratory pathology.

Although the first 24-hour LDH has been shown to be a predictor of "general illness" and a need for NICU support, only a few of the admitted IRDS infants had initially elevated LDH values, and IRDS infants were almost always admitted early.[2] This is important, because Lackmann et al.[8] normal values show a significant increase in LDH from birth to the 12-hour measurement. In our study, most newborns were born at another institution, so their admission LDH levels, although all within the first 24 hours of age, were not taken soon after birth. Additionally, their respiratory status may not have been ideal prior to admission at our institution. Our initial LDH levels on admission may thus, not reflect the condition of the patients at birth, but rather the condition secondary to the (possibly sub-optimally treated) respiratory condition.

Thus, can an elevated LDH be due to respiratory distress, or is it simply a reflection of preceding perinatal hypoxia? Aydogdu et al.[19] animal model showed that serum enzymes, including LDH, are elevated in cases of respiratory distress syndrome without preceding perinatal hypoxia. Ozkiraz et al.[5] showed that an initial LDH level, with cut-off value of 750 U/L, offered good predictive value for prolonged oxygen requirement in TTN patients. These authors suggested that initial lactate and LDH values may be useful to identify newborns who may subsequently deteriorate and require respiratory support. Lim's animal model demonstrated a progressive increase of LDH during ventilator-induced lung injury, showing a negative correlation with oxygenation, suggesting that LDH levels could be an early marker of primary lung injury.[20]

Our term newborns had no measurable perinatal asphyxia, but we cannot exclude some degree of possible mild perinatal hypoxia. LDH levels increasing in the first three days (an expected trend in moderate/severe perinatal asphyxia) in our cohort must clearly be due to postnatal direct lung cellular injury, or to generalized hypoxic effects (including changes in circulation) secondary to the respiratory disease process.

The weaknesses of our study are inherent to its retrospective nature. As standard protocol in most institutions did not include routine measurement of cord arterial lactate levels, we could not definitively assess perinatal hypoxia using this parameter. Cord arterial lactate would be an ideal parameter to include in future studies.

Our study suggests that a raised LDH on day 1 may reflect respiratory distress in the absence of perinatal asphyxia. The day 1 LDH levels were 1337.2 ± 285.3 for RDS and 1205.6 ± 399.0 for TTN, suggesting a range of 1051.9–1622.5 and 806.6–1604.6, respectively. Since there is considerable overlap in these ranges, LDH levels alone are not clinically useful to differentiate RDS from TTN on day 1, even if there was a statistically significant difference between these groups. However, LDH levels on day 1 in both groups of patients with respiratory distress may predict the degree and duration of the required respiratory support and hospital stay. The significantly higher day 3 LDH in RDS compared to TTN may be predictive of a longer expected duration of respiratory support and hospital stay in the RDS group. The higher LDH in the RDS group may also reflect the degree of ongoing injury on day 3, as these infants usually take a few days to improve.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Young Mi Han, Choongrak Kim; data collection: Miran Lee, Narae Lee, Mi Hye Bae; analysis and interpretation of results: Miran Lee, Young Mi Han, Kyung Hee Park, Shin Yun Byun, Choongrak Kim; draft manuscript preparation: Miran Lee, Young Mi Han, Choongrak Kim. All authors reviewed the results and approved the final version of the manuscript.

### Ethical approval

This study was approved by the Institutional Review Board of our hospital and the requirement for informed consent was waived (IRB No. 05-2019-126).

### Source of funding

There is no specific funding.

### Conflict of interest

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 404-416
https://doi.org/10.24953/turkjped.2021.03.007

**Original Article**

# Evaluation of serum/salivary levels of carnosine and cotinine in recurrent wheezing of young children with passive smoking

Mehmet Kılıç[1], Şilem Özdem Alataş[2], Erdal Taşkın[3], Süleyman Aydın[4], Mehmet Onur Kaya[5]

*Departments of [1]Pediatric Allergy and Immunology, [2]Pediatrics, [3]Neonatology, [4]Biochemistry and [5]Biostatistics, Fırat University Faculty of Medicine, Elazığ, Turkey.*

## ABSTRACT

**Background.** Recurrent wheezing is common in young children, with a cumulative prevalence of up to 40 % in the first 6 years of life. In this study, we aimed to evaluate the relationship between the number of wheezing episodes and the number of cigarettes smoked at home and serum / saliva cotinine and carnosine levels in children with recurrent wheezing.

**Methods.** This study was conducted with 80 young children with recurrent wheezing, aged between 1-4 years and 50 healthy control groups. Patient population was divided into three groups depending on the number of their exposure to cigarette smoke and wheezing attacks. Serum cotinine, saliva cotinine, serum carnosine, saliva carnosine, vitamin D levels were measured by using the ELISA method.

**Results.** A significant relationship for serum cotinine and saliva cotinine levels was found between groups ($p<0.05$). It was determined that as the number of exposure to cigarette smoke and number of wheezing episodes in young children with recurrent wheezing increased, the level of serum/saliva cotinine levels increased significantly, compared to the control group. In contrast, it was determined that as the number of exposure to cigarette smoke and number of wheezing episodes in young children with recurrent wheezing increased, serum/saliva carnosine levels decreased significantly, compared to the control group. In addition, a significant difference in serum vitamin D levels was found between healthy young children and young children with recurrent wheezing ($p<0.05$).

**Conclusions.** We think that the measurement of salivary cotinine is a useful and noninvasive marker to evaluate passive smoking exposure in the etiology of recurrent wheezing in young children.

**Key words:** recurrent wheezing, cotinine, carnosine, serum, saliva, vitamin D, exposure smoke.

Recurrent wheezing is common in young children, with a cumulative prevalence of up to 40% in the first six years of life. It is an important cause of diminished health-related quality of life in infancy.[1] The etiology of wheezing is usually difficult to ascertain, and wheezing is not specific for asthma and can be due to viral infection or environmental exposure (e.g.,smoking).[2]

✉ Mehmet Kılıç
drmkilic@gmail.com

Received 23th October 2020, revised 9th December 2020, accepted 25th December 2020.

Children are commonly exposed to passive cigarette smoke at an early age. Children are one of the groups mostly affected by exposure to environmental cigarette smoke. As children breathe more frequently than adults, they inhale environmental cigarette smoke more often. In addition, children cannot protect themselves from exposure to cigarette smoke.[3] Smoking during pregnancy has a devastating effect on the fetal lung and suppresses the development of airways. Passive smoking after birth has been reported to cause a decrease in ciliary movements, an increase in mucus production, impairment in lung functions, inflammation in

the mucosa, degeneration in epithelial cells, and an increase in the incidence of lower respiratory tract infections.[4]

Two methods can be used to evaluate the exposure to passive cigarette smoke in children: a questionnaire answered by the parents and the measurement of saliva, urine, and serum cotinine levels. Studies have reported that the information given by parents about the exposure of their children to cigarette smoke does not correlate with the level of cotinine measured in children, and thus this information alone is not sufficient.[5,6] Measuring the cotinine level indicates recent smoking or passive exposure to cigarette smoke (e.g., 2–3 days). The half-life of cotinine in biological fluids (i.e., urine, serum, and saliva) is the same at 15–19 hours. Its half-life in children is higher than that in adults (65 h in neonates, < 18 months 60 hours, ≥ 18 months 40 hours). The cotinine levels in serum and saliva are similar, and urine / serum ratio is 5/6. The measurement of cotinine in saliva and urine is preferred, especially in epidemiological studies, because it is not invasive and shows a relatively long duration of exposure. Although the cotinine values in urine change depending on age, renal functions are also affected by the amount of urine output and urine pH. For this reason, measuring the level of cotinine in saliva is preferred.[6]

The lungs have an antioxidant system to minimize exposure to the oxidant, but due to the overproduction of oxidant molecules, this protective system falls short and damage occurs. An increase in reactive oxygen products caused by cigarette smoke exceeding the protective capacity of the oxidant defense system has been reported to cause oxidative damage in proteins, lipids, and DNA.[7] Moreover, exposure to cigarette smoke causes a decrease in antioxidant levels.[8] Also, it has been shown that levels of antioxidants are reduced in children with wheezing episodes.[9] Carnosine, one of the antioxidant molecules, is endogenously synthesized by binding the amino acids β-alanine and L-histidine through a reaction catalyzed by carnosine synthase. Carnosine is found mainly in the kidneys, lungs, liver, and blood. Similar to many molecules synthesized from biological tissues, carnosine can pass through circulation and saliva. The antioxidant activity of carnosine was examined by Boldyrev et al.[10] in the 1980s. The antioxidant activity of carnosine is mediated by different mechanisms, including metal ion chelation, scavenging of reactive oxygen radicals, and protection of the cell membrane from lipid peroxidation.[10-12] In addition, there is an increasing awareness of the important role of vitamin D in the maintenance of general immune and respiratory health. The growing data suggests that vitamin D plays an important role in the lung development and it is discussed as a risk factor for respiratory infections andrecurrent wheezing.[13,14]

In this study, we aimed to evaluate the relationship between the serum/saliva cotinine and carnosine levels and the number of wheezing episodes and the number of cigarettes smoked at home in young children with recurrent wheezing.

## Material and Methods

Eighty children aged 1-4 years with a diagnosis of recurrent wheezing and passive smoking were included in the study. The attacks of wheezing were diagnosed by a doctor. In addition, 50 healthy children who were not exposed to passive smoking at home were included in the control group. Cases with prematurity, malnutrition, cystic fibrosis, primary ciliary dyskinesia, bronchopulmonary dyskinesia, bronchiectasis, congenital anomalies of the respiratory system, chest deformity and interstitial lung diseases, gastroesophageal reflux, swallowing dysfunction, foreign body aspiration, immunodeficiency, malignancy, congenital heart disease, congenital syndromes, and chronic hematological, renal, hepatic, and neurological diseases were not included in the study and the control group. Detailed questionnaire data were obtained from parents of all the young children participating in the study. The questionnaire consisted of questions

about the young children (age, gender, modes of delivery, birth weight, breastfeeding duration, sibling attending school or nursery, number of wheezing episodes diagnosed by the doctor), his/her family (number of peoples living at home, maternal education, age of mother at birth, father education, monthly income status, the number of cigarettes smoked daily by individuals living at home while the child is at home, mother's smoking during pregnancy, mother's smoking in postpartum period, father's smoking, smoking by other members of the household).

The amount of passive smoking was defined as the number of cigarettes smoked daily by individuals living at home (mother, father, siblings, and other members of the family) while the child is at home. The number of cigarettes smoked when they are away from the child during the day (e.g., workplace, outdoor environment) was not included in this grouping. The definition of recurrent wheezing was accepted as at least ≥ 4 wheezing episodes requiring bronchodilator medication (salbutamol). These patients were classified according to the number of episodes they had experienced in the previous year: group I, 4–6 episodes; group II, 7–10 episodes; and group III, > 10 episodes. Moreover, the patients with recurrent wheezing were divided into three groups according to the number of cigarettes smoked daily at home: group A, ≤ 5; group B, 6–9; and group C, ≥ 10 cigarettes.

To clarify the etiology of wheezing in all patients included in the study, complete blood count, eosinophil count in peripheral blood, serum immunoglobulin levels, evaluation of lymphocyte subgroups, sweat test, purified protein derivative test, chest radiography, QuantiFERON test, gastroesophageal reflux scintigraphy, fx5-specific IgE, Phadiatop test, and food and inhaled allergens skin prick test were performed. In case of an indication, 24-h esophageal pH monitoring, barium esophagus-stomach-duodenum radiography, analysis of acid-resistant bacteria and hemosiderin-loaded macrophage in fasting gastric juice, IgG subgroup measurement, tuberculosis culture, bronchoscopy, echocardiography, measurement of alpha-1 antitrypsin, electron microscopic examination, nasal biopsy, and radiological imaging studies, such as high resolution computed tomography and thorax magnetic resonance were also performed.

The serum carnosine and cotinine levels were analyzed in 1 cc venous blood samples remaining from the blood taken from the cases in the study and control groups for routine tests. The venous blood samples were centrifuged and stored at −80°C. In addition, the saliva was obtained from the patients in the study and control groups to analyze the cotinine and carnosine levels. All saliva collections were performed by suctioning saliva from the buccal cavity in the infant's mouth with a soft plastic transfer pipette, attempting to collect 0.5-1 mL of saliva. All saliva specimens were performed when the child's mouth was free of food or milk. After centrifugation for 5 min at 2000 rpm, the samples were stored at −80°C. The serum and saliva cotinine levels were measured using an ELISA kit according to the manufacturer's instructions. The detailed description of the sample collection and analysis has been published elsewhere.[15] In addition, serum 25 (OH) D3 level was studied from the blood taken for routine biochemical tests in both the control and wheezing groups. Serum 25 (OH) D3 levels were analyzed with chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT c8000, Abbott, Abbott Park, Illinois, USA). The 25 (OH) D3 level above 30 ng/ml is accepted sufficient; the level between 20 and 30 ng/ml insufficient; <20 ng/ml deficient and under 10 ng/ml is extremely deficient.

The necessary approval for the study was obtained from the clinical research ethics committee of Firat University (Ethical board date/number: 05.09.2013/03-07). The families invited to participate in the study were informed about the research, and their written consent was obtained.

### *Statistical methods*

All data were analyzed using the SPSS version 22.0 (IBM, Chicago, IL, USA) program. The compliance of the continuous variables determined by the measurement of a normal distribution was evaluated using the Shapiro-Wilk test. Nonparametric tests were used to analyze the data. The results were given as the median (minimum-maximum). The Mann-Whitney U test was used for the comparison of the mean values, the Kruskal-Wallis test for the comparison of more than two groups, the post-hoc Dunn test with Bonferroni correction for pairwise comparisons, and the Pearson chi-square test for the comparison of percentages. The cutoff points and the rates of sensitivity and specificity were evaluated with the receiver operating characteristic (ROC) analysis to determine the diagnostic power of the quantitative data measured. A p-value of $< 0.05$ was considered statistically significant.

## Results

Twenty-eight (35%) girls and 52 (65%) boys, with an overall mean age of 25.5 (12–46) months, had a history of recurrent wheezing episodes. In the control group, 22 (44%) were girls and 28 (56%) were boys, with an overall mean age of 24 (12–47) months. No difference was found between the patient and control groups in terms of age and gender ($p > 0.05$). In the wheezing group, the levels of serum cotinine (2.01 [0.78–3.44] pg/ml), salivary cotinine (2.00 [0.66–3.04] pg/ml), serum carmosine (129 [96–191] ng/ml), salivary carnosine (124 [93–186] ng/ml), and vitamin D (19.5 [3–63] ng / ml) were determined as indicated. In the control group, the levels of serum cotinine (0.59 [0.18–1.61] pg/ml), salivary cotinine (0.46 [0.06–1.28] pg/ml), serum carmosine (166 [96–192] ng/ml), salivary carnosine (148 [103–190] ng/ml), and vitamin D (24.6 [11–52] ng/ml) were determined as indicated.

A statistically significant difference was found between the recurrent wheezing and control groups in terms of serum vitamin D, serum/salivary cotinine, and serum/salivary carnosine levels ($p < 0.05$) (Table I). A statistically significant difference was also found between the recurrent wheezing and control groups in terms of total IgE levels, maternal age at birth, duration of breastfeeding, maternal education, and number of individuals living at home ($p < 0.05$). However, there was no difference between the patient and control groups in terms of birth weight, absolute eosinophil count in peripheral blood, history of siblings attending school or nursery, family income, and father's education level ($p > 0.05$) (Table I).

Among the 32 patients in group I, 10 (31.3%) were girls and 22 (68.8%) were boys, with an overall average age of 24 (12–44) months. Among the 30 patients in group II, 12 (40%) were boys and 18 (60%) were girls, with an overall average age of 30 (15–46) months. Among the 18 patients in group III, 6 (33.3%) were girls and 12 (66.7%) were boys, with an overall average age of 33 (12–46) months. No statistically significant difference was found between the groups in terms of gender ($p > 0.05$) (Table II). However, a weak relationship was found between group I and group II when the groups were evaluated in terms of age. The number of wheezing episodes increased with age ($p = 0.01$, $r = 0.28$).

When the children in the wheezing group were compared in terms of serum and salivary cotinine levels, a statistically significant difference was found only between groups I and III ($p = 0.002$, $p = 0.001$, respectively) (Table II, Fig. 1). In addition, when the cases in the wheezing group were compared in terms of the number of cigarettes smoked daily at home, a significant difference was found between groups I and II and between groups I and III ($p = 0.001$, $p = 0.0001$, respectively).

When the wheezing groups were compared in terms of father's smoking and mother's smoking during pregnancy, during the breastfeeding period, and after the breastfeeding period, no statistical difference was found ($p > 0.05$).

**Table I.** Comparison of demographic and laboratory characteristics of the patient and control groups.

| Features | Control group (n: 50) | Wheezing group (n: 80) | p value |
|---|---|---|---|
| Age (months) * | 24 (12-47) | 25.5 (12-46) | 0.08 |
| Gender n (%) | | | |
| Girl | 22 (44%) | 28 (35%) | 0.3 |
| Boy | 28 (56%) | 52 (65%) | |
| Absolute eosinophil count (/mm$^3$) * | 205 (40-890) | 260 (10-3230) | 0.2 |
| Total IgE level (IU / ml) | | | |
| Normal | 42 (84%) | 35(43.8%) | 0.03 |
| High | 8 (16%) | 45 (56.3%) | |
| Vitamin D level (ng / ml)* | 24.6 (11-52) | 19.5 (3-63) | 0.005 |
| Serum cotinine level (pg / ml) * | 0.59 (0.18-1.61) | 2.01 (0.78-3.44) | 0.0001 |
| Saliva cotinine level (pg / ml) * | 0.46 (0.06-1.28) | 2.00 (0.66-3.04) | 0.0001 |
| Serum carnosine level (ng / ml) | 166 (96-192) | 129 (96-191) | 0.0001 |
| Saliva carnosine level (ng / ml) * | 148 (103-190) | 124 (93-186) | 0.0001 |
| Age of mother at birth (years) * | 29 (21-40) | 25 (20-39) | 0.0001 |
| Monthly income status (dollars) n (%) | | | |
| <300 $ | 19 (38%) | 23 (28.7%) | 0.1 |
| 300-499 $ | 24 (48%) | 51 (63.7%) | |
| 500-999 $ | 6 (12%) | 3 (3%) | |
| 1000-1499 $ | 1 (2%) | 1 (1.3%) | |
| >1500 $ | - | 2 (2.5%) | |
| Maternal education n (%) | | | |
| Primary education | 35 (70%) | 70 (87.5%) | 0.004 |
| High school | 15 (30%) | 7 (8.8%) | |
| University | - | 3 (3.8%) | |
| Father education n (%) | | | |
| Primary education | 36 (72%) | 56 (70%) | 0.9 |
| High school | 12 (24%) | 21 (26.3%) | |
| University | 2 (4%) | 3 (3.8%) | |
| Breastfeeding duration n (%) | | | 0.007 |
| No | - | 6 (7.5) | |
| <6 months | 11 (22%) | 32 (46%) | |
| ≥ 6 months | 39 (78%) | 42 (52.5) | |
| Sibling attending school or nursery n (%) | | | |
| Yes | 44 (88%) | 65 (81.3) | 0.3 |
| No | 6 (12%) | 15 (18.7) | |
| Birth weight (kg) | | | |
| ≤2500 | 9 (18%) | 23 (28.1%) | 0.06 |
| >2500 | 41 (82%) | 57 (71.3%) | |
| Modes of delivery n (%) | | | |
| Vaginal delivery | 40 (80%) | 62 (77.5%) | 0.1 |
| Cesarean section | 10 (20%) | 18 (22.5%) | |
| Number of peoples living at home * | 4 (3-6) | 5 (3-7) | 0.002 |

* Median (minimum-maximum)

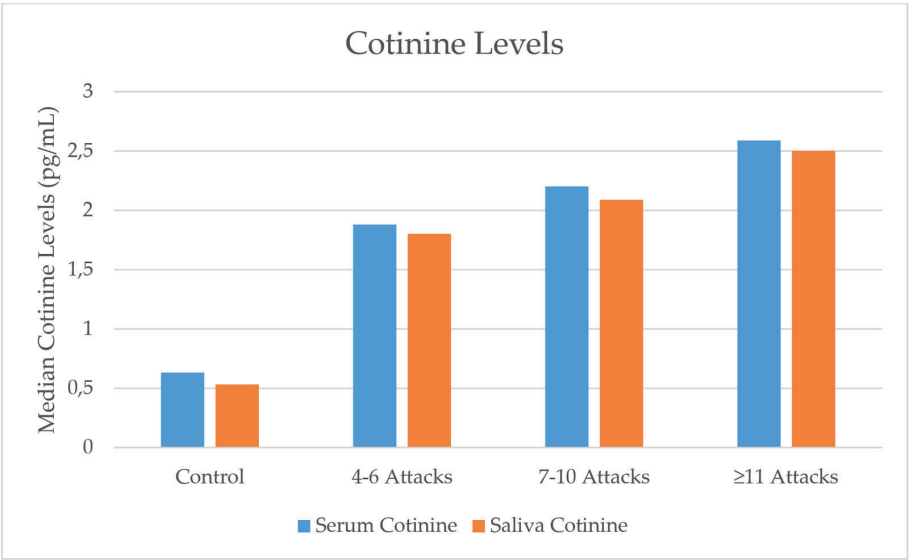


**Fig. 1.** Comparison of serum / salivary cotinine levels between control group and group I-II-III.

However, a statistically significant difference was found (p = 0.003) when the wheezing group was evaluated in terms of the smoking history of other family members. We found that in 22 (27.5%) of the 80 patients with wheezing, household members other than parents at home were smoking. In four of the cases (18.2%), other family members except both parents smoked at home, and in 18 cases (81.8), the mother and/or father of the patients smoked together with the other family members. In addition, when the wheezing groups were compared in terms of serum and salivary carnosine levels, a statistically significant difference was found only between groups I and III (p = 0.01, p = 0.01, respectively) (Table II, Fig. 2).

When groups A, B, and C, which were classified according to the number of cigarettes smoked daily that the child was exposed to at home, were compared in terms of serum cotinine levels, a statistical difference was found between groups A and B, between groups A and C, and between groups B and C (p = 0.03, p = 0.0001, p = 0.01, respectively). In terms of cotinine levels in saliva, a statistically significant difference was found between groups A and C and between groups B and C (p = 0.0001, p = 0.01, respectively) (Table III, Fig. 3).

When the groups classified according to the number of cigarettes smoked daily that the child was exposed to at home were evaluated in terms of serum carnosine levels, a statistically significant difference was found between groups A and B, between groups A and C, and between groups B and C (p = 0.04, p = 0.0001, p = 0.01, respectively). When the salivary carnosine levels were evaluated, a statistically significant difference was found between groups A and C and between groups B and C (p = 0.0001, p = 0.0001, respectively) (Table III). When the groups classified according to the number of cigarettes smoked daily that the child was exposed to at home were evaluated in terms of mother's education, father's education, monthly income of the family, and number of people living at home, no statistically significant difference was found (p = 0.2, p = 0.3, p = 0.7, p = 0.5, respectively).

To predict the exposure to passive smoking in children with a history of recurrent wheezing based on the ROC curve analysis for the serum cotinine level, we used the cutoff value of > 1.26 pg/ml, with 97.5% sensitivity and 98% specificity. Moreover, the cutoff value for the salivary cotinine level was > 1.18 pg/ml, with 96.2% sensitivity and 98% specificity.

**Table II.** Comparison of demographic and laboratory characteristics of cases classified according to the number of wheezing attacks.

| Features | Group I (n: 32) | Group II (n: 30) | Group III (n: 18) | p value |
|---|---|---|---|---|
| Age (month)* | 24 (12-44) | 30 (15-46) | 33 (12-46) | 0.01** |
| Gender n (%) | | | | |
| Girl | 10 (%31.3) | 12 (%40) | 6 (%33.3) | 0.8 |
| Boy | 22 (%68.8) | 18 (%60) | 12 (%66.7) | |
| Serum vitamin D level (ng / ml) * | 19.3 (3-63) | 19.1 (3-45) | 20 (7-43) | 0.8 |
| Serum cotinine level (pg / ml) * | 1.93 (0.78-2.92) | 2.09 (0.78-3.31) | 2.84 (1.66-3.44) | 0.002*** |
| Saliva cotinine level (pg / ml) * | 1.86 (0.69-2.91) | 2.0 (0.66-3.04) | 2.73 (1.60-3.01) | 0.001*** |
| The number of cigarettes smoked at home (pieces / day) * | 4 (2-11) | 9 (2-40) | 15 (3-30) | 0.001** 0.0001*** |
| Number of peoples living at home* | 5 (4-7) | 5 (3-7) | 5 (3-6) | 0.9 |
| Maternal smoking during pregnancy n (%) | | | | |
| No | 27 (87.1%) | 29 (80.6%) | 9 (69.2%) | 0.3 |
| Yes | 4 (12.9%) | 7 (19.4%) | 4 (30.8%) | |
| Mother's smoking during breastfeeding period n (%) | | | | |
| No | 23 (74.2%) | 24 (66.7%) | 8 (61.5%) | 0.6 |
| Yes | 8 (25.8) | 12 (33.3%) | 5 (38.5%) | |
| Mother's smoking after breastfeeding period n (%) | | | | |
| No | 20 (64.5%) | 23 (63.9%) | 6 (46.2%) | 0.4 |
| Yes | 11 (35.5%) | 13 (36.1%) | 7 (53.8%) | |
| Father's smoking n (%) | | | | |
| No | 7 (22.6%) | 6 (16.7%) | 2 (15.4%) | 0.7 |
| Yes | 24 (77.4%) | 30 (83.3%) | 11 (84.6%) | |
| Smoking of other family members n (%) | | | | |
| No | 27 (87.1%) | 23 (63.9%) | 7 (53.8%) | 0.003 |
| Yes | 4 (12.9%) | 13 (36.1%) | 6 (46.2%) | |
| Serum carnosine level (ng / ml) * | 131 (118-191) | 133.5(96-165) | 120 (108-161) | 0.01*** |
| Saliva carnosine level (ng / ml) * | 128 (110-186) | 126 (93-146) | 118 (100-141) | 0.01*** |

* Median (minimum-maximum)
** Comparison of group I and II
*** Comparison of group I and III
**** Comparison of group II and III

## Discussion

Various risk factors have been identified for recurrent wheezing in the first year of life. These risk factors may include gestational age, maternal age at birth, type of delivery, mother's smoking during pregnancy and postnatal period, exposure to cigarette smoke at home, environmental air pollution, breastfeeding duration, number of people living in the family, place of residence, male gender, going to nursery, viral respiratory tract infections, socio-economic status, exposure to allergens, use of antibiotics or paracetamol, feeding animals at home, and history of atopic dermatitis.[16-20]

In our study, we did not find a statistically significant difference between the wheezing group and the control group in terms of age, gender, presence of siblings attending school or

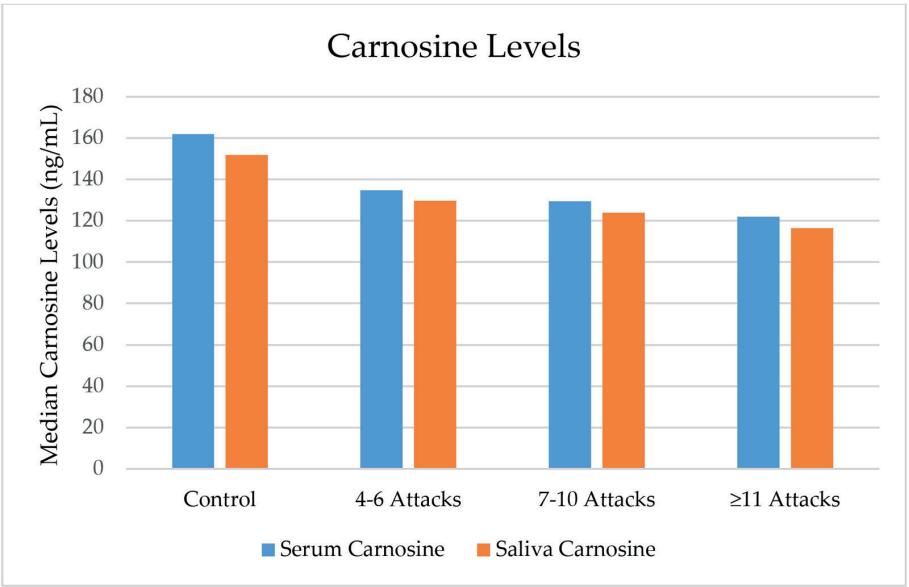


**Fig. 2.** Comparison of serum/salivary carnosine levels between control group and group I-II-III.

**Table III.** Comparison of serum/salivary cotinine and carnosine levels between control group and groups A-B-C.

| Features | Control group | Group A (n: 32) | Group B (n: 23) | Group C (n: 25) | p value |
|---|---|---|---|---|---|
| Serum cotinine level (pg/ml) ≠ | 0.59 (0.18-1.61) | 1.78 (0.78-2.20) | 2.05 (1.28-2.92) | 2.85 (1.48-3.44) | 0.03* |
| | | | | | 0.0001** |
| | | | | | 0.01*** |
| | | | | | 0.0001**** |
| Saliva cotinine level (pg/ml) ≠ | 0.46 (0.06-1.28) | 1.72 (0.66-2.12) | 2.00 (1.31-2.91) | 2.76 (1.18-3.04) | 0.0001** |
| | | | | | 0.01*** |
| | | | | | 0.0001**** |
| Serum carnosine level (ng/ml) ≠ | 166 (96-192) | 138 (125-165) | 128.5 (120-191) | 119.5 (96-161) | 0.04* |
| | | | | | 0.0001** |
| | | | | | 0.01*** |
| | | | | | 0.0001**** |
| Saliva carnosine level (ng/ml) ≠ | 148 (103-190) | 129 (110-148) | 128 (118-186) | 118 (93-146) | 0.0001** |
| | | | | | 0.0001*** |
| | | | | | 0.0001**** |

≠ Median (minimum-maximum)
* Comparison of group IV and V
** Comparison of group IV and VI
*** Comparison of group V and VI
**** Comparison of control and group IV, V, VI

kindergarten, birth weight, birth type, monthly income of the family, father's education status, and absolute eosinophil count. However, we found a statistically significant difference between the wheezing group and the control group in terms of breastfeeding period time, number of people living at home, maternal education level, maternal age at birth, and total IgE level. Exposure to passive smoking increases mucus production in the lungs up to seven

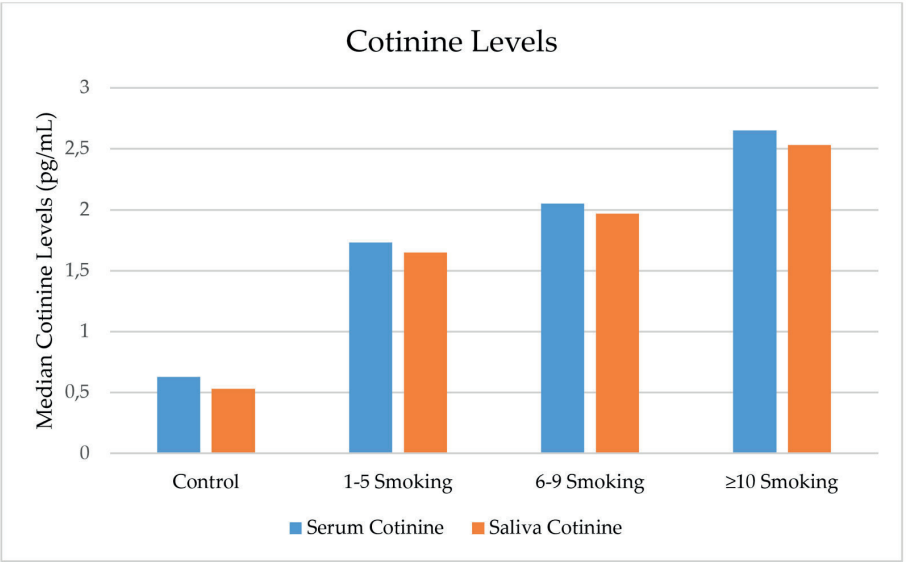


**Fig. 3.** Comparison of serum / salivary cotinine levels between control group and group A-B-C.

times, causing a decrease in ciliary movements and an increase in white blood cell count, total and specific IgE production, and eosinophil count in the blood. In addition, previous studies consistently linked younger maternal age with an increased risk of wheezing in childhood, suggesting that this could be explained by both social and biological factors.[17,18] Moreover, Caudri et al.[19] found a significant decrease in the frequency of wheezing in breastfed children. In our study, we found that the patients in the wheezing group were breastfed for a lesser time period than those in the control group and that the maternal age at birth was lower than that in the control group. Benicio et al.[20] reported that a crowded home environment and poor socio-economic conditions are risk factors for recurrent wheezing. In our study, we did not find a difference between the wheezing and the control group in terms of monthly income of the family, but we found that more people were living at home with the children in the wheezing group. Unfortunately, we were unable to assess parental asthma or atopy history among risk factors for wheezing in young children. This, in assessing risk factors of recurrent wheezing can also be considered as a limitation.

There are some studies investigating the relationship between wheezing and vitamin D. Demirel et al.[21] detected lower vitamin D levels in children with recurrent wheezing than the controls. In addition, in many studies it has been reported that low levels of vitamin D may be a risk factor for recurrent wheezing.[22,23] Similarly, we found significantly lower vitamin D levels in children with recurrent wheezing compared to the control group.

It has been reported that there is an increase in the frequency of cough, wheezing, and respiratory tract infections in children exposed to cigarette smoke at home and that this increase is directly proportional to the number of cigarettes smoked.[24-26] Small children are particularly affected by their mothers' smoking.[24,27] Epidemiological studies have reported that children are exposed to second-hand smoking because they stay with their parents for longer periods of time, and thus they experience respiratory diseases more frequently and severely.[24,27] Atay et al.[28] shown that the frequency of bronchiolitis was significantly higher in patients living in smoking homes. They also found that breathing second-hand smoke, living in a crowded house and heating with a stove were factors affecting the severity of bronchiolitis in patient. In our study, we did not find any statistical difference between the groups classified according to

the number of wheezing episodes in terms of mother's smoking status during pregnancy, during the breastfeeding period, and after the breastfeeding period, and the father's smoking at home. However, we found a statistically significant difference between the groups classified according to the number of wheezing episodes in terms of smoking at home among other members of the family. We also found a difference between the wheezing groups in terms of the number of cigarettes smoked daily at home. As the number of cigarettes smoked at home increased, the number of wheezing episodes experienced by children increased. These findings indicate that exposure to cigarette smoke has an important place in the etiology of wheezing. In our study, we found that smoking by the mother and/or father and other members of the household increased the number of cigarettes smoked daily in the house, thus increasing the intensity of exposure of children to cigarette smoke in the house. This is supported by our finding that only four (18.2%) homes had both mother and father who did not smoke, whereas the other members of the household were smokers. Conversely, in 18 cases (81.8%), the mother and/or father smoked together with the other members of the household. In a study conducted in China, a positive relationship was found between the number of people who smoke at home and the hospitalization of children due to respiratory infections.[29] Mannino et al.[30] reported that children living with crowded families with low income levels and in small houses with fewer rooms were more likely to be exposed to passive smoking.

Irvine et al.[31] revealed a positive correlation between the number of cigarettes smoked at home and the number of people who smoked at home and the cotinine levels in children. In Sweden, the cotinine levels were measured in the serum, saliva, and urine samples of 2,684 children exposed to passive smoking. The cotinine values in urine, blood, and saliva of children whose both parents smoked were found to be higher than those of children who were not exposed to passive smoking. The respiratory symptoms of these children were detected at a higher frequency compared with other children.[32] Chang et al.[33] measured the salivary cotinine levels of children who presented to the emergency department and were diagnosed with wheezing. This study showed that measuring the level of cotinine in saliva was a valuable test to monitor the prevalence and intensity of passive exposure to cigarette smoke at home in wheezing children under four years of age. Moreover, the cotinine levels in saliva and serum were similar in children under the age of four years and were significantly correlated with each other. In our study, similar to the literature, we found that as the number of cigarettes smoked daily at home increased, the serum and saliva cotinine levels also increased. In the groups classified according to the number of wheezing episodes, we found that as the number of wheezing episodes increased, the serum and salivary cotinine levels also increased. The serum and salivary cotinine levels we measured to evaluate exposure to second-hand smoking at home were similar, with a positive correlation between them. In children with a history of recurrent wheezing episodes, the cutoff values for serum and salivary cotinine levels were > 1.26 pg/mL and > 1.18 pg/ml, respectively. In light of these results, examining the level of cotinine in saliva, which is a noninvasive method, to evaluate passive smoking exposure in young children presenting with recurrent wheezing is recommended.

The carnosine has been proven to scavenge reactive oxygen species. Aside from its antioxidant properties, carnosine protects the cell membrane from lipid peroxidation. The function of most antioxidants is to prevent the entry of free oxygen radicals into tissues. However, if the oxidant molecules overcome this initial defense, these antioxidant molecules are not effective. The most important feature that distinguishes carnosine from other antioxidants is that it can also be protective at the cellular level, as it functions in the cytosol. Therefore,

it protects the tissues from a second oxidant effect. Owing to these properties, carnosine stands out among other antioxidants.[7,8] In our study, there was a negative correlation between the serum/salivary carnosine levels and the number of cigarette exposure. Moreover, as the number of wheezing episodes increased, the serum/salivary carnosine levels of the patients significantly decreased. A positive correlation was found between the saliva and serum carnosine levels. In our patients, the possible reason for the decrease in carnosine levels as the number of wheezing episodes increased is related to the utilization of carnosine to reduce oxidant molecules.

This study has several limitations. First, the patient and control groups had a limited number of cases. Moreover, the serum/salivary cotinine level together with the urine cotinine level were not measured. Second, the total antioxidant capacity of the patients in the patient and control groups were not measured. Third, the antioxidant properties of carnosine and the changes due to histopathological oxidative stress at the cellular and tissue levels were not demonstrated.

In conclusion, we found that the serum and salivary cotinine levels increased in parallel with each other in the assessment of passive smoking exposure in young children. Therefore, the measurement of salivary cotinine is a useful and noninvasive marker to evaluate passive smoking exposure in the etiology of recurrent wheezing in young children. Moreover, the serum/salivary carnosine levels decreased in young children as a result of recurrent episodes of wheezing and exposure to second-hand smoking. However, we consider that these results we obtained for carnosine should be investigated in future studies.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: Mehmet Kılıç, Erdal Taşkın; data collection: Şilem Özdem Alataş; analysis and interpretation of results: Mehmet Kılıç, Şilem Özdem Alataş, Süleyman Aydın, Erdal Taşkın, Mehmet Onur Kaya; draft manuscript preparation: Mehmet Kılıç, Erdal Taşkın. All authors reviewed the results and approved the final version of the manuscript.

**Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the clinic.

The necessary approval for the study was obtained from the clinical research ethics committee of Firat University (Ethical board date/number: 05.09.2013/03-07). The families invited to participate in the study were informed about the research, and their written consent was obtained.

**Source of funding**

No financial conflicts to disclose.

**Conflict of interest**

The authors declare that they have no conflict of interests.

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The Turkish Journal of Pediatrics 2021; 63: 417-426
https://doi.org/10.24953/turkjped.2021.03.008

**Original Article**

# Clinical and demographic characteristics of childhood neuro-ophthalmology diseases at a tertiary eye care center

Sevim Kuyumcu Kavuncu[1], Pınar Nalçacıoğlu[2], Gölge Acaroğlu[3]

*[1]Ankara Ulucanlar Eye Training and Research Hospital, Ankara; [2]Department of Ophthalmology, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara; [3]Private Practice, Ankara, Turkey.*

## ABSTRACT

**Background.** To evaluate the demographic, etiological, and clinical properties, as well as the treatment modalities of neuro-ophthalmological diseases in childhood.

**Methods.** We retrospectively analyzed the clinical data of patients younger than 18 years old who were referred to the Neuro-Ophthalmology Department of Ulucanlar Eye Hospital from 2004 to 2019.

**Results.** Of 1,910 patients who presented to the Neuro-Ophthalmology Department, 128 (6.7%) were younger than 18 years old at diagnosis, and their data were analyzed. The three most common diagnoses were congenital optic disc (OD) abnormalities in 43 (33.5%), optic neuropathies in 42 (32.8%), and idiopathic intracranial hypertension in 11 (8.5%) patients. The most frequent symptoms were as follows: decreased visual acuity in 36 (28.1%), headache in 32 (25%), and no symptoms in 19 (14.8%) patients. The best visual prognosis was associated with inflammatory optic neuritis, while hereditary and compressive optic neuropathy resulted in poor visual acuity outcomes.

**Conclusions.** Congenital OD abnormalities and optic neuropathies are the most frequently seen disorders among children with neuro-ophthalmological diseases. Clinicians should also be aware that children without any symptoms may also have neuro-ophthalmological disorders.

**Key words:** Children, congenital optic disc abnormalities, neuro-ophthalmological diseases, optic neuropathies.

Neuro-ophthalmologic diseases vary from disorders of life-threatening intracranial or systemic diseases to congenital disc anomalies. Moreover, they also have a long-term effect on the visual system and overall development of a child.[1] Loss of vision at the early stage of life has a negative effect on neurobehavioral development.[2] Early diagnosis of neuro-ophthalmological diseases is significant because some of these disorders are treatable and preventable.[3] Mirdehghan et al.[4] reported the rate of the prevalence of treatable diseases, such as secondary atrophy of the optic nerve, cortical blindness due to trauma, retinal detachment, and cataract secondary to intrauterine infections, as 25.7% in school-aged children. Dhiman et al.[5] suggested that more than half of optic nerve disease cases could be treatable at an early stage of the disease to prevent visual acuity loss.

There is limited information regarding the various clinical patterns of neuro-ophthalmological diseases in young ages. It is possible that genetics, race or ethnicity, regional traditions or cultural behaviors, different social and economic conditions, and limited access to medical care centers may result in variability in the diagnosis of neuro-ophthalmological disorders.[6-7]

In this study, we evaluated the causes and clinical characteristics and natural courses of neuro-ophthalmological diseases in children who presented at a neuro-ophthalmology specialty clinic of a tertiary referral eye center in Turkey.

✉ Sevim Kuyumcu Kavuncu
sevimkavuncu@hotmail.com

Received 21st June 2020, revised 26th August 2020, 22nd October 2020, accepted 24th December 2020.

## Material and Methods

Of the 1910 patients who presented to the Neuro-ophthalmology Department of the Ulucanlar Eye Hospital from 2004 to 2019, the medical records of the 128 (6.7%) patients younger than 18 years old at diagnosis were analyzed retrospectively. The study protocol was approved by the Ethics Committee of Ankara Kecioren Training and Research Hospital. All study procedures were carried out in accordance with the Declaration of Helsinki.

A comprehensive medical history was taken from each participant. Demographic and clinical data were recorded. The mean age of onset of disease, gender, laterality of eye involvement, etiology, complaints at presentation, clinical findings, follow-up period, best-corrected visual acuity (BCVA) with Snellen chart at the initial and final visits, number of visits during the follow-up period, associated systemic diseases and treatment protocols were recorded. All patients' diagnoses and follow-up visits were conducted in the Neuro-Ophthalmology Department by three clinicians (SK, PN, and GA).

During the neuro-ophthalmological examination, we evaluated the color vision using Ishihara's pseudoisochromatic color vision chart. Pupillary light reflexes were checked with a penlight to find any relative afferent pupillary defect (RAPD) and pupillary diameters were measured with the pupil gauge chart in both dim and bright light. Extraocular eye movements were recorded in nine cardinal positions. In addition, anterior segment examination by slit-lamp biomicroscopy and dilated fundus examination with a 90-diopter lens or indirect ophthalmoscopic examination with a 20-diopter lens were performed. If children were unable to read the Snellen E chart, Allen's figures, HOTV, Lea tests or Toddler carts, the visual acuity (VA) was evaluated by counting fingers or from the ability to fix and follow.

Ancillary tests, including a 30-2 or 24-2 visual field test (VFT; Carl Zeiss Humphrey 750 Field Analyzer, Germany), were performed by automatic perimetry. If VFT could not be performed due to patient age and cooperation problems, it was assessed by a confrontation test.

Electrodiagnostic testing (visual evoked potential(VEP)) was performed in patients suspected of optic nerve and visual pathway damage. To make the correct diagnosis, cranial and orbital magnetic resonance imaging (MRI) and cranial MR venography were performed if needed. In addition, if necessary, diagnostic tests like lumbar puncture (LP) were done by a pediatric neurologist.

Especially for diagnosis of congenital optic disc (OD) abnormalities, the patients were examined by B-scan ocular ultrasonography (USG) and when available; optic nerve head autofluorescence imaging by fundus camera or spectral domain optical coherence tomography (SD-OCT). In addition, volumetric OCT scans through the optic nerve head were carried out, and peripapillary retina nerve fiber layer thickness was evaluated by SD-OCT. Anterior segment and fundus photographs were taken when needed.

Optic disc drusen (ODD) was diagnosed as showing refractile calcified hyaline nodules located in the optic nerve head. We accepted significant results in terms of the presented ODD as reported previously.[8-10] Also, the diagnostic criteria for other congenital optic disc abnormalities (crowded optic disc anomalies, tilted disc syndrome and optic nerve hypoplasia) are as follows the study from Brodsky.[11] A cranial MRI is ordered in all cases with optic nerve hypoplasia to exclude associated intracranial pathologies.

Optic neuritis was determined as acute or subacute unilateral or bilateral visual loss, abnormal color vision, the presence of the RAPD if unilateral or asymmetric involvement, and normal or swollen OD on fundus examination that could not be explained by any other disorder. Bilateral involvement of disease was

defined as both eyes affected within 1 month, and bilateral recurrent disease was defined as one or both eyes affected more than once. Chronic relapsing inflammatory optic neuritis (CRION) was diagnosed depending on the reported study.[12]

Idiopathic intracranial hypertension (IIH) was determined as elevated intracranial pressure (higher than ≥ 28 cm $H_2O$ if the patient was obese or sedated for the process, or ≥ 25 cm $H_2O$ if non-obese and non-sedated) for children with normal CSF in combination with normal brain neuroimaging.[13]

Hereditary optic neuropathies were diagnosed with painless, progressive, and permanent bilateral visual loss with central or cecocentral visual field defects and dyschromatopsia after excluding compressive, infiltrative, or infective causes by normal MRI and normal CSF examination if necessary.[14] Wolfram syndrome is also called DIDMOAD, a term that indicates the components of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness.[15]

Traumatic optic neuropathy was diagnosed as optic nerve dysfunction, including decreased visual loss, RAPD, or dyschromatopsia, following direct or indirect ocular and head trauma that could not be explained by other causes. Furthermore, patients were considered to have acute disseminated encephalomyelitis (ADEM) if they fulfilled the criteria previously reported by the International Pediatric Multiple Sclerosis Study Group.[16]

Compressive or infiltrative optic neuropathy or tumor-related optic neuropathy diagnosis depended on a slow progression of vision loss, the presence of RAPD, abnormal color vision, visual field defect and OD pallor or optic disk edema. The lesions may compress or infiltrate the anterior visual pathway, which is confirmed via cranial and orbital MRI.

Simultaneous changes in pupillary diameter were measured in both bright and dim light to assess anisocoria by using a pupil gauge chart. The diagnosis of pupillary abnormalities, Adie's pupil, and Horner syndrome or physiological anisocoria have been described in a previous study.[17] Due to the exclusion of the causes of Horner syndrome, we ordered neck and abdominal ultrasonography, cranial and neck MRI, and MRI angiography, as well as enhanced thoracic computed tomography (CT) scanning if needed.[18]

### *Statistical Analysis*

All statistical analyses were carried out using the SPSS 21.0 statistical analysis program. The descriptive statistics mean or median values, and percentages were obtained in the analysis.

## Results

We identified 128 patients, 6.7% of our total neuro-ophthalmological patient group, aged less than 18 years. Mean age at onset of disease was 11.9 ± 3.5 years (range, 1–18 years), and median follow-up time was 6 months (range, 3-101 months).

All patients were Turkish Caucasians. There were 71 (57.5%) girls and 57 (44.5%) boys. Among the 128 patients, 83 (64.8%) presented with bilateral involvement, 43 (33.6%) presented with unilateral involvement, and 2 (1.6%) did not exhibit ocular involvement. Median number of visits was 3 (1-35). Table I shows the list of diagnoses of neuro-ophthalmological diseases in children and the distribution of gender, ocular involvement, and median age of onset at presentation. We could not find the cause of one case with optic atrophy. This case had no complaint, and optic atrophy was found incidentally on routine eye examination.

Among the compressive etiology of optic neuropathy, the most common presentation was optic nerve glioma ($n$ = 6, 66.7%) associated with neurofibromatosis (NF) type 1, followed by pituitary adenoma ($n$ = 2, 22.2%) and craniopharyngioma ($n$ = 1, 11.1%). Ocular findings of NF type-1 cases are shown in Table II.

**Table I.** The specific diagnosis of children and distrubition of gender, ocular involvement and the mean age at onset.

| Diagnosis | No. of patients (%) | Gender (F/M) (%) | Unilateral (n,%) | Bilateral (n,%) | Age at onset, years[$] |
|---|---|---|---|---|---|
| *Congenital OD anom.* | 43 (33.5%) | | | | |
| OD drusen | 30 (68.9%) | 17 (56.7%) / 13 (43.3%) | 7 (23.3%) | 23 (76.7%) | 12.0±3.0 |
| Crowded disc | 11 (25%) | 5(45.5%)/6(54.5%) | 2 (18.2%) | 9 (81.8%) | 9.8±2.4 |
| OD Hypoplasia | 1 (4.5%) | 1 F | -- | 1 (100%) | 13 |
| Tilted disc | 1 (2.3%) | 1 M | -- | 1 (100%) | 14 |
| *Optic neuropathy* | 42 (32.8%) | | | | |
| Optic neuritis | 14 (33.3%) | | | | |
| Demiyelizan ON | 10 (76.9%) | | | | |
| Isolated ON | 5 (38.5%) | 2 (40%) / 3 (60%) | 3 (60%) | 2 (40%) | 14 (12-18) |
| MS-related ON | 3 (23.1%) | 3 F | 3 (100%) | --- | 14 (10-15) |
| CRION | 1 (7.6%) | 1 M | --- | 1 (100%) | 15 |
| ADEM | 1 (7.6%) | 1 M | --- | 1 (100%) | 16 |
| Infectious ON | 4 (9.5%) | 1 (25%) /3 (75%) | --- | 4 (100%) | 11.5 (10-13) |
| Herediter ON | 10 (23.8%) | 6 (60%) / 4 (40%) | --- | 10 (100%) | 11.9±4.5 |
| Compressive ON | 9 (21.4%) | 6 (66.7%)/3 (33.3%) | 5 (55.6%) | 4 (44.4%) | 11.6±3.7 |
| Traumatic ON | 7 (16.7%) | 4 (57.1%)/3 (42.9%) | 7 (100%) | --- | 12 (10-16) |
| Optic atrophy | 1 (2.4%) | 1 M | 1 (100%) | --- | 10 |
| Toxic ON | 1 (2.4%) | 1 M | --- | 1 (100%) | 10 |
| *IIH** | 11 (8.6%) | 5(45.5%) /6(54.5%) | --- | 11 (100%) | 11.7±3.9 |
| *Pupillary Anomalies* | 7 (5.5%) | | | | |
| Horner syndrome | 4 (57.1%) | 1 (25%) / 3(75%) | 4 (100%) | --- | 9.5 (1-15) |
| Adie pupilla | 1 (14.3%) | 1M | 1 (100%) | --- | 12 |
| Physiologic anisocoria | 1 (14.3%) | 1M | 1 (100%) | --- | 9 |
| Post-traumatic Mydr. | 1 (14.3%) | 1 M | --- | 1 (100%) | 15 |
| *Cranial Nerve Palsies* | 6 (4.7%) | | | | |
| 4th nerve palsy | 3 (50%) | 2 (66.7%) /1 (33.3%) | 2 (66.7%) | 1 (33.3%) | 10 (8-11) |
| 3th nerve palsy | 2 (33.3%) | 2 F (100%) | 2 (100%) | --- | 4.5 (2-7) |
| 6th nerve palsy | 1 (16.7%) | 1 F | 1 (100%) | --- | 7 |
| *Migraine* | 3 (2.3%) | 3 F (100%) | ---- | 3 (100%) | 15 (11-15) |
| *Thyroid Ophthalmopathy* | 3 (2.3%) | 3 F (100%) | ---- | 3 (100%) | 16 (8-16) |
| *Secondary IH* | 3 (2.3%) | 2 (66.7%) /1 (33.3%) | --- | 3 (100%) | 13 (10-16) |
| *Intracranial Tumor* | 2 (1.6%) | 2 M (100%) | --- | --- | 15 (13-17) |
| *Non-organic VFL* | 2 (1.6%) | 2 F(100%) | --- | 2 (100%) | 12 (12) |
| Other diseases** | 6 (4.7%) | 5 (83.3%)/ 1 (16.7%) | 4 (66.7%) | 2 (33.3%) | 14 (11-17) |

*: Two cases with idiopathic intracranial hypertension also had optic disc drusen in both eye.
**: The number of cases is one for each diagnosis: blepharospasm, nonspesific orbital inflammatory disease, accommodation spasm, oculomotor apraxia, isolated inferior rectus paralysis, carotid-cavernous fistula
[$] : mean±standard deviation (min-max), median (min-max)
Anom: anomaly, ON: optic neuropathy, CRION: chronic relapsing inflammatory optic neuropathy, ADEM: acute disseminated encephalomyelitis, IIH: idiopathic intracranial hypertension, Mydr: mydriasis, VFL: visual field loss

**Table II.** The clinical characteristics of cases with Neurofibromatosis Type-1.

| Patient | Optic nerve glioma | Cranial glioma | Lisch nodule |
|---|---|---|---|
| Case 1 | Unilateral | -- | + |
| Case 2 | Bilateral | -- | + |
| Case 3 | Bilateral | -- | + |
| Case 4 | Bilateral | -- | -- |
| Case 5 | -- | + | + |
| Case 6 | -- | + | -- |

**Table III.** The clinical features of optic disc drusen.

| Charecteristics | n (%) | Age, y, mean±SD (min-max) |
|---|---|---|
| Unilateral/Bilateral | 7(21.9) / 25 (78.1) | --- |
| Superficial cases | 15 (46.9) | 11.7±3.63 (6-17) y |
| Buried cases | 9 (28.1) | 10.5±3.1 (5-14) y |
| Superficial and buried cases | 8 (25) | 11.5±1.8 (9-14) y |

n: number of cases, y: years

**Table IV.** The distribution of causes of ocular motor nerve palsies.

| Etiology | 3th nerve palsy (n,%) | 4th nerve palsy (n,%) | 6th nerve palsy (n,%) |
|---|---|---|---|
| Idiopathic | -- | 1 (14.3) | -- |
| Congenital | -- | 1 (14.3) | -- |
| Trauma | -- | -- | 1 (14.3) |
| Vasculitis | -- | 2 (28.6) $ | -- |
| Enfection | 1(14.3)* | -- | -- |
| Migraine | 1 (14.3)** | -- | -- |

n: number of eye involvement, $ : The case with left central Horner syndrome , *: Pupillary sparing 3th nerve palsy, **: 3th nerve palsy with pupillary involvement

Diagnosis of hereditary optic neuropathy was made with evaluation of clinical findings, and if Leber's hereditary optic neuropathy (LHON) was suspected, genetic testing was ordered. Five cases (50%) of our series had a diagnosis of Wolfram syndrome, and five (50%) were classified as hereditary optic neuropathy without primary mutations of LHON.

We evaluated 32 cases with OD drusen, with unilateral involvement in 7 (23.3%), bilateral involvement in 25 (78.1%) cases. Two cases of IIH also had OD drusen in both eyes. The clinical features of OD drusen are shown in Table III. All paralytic cranial nerve cases completely recovered during follow-up period. Causes of ocular motor nerve palsies are summarized in Table IV.

We had four cases with pupillary abnormalities including anisocoria and miosis. All of them had Horner syndrome; one (25%) congenital case and the other three (75%) cases related to migraine. All seven patients with traumatic optic neuropathy were caused by blunt trauma.

Six (4.7%) children presented with at least two or three complaints simultaneously at the onset of the disease, and six (4.7%) children were referred to our clinic by a pediatrician or pediatric neurologist. Table V summarizes the presenting complaints of children with neuro-ophthalmologic diseases. Of the patients with IIH, 72.7% had a headache as the primary complaint. Other symptoms at the onset of disease were blurred vision (18.1%) and strabismus (18.1%). Unlikely, 1 (9%) of our

**Table V.** The presenting complaints at the onset of the neuro-ophthalmological diseases.

| Presenting complaints* | Number of subjects (n,%) |
|---|---|
| Vision loss | 36 (28.1%) |
| Headache | 32 (25 %) |
| Without compliants | 25 (19.5%) |
| Double vision | 13 (10.2%) |
| Blurry vision | 10 (7.8%) |
| Ptosis | 3 (2.3%) |
| Visual Field loss | 2 (1.6%) |
| Eye lid retraction | 2 (1.6%) |
| Anisocoria | 2 (1.6%) |
| Others** | 3 (3.9%) |

*: Some subjects had more than one complaint.
**: Others: Proptosis (n=1,0.8%), nistagmus (n=1, 0.8%) and heterocromia (n=1,0.8%).

**Table VI.** The visual acuity distrubition of 213 affected eyes in children with neuro-ophthalmological disorders.

| Visual acuity | Number of affected eyes (n; %) | |
|---|---|---|
| | Initial visit | Final visit |
| ≤ 0.1 | 28 (13.1%) | 20 (9.4%) |
| 0.2-0.5 | 26 (12.2%) | 24 (11.3%) |
| ≥ 0.6 | 159 (74.6%) | 169 (79.3%) |

patients, who was 9 years old, had presented with anisocoria noticed by his parents.

Outcomes of VA of the affected eyes are shown in Table VI. When compared with the results from the initial visit, the final visit showed improvement by at least two lines of Snellen visual acuity in 25 (11.7%) eyes. The VA decreased in 16 (7.5%) and remained unchanged in the other 172 (80.7%) eyes. The causes of decreased VA were hereditary optic neuropathy in 10 (66.6%) eyes, toxic optic neuropathy resulting from Vigabatrin in 2 (13.3%) eyes, compressive optic neuropathy due to optic nerve glioma in 2 (13.3%) eyes, and amblyopia of strabismus with optic nerve drusen in 2 (13.3%) eyes.

We used ancillary tests to establish a certain diagnosis following the neuroimaging (including cranial and orbital MRI or CT and cranial or MR venography) tests in 92 (71.9%) cases, LP in 30 (23.4%) cases, and electrophysiological tests (including VEP) in 12 (9.4%) cases. In our series, we had difficulty in making a correct diagnosis in two cases in which LP was performed with normal pressure. We noticed that we had difficulty differentiating papilledema from anomalous OD in 2 (6.6%) cases in which LP was performed with normal opening pressure (<20 mm Hg) when we retrospectively evaluated 30 patients who underwent LP. One of these was a 10-year-old boy with Down syndrome and another patient was a 10-year-old boy with morbid obesity and blurred vision. Two cases were re-evaluated and diagnosed as crowded OD. The treatment methods employed during the follow-up period are summarized in Table VII.

## Discussion

The present study reports on the demographics, clinical characteristics, and natural courses of neuro-ophthalmological diseases in a population of patients under the age of 18 years. In particular, studies have focused on and describing the etiology of optic atrophy or causes of blindness in children.[1,4,6,19] The major cause of ophthalmological examination in 48% of the

**Table VII.** Treatment methods of children with neuro-ophthalmological diseases.

| Treatment methods | Diagnosis | Number of cases (n) |
|---|---|---|
| IV methylprednisolone[a] | Acute optic neuritis | 10 |
| Oral acetazolamide | IHH | 8 |
| Oral acetazolamide+ topiramate | IHH | 3 |
| Radiotherapy+ chemotherapy | NF-1 | 2 |
| Cycloplegic drop | Accommodation spasm | 2 |
| Oral acetazolamide+ IV antibiotics[b] | Bacterial meningitis | 1 |
| LP shunt[c] | IHH | 1 |
| Tumor excision+ VP shunt | Pinealoma | 1 |
| Tumor excision surgery | Craniopharyngioma | 1 |
| Oral CS+ immunosuppressive agent[d] | Autoimmune Vasculitis | 1 |
| Total thyroidectomy | Thyroid ophthalmopathy | 1 |
| Covered stent | Carotico-cavernous fistula | 1 |

[a] : Treatment protocol for this cases consisted of 10 to 30 mg / kg / day IV pulse methylprednisolone for 3-5days, followed by oral steroids for 11 days (at a starting dose of 1 mg/kg)
[b] : This case resulted in bilateral optic atrophy
[c] :Symptoms were not controlled by medical treatment and the patient required Lumboperitoneal shunt surgery
[d] : 30 mg/kg/day oral corticosteroid, and Azathioprine 2.5 mg/kg/day as immunosuppressive agent
CS: Corticosteroid, IHH: Idiopathic intracranial hypertension, NF-1: Neurofibromatosis type 1, LP shunt: Lumboperitoneal shunt, VP shunt: Ventriculoperitoneal shunt, IV: İntravenous

children was related to systemic symptoms like seizure, headache, and vomiting.[20] The others were examined for different kinds of ocular problems, such as strabismus, or underwent routine examination.[21]

In the present study, 28.1% of the patients applied to our clinic due to visual loss, 25% for headache, and 19.5% with no complaints but having abnormalities that were observed in routine eye examination. The most common diagnosis was congenital OD anomalies (33.6%), and 69.6% of these patients involved ODD cases in our series. ODD occurs in about 0.4% of children.[20] It is often diagnosed incidentally during routine ophthalmological examination, when there are no related symptoms. Some symptoms, such as temporary visual loss.[22] and visual field loss.[23] associated with ODD, were reported in studies with data from adult patients rather than children. We observed that the diagnosis of ODD was made incidentally in 54.6% of cases in our series. However, 45.4% of patients with ODD applied to our clinic with a headache.

During the childhood period, ODD is usually buried within the optic nerve, but it becomes more superficial as time progresses. Because of this, it is often difficult to diagnose buried drusen properly and differentiate it from papilledema in children.[20] Hoover et al.[24] reported that the mean age at which drusen becomes more superficial and visible is 12 years old. The results of our study support this claim; we estimated a mean age of 10.5 years in buried cases and a mean age of 11.7 years in superficial cases.

Some studies have investigated whether the presence of some symptoms and the character of the headache in patients could lead clinicians to perform LP in patients with IIH. The study of the Idiopathic Intracranial Hypertension Treatment Trial Group revealed that headache is the most frequent (84%) symptom among patients with IIH.[25] In contrast, Hamedani et al.[26] reported that only 10% of children with IIH had a symptom of headache in their study. We observed that 72.7% of our patients had a headache as a primary symptom. As in our study, it has been revealed that diagnosing

ODD depending only on symptoms is not safe because a headache may be present in patients with ODD or IIH. Furthermore, it has been emphasized that the final diagnosis based on a single fundus examination or LP may cause misdiagnosis.[27] Therefore, using other diagnostic methods, such as OCT, FAF, and B-scan USG, may decrease the possibility of false diagnosis.[19,27,28] Krishnakumar et al.[27] examined 15 children with a suspected diagnosis of IIH and reported that only 6 (40%) out of 15 patients had IIH. In our series, we had difficulty making a correct diagnosis in two cases with crowded disc and performed LP with normal pressure, although both were evaluated and examined by experienced neuro-ophthalmologists with additional OCT and USG findings. Cases with OD abnormality accompanying IIH are also challenging, although this is rarely seen. In addition, it is more likely to develop papilledema with coexisting risk factors for IIH when ODD or a small cup-to-disc ratio is present.[29] We had two cases with buried ODD and IIH together.

NF type 1 is one of the most common syndromes coexisting with brain tumors, affecting approximately 1 in 2,500–3,000 people worldwide.[30] Malignancies of the peripheral nerve sheaths or central nervous system, such as optic nerve glioma or astrocytoma, are often related to NF-1 associated tumors.[31] Although gliomas frequently settle through the optic pathway (optic nerves, chiasm, tracks, and radiation), they may also arise anywhere in the brain.[32] Mbekeani et al.[6] reported that optic glioma was the major reason for children having optic atrophy related to malignancy. Chinta et al.[1] found that craniopharyngioma (8 patients; 44% of children with tumors) was the most common cause of compressive optic neuropathy leading to optic atrophy in children. In our series, the most common compressive optic neuropathy was optic nerve glioma due to NF type 1, followed by pituitary adenoma.

Observation of Lisch nodules in the biomicroscopic examination is an important key point for diagnosis. In our series, one of the NF cases presented with bilateral Lisch nodules and intracranial glioma but no optic pathway glioma involvement; this patient was diagnosed via neuroradiological imaging.

The disease with the best visual prognosis was inflammatory optic neuritis in our cases. A few specific studies have focused on optic neuritis in children; most of the studies have been observational case reports with a limited number of patients. Generally, in the pediatric population, optic neuritis has a good prognosis. Its presentation is usually bilateral, and it occurs after viral infection. In our series, we had cases with mostly bilateral (67%) rather than unilateral (33%) involvement, with an equal gender predominance. Optic neuritis is an isolated condition or a component of systemic autoimmune disorders, such as multiple sclerosis (MS), neuromyelitis optica, or ADEM. Some studies have evaluated risk factors in the development of MS. Different studies have reported the rate of development of MS in children, ranging from 4% to 36%.[33] Only three of the cases (27.3%) in our series were associated with MS, while others exhibited isolated optic neuritis. This result may depend on the follow-up time of those patients. Increased age, ethnicity, recurring optic neuritis in both eyes or either eye,[34,35] and the presence of one or more white matter lesions on the brain MRI[36] in the first episode of optic neuritis were accepted as related to a higher risk of MS development. All three cases associated with MS were female and had recurrent attacks in both eyes, as well as white matter lesions on the brain MRI at the presentation of optic neuritis. In our series, a high dosage of intravenous methylprednisolone (10–30 mg/kg/day) for 3–5 days, followed by oral steroid for 11 days (at a starting dose of 1 mg/kg), was given in patients with acute inflammatory optic neuritis; we observed a rapid visual recovery after the treatment. In our series, the final visual acuity of the patients with demyelinating optic neuritis was ≥ 0.6 in all cases except one which was the case with CRION who had optic atrophy and low visual acuity < 0.1

at the first presentation. A similar observation was also reported by Sun et al.,[37] and this was related to poor visual outcomes and a pale disc at the presentation of the disease. Therefore, it is essential to diagnose diseases early that could present as optic neuritis to prevent permanent visual and neurological dysfunction.

In the present study, most of our cases were involved congenital OD abnormalities and optic neuropathies. The best response to the treatment was in patients with inflammatory optic neuritis. However, the worst prognosis was in cases with hereditary and compressive optic neuropathies. Cases with pupillary abnormalities mostly had migraine, but this symptom may also arise in patients with IIH. It should also be kept in mind that children may exhibit cooperation problems during the examination; moreover, they may not have any complaints even if they have a serious neuro-ophthalmological disease.

In conclusion, knowledge of the etiology of childhood neuro-ophthalmologic diseases is essential in terms of the clinician's ability to diagnose and treat children with preventable and curable diseases as early as possible. In addition, knowledge of the clinical progression of these disorders will give clinicians the chance to contribute to families' actions to support their children's early rehabilitation and functional improvement.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Sevim Kuyumcu Kavuncu, Pınar Nalçacıoğlu; data collection: Sevim Kuyumcu Kavuncu, Pınar Nalçacıoğlu; analysis and interpretation of results: Sevim Kuyumcu Kavuncu, Pınar Nalçacıoğlu; draft manuscript preparation: Sevim Kuyumcu Kavuncu, Pınar Nalçacıoğlu, Gölge Acaroğlu. All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

The study protocol was approved by the Ethics Committee of Ankara Kecioren Training and Research Hospital. (Number:09.10.2019/1980 )

## Source of funding

No financial support was received for this manuscript.

## Conflict of interest

The authors report no conflicts of interest and have no proprietary interest any of the materials mentioned in this article.

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The Turkish Journal of Pediatrics 2021; 63: 427-433
https://doi.org/10.24953/turkjped.2021.03.009

Original Article

# Relationship between tumor viability during treatment and the clinical outcomes of patients with bladder/prostate rhabdomyosarcoma: a single-center experience

Yun-peng Li[1], Hong-cheng Song[1], Xiao-li Ma[2], Le-jian He[3], Wei-ping Zhang[1]

*Departments of [1]Urology, [2]Hematology Oncology Center and [3]Pathology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China.*

## ABSTRACT

**Background.** To analyze the relationship between tumor viability in specimens retrieved at second-look procedures (SLPs) and clinical outcomes in patients with bladder/prostate rhabdomyosarcoma (BP RMS).

**Methods.** We retrospectively analyzed patients' characteristics, times between diagnoses and SLPs, types of SLPs, the pathological findings, and clinical outcomes between January 2003 and May 2014.

**Results.** A total of 29 patients underwent at least one SLP before completing chemotherapy, including 24 boys and 5 girls. The mean age was 36 months. No patients with clinical/radiographic complete responses (CRs) had viable tumor cells and 7/18 patients (38.9%) without CR had no viable tumor cells. Seven patients experienced tumor relapse, progression, and metastasis, and three of these survived. Five-year event-free survival (EFS) rates were 88.5% in 18 patients without viable tumor at SLPs and 54.5% in 11 patients with viable tumor (Cox proportional hazards adjusted P=0.045). The respective five-year overall survival (OS) rates were 94.1% and 72.7% (Cox proportional hazards adjusted P=0.175).

**Conclusions.** EFS was increased in patients with BP RMS having no viable tumor cells; however, OS was comparable in patients with and without viable tumor cells. Patients who achieved CR during the treatment generally had no viable tumor cells.

**Key words:** bladder/prostate, outcome, rhabdomyosarcoma, second-look procedures.

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, with an incidence of 4.4-6/1000000 per year.[1,2] The prognosis of patients with bladder/prostate rhabdomyosarcoma (BP RMS) has improved in recent decades, and the overall survival rate for localized disease is approximately 84%.[3]

During treatment, the response to previous therapy may vary in patients with RMS. Some patients achieved CR while others had residual tumor mass on imaging.[4,5] Second-look procedures (SLPs) are recommended to evaluate the response to previous therapy, to control the progression of disease or to eliminate residual tumor tissue.[6-8] Some reports found viable tumor cells in specimens retrieved at SLPs,[9] causing confusion as to whether this affected survival, and sometimes this led to radical surgery.[10] We hypothesized that the presence of viable tumor cells would indicate that the tumor responded poorly to previous therapy, thereby having an adverse effect on treatment outcome.

## Material and Methods

We retrospectively analyzed the medical records of patients with BP RMS treated in our institution between January 2003 and May 2014. Patients who underwent SLPs were included and those diagnosed with metastatic disease

✉ Wei-ping Zhang
zwp59616406@163.com

Received 31st May 2020, revised 17th July 2020, accepted 25th August 2020.

prior to treatment were excluded. Demographic data, types of SLPs, tumor viability and survival status were documented. The study was approved by the Medical Ethics Committee of our center (No. 2020-Z-016).

Our treatment protocol was derived from the Third Intergroup Rhabdomyosarcoma study (IRS-III).[11] The chemotherapy regimens included vincristine, actinomycin D, and cyclophosphamide (VAC). According to the treatment protocol, we performed SLPs at around 20 weeks to confirm the response to previous therapy, so as to facilitate subsequent treatment based on pathological findings. SLPs were defined as any procedures conducted after the initial biopsy, and open procedures were advised. Radiotherapy (RT) was administered based on the tumor response to prior chemotherapy and the pathological findings at SLPs. Tumor responses were categorized as complete response (CR, no residual mass detected on radiographic films or during the SLPs), partial response (PR, a more than 50% decrease in tumor mass), no response (NR, a decrease of <50% and an increase of <25% of tumor tissues), and progressive disease (PD, an increment of more than 25% in tumor mass). Only cells consistent with RMS on histology were identified as viable tumor cells.[12] Mature rhabdomyoblastic cells were not considered evidence of viable tumor cells.[13] The final pathological results were confirmed by two expert pathologists.

In situations where patients underwent more than one procedure after the initial biopsy, tumor viability at the first surgery was documented, because we believed this was a relatively accurate reflection of previous therapy and it might influence the subsequent treatment plan.

Overall survival (OS) was defined as the time from diagnosis to death from any cause. Event-free survival (EFS) was defined as the time from diagnosis to relapse, progression, metastasis or death. There were no significant differences in OS or EFS using the time at SLPs as the initial time of follow-up when compared with values using the time at diagnosis. Therefore, the time at which SLPs were performed was used as the initial value for comparing the EFS and OS distributions, rather than the time at diagnosis, in order to avoid potential bias when comparing outcomes based on patient and disease characteristics.[6] Kaplan-Meier curves were used to calculate the distributions of OS and EFS. Discrepancies between survival curves, based on whether viable tumor cells were present and whether the patients achieved CR, were analyzed using the log-rank test. The chi-square test was used to compare the distributions of categorical characteristics. Previous studies found tumor size and invasive tumors were independent predictors for EFS and OS.[3] To avoid other confounding factors, we used a Cox proportional hazards regression model, which incorporated age, tumor size, and invasiveness, to assess the differences of EFS and OS between different groups, if appropriate. Statistical analysis was conducted using SPSS 25.0 statistics software. Differences were considered statistically significant when $p < 0.05$.

## Results

Fifty patients were treated in our center during the study period, and 29 (58%) met the inclusion criterion, including 24 boys and five girls. The mean age at diagnosis was 36 months ± 33 months. Patient characteristics are illustrated in Table I. All patients were diagnosed with gross residual masses, and localized embryonal BP RMS, which were classified as IRS-III RMS based on the surgical-pathologic group system. The median time between diagnosis and SLPs was 5 months (range: 4-7 months), and the median time of follow-up was 7.5 years (range: 0.9-14.3 years).

Biopsy was performed in 15 patients, tumor resection in eight patients, partial cystectomy in one patient, and cystectomy in five patients. Eleven patients achieved CR on imaging before SLPs. None had viable tumor cells and none

relapsed subsequently. Three patients received RT before SLPs and the remainder had never received RT. Seven out of 18 (38.9%) patients without CR on imaging had no viable tumor cells (Table II). Two patients received RT before SLPs and two received RT subsequently. Among the remaining patients with viable tumor cells, three received RT before SLPs and four underwent RT subsequently. All patients continued chemotherapy after SLPs. The proportion of patients with no viable tumor cells was 62.1% (18/29). Among the patients with viable tumor cells, cells with rhabdomyoblastic differentiation were observed in the specimens retrieved from three patients. However, mature rhabdomyoblasts were observed in none of the patients without viable tumor cells. There was no significant difference in tumor viability between patients who received RT before SLPs and those who did not (p=1.000).

**Table I.** Characteristics of patients who underwent SLPs during treatment.

| Variables | Number |
|---|---|
| Age | |
| <1 year | 4 |
| >1 year | 25 |
| Gender | |
| Male | 24 |
| Female | 5 |
| Tumor size | |
| <5cm | 20 |
| >5cm | 9 |
| Types of SLPs | |
| Biopsy | 15 |
| Tumor resection | 8 |
| Partial cystectomy | 1 |
| Radical surgery | 5 |

SLPs= second-look procedures.

**Table II.** Tumor viability, and tumor response to previous treatment.

| Tumor Response | No Viable Tumor | Viable Tumor |
|---|---|---|
| CR | 11 | 0 |
| PR | 5 | 2 |
| NR | 2 | 8 |
| PD | 0 | 1 |

RT: radition therapy, CR: complete response, PR: partial response, NR: no response, PD: progression disease, SLPs: second-look procedures.

Tumor progression, relapse and/or distant metastasis occurred in two patients with PR, four patients with NR and one patient with PD after SLPs. Four of them received RT and five had viable tumor cells. Three patients experienced local recurrence after radical surgery and all of them had viable tumor cells. One patient died 6 months after the SLP and the other two patients survived event-free. One patient without viable tumor cells relapsed 5 months after completion of treatment, and was successfully treated with chemotherapy and RT. Two patients developed metastases, and both subsequently died including one patient without viable tumor cells. One patient with viable tumor cells died of disease progression. None of the patients with CR experienced any adverse events after SLPs.

The five-year EFS rates were 88.5% for patients without viable tumor cells and 54.5% for patients with viable tumor cells (p= 0.030) using the time at diagnosis as the beginning time of follow-up, while the five-year EFS rates were 88.5% for patients without viable tumor tissues and 54.5% for those with viable tumor tissues (p= 0.031) (Fig. 1) when using the time at SLPs as the beginning time of follow-up. After adjusting the data in terms of age, tumor size, and invasiveness, the EFS was prolonged significantly in patients without viable tumor cells (HR=0.14 for no viable tumor cells vs. viable tumor cells, p=0.045, 95%CI=0.02-0.96). The five-year OS rates were 94.1% and 72.7% among patients without viable tumor cells and those with viable cells, respectively (p=0.097), when using the time at diagnosis as the beginning time of follow-up. The five-year OS rates were 94.1% and 72.7% among patients without viable tumor cells and those with viable tumor cells, respectively (p=0.094) (Fig. 2), when using the time at SLPs as the beginning time of follow-up. OS was also extended, but not significantly based on the comparisons adjusted for age, tumor size, and invasiveness (HR=0.17 for

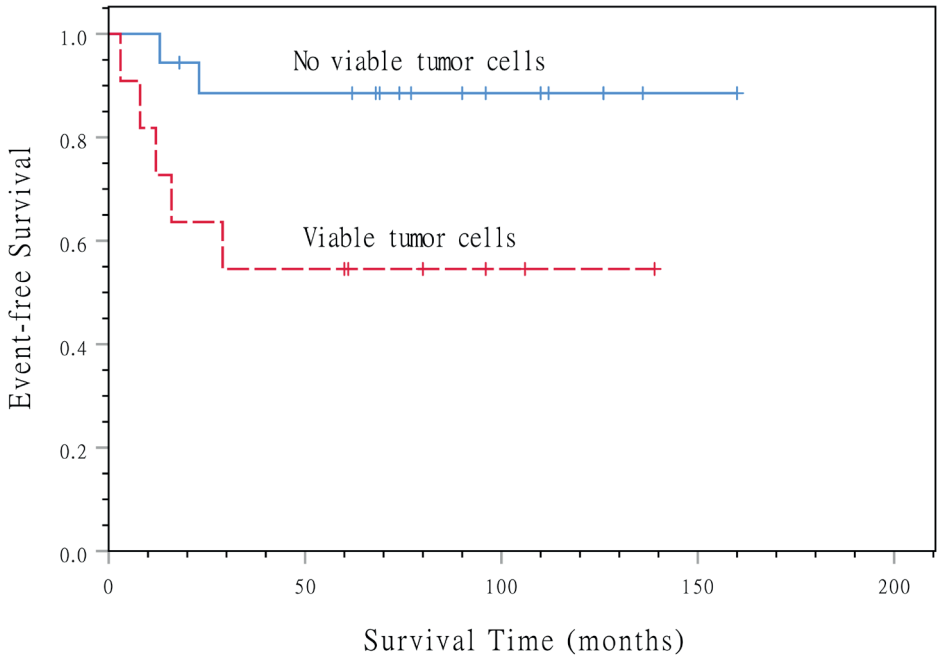


**Fig. 1.** Kaplan-Meier curve for EFS of patients who underwent SLPs during the treatment.

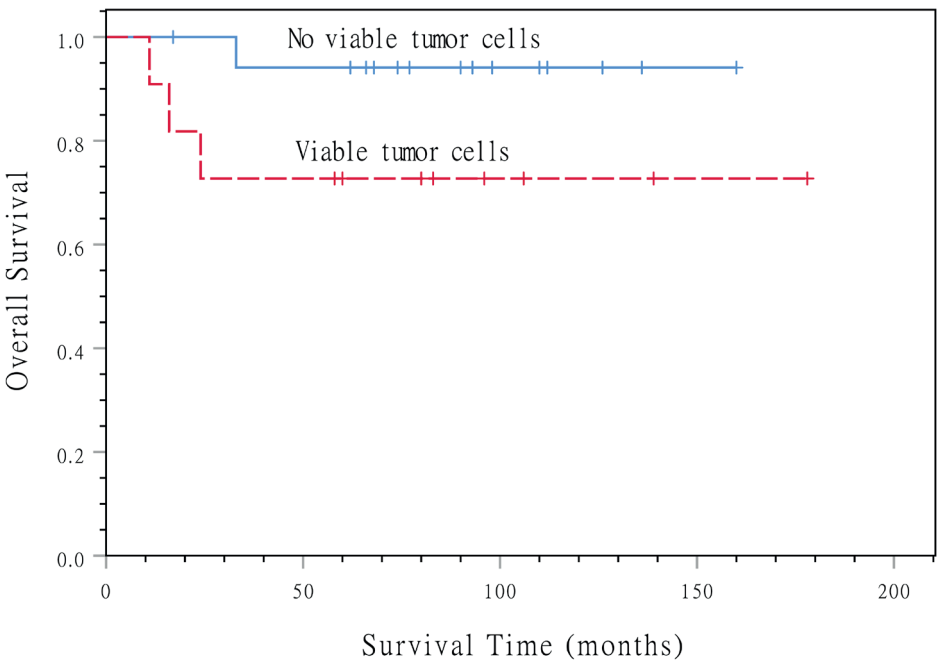


**Fig. 2.** Kaplan-Meier curve for OS of patients who underwent SLPs during the treatment.

no viable tumor cells vs. viable tumor cells, p=0.175, 95%CI=0.01-2.19). We also compared the differences of EFS and OS between patients with CR and those without CR. The 5-year EFS rates were 100% in patients with CR and 61.1% in those without CR (p=0.025). The 5-year OS rates were 100% and 77.8%, respectively (p= 0.11).

## Discussion

Determining whether viable tumor cells existed in the residual masses and whether tumor viability would influence the clinical outcome appeared important for subsequent treatment. Our experience with BP RMS patients demonstrated that all the participants with CR were free from viable tumor cells and 38.9% of patients without CR had no viable tumor cells. The overall outcome was comparable between patients with viable tumor cells and those without viable tumor cells.

Our results were similar to those of previous reports.[6] In the IRS-III study, SLPs were often performed around 20 weeks following chemotherapy and RT.[11] Among the 109 patients who received SLPs in the IRS-III study, 88% of those with CR did not have viable tumor cells and the 3-year survival rate was 85%. The respective percentage of patients without viable tumor cells was 75% in patients with PR and 28% in patients with less than PR; their 3-year survival rate was 83%. Among the 14 patients who had viable tumor cells, the 3-year survival rate was 62%.[6] However, the authors did not assess the EFS nor compare the differences in OS. In the IRS-IV study, SLPs were recommended to be undergone at 46 or 47 weeks in patients still having tumor masses.[6] A total of 13/14 (93%) patients with CR presented no viable tumor cells and 24/59 (41%) patients without CR had no viable tumor cells in the IRS-IV study.[6] Both our study and the IRS-IV study showed patients without viable tumor cells had increased EFS, not OS; we further demonstrated that the tumor viability did not influence the outcome of patients with BP RMS.

Whether the response to the previous treatment modality affects the outcome and whether it influences further treatment plans are becoming important concerns. In a report from the Children's Oncology Group (COG), the authors discovered that, among the 338 patients with Group III RMS, the 5-year FFS was similar between patients with CR, PR, and NR.[5] Another report from the International Society of Pediatric Oncology (SIOP) MMT-95 also reported no significant differences in FFS and OS between different response groups.[4] Therefore, the authors proposed that treatment adaptations based on early response should not be incorporated into future studies.[4] However, patients with BP RMS in our study who achieved CR had longer EFS, than those without CR. These inconsistencies can be explained by the fact that our study's sample size was relatively small and that confounding factors were not controlled for. The results agreed with our pathological findings at SLPs, which found EFS was significantly prolonged in patients without viable tumor cells.

The disappearance of tumor masses on imaging or viable tumor cells on the specimens are not always reliable factors to predict any potential relapse. In a study from SIOP MMT-84,[7] the authors discovered 51% (27/52) of patients with RMS developed localized recurrence despite biological confirmation of complete remission during the treatment, in contrast to 48% (19/39) in non-biopsied patients.[7] Nonetheless, the recurrence rate was 14% (1/7) in patients with BP RMS.[7] The low recurrence rate in BP RMS patients with CR on pathologic screening was confirmed in our study, suggesting that none of the patients who attained CR without viable tumor cells relapsed, while another study found that only 22% (2/9) of patients with BP RMS who achieved CR on pathologic screening experienced tumor relapse.[6] It appears that the recurrence rates in different sites might vary for patients with CR on pathologic finding. Hays et al.[9] found that seven of 18 patients without viable tumor cells at second surgeries experienced tumor relapse; the authors argued that negative pathological findings did not predict any subsequent recurrence, given that previous therapy made it difficult to identify viable tumor cells.

RT seemed to have a pivotal role in local control of the disease. Cecchetto et al.[8] examined the relationship between radiation

and tumor relapse in patients who underwent microscopically complete second look operations of gross residual masses and found that two of 12 patients with RT relapsed; by contrast, relapse occurred in 11 out of 27 patients without RT and five-year progression-free survival rates were 83% and 58% in patients with RT and those without RT, respectively (No p value was provided).[8] Raney et al.[6] reported that 32% (13/41) of patients with RT administered before SLPs had viable tumor cells, compared to 78% (14/18) in patients without RT (p= 0.001). In our study, we found there was no significant difference in tumor viability at SLPs between patients who received RT and those who did not. The variation might be explained by the fact that the results of these studies came from different timing. Unlike the study carried out by Raney et al.[6], whose results were obtained from final procedures, we used pathological findings at initial SLPs. Despite these negative findings, we continue to hold the opinion that RT permits better local control, and nearly half of the patients received RT in our study.

It is worth mentioning that there are some limitations in our study. This study was a retrospective review with a small number of patients. There were several factors that could possibly affect the prognosis. That said, the adjusted model only incorporated patients' age, tumor size, and invasiveness due to the small number of patients; therefore, there might be some other variables such as lymph node involvement and the status of radiotherapy that could have influenced the outcomes. The timing and reasons for SLPs may vary from one patient to another. Nevertheless, our study shed light on the effect of tumor viability on the survival of patients with BP RMS. Further functional imaging studies such as F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) are required to investigate the biological activity of residual masses. In this way, selected patients may be spared additional surgery.[14]

Among patients with BP RMS, the five-year EFS was increased in patients without viable tumor cells. OS was also increased, but not significantly. Disappearance of tumor masses was always associated with the absence of viable tumor cells at SLPs. Thus, avoiding an additional SLP to confirm the results in patients with CR appears reasonable. Notably, 38.9% of patients with residual masses had no viable tumor cells at SLPs. Further studies are necessary to verify these results.

**Acknowledgement**

The authors would like to thank Dr. Libing Fu and Dr. Nan Zhang from the Department of Pathology of our center for reviewing the specimens.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: Hong-cheng Song, Wei-ping Zhang; data collection: Yun-peng Li, Le-jian He; analysis and interpretation of results: Xiao-li Ma, Le-jian He, Wei-ping Zhang; draft manuscript preparation: Yun-peng Li, Hong-cheng Song. All authors reviewed the results and approved the final version of the manuscript.

**Ethical approval**

The study was approved by the Medical Ethics Committee of our center (No. 2020-Z-016).

**Sources of financial**

None.

**Conflicts of interest**

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 434-442
https://doi.org/10.24953/turkjped.2021.03.010

**Original Article**

# The comparison and diagnostic accuracy of different types of thermometers

Nurettin Erdem[1], Tuğba Bedir Demirdağ[2], Hasan Tezer[2], Burcu Ceylan Cura Yayla[2], Fatma Nur Baran Aksakal[3], Anıl Tapısız[2], Okşan Derinöz[4], Arzu Okur[5], Faruk Güçlü Pınarlı[5], Ülker Koçak[5], Aysun Bideci[6]

*Departments of [1]Pediatrics, [2]Pediatic Infectious Diseases, [3]Public Health, [4]Pediatric Emergency, [5]Pediatric Hematology and Oncology, and [6]Pediatric Endocrinology Gazi University Faculty of Medicine, Ankara, Turkey.*

## ABSTRACT

**Background.** Fever is one of the leading causes of hospital admissions in children. Although there are many ways to measure body temperature, the optimal method and the anatomic site are still controversial. In this study, we aimed to evaluate the performance of new methods of measuring body temperature and to compare the accuracy, sensitivity and specificity of these methods.

**Methods.** The body temperatures of the patients who were hospitalized as inpatients or who presented to the emergency room as outpatients between November 2014- March 2015 were measured and recorded. Mercury and digital axillary measurements, tympanic, temporal artery and non-contact skin temperatures were measured. Measurements were compared with each other.

**Results.** According to our results temperature tends to increase over time for up to 8 minutes after placement when using axillary thermometers. Non-contact skin thermometers should be used only for follow-up of patients with fever, because of their low sensitivity and low negative predictivity. At the first examination, tympanic thermometers and axillary thermometers may be preferable for the diagnosis of fever.

**Conclusions.** According to our results, using non-contact thermometers seems feasible and logical during the follow-up ofpatients with fever, but not in cases whose exact body temperature should be known. For the first examination of the patient to diagnose fever, tympanic thermometers and axillary thermometers may be preferable. Future studies are warranted to expose the optimum way of measuring body temperature in children.

**Key words:** body temperature, thermometers, pediatrics.

Fever is one of the leading causes of hospital admissions for children.[1] Although it is an important "self-defense" mechanism, it often triggers significant fear and anxiety among caregivers.[2]

The purpose of body temperature monitoring is to approximate the core temperature and the temperature of the pulmonary artery, as accurately as possible.[3] Rectal measurement is practically accepted as the core temperature.[1] Although rectal measurement is known to be the gold standard method of measuring body temperature; it may cause traumatic or infectious complications.[1,4]

Tympanic and non-contact measurements of body-temperature from the skin are new alternatives to mercury thermometers. Although there are many advantages and disadvantages to each method of measuring body temperature, the selection of the appropriate method and anatomic site still remains controversial. An

✉ Tuğba Bedir Demirdağ
tugbabedir@gmail.com

Received 16th October 2019, revised 26th August 2020, accepted 12th September 2020.

ideal method should make measurements as close as possible to core temperature rapidly and accurately across all age groups, should minimize cross-contamination, and should have an excellent safety profile. Cost and ease of calibration are also important concerns.[1]

Herein we aimed to evaluate the performance of new methods for measuring body temperature and to compare the sensitivity, specificity, and accuracy of these methods. For this purpose, we applied axillary mercury thermometers, axillary digital thermometers, tympanic thermometers, temporal artery thermometers and non-contact skin thermometers for measuring body temperature to our patient cohort and compared the results obtained using each.

## Material and Methods

Body temperatures of all pediatric patients who were admitted as inpatients or applied to the emergency room as outpatients were recorded between the dates of November 1, 2014 and February 28, 2015. Informed consent was provided by the parents of all patients.

Patient demographics, social status, and physical examination findings were recorded for each patient. The final study group consisted of a total of 151 patients and 2265 body temperature measurements were collected (15 times for each patient).

Fever was defined as a rectal temperature ≥38°C.[5] But rectal measurement was not used in this study and body temperature ≥38°C was accepted as fever for all methods.

The thermometer accuracy was checked using a 38°C water bath before each measurement.

Thermometers were calibrated according to the product manual. The measurements were taken at 24°C room temperature (24°C), allowing 10 minutes for the patient to become acclimated to ambient temperature before the measurement.

Body temperatures were measured by axillary mercury thermometers, axillary digital thermometers, tympanic thermometers, temporal artery thermometers and non-contact skin thermometers for each patient. The axilla and forehead were dried before the measurement with non-contact thermometers. A tympanic thermometer was placed in the outer third of the external auditory canal. The tragus was pulled down and back in children aged less than three years, and up and back in children aged greater than three years. After placement of the thermometer, a signal from the thermometer indicated completion of the measurement. Non-contact skin thermometers were also used according to the instructions and body temperature was measured from the forehead. Similarly, body temperature was measured with temporal artery thermometers(TAT) according to the instructions from the manufacturer. Temperatures of the skin over the temporal artery, on the temple, and on the mastoid process were measured. Mercury and digital thermometers were placed at the axilla and for eight minutes. The temperatures were recorded at the end of the 3rd, 5th, and 8th minute. For each kind of thermometer, these measurements were repeated three times with a total number of 15 measurements for each patient.

All statistical analyses were carried out using IBM SPSS Statistics, version 20(SPSS Inc, Chicago, IL, USA). Homogeneity of the distribution of variables was evaluated using the chi-square test. Non-parametric tests were used in cases of non-normal variable distribution. Comparison of the methods was done using the Spearman correlation test and Bland-Altman test.

The study protocol was approved by the ethics committee of Gazi University, with the reference number 2014/112.

## Results

One hundred and fifty-one patients were included in the study. A total of 2,265 body temperature measurements were collected (15 times for each patient). Eighty-one of the patients were male (54%) and 70 patients were

female (46%). The male/female ratio was 1.15. There was no significant difference in gender distribution (P= 0.374).

The age range of the study group was 2-18 years. The mean age was 7.95±4.48 years. The median age was 7.4 (2.5-17) years. The distribution of patients by age and sex was homogeneous.

While 83 patients were hospitalized, 68 patients were admitted to the emergency service as outpatients.

The clinical diagnoses of the patients were as follows: upper respiratory infection (n=83, 55%), lower respiratory infection (n=18, 11.9%), gastrointestinal diseases (n=12, 7.9%), haematologic diseases (n=9, 6%), urinary tract infections (n=7, 4.6%), oncologic diseases (n=6, 4%), cellulitis (n=5, 3.3%), lymphadenitis (n=5, 3.3%) and nephrologic diseases (n=5, 3.3%).

The 3rd, 5th and 8th measurements of each method (mercury thermometer, digital axillary thermometer (AT), TAT, and non-contact skin thermometer) were compared individually, using the Friedman test. The 8th minute measurements of digital axillary and mercury thermometer measurements were significantly higher than 3rd and 5th minute measurements of these methods (p=0.00; Table I-II-III).

The values measured using a tympanic thermometer were tested using the Friedman test.

There was no significant difference between the measurement methods among patients more

**Table I.** Comparison of methods of body-temperature measurement.

| | | 3rd minute | 5th minute | 8th minute |
|---|---|---|---|---|
| Mercury Thermometer | Mean | 37.52 | 37.67 | 37.81 |
| | Median | 37.80 | 37.80 | 38.00 |
| | Standart deviation | 1.16 | 1.17 | 1.20 |
| | Min | 35.20 | 35.10 | 35.50 |
| | Max | 40.00 | 40.30 | 40.60 |
| Digital Axillary Thermometer | Mean | 37.50 | 37.61 | 37.71 |
| | Median | 37.80 | 37.80 | 37.80 |
| | Standart deviation | 1.24 | 1.26 | 1.30 |
| | Min | 35.50 | 34.50 | 34.30 |
| | Max | 40.10 | 40.30 | 40.30 |
| Temporal Artery Thermometer | Mean | 38.2146 | 38.3007 | 38.3291 |
| | Median | 37.90 | 37.90 | 38.10 |
| | Standart deviation | 1.2148 | 1.2886 | 1.2790 |
| | Min | 35.90 | 35.90 | 35.90 |
| | Max | 41.80 | 41.70 | 42.10 |
| Non-contact Skin Thermometer | Mean | 37.5026 | 37.5444 | 37.5603 |
| | Median | 37.30 | 37.40 | 37.40 |
| | Standart deviation | 1.0075 | 1.0153 | 1.0263 |
| | Min | 36.00 | 36.10 | 35.90 |
| | Max | 40.00 | 39.90 | 40.10 |
| Tympanic Thermometer | Mean | 37.64 | 37.56 | 37.56 |
| | Median | 37.70 | 37.60 | 37.70 |
| | Standart deviation | 1.10 | 1.07 | 1.07 |
| | Min | 35.20 | 35.10 | 35.10 |
| | Max | 40.20 | 40.20 | 40.30 |

**Table II.** Comparison of methods of body-temperature measurement.

| | Digital | Non-contact | Tympanic | Mercury | Temporal artery thermometer |
|---|---|---|---|---|---|
| Mean | 37.71 | 37.56 | 37.56 | 37.81 | 38.32 |
| Median | 37.80 | 37.40 | 37.70 | 38.00 | 38.10 |
| SD | 1.30 | 1.02 | 1.07 | 1.20 | 1.27 |
| Min | 34.30 | 35.90 | 35.10 | 35.50 | 35.90 |
| Max | 40.30 | 40.10 | 40.30 | 40.60 | 42.10 |

**Table III.** The comparison of mercury thermometer values with other methods.

| | Specifity | Senstivity | Positive predictivity | Negative predictivity |
|---|---|---|---|---|
| Digital axillary thermometer | 98% | 85% | 98% | 92% |
| Temporal artery thermometer | 94% | 93% | 94% | 88% |
| Non-contact thermometer | 100% | 69% | 100% | 93% |
| Tympanic thermometer | 98% | 87% | 98% | 92% |

than 12 years of age(p=0.64). However, among patients under 12 years of age the difference betweenthe measurement methods was significant (p=0.04), (Table I).

The mean and the median 8th minute measurements of mercury, digital, non-contact skin,and TAT were compared. TAT measurements were significantly higher than othermethods (Table I).

Defining fever as an axillary temperature of >38°C the specificity,sensitivity, positive and negative predictive values of each measurement technique were calculated (Table III).

The distribution of temperature measurements did not conform to a normal distribution and; therefore, the spearman correlation test was used. There was a strong positive correlation between the mean 8th minute measurementof the mercury thermometer and that of the tympanic thermometer (Fig 1), the non-contact skin thermometer (Fig. 2), the TAT (Fig. 3) and the digital AT (Fig. 4), (Sperman's rho: 0.77, p=0.001) within a confidence interval of 95%.

## Discussion

The aim of this study was to compare body temperature measurement methods. To the best of our knowledge, this study includes one of the largest pediatric data. One of the most important points of this study is that temperature tends to increase over time for up to 8 minutes after placement when using axillary mercury and axillary digital thermometers. Secondly, according to our results non-contact skin thermometers should be used only for the follow-up of patients with fever, because of their low sensitivity and low negative predictivity.

The first method used to measure body temperature was an AT. The American Academy of Pediatrics recommends the use of ATs to measure body temperature, despite its relatively low sensitivity and specificity.[1] In a review, Craig et al.[4] concluded that axillary measurement might be inaccurate and was not ideal for body temperature measurement.

There is no consensus on the optimal duration for retaining ATs in the armpit. Common protocols vary from 1-15 minutes, but 4-7 minutes are generally accepted assufficient.[6-8] Chaturvedi et al.[8] showed that temperature was stable after five minutes after the placement of the thermometer in 76% of patients. When we evaluated axillary measurements, the mean temperature at the 8th minutes (37.8°C) was greater than the means of the 3rd (37.5°C) and 5th minutes (37.6°C). Erdal et al.[9] have claimed that mercury thermometer measurements stabilize at 4-6 minutes. Greylinget al.[10]

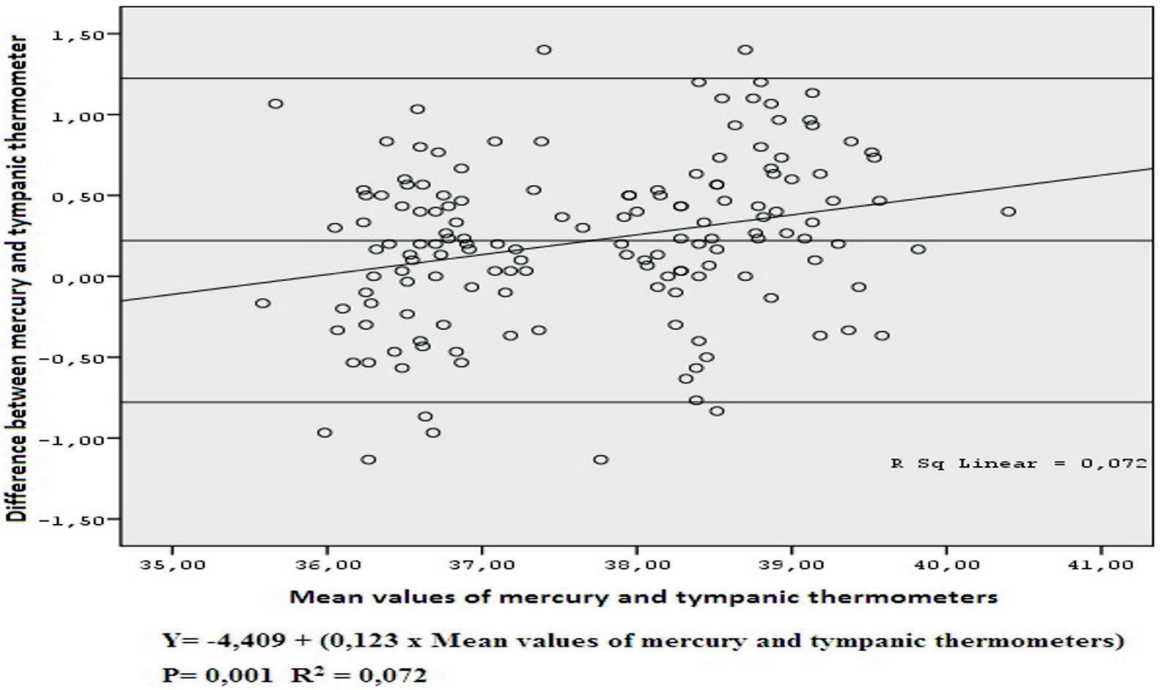


**Fig. 1.** Tympanic thermometer Bland altman test results.

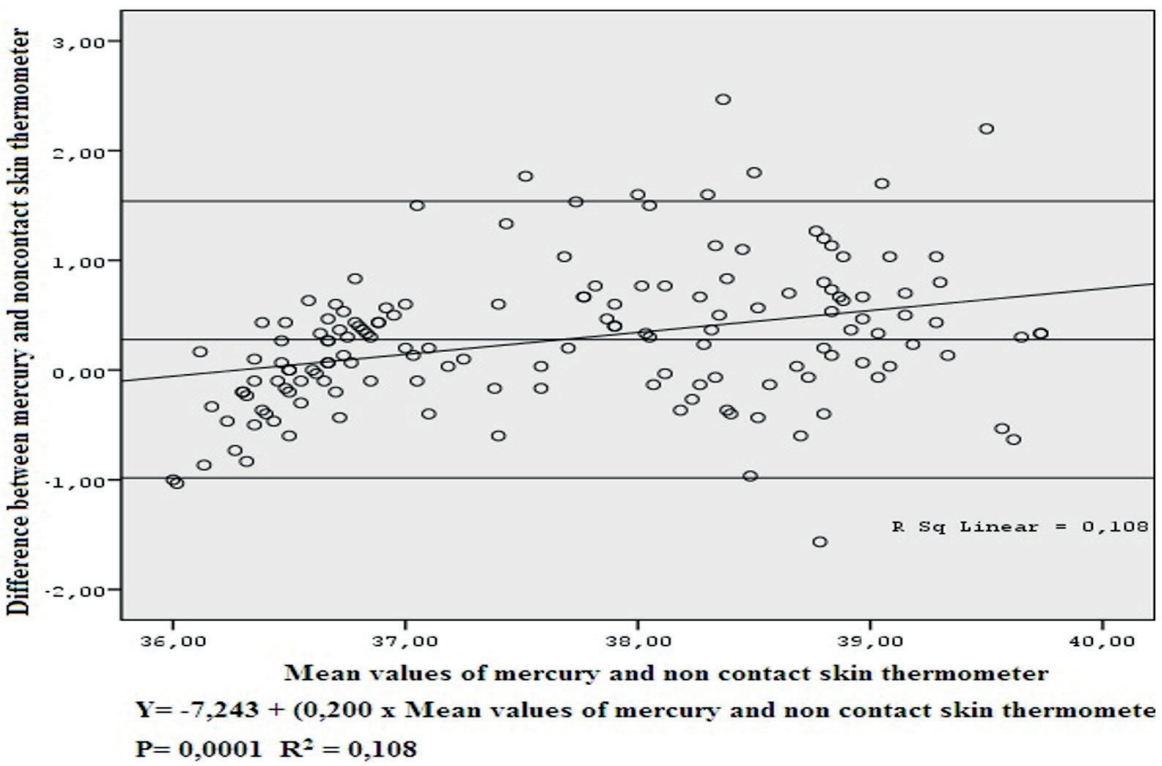


**Fig. 2.** Non-contact skin thermometer Bland altman test results.

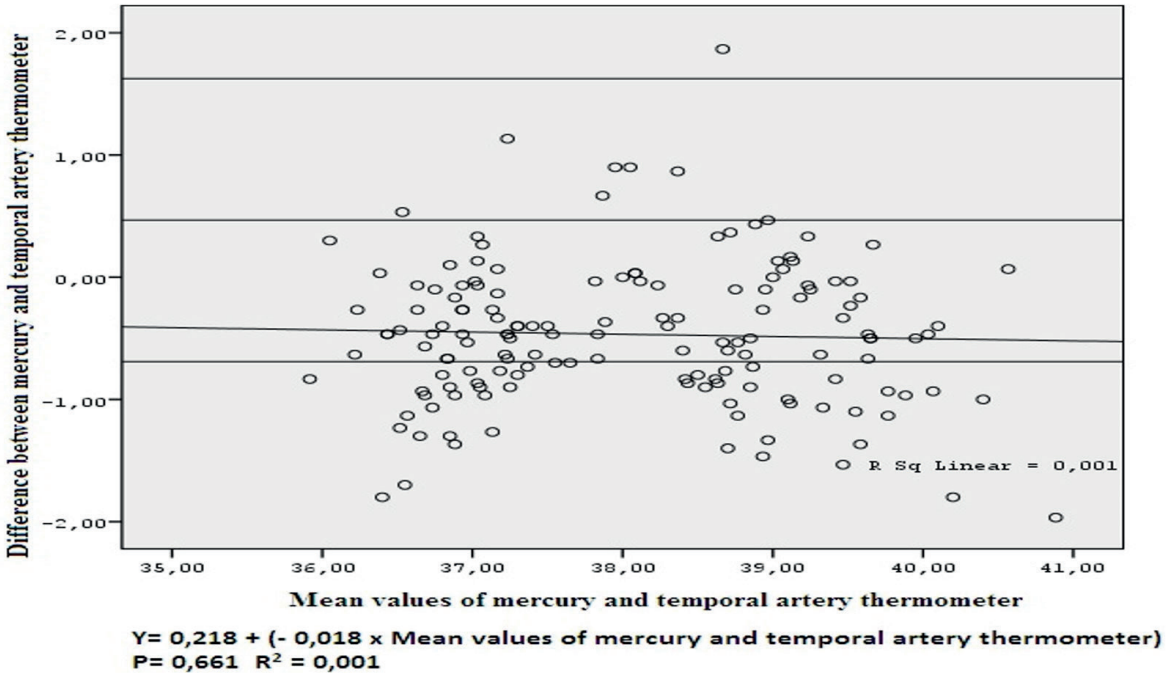


**Fig. 3.** Temporal artery thermometers Bland altman test results.

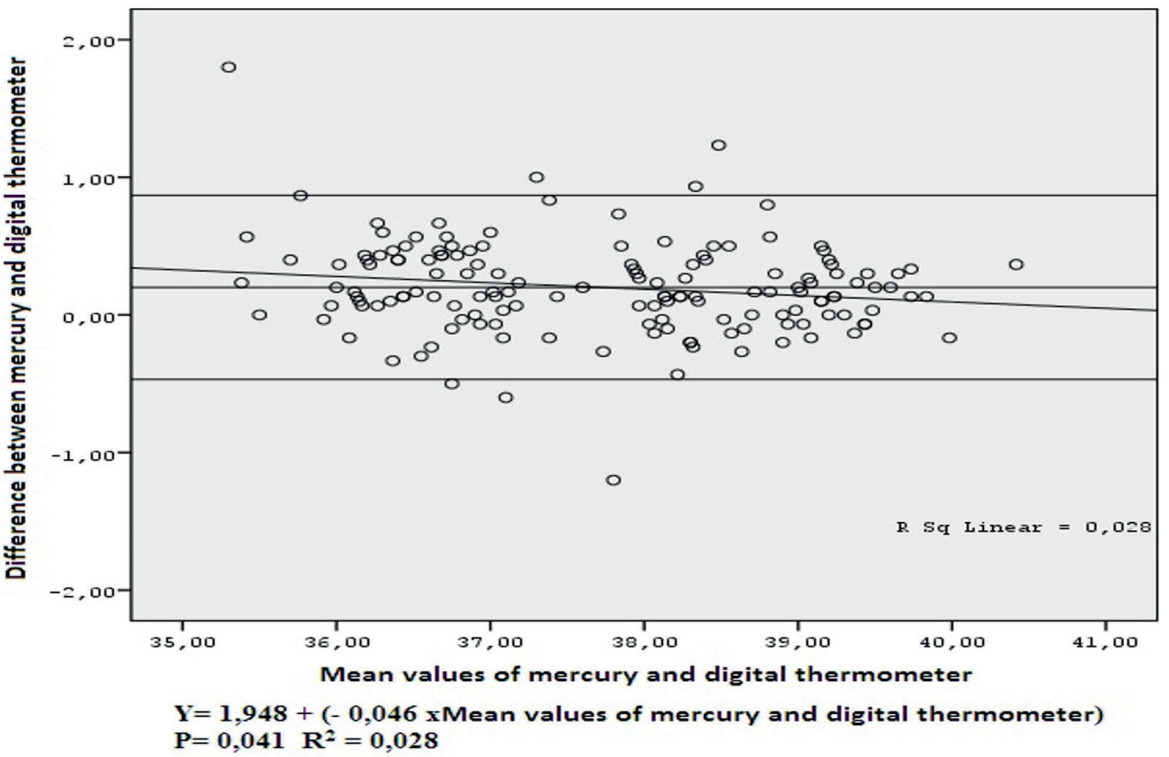


**Fig. 4.** Digital thermometer Bland altman test results.

concluded that the 9th minute value had the greatest sensitivity. Khordish et al.[6] indicated that the increase in temperature after eight minutes was not statistically significant. Similar to the literature, we found that temperature tends to increase over time for up to the 8th minute after placement when using axillary mercury and digital thermometers.

In 2001 Latman et al.[11] proposed that digital thermometers may be used as a replacement for mercury thermometers. According to the sensitivity and specificity of ATs, our study supports the use of digital thermometers in place of mercury thermometers.[4]

The TAT is a newer method for measuring body temperature ultra-red waves and measuring

the highest temperature on the temporal artery trace. Batra et al.[12] established that temporal artery temperature is similar to rectal body temperature and is better than axillary or tympanic temperature at approximating core temperature. In 2014, Isler et al.[13] stated that the TATs can be safely used instead of ATs. Besides positive aspects of TATs, there are some studies indicating that the sensitivity of temporal artery temperature measurement is low. Siberry and Hoffman[14,15] claimed that TATs had low sensitivity. Greenes et al.[16] showed that TATs were more reliable than ATs, but their sensitivity became lower at high temperatures. In our study, the difference between the temporal artery temperature and the axillary temperature was 0.7°C at 3 minutes and 0.5°C at 8 minutes. We found that the 3rd minute values were similar to reports in the literature, although 8th minute values differed from previous reports. We demonstrated that if the waiting period (8 minute) and technique were appropriate, ATs resulted in temperature measurements comparable to TATs.

Non-contact skin thermometers resulted in 0.7°C lower body temperature measurements when compared to ATs. In a study involving 179 newborns, Can et al.[17] showed that non-contact thermometers measured body temperature 0.5°C higher than digital or mercury ATs. Our findings probably differ as a result of the older patient population that we examined. Batra and Goyal[12] found that non-contact skin temperature was the closest approximation to rectal body temperature. Conversely, Fortuna et al.[18] found that non-contact skin thermometers correlated poorly with rectal thermometers and should not be used routinely in pediatric patients. Paes etal.[19] claimed that non-contact skin thermometers could be used when rectal thermometers are not feasible. We compared non-contact skin temperature with axillary temperature and we found a strong, positive and statistically significant correlation between the two measurement techniques. When mercury thermometer measurement > 38 °C is accepted as fever, non-contact skin thermometer measurements had 100% specificity, 69% sensitivity, 69% positive predictivity and 93% negative predictivity. Thus, we recommend that non-contact thermometers should be used only for the follow-up of patients with fever, because of their low sensitivity and low negative predictivity.

The tympanic membrane accurately reflects core temperature. Barton and Kocaoğlu[20,21] determined that the tympanic measurement method had the best correlation with rectal measurement and that it was strongly preferred by patients. However, Lanham et al.[22] showed that the tympanic measurement technique had very low sensitivity and specificity when compared to rectal measurement. According to our study, tympanic thermometers have a strong correlation with ATs and have acceptable sensitivity and specificity. However, the fact that recurrent measurements result in different values among the patients under 12 may raise doubts about the reliability of this method. This technique is more difficult in younger children resulting in reduced reliability.[23] Tympanic thermometers may be preferred due to ease of use and correlation with rectal measurements, however practitioners should keep in mind that this method may not be the most accurate approach in young children.

In conclusion, there is still no consensus concerning the best way of measuring body temperature and it is still an unclear issue in pediatrics. This study aimed to present the most appropriate method including the newer techniques. According to our results, using non-contact thermometers seems feasible and logical in the follow-up of the patients with fever, but not in cases whose exact body temperature should be known. Tympanic thermometers and axillary thermometers may be preferable for the initial diagnosis of fever. Future studies are warranted to expose the optimum way of measuring body temperature in children.

### Author contribution

The contributions of all authors must be described in the following manner: The authors confirm contribution to the paper as follows: study conception and design: Hasan Tezer, Tuğba Bedir Demirdağ; data collection: Nurettin Erdem, Tuğba Bedir Demirdağ, Burcu Ceylan Cura Yayla, Anıl Tapısız, Arzu Okur, Okşan Derinöz, Faruk Güçlü Pınarlı, Ülker Koçak, Aysun Bideci; analysis and interpretation of results: Hasan Tezer, Fatma Nur Baran Aksakal; draft manuscript preparation: Tuğba Bedir Demirdağ, Nurettin Erdem. All authors reviewed the results and approved the final version of the manuscript.

### Ethical approval

The study protocol was approved by the ethics committee of Gazi University, with the reference number 2014/112.

### Source of funding

No funding resources.

### Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose.

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The Turkish Journal of Pediatrics 2021; 63: 443-449
https://doi.org/10.24953/turkjped.2021.03.011

**Original Article**

# Oral health status in children with familial Mediterranean fever

Pelin Esmeray[1], Tülin İleri Keçeli[2], Meryem Tekçiçek[2], Ezgi Deniz Batu[3], Zehra Serap Arıcı[4], Hande Konşuk Ünlü[5], Seza Özen[3], Yelda Bilginer[3]

*[1]Department of Pediatrics, Erzurum Training and Research Hospital, Erzurum; [2]Department of Pediatric Dentistry, Hacettepe University Faculty of Dentistry, Ankara; [3]Department of Pediatrics, Division of Pediatric Rheumatology, Hacettepe University Faculty of Medicine, Ankara; [4]Şanlıurfa Training and Research Hospital, Department of Pediatric Rheumatology, Şanlıurfa; [5]Hacettepe University Institute of Public Health, Ankara, Turkey.*

## ABSTRACT

**Background.** Familial Mediterranean fever (FMF) is the most common hereditary autoinflammatory disease. We aimed to investigate the oral health status and oral hygiene habits in children with FMF.

**Methods.** In this cross-sectional study, 199 children with FMF, aged between 3-18 years, were included. Demographic findings and oral hygiene habits of children were questioned by face-to-face interview. Oral health status of patients was evaluated using decay-missing-filled index [DMFT (decay-missing-filled teeth), DMFS (decay-missing-filled teeth) for permanent; dmft, dmfs for primary teeth], the International Caries Detection and Assessment System (ICDAS-II) index, PUFA / pufa index [the presence of severely decayed teeth with visible pulpal involvement (P/p), ulceration caused by dislocated tooth fragments (U/u), fistula (F/f) and abscess (A/a)], gingival (GI) and plaque index (PI). In addition to these, occlusion, oral soft and hard tissues were examined.

**Results.** One-hundred-nine (54.8%) of children had at least one decayed permanent tooth and 81.2% of children had at least one decayed primary tooth. The mean DMFT was 1.91±2.45, DMFS was 3.1±4.49, dmft was 3.95±3.54, dmfs was 8.62±8.88, PI was 1.17±0.44, GI was 0.85±0.39. Aphthous mouth ulcer occurred in 19 (9.5%) patients. Recurrent aphthous mouth ulcers were more frequent among patients with one exon-ten and one exon-two mutations than patients with one exon-10 mutation, two exon-ten mutations, or two exon-2 mutations (61.1% vs. 47.9%, 26.1%, 20%, respectively p<0.001). Tooth decay was more frequent among patients who had attacks in the last six months than those who did not have any attacks during the last six months (97.4% vs. 87.7%, p=0.017).

**Conclusion.** Dental caries and periodontal disease, which are public health problems, were seen at a high percentage of children with FMF in our study.

**Key words:** familial Mediterranean fever, dental caries, periodontal disease, oral hygiene.

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease worldwide with an increased incidence among Arabs, Jews, Turks, and Armenians.[1,2] FMF is characterized by recurrent, un-provoked, and self-limited attacks of fever (6-72 hours) resulting from mutations in the *MEFV* (*MEditerranean FeVer*) gene on chromosome 16p.[3] The attacks are usually accompanied by serositis. Oral manifestations such as recurrent aphthous mouth ulcers are commonly observed in autoinflammatory diseases such as PFAPA (periodic fever aphthous stomatitis pharyngitis adenitis) syndrome and mevalonate kinase deficiency;[4,5] however, these along with periodontitis may also occur in FMF. The dysregulation of cellular immunity is blamed to be underlying etiology of recurrent aphthous mouth ulcer and colchicine is suggested to be

✉ Yelda Bilginer
yeldabilginer@yahoo.com

Received 23rd September 2019, revised 26th May 2020, accepted 12th September 2020.

effective in aphthous mouth ulcer treatment, since it binds to microtubular proteins and impairs the movement and phagocytosis of granulocytes. However, the studies about oral findings in FMF patients are scarce, and the real frequency of aphthous mouth ulcer remains unknown. Therefore, we aimed to evaluate oral health status and oral hygiene habits of the pediatric FMF patients and the effect of oral and dental health throughout the course of the disease.

## Material and Methods

The study was designed as a cross-sectional survey between May and October 2016. In this study, pediatric FMF patients were referred consecutively to the Pediatric Rheumatology and Department of Pediatric Dentistry outpatient clinics. The study was approved by the Hacettepe University Non-Interventional Clinical Researches Ethics Board (GO-16/187-48; approval date, 10th May 2016). Informed consent was obtained from the all patients. Patients were classified as having FMF according to pediatric FMF criteria.[6] Demographic data, clinical manifestations, and *MEFV* variant analysis were documented by medical file screening and face-to-face interviews. *MEFV* gene variant analysis was performed with Sanger sequencing and 12 variants (E148Q, P369S, F479I, M680I (G-C), M680I (G-A), I692del, M694V, M694I, K695R, V726A, A744S, R761H) were checked in the *MEFV* gene in the Department of Medical Biology. The disease severity and activity were assessed by the International Severity Scoring system for FMF (ISSF)[7] and autoinflammatory disease activity index (AIDAI), respectively.[8] Furthermore, parents were questioned in terms of socioeconomic status, the level of education, oral hygiene, and oral hygiene habits. Oral health status of patients was evaluated from one operator, using decay-missing-filled index (DMFT, DMFS, dmft, dmfs), the International Caries Detection and Assessment System (ICDAS-II) index, PUFA/pufa indices, gingival index (GI) and plaque index (PI). Oral soft tissues were also examined. Decay-missing-filled index describes the prevalence of dental caries in an individual.[9,10] While DMFT (decay-missing-filled teeth)/DMFS (decay-missing-filled surfaces) is used for permanent teeth, dmft/dmfs is used for primary teeth. ICDAS-II is a clinical scoring system which allows detection and assessment of caries activity.[11,12] The PUFA index which is used for assessing the presence of oral conditions resulting from untreated caries, records the presence of severely decayed teeth with visible pulpal involvement (P/p), ulceration caused by dislocated tooth fragments (U/u), fistula (F/f) and abscess (A/a).[13] Plaque index estimates the status of oral hygiene by measuring dental plaque and gingival index (GI) scores gingival inflammation.[14,15]

### *Statistical analyses*

Statistical analysis was performed using IBM SPSS Statistics Version 23.0. The goodness of fit test of numeric variables to normal distribution was determined using Shapiro–Wilk test ($n \leq 50$) and Kolmogorov-Smirnov test ($n > 50$). Equality of variances of numeric variables was tested by Levene's test. Numeric variables were given as mean ± standard deviation (SD) if normality assumption was satisfied; median (minimum-maximum) was presented, otherwise. Categorical variables were reported as count and percentages. One-way ANOVA (when homogeneity of variances assumption was satisfied) was performed to test the difference between more than two independent groups for normally-distributed data. When parametric conditions were not met, Kruskal-Wallis test was used to assess the difference between more than two independent groups. Pearson chi-square tests were conducted to examine bivariate differences between categorical variables where the assumption of the expected count less than 5 should not exceed 20% for variables was satisfied, if otherwise exact chi-square test was used. A p-value below 0.05 was accepted as significant.

## Results

One-hundred-ninety-nine children, aged between 3 and 18 years, participated in the study. The mean age of the children was 10.82±4.07 years and 115 (57.8%) of them were male. The mean age at symptom onset, and current age was 4.79±3.73 and 6.05±3.6 years, respectively. Among 199 patients, age at symptom onset was ≤5 years in 10.6% (n=21), 6-11 years in 46.2% (n=92) and ≥12 years in 43.2% (n=86); 84.4% (n=168) of patients had fever, 78.4% (n=156) had abdominal pain, 57.8% (n=115) had arthralgia, 20.6% (n=41) had arthritis, 12.6% (n=25) had chest pain, and 7.5% (n=15) had erysipelas-like erythema. The median AIDAI score was 0 (range: 0-9). The median number of attacks during the last six months and attack duration was 0 (range: 0-19) and 2 days (range: 0-7), respectively. At the attack free period, the median leukocyte count, erythrocyte sedimentation rate, and CRP levels were 6,900/mm$^3$ (range: 3,500-14,600), 7 mm/hour (range: 0-79), and 0.2 mg/dl (range: 0.1-19), respectively. The descriptive characteristics of the parents are summarized in Table I.

**Table I.** Descriptive characteristics of parents.

| Characteristics | n (%) |
|---|---|
| Parents' education status (mother, N=197) | |
| Illiterate | 5 (2.6) |
| Literate | 4 (3.0) |
| Primary school | 72 (36.4) |
| Middle school | 44 (22.7) |
| High school | 52 (26.3) |
| University | 18 (9.1) |
| Post graduate | 2 (1.0) |
| Parents' education status (father, N=196) | |
| Illiterate | 5 (2.5) |
| Literate | 54 (27.4) |
| Primary school | 35 (17.8) |
| Middle school | 66 (33.5) |
| High school | 28 (14.7) |
| University | 8 (4.1) |
| Working status of mother (N=197) | |
| Yes | 41 (20.7) |
| No | 156 (79.3) |
| Working status of father (N=196) | |
| Yes | 180 (91.9) |
| No | 16 (8.1) |

Among 199 patients, 96.5% (n=192) had their own toothbrushes. However, 49.7% (n= 99) of them did not brush regularly. 64 (32.2%) patients brushed their teeth once a day, while only 35 (17.6%) patients brushed their teeth two or more times a day. One-hundred-sixty-seven (83.9%) children reported that they did not have regular dentist visits and they visited the dentist only when they had a complaint. There was no statistically significant difference between the educational status of the mother or father and going to the dentist (p=0.055 and p=0.36, respectively). There was a statistically significant high rate of going to the dentist in employed mothers but a similar relationship was not established for employed fathers (12.8% vs. 26.8%; p=0.028, and 6.3% vs. 16.1%; p=0.474, respectively).

Tongue pathologies were observed in 65 (32.7%) patients. The most common tongue pathologies were as follows: coated tongue (56.9%), fissured tongue (27.7%), and geographic tongue (9.2%). The mean DMFT, DMFS, dmft, dmfs indices were 1.91±2.45, 3.1±4.49, 3.95±3.54, 8.62±8.88, respectively. One-hundred-nine (54.8%) children had at least one decayed permanent tooth and 81.2% of children had at least one decayed primary tooth. According to ICDAS II index, 54.3% (n= 108) of the patients had a score of 5 and 29.1% (n= 58) had a score of 2 (score 5 presents distinct cavity with visible dentin and score 2 presents distinct visual changes in enamel). According to the PUFA/pufa index, in ≤5 years only 1 tooth had "p score" (4.8%) and 1 tooth had "f score" (4.8%), in ≥12 years 2 teeth had "P score" (2.3%) and only 1 tooth had "F score" (1.2%), in 6-11 years who were in mixed dentition 9 teeth (9.8%) had "p score", 7 teeth (7.6%) had "f score" and 4 teeth had (4.3%) "a score" resulting from untreated caries.

The mean of PI was 1.17±0.44 and the mean of GI was 0.85±0.39. During clinical examinations, aphthous mouth ulcer were observed among 9.5% (n=19) of patients. Furthermore, parents

reported recurrent aphthous mouth ulcer in 77 (38.7%) children; 91.5% (n=182) of children did not have dental trauma in the past. Flush terminal plane in primary dentition and Class 1 molar occlusion in permanent dentition are normal occlusion types in posterior teeth. Flush terminal plane was the most common occlusion relation in primary dentition (55.3%, 21/38) and Class 1 molar occlusion relation (73.9%, 105/142) in permanent dentition in the right side. However, in the left side, 16 patients (44.4%; 16/36) had flush terminal occlusion relation in primary dentition and 88 patients (62.8%; 88/140) had Class 1 molar occlusion relation in permanent dentition.

The most prevalent parafunctional oral habit was nail biting (20.6%; n=41) and 7.5% (n=15) of patients had chromogenic bacteria discoloration on their teeth.

When patients were compared according to *MEFV* mutations, there was no differences in terms of tooth decay, presence of aphthous mouth ulcer during the examination, or DMFT, DMFS, dmft, dmfs parameters, PI, and GI scores between groups. However, recurrent aphthous mouth ulcers were more frequent among patients with one exon-ten and one exon-two mutations than patients with one exon-10 mutation, two exon-ten mutations, or two exon-2 mutations (61.1% vs. 47.9%, 26.1%, 20%, respectively p<0.001). (Table II). There was no significant difference with regards to oral health when we compared the patients according to socioeconomic and educational status of their parents. Tooth decay was more frequent among patients who had attacks in the last six months than those who did not have any attacks during the last six months (97.4% vs. 87.7%, p=0.017). However, aphthosis during clinical examination and recurrent aphthous mouth ulcers were not significantly associated with attack frequency in the last six months (37.9% vs. 47.6%, p=0.385, and 38.9% vs. 38.8%, p=0.986, respectively).

**Table II.** Comparison of oral and dental findings of children with familial Mediterranean fever (FMF) according to *MEFV* mutations (N=187[#])

| Findings | Groups according to presence of mutations | | | | P value |
|---|---|---|---|---|---|
| | Two exon-10 mutations (n=111) | One exon-10 and one exon-2 mutations (n=18) | Two exon-2 mutations (n=10) | One exon-10 mutation (n=48) | |
| Tooth decay, n (%) | 76 (68.5) | 12 (66.7) | 8 (80) | 30 (62.5) | 0.445 |
| Number of attacks during last six months* | 0 (0-15) | 0 (0-6) | 0 (0-3) | 0 (0-19) | 0.586 |
| DMFT* | 1 (0-9) | 2 (0-8) | 2 (0-10) | 0 (0-12) | 0.091 |
| DMFS* | 1 (0-23) | 2 (0-9) | 2 (0-10) | 0 (0-23) | 0.132 |
| dmft* | 3 (10-15) | 1.5 (0-7) | 3 (21-11) | 4 (0-13) | 0.266 |
| dmfs* | 6 (0-43) | 3.5 (20-22) | 4 (1-30) | 7 (0-35) | 0.815 |
| Plaque index* | 1.23 (0.00-2.25) | 1.16 (0.35-1.74) | 1.23 (0.00-1.50) | 1.27 (0.00-2.17) | 0.949 |
| Gingival index** | 0.86 ± 0.41 | 0.82 ± 0.33 | 0.86 ± 0.39 | 0.80 ± 0.39 | 0.831 |
| ISSF* | 1 (0-6) | 0.5 (0-3) | 0.5 (0-2) | 0 (0-2) | 0.102 |
| AIDAI* | 0 (0-9) | 0 (0-6) | 0 (0-2) | 0 (0-8) | 0.181 |
| Recurrent aphthous mouth ulcer, n (%) | 29 (26.1) | 11 (61.1) | 2 (20.0) | 23 (47.9) | <0.001 |

[#]: Mutation analysis revealed no mutation in 12 patients.
* Values are expressed as median (minimum-maximum).
** Values are expressed as mean (standard deviation)
AIDAI: autoinflammatory disease activity index, DMFT: decay-missing-filled-teeth for permanent teeth, DMFS: decay-missing-filled-surfaces for permanent teeth, dmft: decay-missing-filled-teeth for temporary teeth, dmfs: decay-missing-filled-surfaces for temporary teeth, ISSF: international severity scoring system for familial Mediterranean fever.

## Discussion

We found that recurrent aphthous mouth ulcer was more common in patients who had one exon-10 and one exon-2 mutations and patients who had attacks at the last six months had significantly higher frequency of dental caries.

There are only a few studies on oral health in adult FMF patients and these are mostly focused on periodontitis.[16,17] In a study by Sogur et al.[18] numbers of extracted teeth and filled teeth were found to be significantly higher in the FMF group compared to healthy controls and a study by Cengiz et al.[19] showed that GI and PI indices were higher in FMF patients with amyloidosis compared to patients without amyloidosis.

According to the oral-health-related survey in our country, caries prevalence was 69.8% in 5-year-olds, 61.1% in 12-years-olds and 61.2% in 15-years-olds while dmft was 3.7, DMFT was 2.7, and DMFT was 2.3, respectively among these ages.[20,21] In our FMF patients, frequency of tooth decay was similar to that in healthy children (54.8% had at least one decayed permanent tooth and 81.2 had at least one decayed primary tooth). In healthy populations, 45% of 5-year-olds, 44.6% of 12-year-olds and 38.2% of 15-year-olds report not brushing their teeth regularly. Moreover, in healthy populations only 4.4% of 5-year-olds, 1.4% of 12-year-olds and 1.8% of 15-year-olds had visited a dentist regularly.[21] Similarly, most of our patients (83.9%) reported that they did not have regular dentist visits and did not brush regularly (49.8%). Interestingly, neither the oral health nor the dentist visits differed according to the educational status of parents. It is possible that we could not demonstrate a significant difference since the number of patients was limited.

Previously, Kone Paut et al.[22] compared patients with different *MEFV* mutations with regards to mucocutaneous findings and did not find any significant difference in the frequency of oral aphthous lesions. Contrary to this study, we have demonstrated that patients who had one exon-10 and one exon-2 mutations had recurrent aphthous mouth ulcers more frequently. Ours is an interesting finding since oral aphthous ulcer was thought to be a significant component of PFAPA syndrome rather than FMF. However, in Mediterranean countries, we are aware that FMF patients may present with a PFAPA-like phenotype at early ages.[22]

FMF is a monogenic disease, however the course of the disease varies among the patients.[24] In recent years, researches have focused on the epigenetic factors affecting the phenotype of the disease. Microbiota is one of the epigenetic factors probably contributing to disease severity. A study by Khachatryan et al.[25] previously demonstrated that the gut microbiota were different during attack and attack-free periods as well as between FMF patients and healthy controls. However, the effect of oral microbiota is still unknown. In the presented study, we observed higher frequency of tooth decay among patients that have had attacks in the last six months. It is tempting to speculate the effect of oral microbiota.

Our study is limited by the confounding factors associated with a small sample size and lack of aged-matched healthy controls.

In conclusion, we evaluated the oral health in children with FMF. We have observed that dental caries was more frequent in patients with a more active disease (with attacks in the last six months). It should be kept in mind that in all age groups and in all kinds of chronic diseases, oral health is affected by underlying disease, and oral health could also affect the course of the underlying disease. Therefore, it is critical for physicians to have a holistic approach while evaluating patients with chronic diseases.

## Acknowledgement

None.

## Author contribution

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

Özen, Yelda Bilginer and Meryem Tekçiçek; data collection Pelin Esmeray, Ezgi D. Batu, Zehra S. Arıcı and Tülin İ. Keçeli, analysis and interpretation of results: Hande K. Ünlü, Pelin Esmeray, Ezgi D. Batu, Yelda Bilginer. All authors reviewed the results and approved the final version of the manuscript.

**Source of funding**

No funding support was received for this study.

**Conflict of interest**

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 450-460
https://doi.org/10.24953/turkjped.2021.03.012

**Original Article**

# Early-auditory intervention in children with hearing loss and neurodevelopmental outcomes: cognitive, motor and language development

Pelin Çelik[1], Kemal Keseroğlu[2], Serap Er[3], İclal Ayrancı Sucaklı[1], Güleser Saylam[2], Halil İbrahim Yakut[4]

*[1]Department of Pediatrics, Division of Developmental and Behavioral Pediatrics, [4]Department of Pediatrics, Ankara City Hospital, Ankara; [2]Department of Otorhinolaryngology, Head and Neck Surgery, Dışkapı Yıldırım Beyazıt Training and Research Hospital, University of Health Sciences Turkey, Ankara; [3]Department of Otorhinolaryngology, Head and Neck Surgery, Hearing-Speech-Balance Unit, Dışkapı Yıldırım Beyazıt Training and Research Hospital, University of Health Sciences Turkey, Ankara, Turkey.*

## ABSTRACT

**Background.** To date, studies have mostly focused on the language outcome of early-auditory interventions including amplification for congenital hearing loss within the first 6 months. We aimed to examine the effect of early-auditory intervention in patients with congenital hearing loss on cognitive, motor and language outcomes, and determine the clinical variables that affect developmental outcomes.

**Methods.** The medical records of 104 patients were retrospectively reviewed. Children were evaluated by the Bayley Scales of Infant and Toddler Development, Third Edition.

**Results.** The median ages of confirmation of hearing loss, amplification, starting auditory-verbal intervention and cochlear implantation were 9, 10, 13 and 19 months, respectively. Of the patients, 26% received a hearing-aid fitting ≤6 months of age. Fifty-one children (49%) had additional disabilities. The median cognitive, language and motor scores of children with no additional disabilities were 95 (65-115), 68 (47-103) and 97 (58-130), respectively and children with early-auditory intervention (≤6 months) demonstrated higher cognitive, receptive and expressive language subscale scores than late-auditory intervention group ($p<0.05$) whereas there was no significant difference in motor scores ($p>0.05$). A significant negative correlation was found between additional disability and cognitive, language and motor outcomes ($r=-0.78$, $r=-0.54$ and $r=-0.75$, respectively $p<0.01$). There was a significant negative correlation between language outcomes and the degree of hearing loss ($r=-0.20$, $p<0.05$). Multiple regression analyses revealed that additional disability and early-auditory intervention showed a significant amount of variance in cognitive and language scores. The early intervention did not make a significant, independent contribution on motor outcomes whereas additional disability did.

**Conclusions.** Presence of additional disability was the strongest significant variable on developmental outcomes in hearing-impaired children. In children with no additional disability, significantly better cognitive and language scores were associated with the early-auditory intervention. Motor skills were not affected by the early-auditory intervention.

**Key words:** congenital hearing loss, early-auditory intervention, cognitive outcome, language outcome, motor outcome.

More than 250 million children (43%) under 5 years of age living in low- and middle-income countries are at risk of not reaching their optimal neurodevelopment.[1] The first years of life are critically important for cognitive, linguistic, social, emotional and motor development.[2] During this period, congenital hearing loss has a negative impact on development. The Joint Committee on Infant Hearing (JCIH) recommends that children should be screened for hearing loss by 1 month, diagnosed by 3

✉ Pelin Çelik
drpelincelik06@gmail.com

Received 20th July 2020, accepted 14th December 2020.

months and should be received appropriate intervention by 6 months of age to reduce the negative effects of hearing loss on optimal development.[3] These recommendations are also named as the Early Hearing Detection and Intervention (EHDI) guidelines. The age of amplification and early intervention commencement is decreasing due to newborn hearing screening programs (NHS) soon after birth.

Congenital hearing loss affects speech and language development negatively.[4-6] Studies showed that children who were diagnosed and accessed auditory stimulation through hearing aids within the first six months of life, have significantly better language acquisition.[7,8] Although, the impact of auditory deprivation on language development has been extensively studied, comparably less research has been focused on the effect of hearing loss on non-verbal skills including cognitive and motor development.[9] As speech and language development are prerequisites for cognitive development, an auditory defect may have a negative effect on the hearing-impaired child's cognitive ability.[10-12] Some studies showed that hearing impairment was associated with impaired motor development especially gross motor skills[13-15] whereas some authors reported motor scores of hearing impaired children to be within typical ranges.[16,17]

The primary purpose of this study was to evaluate the effects of the early auditory intervention (fitting of amplification) on cognitive and motor outcomes as well as language outcomes. The secondary purpose was to determine clinical and sociodemographic variables that influence the language, cognitive and motor outcomes in children with congenital hearing loss.

## Material and Methods

### *Procedure and Participants*

This retrospective study was conducted at the Department of Developmental and Behavioral Pediatrics, Ankara Child Health and Diseases Hematology and Oncology Training and Research Hospital, University of Health Sciences Turkey, and Department of Otorhinolaryngology, Head and Neck Surgery of Dışkapı Yıldırım Beyazit Training and Research Hospital, University of Health Sciences Turkey. Participants were children with congenital hearing loss ranging from mild to profound who were followed by both departments between January 2018 and June 2019.

This retrospective research was reviewed and approved by the Ethical Committee of Ankara City Hospital, Turkey (24.12.2019-E1/235/2019) and also reviewed and approved by the institutional review board of Ankara Child Health and Diseases Hematology and Oncology Training and Research Hospital, University of Health Sciences Turkey (18.07.2019/17). Informed consent was not taken because of retrospective design of the study.

Inclusion criteria were: 1) bilateral, congenital sensorineural hearing loss ranging from mild to profound requiring amplification 2) living in a Turkish-speaking home 3) chronological age between 8-42 months 4) children without auditory neuropathy.

Clinical records were retrospectively reviewed. Sociodemographic data, presence or absence of additional disability, gender, age at diagnosis, degree of hearing loss, age at amplification or cochlear implantation, age of enrollment for auditory-verbal therapy, communication mode used by the family, parental education status, household income, parental consanguinity, the hearing status of the family members were extracted from clinical charts.

### *Additional Disability*

Additional disability was defined as cerebral palsy, visual impairment, autism spectrum disorder, extreme prematurity, genetic, metabolic or neurological diseases or other medical conditions that may affect cognitive, language or motor outcomes.

### *Degree of Hearing Loss*

The degree of hearing loss was determined by using the better-ear pure tone average which was calculated for the thresholds at 500 Hz, 1 kHz, and 2 kHz. It was classified according to American Speech-Language-Hearing Association.[18]

### *Auditory Intervention*

The early-auditory intervention was described as diagnosis of hearing loss and fitting of a hearing aid by 6 months of age, and late-auditory intervention was described as fitting of a hearing aids >6 months age or not fitted yet. All patients received auditory-verbal therapy which was provided by the government health insurance as 2 hours per week.

### *Developmental Assessment*

Cognitive, language and motor function were evaluated by the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III).[19] This was designed to measure the child's level of development in three scales; cognitive, language and motor (expected population mean 100; standard deviation (SD) 15). The language scale is composed of receptive communication and expressive communication subscales. The motor scale is composed of fine and gross motor subscales (mean 10, SD 3). During the assessment, it was made sure that the child used the hearing aid or cochlear implant correctly; ambient noise was minimized and the child was spoken to clearly and naturally with a parent present.

### *Statistical Analysis*

Statistical analyses were performed using the SPSS statistical package (v. 20.0 for MAC). Categorical variables between groups were analyzed using the $\chi 2$ test. Comparison of means between two groups was examined using a t-test, where the data fit a normal distribution. For comparison of more than two groups, ANOVA was used for normal distributions and the Kruskal-Wallis test for nonnormal distributions. A p-value of <0.05 was deemed to indicate statistical significance. To explore the relationships Spearman's correlation test was performed. Multiple regression analysis was used to investigate the effect of predictor variables on outcomes, after controlling for the effects of other variables.

## Results

One hundred and four children were enrolled in the study. The median age was 25.5 (8-42) months. Of the children, 79.8% were diagnosed as a result of the NHS. The median ages of confirmation of hearing loss, amplification, auditory-verbal therapy and cochlear implantation were 9 (0– 42), 10 (3– 36), 13 (3-35) and 19 (12–40) months, respectively.

Sociodemographic and clinical characteristics are presented in Table I. Moderately severe to profound hearing loss was observed in 85 (81.7%) children. While 27 (26%) children were identified and instrumented with hearing aids by 0-6 months of age, 56 (53.8 %) children fitted their first hearing aid after 6 months of age. Only 9 (8.6%) children met all 3 components of the EHDI guidelines. Twenty-one (20.2%) children had unmet needs in terms of amplification. Of children with hearing aids 53.7% and of children with cochlear implants 76% were wearing the device all waking hours without resistance.

The median Bayley-III cognitive, language and motor scores of the study group were 85 (55-115), 59 (47-103) and 82 (46-130) respectively. Fifty-one of the 104 children (49%) had one or more additional disability other than their hearing loss including visual impairment, autism spectrum disorder, neurological, metabolic or genetic diseases (Table II). Of children with an additional disability 68.7% were fitted with a hearing aid or cochlear implant, and 82% of them were fitted >6 months of age.

When considering the 53 children with no additional disability, the median cognitive, language and motor scores were 95 (65-115), 68 (47-103), 97 (58-130) respectively. Table III

**Table I.** Sociodemographic and clinical characteristics.

| Characteristics | n (%) |
|---|---|
| Gender | |
| Male | 64 (61.5) |
| Female | 40 (38.5) |
| Degree of hearing loss | |
| Mild (26-40 dB HL ) | 2 (1.9) |
| Moderate (41-55 dB HL ) | 13 (12.5) |
| Moderately severe (56-70 dB HL ) | 22 (21.2) |
| Severe (71-90 dB HL ) | 19 (18.2) |
| Profound (>90 dB HL ) | 44 (42.3) |
| Unknown (not reported) | 4 (3.8) |
| Universal newborn hearing screening | |
| Passed | 11 (10.6) |
| Failed | 83 (79.8) |
| Not screened | 2 (1.9) |
| Unknown (family does not remember) | 8 (7.7) |
| Age of onset of hearing loss | |
| Congenital | 93 (89.4) |
| Late onset (before 2 years) | 11 (10.6) |
| Type of amplification | |
| None | 21 (20.2) |
| Hearing aids | 57 (54.8) |
| Cochlear implant | 1 (1) |
| Cochlear implant after hearing aid | 25 (24) |
| Identified and intervention of hearing loss | |
| Early (diagnosed and instrumented with hearing aids by 6 months of age) | 27 (26) |
| Late (instrumented >6 months of age or not instrumented) | 77 (74) |
| Communication mode used with the child | |
| Spoken language only | 60 (57.6) |
| Spoken language with occasional use of sign language | 26 (25) |
| Sign language only | 5 (4.8) |
| Unable to communicate because of severe neurologic impairment | 13 (12.5) |
| Language at home | |
| Monolingual (Turkish) | 82 (78.9) |
| Bilingual | 22 (21.1) |
| Consanguinity | |
| Consanguineous marriages | 51 (49) |
| First degree cousin marriage | 22 (21.2) |
| Second degree cousin marriage | 14 (13.5) |
| Third degree cousin marriage | 2 (1.9) |
| Same village | 13 (12.5) |
| No consanguinity | 53 (50.9) |

**Table I.** Continued.

| Characteristics | n (%) |
|---|---|
| Hearing status of the family members | |
| One or both parents or siblings deaf | 23 (22.1) |
| Deafness and/or hard of hearing in any other family members | 19 (18.3) |
| Parents, siblings and other family members hearing | 62 (59.6) |
| Mother's education | |
| ≤12 years | 95 (91.3) |
| >12 years | 9 (8.7) |
| Father's education | |
| ≤12 years | 85 (81.7) |
| >12 years | 19 (18.3) |
| Annual income (USD) | |
| No reguler income | 21 (20.2) |
| <3000 USD | 37 (35.6) |
| 3001-5000 USD | 23 (22.1) |
| 5001-8000 USD | 12 (11.5) |
| ≥8001 USD | 11 (10.5) |

**Table II.** Additional disability.

| Additional disability (n: 51)* | n (%) |
|---|---|
| Cerebral palsy | 24 (23.1) |
| Seizures | 18 (17.3) |
| Cleft palate | 1 (1) |
| Metabolic disesases (Mucopolysaccharidosis type 1, Mucopolysaccharidosis type 2, Tay-Sachs, mannosidosis, fatty acid oxidation defect) | 5 (5) |
| Genetic syndromes (Down syndrome, Pendred syndrome, Beckwith-Wiedemann syndrome, Waardenburg syndrome, Cornelia de Lange syndrome, Pierre Robin sequence, 1p36 duplication syndrome, Kleefstra syndrome, CHARGE syndrome, Holt-Oram syndrome) | 13 (12.5) |
| Visual impairment | 32 (30.7) |
| Autism spectrum disorder | 5 (4.8) |
| Other | 4 (3.8) |

*Some children had more than 1 additional disability

illustrates the Bayley-III scores of the children with no additional disability according to the early or late-auditory intervention. In the early-auditory intervention group, cognitive and language scores were significantly higher than the late intervention group ($p < 0.01$), whereas there was no significant difference in motor scores. Comparison of Bayley-III language subscaled scores showed that both receptive and expressive communication scaled scores were significantly higher in the early intervention group ($p<0.01$).

Table IV provides the Spearman's rho correlations between the Bayley-III language, cognitive and motor scores and the independent variables: gender, age, presence of additional disability, the early-auditory intervention, degree of hearing loss, hearing loss in first-degree family members, mother's education >8 years, and household income. Of the variables examined, the strongest significant correlation was obtained between additional disability and Bayley-III cognitive, language and motor scores ($r= -0.78$, $r=-0.54$ and $r=-0.75$, respectively

**Table III.** Bayley-III scores of the children with no additional disability according to identification and intervention age.

| Bayley-III scores | Early intervention Group[a] (n= 21) | Late intervention Group[a] (n=32) | Total [a] (n= 53) | p-value |
|---|---|---|---|---|
| Cognitive composite score | 100 (90-115) | 92 (65-115) | 95 (65-115) | 0.000 |
| Language composite score | 86 (50-103) | 63.5 (47-91) | 68 (47-103) | 0.002 |
| Motor composite score | 100 (73-112) | 94 (58-130) | 97 (58-130) | 0.068 |
| Cognitive scaled score | 10 (8-13) | 8.5 (3-13) | 9 (3-13) | 0.000 |
| Receptive communication scaled score | 6 (1-10) | 3 (1-9) | 4 (1-10) | 0.007 |
| Expressive communication scaled score | 8 (3-11) | 5 (1-9) | 6 (1-11) | 0.000 |
| Fine motor scaled score | 10 (6-14) | 10 (2-15) | 10 (2-15) | 0.230 |
| Gross motor scaled score | 9 (1-15) | 8 (1-15) | 9 (1-15) | 0.075 |

[a]Median values and minimum-maximum values are presented

**Table IV.** Correlations between the Bayley-III language, cognitive and motor scores and independent variables.

| | Cognitive composite score | Language composite score | Motor composite score | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Gender | 0.08 | 0.00 | 0.09 | - | - | - | - | - | - | - | - |
| 2. Age | -0.15 | -0.01 | 0.08 | 0.00 | - | - | - | - | - | - | - |
| 3. Additional disability | -0.78** | -0.54** | -0.75** | -0.13 | 0.04 | - | - | - | - | - | - |
| 4. Early auditory intervention | 0.44** | 0.39** | 0.35** | -0.02 | -0.20* | -0.31** | - | - | - | - | - |
| 5. Degree of hearing loss | 0.05 | -0.20* | -0.00 | -0.15 | -0.20* | -0.05 | 0.06 | - | - | - | - |
| 6. Hearing loss in first degree family member | 0.14 | 0.05 | 0.16 | -0.19 | 0.04 | -0.26* | 0.02 | 0.10 | - | - | - |
| 7. Mother's education | -0.02 | -0.01 | -0.05 | 0.00 | -0.06 | 0.00 | 0.07 | 0.00 | -0.07 | - | - |
| 8. Income | -0.01 | 0.11 | -0.07 | -0.09 | -0.04 | 0.15 | 0.00 | -0.01 | -0.29* | 0.31* | - |

Degree of hearing loss: mild to moderate versus moderately severe to profound
Mother's level of education: ≤12 years versus >12 years
* p<0.05, 2-tailed.
** p<0.01, 2-tailed.

p<0.01). Cognitive, language and motor scores were found to be significantly increased with the early-auditory intervention (cognitive; r=0.44, language; r=0.39, and motor; r=0.35, p<0.01). A significant negative correlation was found between language outcomes and degree of hearing loss (r=-0.20, p<0.05). But there was no association between cognitive, language and motor outcomes and other demografic variables. The interaction between age at amplification and presence/absence of additional disability was significant (r=-0.31, p<0.01). There was also a correlation between income and mothers' education (r=0.31, p<0.05).

Multiple regression analyses were conducted for further exploration of the relationships between age, sex, additional disability, early-auditory intervention, degree of hearing loss, hearing loss in first degree family members, mother's education, household income and cognitive, language and motor scores (Table V). The presence of additional disability made the strongest significant contribution on cognitive, language and motor outcomes. The early-auditory intervention also made a significant, independent contribution to both the cognitive and language outcomes. Higher cognitive and language scores were predicted

**Table V.** Multiple Regression Models for predicting cognitive, language and motor composite scores.

| | $R^2$ | F | p | | Unstandardized coefficient | Standardized coefficient | t test value | p |
|---|---|---|---|---|---|---|---|---|
| Cognitive composite score | 0.633 | 31.44 | <0.0001 | Additional disability | -25.49 | -0.69 | -9.09 | <0.0001 |
| | | | | Early auditory intervention | 8.79 | 0.21 | 2.88 | <0.01 |
| | | | | Household income | 1.89 | 0.12 | 1.62 | 0.109 |
| | | | | Mother's education | 10.03 | 0.14 | 1.86 | 0.067 |
| Language composite score | 0.443 | 9.27 | <0.0001 | Gender | -4.15 | -0.12 | -1.33 | 0.187 |
| | | | | Additional disability | -12.77 | -0.39 | -4.08 | <0.0001 |
| | | | | Early auditory intervention | 10.54 | 0.29 | 3.12 | <0.01 |
| | | | | Degree of hearing loss | -14.56 | -0.31 | -3.49 | <0.01 |
| | | | | Household income | 3.14 | 0.23 | 2.41 | 0.018 |
| | | | | Mother's education | 9.61 | 0.15 | 1.61 | 0.110 |
| Motor composite score | 0.606 | 26.64 | <0.0001 | Age | 0.40 | 0.17 | 2.18 | 0.032 |
| | | | | Additional disability | -32.75 | -0.69 | -8.62 | <0.0001 |
| | | | | Early auditory intervention | 7.98 | 0.15 | 1.92 | 0.058 |
| | | | | Mother's education | -11.88 | 0.13 | 1.78 | 0.079 |

by the early-auditory intervention. But the early-auditory intervention did not make a significant, independent contribution to the motor outcomes. Lower language scores were predicted by the higher degree of hearing loss and lower levels of household income. Motor scores also increased as chronological age increased.

## Discussion

This study, which examined the effect of early-auditory intervention on developmental outcomes in children with congenital hearing loss revealed that early-auditory intervention was associated with higher Bayley-III cognitive and language scores, but not motor scores. The presence of additional disability was also found to be the strongest significant variable on all developmental domains in hearing-impaired children.

Despite the benefits of early-auditory intervention, the median language score of children with no additional disability and early-auditory intervention was 86 (<1 SD of the expected mean of 100) in our study. Yoshinaga-Itano et al.[8] and Ching et al.[20] similary showed that hearing impaired children who were detected early and treated with amplification had language scores at or below 1 SD of the normative mean. Although our study was one of the rare studies[21] evaluating the language skills of children with hearing loss with Bayley-III, the language score was similar to other studies using different language assessment tools including MacArtur-Bates Communicative Development Inventories, Preschool Language Scale v.4, Child Development Inventory, Peabody Picture vocabulary test.[8,20] Multiple regression analysis showed that early auditory intervention, absence of additional disability, a lesser degree of hearing loss and a higher level of household income were associated with better language scores in the current study, consistent with previous studies.[5,8,20,22]

To date, few studies have investigated the cognitive skills of hearing impaired toddlers and young children.[11] Kutz et al.[11] revealed the overall poor performance of cognitive skills in a small number of hearing-impaired toddlers and young children. However, most research was conducted in school-aged children[23,24] and adolescents.[25,26] Martinez-Cruz et al.[23] showed that children with unilateral severe to profound sensorineural hearing loss had significantly lower intelligence coefficients

than healthy children. Emmett et al.[26] reported that hearing loss in adolescents and young adults would be associated with decreased nonverbal intelligence. Academic achievement of children with severe to profound hearing loss was significantly impaired relative to peers.[24] Teasdale et al.[25] found that mean intelligence quotients (IQ) of adolescents with normal hearing, mild hearing loss and more severe hearing loss were 101, 98 and 94, respectively. In our study, the median cognitive score of toddlers without an additional disability was 95 in consistent with Teasdale's study. It is important to note that our study was performed at a younger age and the measurement derives a developmental quotient (DQ), not IQ. Additionally, the median cognitive score of children with early diagnosis and fitting hearing aid completed by 6 months of age was 100, within the normative population and statistically significantly higher than the late intervention group. Because of impaired auditory functions in the prelingual period, the nervous system can not get enough information and input, which may affect cognitive development. Hearing impaired children obtain sound, enrich their knowledge, boost their confidence by early-auditory intervention and, cognitive development is promoted. Therefore, the earlier the diagnosis is made and the intervention is started, the better the intelligence development gets.

Motor outcomes have received less attention in the literature for hearing impaired children. A systematic review reported that these children had difficulties especially in balance function.[27] Schlumberger et al.[12] found that hearing impaired children without neurologic diseases had reduced balance and complex motor movements. In contrast, Leigh et al.[16] showed that fine and gross motor development scores were within the typical range for healthy children. In our study, median fine and gross motor scaled scores of children without additional disabilities were 10 (2-15) and 9 (1-15) respectively and, within normal limits. Several studies have investigated the effect of the early-auditory intervention on motor skills, but the results were controversial. Sahli et al.[28] showed that children who received an early diagnosis and intervention in accordance with EHDI Guidelines had significantly better fine and gross motor skills. Korver et al.[17] reported better gross motor subscales but similar fine motor subscales in early-identified children when comparing with the late-identified group. In contrast, Leigh et al.[16] found that early-auditory intervention was not significantly associated with motor outcomes. In conjunction with the study of Leigh et al.,[16] early-auditory intervention was not found to be an independent factor affecting motor scores in our study. The average age of children at the time of testing in our study was similar to studies of Sahli et al.[28] and Leigh et al.,[16] but younger than the study of Korver et al.[17] Also, we used Bayley-III for developmental assesment whereas other studies used the Child Development Inventory or the Denver Development Screening Test-II. The different results may be associated with different age groups and assessment tools used. Surprisingly it was found that motor scores were increased as chronological age increased. Kegel et al.[29] showed a decrease in gross motor scores in hearing-impaired children within the age period of implantation, and increased motor skills at the age of 2 years in a prospective study. Our result may be related to the tendency to catch-up on motor skills over time as suggested by Kegel et al.[29] But more follow up studies are needed to confirm whether the trajectory of gross motor development changes over time.

In the current study, children with additional disabilities were not excluded in order to determine the effect of the presence of an additional disability on developmental outcomes in hearing impaired children. Additional disability was found to be the strongest significant independent factor affecting cognitive, language and motor outcomes in the multiple regression analysis. It should be noted that amplification rate was lower and a hearing aid was fitted at an older age in hearing-impaired children with an additional

disability in this study. Beer et al.[30] showed that children with additional disabilities can benefit from auditory intervention, albeit at a slower pace and/or lesser degree than children with no additional disabilities. So, clinicians should be aware of the importance of accessing early auditory intervention options such as a hearing aid or cochlear implantation in time to reach their developmental potential in hearing impaired children with an additional disability.

Studies in Australia and the United States have reported that 56-58% of children with congenital hearing loss have had their first hearing aid by 6 months of age.[8,20] According to recent studies from different regions of Turkey, the rate of children being fitted with a hearing aid by 6 months was 18.9-26.4%.[28,31] Similarly, our study showed that the rate of children who were diagnosed and fitted with hearing aids by 6 months of age was only 26%. Also, it should be noted that 20.2% of children had unmet needs in terms of amplification in our study. This means that, although the NHS program has been successfully implemented in Turkey the next steps including fitting hearing aids and auditory-verbal interventions have not been conducted effectively. Transportation difficulties, the inability of the family to understand the importance of hearing loss on the child's development, exhaustion of the family during the diagnostic process, family's resistance to accept that their child has hearing loss and particularly being of low socioeconomic status may negatively affect children to reach early-auditory intervention.

The limitations of our study included the retrospective design of the study, the lack of a control group and long-term follow-up results. Also, the majority of children in our study had moderately severe to profound hearing loss and had additional disabilities, so the results are not generalizable to all children with hearing loss.

In conclusion, to the best of our knowledge, this is the first study exploring all developmental domains in hearing-impaired children. Fitting a hearing aid by 6 months of age in children with congenital hearing loss and no other concomitant disability provided similar cognitive and motor skills to their typically hearing peers but lower language skills. Early-auditory intervention was an independent predictor for language and cognitive scores but not motor scores. The presence of additional disability significantly influence all developmental domains in hearing-impaired children. Hearing-impaired children with additional disabilities tend to have no or late auditory intervention. Professionals should be aware of the importance of early detection and early intervention for hearing-impaired children with or without additional disabilities.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Pelin Çelik, Kemal Keseroğlu, Halil İbrahim Yakut, Güleser Saylam; data collection: Pelin Çelik, Serap Er, İclal Ayrancı Sucaklı; analysis and interpretation of results: Pelin Çelik, İclal Ayrancı Sucaklı; draft manuscript preparation: Pelin Çelik, İclal Ayrancı Sucaklı. All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

This retrospective research was reviewed and approved by the Ethical Committee of Ankara City Hospital, Turkey (24.12.2019-E1/235/2019) and also reviewed and approved by the institutional review board of Ankara Child Health and Diseases Hematology and Oncology Training and Research Hospital, University of Health Sciences Turkey (18.07.2019/17).

## Source of funding

There is no funding source.

## Conflict of interest

The authors declare that they have no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 461-470
https://doi.org/10.24953/turkjped.2021.03.013

**Original Article**

# Interaction of functional gastrointestinal disorders with postpartum conditions related to mother and baby

Özlem Bekem[1], İlker Günay[2], Filiz Çelik[2], Hurşit Apa[2]

*[1]Division of Pediatric Gastroenterology, Hepatology and Nutrition, [2]Department of Pediatrics, Health Sciences University, Dr. Behcet Uz Children's Hospital, İzmir, Turkey.*

**ABSTRACT**

**Background.** Functional gastrointestinal disorders (FGID) may affect or may be affected by postpartum depression (PPD), mode of feeding and postpartum life quality. We aimed to evaluate the interaction between FGID and these parameters in infants.

**Methods.** The study group consisted of babies attending our outpatient clinics. There were three age groups: 4-6 weeks, 3-4 months and 6-7 months. Demographic data of the babies and mothers, and data of feeding were collected. For the diagnosis of FGID, Rome III criteria were used. Mothers were screened with the Edinburgh Depression Scale. Quality of life (QoL) scores were obtained by using the "Maternal Postpartum Quality of Life Questionnaire". Factors affecting the presence of FGID, PPD and quality of life were analyzed.

**Results.** Two hundred thirteen infants were enrolled during the study period. FGID was present with similar rates in both genders and was lower (31.5%) in the 6-7 month-old group (p=0.001). Infantile colic was higher in girls (68.6% vs. 31.4%, p=0.016). In 4-6 week-old infants with infantile dyschesia, the delivery route was mostly cesarean (83.3% vs. 16.7%, p=0.006). Similarly, regurgitation was more frequent in 6-7 month-old infants born by cesarean (88.9% vs. 11.1%, p=0.035), and was more frequent in infants being exclusively breastfed (60.9%, p=0.037). QoL scores were lower in mothers with depression (20.9±3.4 vs. 23.9±3.6, p=0.003). Infantile colic was higher in mothers with depression, but not significantly (29.3% vs. 11.4%, p=0.057).

**Conclusions.** Caesarian section delivery and breastfeeding were influential on infantile dyschesia and regurgitation subgroups. No other studied factor seemed to affect FGID.

**Key words:** functional gastrointestinal disorders, infants, breastfeeding, postpartum depression, life quality.

Functional gastrointestinal disorders (FGID) in infants are chronic or recurrent symptoms that are age-dependent, and cannot be explained by structural or biochemical problems. Regurgitation, rumination syndrome, cyclic vomiting syndrome, infantile colic, functional diarrhea, infant dyschesia and functional constipation are defined under this group of disorders. These symptoms may be a component of normal development or an inappropriate response to different stimuli.[1] Biopsychosocial model is commonly accepted to explain the etiology of FGID.[2] In this model genetics, environment, life stress, psychological state, coping, social support, motility and gut hypersensitivity have reciprocal interaction with symptoms.[2] The effect of parent's psychological state and behaviors are emphasized in many studies.[3,4] These studies are mostly performed in older children. During infancy, some conditions like postpartum depression (PPD), mode of feeding, postpartum life quality may affect or may be affected by FGID.

PPD is an important, frequent and chronic problem if untreated.[5] It affects 9.1-19.2% of mothers in the first months after birth.[6,7] Stressful

✉ Özlem Bekem
obekem@yahoo.com

Received 5th June 2020, revised 30th July 2020, 11st November 2020, accepted 15th November 2020.

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

life events, poor social support, infant health problems, low income and education, previous history of depression are risk factors for PPD.[8] It has been shown that PPD lowers life quality significantly. Mothers with PPD displayed lower quality of life (QoL) scores especially in emotional well-being, mostly at the 2nd and 4th months postpartum.[9] This situation may affect the whole family along with the mother in psychological and physical ways. For example, maternal PPD and attachment problems of the mother and the baby are suggested as reasons for infantile colic.[10-13] However, no data is present with regard to PPD and other infant FGID.

Diet is an important factor in FGID etiopathogenesis. Symptoms are reported to be food-related in most of the children with FGID.[14] Consuming foods like milk, spicy foods, pizza, sodas, fried foods and fast foods may exacerbate the symptoms of FGID in older children.[15,16] This may be explained by foods' ability to alter gut motility, sensitivity and microbiota.[14] There may be a similar effect in FGID of infants. The relationship between infantile colic and breastfeeding is well studied. In these studies, it has been demonstrated that exclusive breastfeeding reduces infantile colic.[17,18] However its effect is not clear in other FGID.

FGID do not only affect the infant's health but also the emotional status and daily life of the family. Parents miss workdays and have less ability to concentrate at work.[19] Infantile colic lowers QoL scores of mothers, especially in physical and social functioning domains.[20] However, data concerning the effects of other FGID on life quality is scarce.

In this study, we aimed to evaluate the interaction between FGID, PPD, maternal quality of life and breastfeeding in infants.

## Material and Methods

This study was designed as a cross-sectional study. The study group was selected from three outpatient clinics (pediatric gastroenterology, social pediatrics, general pediatrics). The sample size for a CI level of 95% and 80% power was calculated to be 189 children with regard to infantile FGID incidence of 77%.[21-24] Considering a 10% sample loss we decided to have a sample size of 200. Data was collected between February and December 2013.

We had three age groups for the sample: infants between 4-6 weeks, 3-4 months and 6-7 months. We aimed to study children under 6 months as this age is the limit for exclusive breastfeeding. The 4-6-week-old group was chosen as this age is the only appropriate time to perform the maternal postpartum QoL scale.[25] As PPD is highest at 3 months after birth we chose 3-4 months old as the second group.[26] All children within the age range of groups were considered for the sample. The questionnaires were filled at the first visit, if a baby was admitted more than once they were not reenrolled to the study for older age groups. Exclusion criteria were severe conditions that require urgent intervention, chronic illnesses and babies brought to the hospital without their mother. Organic diseases presenting like FGID were excluded via history, physical examination and absence of alarm signs.

Demographic data collected for the babies were age (in months), sex, birth weight (as appropriate for gestation or small for gestation), socioeconomic status (SES) (low, intermediate, high) and family type. SES was grouped using a classification developed by Nesanir and Eser.[27] This classification uses the maternal level of education and the occupation of the father. Family types were classified as nuclear or extended. Variables related to the mother and birth were maternal age (in years), maternal education level, type of delivery (caesarian (c) section or vaginal), presence of miscarriages (absent or present) and parity (first or multiple). According to education level, mothers were divided into three groups. First group was illiterate and literate but did not finish elementary school; second group was consisted of elementary school graduates and

the last group was for women who graduated from secondary school or higher.[27] We also collected data on feeding. These were; type (breast milk or other) and time (within 1st hour, after 1st hour within 1st day, later than 1st day) of the first feeding following birth, the current type of feeding (breastfeeding, mixed or non-breastfeeding), presence of breastfeeding problems and use of bottle and/or pacifiers. Any problem interfering with breastfeeding like fissures of areolas, rejection of breastfeeding was included under this title. We asked an open-ended question concerning how well mother and baby were coping with breastfeeding. Anything declared as a problem by the mothers was noted as a breastfeeding problem.

Rome III criteria were used for FGID diagnosis.[1] Emotional status of the mother was screened with Edinburgh Depression Scale validated for Turkish.[28] Cut-off score for PPD was set as 10 points as recommended by the original scale.[29] Mothers were referred for the evaluation of depression if their score was above 10. QoL scores were obtained by using the "Maternal Postpartum Quality of Life Questionnaire", which is validated for Turkish.[25] The scale was scored between 0-30. Higher scores demonstrated a better life quality. This scale was given only to the mothers in the 4-6-week-old baby group as this was the age recommended by the authors.[25]

Statistical analysis was done by using SPSS software. Mean values were given as mean+/- standard deviation. When the distribution of the groups was not normal, Mann-Whitney U and variance analysis were used. Chi-square and t-test were used where distribution was normal to analyze factors affecting the presence of FGID, PPD and low scores on quality of life scores. P value <0.05 was accepted as significant. Ethics Committee approval was obtained from Dr. Behcet Uz Children's Ethics Committee (B-10-4-ISM-4-35-65-72, 28.02.2013). Informed consent was taken from all parents.

## Results

Two hundred thirteen infants were enrolled during the study period. Of the study group, 108 (50.7%) were male. Children were grouped as follows; 4-6 weeks old (n=61, 28.6%), 3-4 months old (n=85, 39.9%) and 6-7 months old (n=67, 31.5%). The mean age of the mothers was 27.7±5.2 years. More than half of the mothers (n=116, 54.5%) had multiple parities, and 45 (21.1%) had a history of miscarriage. The mode of delivery was C-section in 120 (56.6%) mothers. Generally, babies had birth weights appropriate for gestational age (n=203, 96.2%). All the infants were born full term. Most (n=166, 78%) of the families were of intermediate socioeconomic status. Thirty-two (15%) families had high and 15 (7%) had low socioeconomic status. Approximately half of the mothers (n=116, 54.5%) had graduated from secondary school or above, while 17 (8.0%) were illiterate or literate but did not finish elementary school. Most of the babies (n=133, 62.3%) within six months were exclusively breastfed. In the babies older than six months 79.1% were still breastfed besides complementary feeding. Breast milk was the first food given to 165 (77.5%) babies. The first breastfeeding was performed in the first hour of birth in 122 (57.5%) and on the first day of birth in the other 67 (31.6%) babies. One baby was not breastfed at all. A breastfeeding problem was present in 70 (33.3%) mothers. Pacifier/bottle usage rate was high in our study group (n=150, 70.8%). The demographic data are given in Table I.

A FGID was present in 44.1%. The most common FGID was regurgitation (21.6%), infantile colic (16.4%) and infantile dyschesia (11.7%). Two babies (0.9%) had infant rumination syndrome, one baby (0,5%) had cyclic vomiting syndrome, six babies (2.8%) had functional constipation and none had functional diarrhea. FGID was present with a similar rate in both genders (p=0.492), and with a lower rate (13.8%) in the 6-7 month-old group (p=0.001) (Table I).

**Table I.** Demographic data of the children.

| | Total group | Postpartum depression | | Quality of life score | FGID | |
|---|---|---|---|---|---|---|
| | | Present | Absent | | Present | Absent |
| Gender | n=213 | n=99 | n=114 | n=53 | n=94 | n=119 |
| Female | 49.3% | 47.5% | 50.9% | 23.0±4.5 | 46.7% | 53.3% |
| Male | 50.7% | 52.5% | 49.1% | 22.4±3.2 | 41.7% | 58.3% |
| Age groups | n=213 | n=99 | n=114 | n=53 | n=94* | n=119* |
| 4-6 weeks | 28.6% | 25.3% | 31.6% | 22.7±3.8 | 31.9% | 26.0% |
| 3-4 months | 39.9% | 41.4% | 38.6% | - | 54.3% | 28.6% |
| 6-7 months | 31.5% | 33.3% | 29.8% | - | 13.8% | 45.4% |
| Miscarriage | n=213 | n=99 | n=114 | n=53 | n=94 | n=119 |
| Present | 21.1% | 20.2% | 21.9% | 21.8±4.2 | 18.1% | 23.5% |
| Absent | 78.9% | 79.8% | 78.1% | 22.9±3.7 | 81.9% | 76.5% |
| Parity | n=213 | n=99 | n=114 | n=53 | n=94 | n=119 |
| One | 45.5% | 43.4% | 47.4% | 23.1±3.8 | 48.9% | 42.9% |
| Multiple | 54.5% | 56.6% | 52.6% | 22.3±3.8 | 51.1% | 57.1% |
| Birth weight | n=211 | n=99 | n=112 | n=52 | n=93 | n=118 |
| AGA | 96.2% | 96.4% | 96.0% | 22.7±3.9 | 96.8% | 95.8% |
| SGA | 3.8% | 3.6% | 4.0% | 21.2±0.0 | 3.2% | 4.2% |
| Mode of delivery | n=212 | n=99 | n=113 | n=53 | n=93 | n=119 |
| c/s | 56.6% | 52.5% | 60.2% | 22.8±3.1 | 59.1% | 54.6% |
| Vaginally | 43.4% | 47.5% | 39.8% | 22.5±4.6 | 40.9% | 45.4% |
| Maternal age groups | n=213 | n=99 | n=114 | n=53 | n=94 | n=119 |
| ≤18 years | 1.9% | 2.0% | 1.8% | 22.0±0.0 | 2.1% | 1.7% |
| 19-35 years | 90.6% | 90.9% | 90.4% | 22.5±3.7 | 89.4% | 91.6% |
| >35 years | 7.5% | 7.1% | 7.8% | 24.8±5.5 | 8.5% | 6.7% |
| Maternal education level | n=213 | n=99* | n=114* | n=53 | n=94 | n=119 |
| Illiterate/literate | 8.0% | 3.0% | 12.3% | 21.2±0.8 | 10.6% | 5.9% |
| Elementary school | 37.5% | 39.4% | 36.0% | 23.7±4.0 | 31.9% | 42.0% |
| Secondary school or above | 54.5% | 57.6% | 51.7% | 22.4±3.9 | 57.5% | 52.1% |
| Socioeconomic status | n=213 | n=99* | n=114* | n=53 | n=94 | n=119 |
| Low | 7.0% | 2.0% | 11.4% | 21.2±0.8 | 10.6% | 4.2% |
| Intermediate | 77.9% | 78.8% | 77.2% | 23.0±4.0 | 75.6% | 79.8% |
| High | 15.0% | 19.2% | 11.4% | 22.4±4.0 | 13.8% | 16.0% |
| Nuclear family | n=213 | n=99 | n=114 | n=53 | n=94 | n=119 |
| Yes | 83.1% | 86.9% | 79.8% | 22.7±3.6 | 81.9% | 84.0% |
| No | 16.9% | 13.1% | 20.2% | 22.4±5.0 | 18.1% | 16.0% |
| First consumed food at birth | n=213 | n=99 | n=114 | n=53 | n=94 | n=119 |
| Breast milk | 77.5% | 80.8% | 74.6% | 22.6±4.0 | 79.8% | 75.6% |
| Other | 22.5% | 19.2% | 25.4% | 23.1±2.2 | 20.2% | 24.4% |

*$p<0.05$
AGA: appropriate for gestational age, SGA: small for gestational age FGID: functional gastrointestinal disorders, c/s: cesarean section

**Table I.** Continued.

| | Total group | Postpartum depression | | Quality of life score | FGID | |
|---|---|---|---|---|---|---|
| | | Present | Absent | | Present | Absent |
| First breastfeeding time | n=212 | n=98 | n=113 | n=53 | n=93 | n=118 |
| 1st hour | 57.5% | 54.1% | 61.1% | 22.8±4.3 | 50.6% | 63.6% |
| 1st day | 31.6% | 35.7% | 28.3% | 22.5±3.3 | 37.6% | 27.1% |
| Later than 1st day | 10.4% | 10.2% | 10.6% | 22.9±2.9 | 11.8% | 9.3% |
| Feeding type | n=212 | n=99 | n=113 | n=52 | n=93* | n=119* |
| Breastfeeding | 44.4% | 37.4% | 50.5% | 22.5±4.3 | 54.8% | 36.1% |
| Non-breastfeeding | 10.8% | 12.1% | 9.7% | - | 7.5% | 13.4% |
| Mixed | 44.8% | 50.5% | 39.8% | 23.0±3.2 | 37.6% | 50.4% |
| Breastfeeding problem | n=210 | n=98 | n=112 | n=52 | n=93 | n=117 |
| Present | 33.3% | 38.8% | 28.6% | 22.2±3.3 | 29.0% | 36.8% |
| Absent | 66.7% | 61.2% | 71.4% | 22.9±4.0 | 71.0% | 63.2% |
| Pacifier/bottle usage | n=212 | n=99 | n=113 | n=52 | n=93 | n=119 |
| Present | 70.8% | 69.7% | 71.7% | 22.7±3.2 | 68.8% | 72.3% |
| Absent | 29.2% | 30.3% | 28.3% | 22.7±4.8 | 31.2% | 27.7% |

*p<0.05
AGA: appropriate for gestational age, SGA: small for gestational age FGID: functional gastrointestinal disorders, c/s: cesarean section

The education level of the mother or family socioeconomic status was not related to FGID frequency (p=0.203 and p=0.185, respectively). FGID was more frequent in exclusively breastfed infants (54.8% vs 36.1%, p<0.05) (Table I). Other demographic data, maternal, birth or feeding related variables were not related to FGID as given in Table I.

Regarding the FGID subgroups, infantile colic was seen mostly in girls (68.6% vs. 31.4%, p=0.016). No difference was present with regard to gender in other subgroups. Infantile colic and dyschesia were more frequent (2.4 ± 1.0 vs. 4.3 ± 2.2 months, and 2.3 ± 1.5 vs. 4.2 ± 2.1 months, respectively) in younger infants (p<0.05). Regurgitation frequency was not different with regards to age. Variables related to birth such as the number of miscarriages, parity and birth weight did not have an effect on FGID subgroups. In the 4-6 week-old infants with infantile dyschesia, the delivery route was mostly C-section (83.3% vs. 16.7%, p=0.006). Similarly, regurgitation was more frequent in 6-7 month-old infants born by C-section (88.9% vs. 11.1%, p=0.035). Maternal education level or family type had no effect on FGID subgroups. Infantile dyschesia was less frequently (17.5%) seen in families with intermediate SES (p=0.044) in 4-6 week old infants. Type of first consumed food or starting time of breastfeeding did not have an effect on FGID subgroups. Regurgitation was more frequent in infants being exclusively breastfed compared to other types of feeding (breastfed 60.9%, mixed 30.4%, non-breastfed 8.7%, p=0.037). Infants with regurgitation had less breastfeeding problems (82.6% vs. 17.4%, p=0.013). Bottle/pacifier usage did not affect FGID subgroups either.

PPD was detected in 46.5% of the mothers. The rate of depression was not different between age groups or genders (p=0.591, p=0.681). In mothers with depression quality of life scores were lower (20.9±3.4 vs. 23.9±3.6, p=0.003). Infantile colic was seen higher in the presence of depression, but this was not significant (29.3% vs. 11.4%, p=0.057). Edinburg depression scale scores were higher in the presence of infantile colic as well (11.6±5.6 vs. 8.3±4.7, p=0.015). (Table II). PPD was significantly lower in mothers with low education levels (illiterate and literate but

**Table II.** Relation of FGID with postpartum depression and life quality.

| | Postpartum depression | | Edinburgh Score (n=213) | Quality of life score (n=53) |
|---|---|---|---|---|
| | Present (n=99) | Absent (n=114) | | |
| FGID | | | | |
| Present | 46.5% | 42.1% | 9.7±5.2 | 22.0±3.0 |
| Absent | 53.5% | 57.9% | 8.9±5.6 | 23.3±4.4 |
| Regurgitation | | | | |
| Present | 20.2% | 22.8% | 8.9±4.8 | - |
| Absent | 79.8% | 77.2% | 9.4±5.6 | 22.7±3.8 |
| Infantile colic | | | | |
| Present | 21.2% | 12.3% | 11.0±5.2* | 21.8±3.0 |
| Absent | 78.8% | 87.7% | 8.9±5.4* | 23.0±4.0 |
| Dyschesia | | | | |
| Present | 9.1% | 14.0% | 9.6±6.3 | 21.9±3.1 |
| Absent | 90.9% | 86.0% | 9.2±5.3 | 23.0±4.1 |

FGID: functional gastrointestinal disorder
*p<0.05

did not finish primary school) and in families with low SES (p=0.046 and 0.012, respectively). Any other demographic data or variables related to the birth, mother or feeding were not related to PPD (Table I). The mean QoL score was 22.7±3.8. The scores were not different with regards to FGID presence, demographic data, mother, birth or feeding variables (Table I and II).

## Discussion

FGID's are chronic problems that affect the life of patients in many ways. There are also many factors like genetics, environment, life stress, psychological state, motility and gut hypersensitivity that may be related to these disorders.[2] The effect of parent's psychological state and behavior are also emphasized.[3,4] We found that FGID are common in infants; regurgitation, infantile colic and infantile dyschesia being the most common ones. Gender did not have an effect on FGID, except infantile colic. Infantile colic was mostly seen in girls, and it seems to be relevant to PPD.

In Izmir, which is the biggest city in the Aegean region, women are mostly elementary school graduates and the SES of the families is intermediate or high.[30] SES and vital parameters of our study were similar to the Aegean region. Some birth parameters were better in our study group compared to Turkey, such as miscarriage and being small for gestational age.[30] It is assumed that breastfeeding might take a role in FGID as it is a determinant of microbiota.[31] Shorter duration of breastfeeding might cause multiple FGIDs.[32] We found higher exclusive breastfeeding rates (44.8%) compared to Turkish data.[30] This may be due to our approach as a baby-friendly hospital. Additionally, Izmir is a baby-friendly province. Bottle-feeding was reported in 39.7% of the babies younger than 6 months.[30] Bottle or pacifier usage was found in 70.8% of our study group. This rate may be higher due to pacifier usage. Using pacifiers is a factor increasing bottle usage. In time, this increases breast rejection and eventually bottle-feeding.[33] Other data about feeding was similar to Turkish data.[30]

FGID is seen in 27.1% of infants/toddlers between 0-3 years old. It may be as high as 67-87% in 2-4 months of age.[21-24] Regurgitation (25.9%), colic (5.9%) and functional constipation (4.7%) are the most common disorders.[21] In

our study group FGID rate was high (44.1%). Regurgitation, infantile colic and dyschesia were the most frequent disorders with similar rates reported in a review as 17.3-26.0%, 6-19% and 2-5.6%, respectively.[34] Even though FGID frequency did not differ with regard to sex, infantile colic was mostly seen in girls in our study. No gender difference is reported for FGID or infantile colic in the literature.[21,35]

4-6 week-old infants with dyschesia and 6-7 month-old infants with regurgitation were mostly born by cesarean. This may be due to the microbiota differences between vaginal and cesarean delivery. The effect of delivery mode on infant microbiota is well defined.[32,36] It is also documented that microbiota is an important factor in the genesis of FGID.[37] Breastfeeding may also have an impact on FGID by affecting microbiota.[35] One study demonstrated the relation of a shorter duration of breastfeeding and the development of irritable bowel syndrome in adult life.[37] We could not find any relation between FGID and feeding parameters. FGID, predominantly regurgitation, was more frequent in breastfed infants in our study which was contradictory to the literature.[38,39] On the other hand, even regurgitation was higher in breastfed infants, infants with regurgitation had fewer breastfeeding problems. As proposed, infants with regurgitation should continue breastfeeding.[38,39]

PPD is a common disorder affecting almost 9.1-19.2% of mothers.[6,7] In our study we found a much higher frequency than reported earlier in Turkey, which was 23.8%.[40] Many factors are known to affect the incidence. Even though the sex of the baby was not associated with PPD in our study, it is accepted as a factor by some authors.[41] Socioeconomic factors such as low income are also associated with PPD.[42] In our study group, PPD was lower in families with low SES. When analyzed further we saw that depression was less in mothers with a lower educational level. Higher education levels may raise awareness. We speculate that a raise in awareness may lead to an increase in anxiety, which is questioned in the Edinburgh Depression Scale.[28] This might explain lower depression rates in mothers with lower education levels. No other factor was found to influence PPD. Maternal depression was found to influence life quality as expected and has also been presented in previous studies.[9,43]

Regarding the FGID subgroups, infantile colic was related to PPD. This finding is in concordance with other studies. In the presence of infantile colic, PPD frequency is higher indicating an interaction.[20] Behavioral symptoms like depression and anxiety of the mother might cause infantile colic and infantile colic might affect parents negatively leading to maternal depression.[3,10] Depressed mothers may have a lower interaction with their infants and they might not respond to their infants' needs appropriately. When the needs of the infants were not met they may respond by crying to this distress. This may increase the occurrence of infantile colic.[10] In the biopsychosocial model, life stress, psychological state and coping, are some factors that interact with symptoms. As suggested in this model, the psychological state of the baby may influence FGID.[2] PPD was not related to any other FGID subgroup.

Giving birth to a child might cause some physical and psychological changes in mothers' lives. These changes lead to changes in life quality.[9] In our study, QoL scores were 22.7±3.8. The scores are expected between 0-30 on the scale we used. Therefore, scores in our study are not low. On the other hand, QoL scores were lower in depressed mothers with babies 4-6 weeks-old. This result supports the negative effect of PPD on life quality as discussed above.[9,43] However, the life quality of the mothers did not affect or was not affected by the presence of FGID and subtypes in 4-6 week old infants. There is only one study evaluating the life quality of infants with FGID. This study revealed that FGID, especially if there is more than one, lowers life quality of the infant.[32] However, the effect of FGID on mothers' life quality is still not known. Therefore, more studies are needed to evaluate the relation of life quality and FGID, especially in different age groups.

There are some limitations to our study. First of all, our sample was selected from patients admitted to a tertiary level hospital. The SES of the families was above the average in Izmir. Since the sample was not population based, it may have a weakness in representing the population. Secondly, all data was obtained by questionnaires. However, we tried to minimize this limitation by questioning face to face. However, this still might lead to recall bias especially concerning feeding data of the older babies.

In conclusion, FGID (especially regurgitation, infantile colic and infantile dyschesia) and PPD are common problems in early infancy. From the FGID, infantile colic was related to PPD. Another influential factor on some FGID subgroups was C-Section delivery and breastfeeding in our study group. No other factor seems to affect FGID. Environment, life stress, parent's psychological state are some factors suggested to being responsible for the pathogenesis of FGID in older children. However, we suppose that other factors may be responsible for FGID in infancy. More studies are needed to enlighten this issue.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Özlem Bekem , İlker Günay, data collection: Özlem Bekem, İlker Günay, Filiz Çelik, Hurşit Apa; analysis and interpretation of results: Özlem Bekem, İlker Günay; draft manuscript preparation: Özlem Bekem, İlker Günay, Hurşit Apa.

All authors reviewed the results and approved the final version of the manuscript.

### Ethics approval

Approval was obtained from the ethics committee of Dr. Behcet Uz Children's Ethic Committee (B-10-4-ISM-4-35-65-72, 28.02.2013). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

### Source of funding

None declared.

### Conflicts of Interest

The authors declare that they have no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 471-481
https://doi.org/10.24953/turkjped.2021.03.014

**Original Article**

# Tracking postural stability of children and adolescents after a concussion: sport-related versus non-sport-related concussion

Eda Çınar[1], Lisa Grilli[2], Debbie Friedman[3], Isabelle Gagnon[1]

*[1]School of Physical and Occupational Therapy, McGill University, Montreal, QC, Canada; [2]The Montreal Children's Hospital, McGill University Health Center, Montreal, QC, Canada. [3]Department of Pediatrics and Pediatric Surgery, McGill University Faculty of Medicine, Montreal, QC, Canada.*

## ABSTRACT

**Background.** Although postural impairments have long been reported following a concussion in the pediatric population, we still know very little about who is more at risk of presenting those balance problems and how the mechanism of injury (sport vs non-sport) could influence balance problems after concussions.

The purpose of this study was to compare balance function in children having sustained a sport-related (SRC) or non-sport-related (NSRC) concussion, to that of children with an orthopedic injury (OI) and to non-injured (NI), over a one-year period.

**Methods.** One-hundred and twelve participants were included in this study. Among them, 38 were concussed, with 27 having sustained a SRC; and 11 an NSRC, as well as 38 NI, and 36 OI. Balance function was evaluated at 2 weeks, 3 months, 6 months, and 12 months after a concussion, and at the same time intervals for the control groups. The balance subtest of the Bruininks-Oseretsky Test of Motor Proficiency (BOT2) and Timed Foam Test was used to measure postural instability. Concussion related symptoms were measured by the Post Concussion Symptom Scale (PCSS).

**Results.** There was an improvement in tandem standing on the balance beam (P=.02) and in single-leg standing (SL) on foam surface (P=.02) for all groups over a year. At the 2nd week, NSRC had more postural instability than NI during SL on the balance beam when eyes were closed (P =.01), and performed significantly worse than SRC (P =.01) and NI (P =.01) during SL on the foam surface. NSRC also reported more symptoms than SRC on PCSS (P <0.001). In the 3rd month, NSRC still had lower performance than SRC in SL on foam surface (P =.01).

**Conclusions.** Children sustaining a concussion outside of a sport seem to have higher levels of postural instability up to 3 months post-injury when compared to those injured in sport.

**Key words:** mild traumatic brain injury, balance, sport related concussion, non-sport related concussion.

Concussion is a growing public health concern affecting more than 1.2 million people in the USA annually.[1] The reported incidence rate has increased fourfold among high school students between 2000 and 2011 due to public awareness and increased attention to injury.[2] Concussion, also referred to as mild traumatic brain injury (mTBI), results from acceleration and deceleration movement of the brain within the skull through external forces acting on the body.[3] The most common causes of concussions in youth are from falls, collisions or sport related contact impacts.[2] In fact, 53.4 % of the concussions occur in children between the ages 10-14 and concussions account for 9-12% of all injuries in high school athletes.[1] Regardless of age, individuals mainly complain of headaches, fatigue, confusion, dizziness as initial symptoms after sustaining a concussion.[2,3] Moreover, a significant number of people also experience cognitive and motor dysfunctions

✉ Eda Çınar
eda.cinar@mail.mcgill.ca

Received 14th September 2020,
accepted 23rd November 2020.

such as attention deficit, reduced concentration, balance deficit, and gait dysfunction.[4,5] Various measurement methods are used for the assessment of balance in concussion such as patient reported outcomes, measurement scales, and laboratory analysis systems in both adult and pediatric population.[6,7] Using motion analysis systems (MAS), balance dysfunction, such as abnormal body sway, was reported to occur in adults even nine month after the injury[8] and it was reported up to one month in adolescents.[9] Indeed, available studies using MAS did not measure balance deficit in long term in concussed adolescents. Moreover, there is no study measuring postural instability by MAS in children with mTBI. On the other hand, performance based measures evaluating both static and dynamic postural stability have been frequently used in the pediatric population. Such a study showed persistent balance deficit up to three months post-concussion,[10] and it was reported by another study continuing up to six months post-injury.[6] However, the evidence supporting the time to recovery from balance deficit among concussed children still remains inconclusive.

The recovery of post-concussion symptoms has been associated with the mechanism of injury.[7] Many concussions are sustained during a sport activity, and much has been done in the way of prevention, evaluation and management programs of sport-related concussions (SRC).[1,2] However, a significant proportion of pediatric injuries also occurs outside of sports, mainly resulting from falls, assaults, motor vehicle or bicycle collisions.[11] Even though SRC is getting much attention in both clinical and research communities, the severity of symptoms for the non-sport related concussion (NSRC) also needs to be characterized. There might exist some differences in outcome, as athletes may be more aware of the risk of concussions, and informed on how to be react with resilience to a concussive injury. In addition, athletes may have better endurance and body strength owing to their training habits, and may recover differently from the impairments brought upon by the brain injury. Nonetheless, persistence of postural instability could be a potential risk factor for sustaining a second injury both for children with SRC and NSRC. To achieve a better understanding of the recovery of balance deficits we saw a need to measure balance symptoms over the course of one-year post-concussion and examine whether recovery occurred in a similar manner between children with SRC and NSRC and when compared to children without concussions.

This study therefore aimed to estimate the extent to which concussed children and adolescents experience balance deficits up to one year following the injury, when compared to orthopedically-injured and non-injured peers; as well as to uncover the extent to which performance in balance function in NSRC may differ from that in SRC. We hypothesized that children and adolescents having sustained a concussion in a sport event would tend to show faster recovery from imbalance as they may have higher skills when considering that they are under a training program before the injury.

## Material and Methods

### *Participants*

We recruited 38 subjects with a concussion presenting consecutively to the Concussion Clinic of the Trauma Programs at Montreal Children's Hospital of the McGill University Health Center in Montreal, Canada as per the following definition: Glasgow Coma Scale of 13-15 score after 30 minutes post-injury or later upon presentation for the healthcare provider; and presenting with at least one of the following signs : confusion or disorientation, loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery.[12] We excluded children if they had a history of concussion in the previous year. Children within the concussion group were separated into two groups, on the basis of the mechanism of their injury; SRC and NSRC.

As controls, we included 38 non-injured (NI) children and 36 children with a minor upper extremity injury (OI) such as shoulder dislocation, wrist sprain, and finger fracture that would restrict physical activities but not interfere with testing. Controls were matched as much as possible to the concussed children based on the Activity Rating Scale (ARS), a tool developed to assess general level of participation in physical activity in the context of epidemiological studies.[13] We excluded participants if they did not speak in French or English, or had a pre-morbid medical diagnosis of learning disabilities, attention-deficit, and hyperactivity disorder or behavior problems.

### *Outcome Measures*

We measured the balance of the participants with the Bruininks- Oseretsky Test of Motor Proficiency, Second Edition (BOT2) balance subscale[14], a Timed Foam Test[15] and using selected items from Post-Concussion Symptom Scale (PCSS).[16] The BOT2 is a reliable and valid assessment tool for the fine and gross motor skill of children and youth between the age of 4 and 21 years and has eight subtests including a scale for balance function.[14] The balance subtest comprises nine items, and evaluates both dynamic and static balance skills such as tandem walking on a line or tandem standing on a beam.[17] Because the items of the BOT2 vary in difficulty levels, we chose two most difficult items for individual analysis; item 8 (single leg standing on balance beam) and item 9 (heel-to-toe standing on balance beam).

The Timed Foam Test is a reliable, practical and cost-effective way to assess postural stability on an unstable surface.[15] Participants were instructed to stand on a foam pad (Airex balance pad) with their hands on their hips in three different foot positions when eyes were closed: Double-leg stance with feet together (FT), single leg stance (SL) and heel-to-toe stance (HT). Participants were asked to maintain stability in these positions for a maximum of 20 seconds with their eyes closed conditions.[15] The performance was recorded as the number of seconds the positions could be held without moving out of the position.

Self-reported post-concussion symptoms were assessed using the PCSS. The PCSS is composed of 22 items scored using a Likert scale ranging from 0 (no difficulty) to 6 (severe difficulty). The reliability and validity of the PCSS are well-documented.[16,18] For the purpose of this study, along with PCSS total score, we also analyzed the "balance problems" item individually.

### *Procedure*

Approval for this study was obtained from the Research Ethics Board of the McGill University Health Center (12-190-PED). Assessments of children meeting inclusion criteria were performed at the Trauma Center of The Montreal Children's Hospital. Children and their parents were informed about the test procedures, and informed consent was signed before the data collection. All assessments were completed by a pediatric physical therapist who was blind to the group status of the child. Assessments were performed within 2 weeks of injury, and then again 12 weeks, 6months and 12 months post-injury for concussed children and adolescents, and at corresponding time intervals for controls.

### *Data Analyses*

The primary outcome of this study was balance as measured using BOT2-balance scale, Foam test and PCSS-balance score, and the secondary outcome was post concussion symptoms on PCSS. The predictor variable was the type of group (concussed group vs controls). Differences in participant socio-demographic and clinical information (e.g., age, gender, dominant foot, Physical Activity Level, Parent Educational Level) between the experimental and control groups were examined using Chi-Square Test and One Way ANOVA Test. The data was examined for normality using Shapiro-Wilk test. A two-way ANOVA with repeated measures was used to examine main time and group effects, as well as time x group interactions for all outcome measures. When

applicable, pairwise comparisons were used to determine group and time differences. The level of significance was set at $p < 0.05$ for all analyses. All analyses were done using IBM SPSS Statistics version 23.

## Results

A total of 112 (mean age 11.86 ± 2.97 years) participants were enrolled in the study with males being slightly overrepresented across all groups (63.39 %). Among concussed children, 71.01 % had sustained a sport-related concussion (n=27), while others experienced falls or motor vehicle collisions (n=11). There were no differences in gender, dominant foot, physical activity level and parent educational level among the four groups ($p > 0.05$). The only difference was in age ($p=0.003$) where participants with OI and NSRC were slightly younger than those in the NI and SRC groups. Majority of concussed children complained of headache (86.84 %) and dizziness (60.53 %) at the time of injury (Table I). The performance of children on outcome measures at three time points were presented in Table II.

**Table I.** Demographic and clinical characteristics by group.

| Characteristic | Concussed Group | | NI Group | OI Group | p |
|---|---|---|---|---|---|
| | SRC (n= 27) | NSRC (n= 11) | (n= 38) | (n= 26) | |
| | (Mean±Sd) | (Mean±Sd) | (Mean±Sd) | (Mean±Sd) | |
| Age | 13.19±3.1 | 10.45±2.7 | 12.31±2.99 | 10.81±2.47 | 0.03 |
| | Number (%) | Number (%) | Number (%) | Number (%) | |
| Gender | | | | | |
| Female | 10 (37.04) | 5(45.45) | 14 (36.84) | 12 (33.33) | 0.91 |
| Male | 17 (62.96) | 6(54.55) | 24 (63.16) | 24 (66.66) | |
| Physical activity level | | | | | |
| Much more than others | 14 (51.85) | 2 (18.18) | 9 (23.68) | 6 (16.66) | 0.07 |
| More than others | 7 (25.92) | 3 (27.27) | 12 (31.58) | 15 (41.66) | |
| Same as others | 4 (14.81) | 6 (54.54) | 16 (42.11) | 13 (36.11) | |
| Less than others | 2 (7.41) | 0(0.0) | 1 (2.63) | 2 (5.55) | |
| Parent educational level | | | | | |
| Not identified | 4 (14.81) | 0 (0.00) | 0 (0.00) | 1 (2.78) | 0.39 |
| High school | 7 (25.93) | 4 (36.36) | 6 (15.79) | 3 (8.33) | |
| College | 6 (22.22) | 3 (27.27) | 9 (23.68) | 13 (36.11) | |
| University | 3 (11.11) | 5 (45.45) | 21 (55.26) | 19 (52.78) | |
| Dominant foot | | | | | |
| Right | 24 (88.89) | 9 (81.82) | 36 (94.73) | 31 (86.11) | 0.53 |
| Left | 3 (11.11) | 2 (18.18) | 2 (5.26) | 5 (13.88) | |
| Initial symptoms | | | | | |
| Confused | 9 (33.33) | 4 (36.36) | | | 0.57 |
| Vomit | 6 (22.22) | 5 (45.45) | | | 0.15 |
| Headache | 23 (85.19) | 10 (90.90) | | | 0.54 |
| Dizziness | 17 (62.96) | 6 (54.54) | | | 0.45 |
| LOC | | | | | |
| No LOS | 19 (70.37) | 10 (90.91) | | | 0.17 |
| 0-10 mi | 8 (29.63) | 1 (9.09) | | | |

SRC: sport-related concussion, NSRC: non-sport related concussion, NI: non-injured group, OI: orthopedically-injured, LOS: loss of consciousness

**Table II.** Analysis of mean and standard deviation for findings on outcome measures (Mean±Sd).

| Variable | Groups | 2 weeks | 12 week | 6 months | 12 months |
|---|---|---|---|---|---|
| BOT Score (n=109) | | | | | |
| Balance subtest | Concussed group | 15.05±4.14 | 14.84±3.81 | 16.73±3.59 | 15.92±4.27 |
| | SRC | 15.48±3.5 | 15.52±4.10 | 17.41±3.57 | 16.15±4.25 |
| | NSRC | 13.90±5.6 | 13.00±2.10 | 14.90±3.10 | 15.30±4.49 |
| | NI group | 15.46±3.73 | 15.81±5.07 | 15.76±3.92 | 15.86±3.30 |
| | OI group | 14.26±4.54 | 14.34±3.46 | 13.86±4.41 | 15.54±4.05 |
| Item 8 | Concussed group | 3.68±0.66 | 3.86±0.41 | 3.89±0.38 | 3.95±0.22 |
| | SRC | 3.77±0.84 | 3.93±0.26 | 3.96±0.19 | 3.96±0.19 |
| | NSRC | 3.40±0.84 | 3.70±0.67 | 3.70±0.67 | 3.90±0.31 |
| | NI group | 3.89±0.38 | 3.86±0.41 | 3.89±0.38 | 3.97±0.16 |
| | OI group | 3.74±0.55 | 3.74±0.55 | 3.66±0.63 | 3.83±0.51 |
| Item 9 | Concussed group | 2.89±1.10 | 2.62±1.10 | 3.30±1.05 | 3.14±1.35 |
| | SR | 3.00±1.07 | 2.78±1.12 | 3.44±1.12 | 3.30±1.29 |
| | NSR | 2.60±1.17 | 2.20±1.03 | 2.90±0.73 | 2.70±1.49 |
| | NI group | 2.76±1.23 | 2.78±1.25 | 2.78±1.22 | 2.86±1.14 |
| | OI group | 2.54±1.31 | 2.46±0.95 | 2.54±1.14 | 2.83±1.29 |
| TFT (n=103) | | | | | |
| Foot Together | Concussed group | 18.73±3.66 | 20.00±0.00 | 20.00±0.00 | 19.89±0.58 |
| | SRC | 18.24±5.26 | 20.00±0.00 | 20.00±0.00 | 20.00±0.00 |
| | NSRC | 18.89±3.04 | 20.00±0.00 | 20.00±0.00 | 19.85±0.67 |
| | NI group | 19.68±1.72 | 20.00±0.00 | 19.86±0.82 | 20.00±0.00 |
| | OI group | 19.66±2.47 | 20.00±0.00 | 19.91±0.56 | 19.61±1.62 |
| Single Leg | Concussed group | 4.43±2.93 | 6.43 ±5.08 | 5.87±3.86 | 6.10±4.11 |
| | SRC | 5.05±3.13 | 7.33±5.56 | 6.26±4.00 | 6.00±3.73 |
| | NSRC | 2.63±0.95 | 3.79 ±1.48 | 4.74±3.34 | 6.36±5.29 |
| | NI group | 5.03±3.22 | 4.87±3.46 | 5.05±2.77 | 5.36±4.24 |
| | OI group | 3.78±1.58 | 4.51±2.73 | 3.93±1.91 | 3.78±1.86 |
| Heel-to-toe | Concussed group | 8.60±5.96 | 8.32±5.67 | 8.91±5.17 | 9.17±6.10 |
| | SRC | 9.44±5.96 | 8.27±5.30 | 9.12±5.32 | 10.30±6.41 |
| | NSRC | 6.14±5.54 | 8.44±6.98 | 8.30±4.96 | 5.05±2.67 |
| | NI group | 9.28±5.05 | 9.62±5.07 | 8.84±5.15 | 8.55±4.87 |
| | OI group | 6.12±4.16 | 6.57±4.39 | 7.94±4.39 | 7.54±3.98 |
| PCSS (n=97) | | | | | |
| Total score | Concussed group | 9.16±15.40 | 3.16±6.47 | 4.16±10.95 | 1.50±3.81 |
| | SRC | 6.15±7.97 | 3.35±6.91 | 2.58±4.30 | 3.50±7.63 |
| | NSRC | 22.17±30.0 | 2.33±4.41 | 11.00±24.13 | 1.04±2.27 |
| | NI group | 1.67±2.16 | 1.91±4.18 | 1.61± 2.89 | 1.52± 3.75 |
| | OI group | 2.16±6.01 | 1.13±2.74 | 1.09±3.70 | 0.81±3.05 |
| Balance item | Concussed group | 0.28±0.88 | 0.13 ±0.70 | 0.00 ±0.00 | 0.00 ±0.00 |
| | SRC | 0.00±0.00 | 0.15±0.784 | 0.00±0.00 | 0.00±0.00 |
| | NSRC | 1.50 ±1.64 | 0.00 ±0.00 | 0.00 ±0.00 | 0.00 ±0.00 |
| | NI group | 0.03±0.17 | 0.00 ±0.00 | 0.06± 0.34 | 0.00 ±0.00 |
| | OI group | 0.03±0.18 | 0.06 ±0.35 | 0.00 ±0.00 | 0.00 ±0.00 |

BOT: bruininks- oseretsky test of motor proficiency-second edition, TFT: timed foam test, PCSS: post-concussion symptom scale, SRC: sport-related concussion, NSRC: non-sport related concussion, NI: non-injured group, OI: orthopedically-injured

### *Comparison of the Concussed group with NI and OI groups*

*BOT2 -Balance subtest*

Repeated-measures ANOVA of the performances on the BOT2-balance scale showed no significant group x time interaction (p= 0.10, $\eta^2_p$ =0.049), and the main effects for time (p=0.226, $\eta^2_p$ =0.041) or group (p=0.17, $\eta^2_p$=0.033) were not significant. Analysis of two most difficult items (item 8 and item 9) of the balance subscale did not reveal any significant group x time interactions (p=0.44, $\eta^2_p$ =0.027; p= 0.37, $\eta^2_p$ = 0.031 respectively) and no main effects of group (p= 0.10, $\eta^2_p$=0.042; p=0.145,$\eta^2_p$=0.036 respectively). There was, however, a significant effect of time for item 8 (p=0.020, $\eta^2_p$=0. 75), illustrating an overall increase in performance of heel-to-toe standing on balance beam during the testing period for all groups (Fig. 1).

*Timed FOAM Test*

The ANOVA results revealed no significant group x time interactions for FT, SL and HT eyes closed standing positions (p=0.19, $\eta^2_p$=0.042; p=0.160, $\eta^2_p$ =0.046; p=0.39, $\eta^2_p$= 0.031 respectively). However, there was a main effect of time (p=0.027, $\eta^2_p$ = 0.89) for FT position without a significant group effect (p=0.44 $\eta^2_p$ =0.016), indicating improvements in the performance on FT position over the time in all groups. There was no main effect of time for SL position (p= 0.60, $\eta^2_p$ = 0.073), however group effect was significant (p=0.017, $\eta^2_p$ = 0.07), indicating significant differences between the concussed and OI groups. Multiple comparisons showed that the OI group had lower performance than the concussed group over the period (p=0.014). Yet, there were no main effect of group (p=0.081, $\eta^2_p$=0.049) and time (p=0.917, $\eta^2_p$=0.005) for HT position.

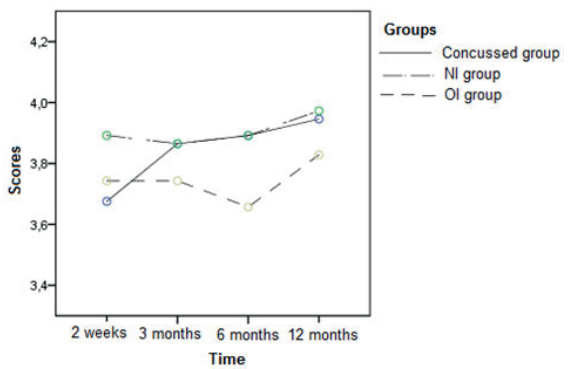


**Fig. 1.** The mean score of heel-to-toe standing position over the period by group.

*PCSS*

The ANOVA results performed on PCSS total score showed significant time x group interactions (p=0.033, $\eta^2_p$ =0.71), as well as significant main effect of time (p= 0.05 $\eta^2_p$ =0.128) and group (p=0.019, $\eta^2_p$= 0.081). Between-group comparisons revealed significant differences in total score at 2 weeks post-injury, where children in the concussed group had more severe symptoms than those of the NI and OI groups (p=0.002, p=0.005 respectively). There were significant improvements in total score after three (p=0.001), six (p=0.00) and 12 (p=0.00) months in the concussed group while no differences were observed in NI and OI groups (p>0.05). Further analysis for the specific "balance problems" item revealed no group x time interaction (p= 0.192, $\eta^2_p$=0.046) and no significant main effects for time (p=0.085, $\eta^2_p$=0.069) or group (p=0.149, $\eta^2_p$=0.040).

### *Comparison of the SRC and the NSRC with the NI and OI groups*

The repeated measures ANOVA was performed again on all the outcomes after separating the concussed participants into two subgroups: SRC and NSRC groups.

*BOT2 -Balance subtest*

There were no significant group x time interactions (p=0.227, $\eta^2_p$=0.036; p=0.671, $\eta^2_p$=0.021) and no significant main effects of time (p=0.226, $\eta^2_p$=0.041; p=0.054; $\eta^2_p$= 0.071) or group (p=0.173, $\eta^2_p$=0.033; p=0.080, $\eta^2_p$=0.062) in the subtest total scale score or for item 9; however, there were main effects of time (p=0.003, $\eta^2_p$ =0.127) and group (p=0.039, $\eta^2_p$=0.076) for item 8. Further analysis for item 8 showed that time was only significant for the NSRC group, where

children showed significant improvements over the 12-month period (p=0.003). Group comparisons also revealed that the NSRC group had poorer balance skills than the NI group (p=0.013) 2 weeks post-injury. At the third month, the SRC group showed better scores than the NI group (p=0.015) (Fig. 2).

*Timed FOAM Test*

There were no significant group x time interactions for FT, SL, and HT eyes closed positions (p=0.40, $\eta^2_p$=0.031; p=0.091, $\eta^2_p$=0.049; p=0.114, $\eta^2_p$=0.047). However, there were main effects of time (p=0.007, $\eta^2_p$=0.117) and group (p=0.009, $\eta^2_p$=0.111) for SL position, illustrating lower performance in NSRC group than the SRC at the 2nd (0.018) and the 12th week (p=0.018) (Fig. 3), and the NI groups at the 2nd week (p=0.016) despite overall improvement in all groups.

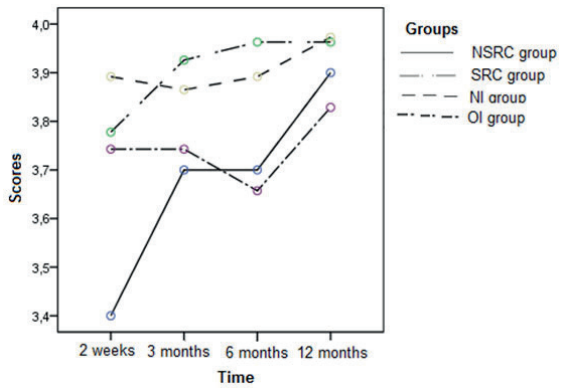


**Fig. 2.** The mean score of heel-to-toe standing on a balance beam (item 8) over the period by group.

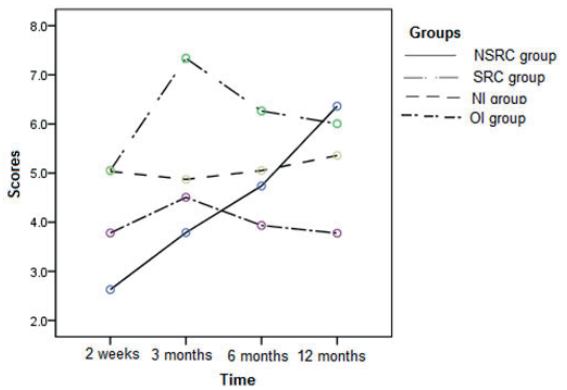


**Fig. 3.** The average durations of single leg standing on foam surface over the period by group.

There were no significant main effects of time (p=0.57, $\eta^2_p$ = 0.020) or group (p=0.67, $\eta^2_p$=0.070) for HT position while there was a significant time effect (p=0.003, $\eta^2_p$=0.072) for FT position unlike group effect (p=0.614, $\eta^2_p$=0.018); demonstrating overall improvement in the HT position in all the groups.

*PCSS*

Time x group interactions were significant for total score and balance item (p=0.00, $\eta^2_p$ =0.105; p=0.00, $\eta^2_p$=0.198 respectively) with significant main effects of time (p=0.00, $\eta^2_p$ =0.279; p=0.00, $\eta^2_p$=0.383 respectively) and groups (p=0.00, $\eta^2_p$ =0.166; p=0.00, $\eta^2_p$ =0.665 respectively). There were significant decreases in total score for NSRC and SRC group after six (p= 0.00, p=0.015 respectively) and twelve months (p=0.00, p=0.002 respectively) contrary to NI and OI groups (p>0.05) (Fig. 4). There were significant improvements in only NSRC group in balance item after three (p=0.00), six (p=0.00) and twelve (p=0.00) months later. Group differences were reported only at 2nd week when NSRC had higher score as compared to SRC (p=0.00), NI (p=0.00), and OI (p=0.00) groups. There were no other group differences at any follow-up time (p>0.05).

## Discussion

We aimed to investigate the long-term consequences of a concussion on balance

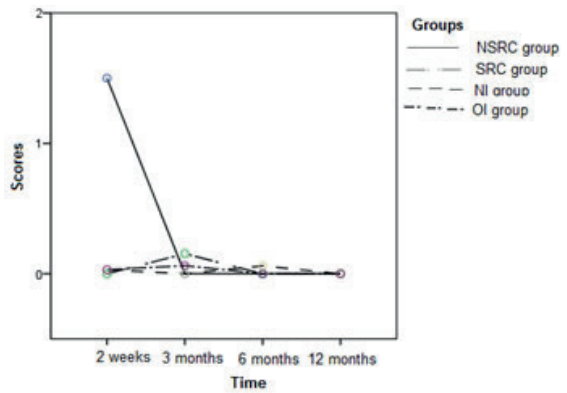


**Fig. 4.** PCSS –balance item scores over the period by group.

function in the pediatric population aged between 6-17 years, as well as to illustrate the potential differences in recovery between sport-related and non-sport-related concussions when compared with non-injured and orthopedically injured children. The results of this study revealed that concussed children improved their postural stability over a one-year period on some challenging activities; however, the improvements were comparable to those of OI and NI groups, for all measurements at each assessment time. On the other hand, children who sustained a concussion outside of sport had lower postural stability than healthy controls at the second week of injury. They also showed slower improvements in maintaining balance on unstable surfaces up to three months, as well as more post-concussion symptoms, particularly about imbalance at the second week when compared to NSRC group.

The BOT2 is one of the most commonly used tests for the assessment of motor proficiency in children after an injury.[10,19,20] Previous studies using the BOT2 test in the concussed population generally reported greater differences in the balance subtest between concussed and uninjured children.[6,10,19] For example, Dahl et al.6 reported reduced postural instability on the BOT2 balance subtest in concussed children three to six months after the injury. Similarly, Sambasivan et al.[20] demonstrated lower performance in concussed children on the BOT2-balance subtest, especially for the most difficult items which were tandem and single leg standing on a balance beam.[20] Our study reported similar findings only for children with NSRC who had lower performance on tandem standing on a balance beam at the second week. The NSRC group also showed higher postural instability on a foam surface while standing on single leg than healthy controls and that was found up to three months following the injury. Measuring balance on a foam surface is a component of the Balance Error Scoring System (BESS), which was originally designed for collegiate athletes to assess balance function, using a cost-effective equipment. It has since widely used in patients with mild TBI and became an important assessment tool for concussion. It was found a valid and reliable tool to detect the differences between concussed and healthy college athletes, especially within a week post-concussion. The BESS has six different positions including double-leg stance, single-leg stance, and tandem stance on 2 different support surfaces (firm and foam) with eyes were closed. In children and adolescent, two positions were highlighted to distinguish the differences between concussed and healthy individuals: single leg standing on firm and foam surfaces.[21] The results of the current study also supported this finding. We only detected postural instability in NSRC group when compared them with SRC group while standing on single-leg condition on the foam when eyes were closed. Moreover, differences were evident not only at the second week but also in the third month after post injury, where NSRC group had lower score than SRC. A previous study in young adults also illustrated that non-athletes had larger distance between the center of mass and center of pressure than the athletes during a one-month period post-concussion, illustrating higher body sway in the non-athletes.[7] However, the same study also reported that athletes had slower gait speed and faster body sway than non-athletes.[7] In fact, there are very few studies in the literature addressing the relationship between the cause of injury and the post-concussion symptoms, so it is not known whether the differences in the injury mechanism lead to diverse pathological changes, consequently various symptoms.

There is a great emphasis on athletes in the concussion literature since participation in a sport-related activity increases the risk of falling and contact head injury. A recent review concluded that athletes generally experience balance impairment lasting three to ten days following a concussion without a persisting deficit.[22] However, our study did not identify any balance deficit in the SRC group; rather they performed better than NSRC and controls in the single leg standing position on foam surface in

the given time frame. This may be because they had better physical fitness and body endurance as they had been training previously in some way to maintain their postural stability in a sport activity. This notion was elucidated from the finding of the review evaluating the balance function of athletes from different sport activities in comparison with non-athletes.[23] It showed that athletes were better than non-athletes to maintain the body in equilibrium, particularly those who were participating in soccer and gym.[23] There might be a relationship between physical activity level and balance ability which is evident in the current study; for example, more than a half of the children in the SRC group defined themselves as "much more active than the others" (52%) while it was much less in the NSRC (18%).

Another considerable difference between the SRC and the NSRC groups arose from PCSS score. The mean score of PCSS was more than twenty for the NSRC group at the second week, indicating "very high level" in severity classification of concussion, whereas SRC group scored less than seven which is "borderline".[16] Interestingly, NSRC group also showed an increase in the total symptom score in the sixth month after a reduction in the third month. It seems that recovery from the symptoms lasted a year in the NSRC group, yet imbalance was only higher at the second week than the other groups. To summarize, this current study did not support the existence of persistent balance deficits in the concussed children that are contrary findings to the previous studies.[6,10,24] This may be because of the inadequacy of the measurements to evaluate the complex balance function. More challenging activities like adding cognitive loads or modifying environmental demands may be more appropriate to measure postural stability in the concussed children and adolescents. This is because maintaining balance in a silent room without any visual interference is much easier than balancing your body outside in a crowded and noisy environment with many visual distractions. Future studies should evaluate balance function in a more dynamic environment or under a dual task condition to examine the real-life performance. Moreover, our current study suggests that non-sport related concussion, which is not emphasized as much as sport related concussion, was deemed a significant risk for postural instability; so, further investigations are needed to estimate the differences in balance function and post-concussion symptoms between the children having a concussion in and out of a sport activity. This study had a few limitations. Non-random sampling resulted in the differences in the age among the groups. The children having an orthopedic minor injury and sustaining a concussion outside of the sport activity were younger than the other groups, yet average ages in all groups were older than 10 years old. Since the maturation in balance and gait function is completed by the age of seven,[25,26] all groups are treated as equal. This study had also limited sample size for NSRC group. In order to generalize the findings for the balance deficits observed in NSRC, studies with a larger sample size are required.

Children having a concussion outside of sport had balance impairment up to three months compared to those getting injured in sport and non-injured subjects, but all concussed children gradually improved their function up to three months following concussion and performed better than non-injured group after a year.

**Acknowledgements**

The authors wish to thank the clinicians from the Trauma Center of The Montreal Children's Hospital, McGill University Health Center, the children and their families for their participation.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: Eda Çınar, Isabelle Gagnon, Lisa Grilli, Debbie Friedman; data collection: Isabelle Gagnon,Lisa Grilli, Debbie Friedman; analysis

and interpretation of results: Eda Çınar, Isabelle Gagnon, Lisa Grilli, Debbie Friedman; draft manuscript preparation: Eda Çınar, Isabelle Gagnon. All authors reviewed the results and approved the final version of the manuscript.

### Ethical approval

Research Ethics Board of the McGill University Health Center (12-190-PED).

### Source of funding

This study was funded by the SickKids Foundation and the Canadian Institutes of Health Research.

### Conflict of interest

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 482-489
https://doi.org/10.24953/turkjped.2021.03.015

**Original Article**

# Can early hyperglycemia affect the morbidity/mortality of very low birth weight premature infants?

Aleksandra Simovic[1], Admir Kuc[1], Ema Jevtic[1], Aleksandar Kocovic[2], Slavica Markovic[1], Marina Stanojevic[1], Maja Jakovcevski[3], Dejan Jeremic[3]

*Departments of [1]Pediatrics, [2]Pharmacy and [3]Anatomy, University of Kragujevac, Faculty of Medical Sciences, Serbia.*

## ABSTRACT

**Background.** The study aimed to examine the effect of early hyperglycemia on the morbidity/mortality of very low birth weight premature infants.

**Methods.** This retrospective study included all premature infants with gestational age ≤32 gestational weeks, hospitalized at the Department of Intensive Neonatal Care, Clinical Center Kragujevac, during the period 2017-2019. Hyperglycemia was defined as glycemia of ≥12 mmol/l in one measurement, or >10 mmol/l in two measurements, at repeated intervals of 2-4 hours. Glycemia was determined from capillary blood, using a gas analyzer of *Gem Premier* 3000, during the first 7 days of life. Continuous intravenous insulin infusion was administered after ineffective glucose restriction at glycemic values of >14 mmol/l.

**Results.** Patients with normoglycemia (41/72 (56.94%)) and hyperglycemia (31/72 (43.06%)) did not differ in gender, gestational age, mode of delivery and antenatal administration of steroids, while birth weight had a tendency to be lower in the hyperglycemic group ($p$=0.052). Hyperglycemia was significantly associated with a low APGAR score at the fifth minute ($p$=0.048), necrotizing enterocolitis ($p$=0.011), and shorter duration of mechanical ventilation ($p$=0.006). Hyperglycemia was associated with significantly more frequent fatal outcomes (35.5%) when compared with the normoglycemic group (4.9%). Accordingly, these patients required inotropic (r=0.036) and insulin therapy (r<0.001) more often. Retinopathy of prematurity, bronchopulmonary dysplasia and sepsis did not correlate with hyperglycemia in our study. Intraventricular hemorrhage of the first degree was more often associated with normoglycemia in premature infants on prolonged mechanical ventilation while more severe intracranial hemorrhage was more common in the hyperglycemic group but did not result in statistical significance due to the small number of patients.

**Conclusions.** Monitoring glucose levels in the blood of very low birth weight premature infants is clinically important because abnormalities in glucose homeostasis can have serious short-term and long-term consequences.

**Key words:** hyperglycemia, newborn, insulin, prognosis.

Hyperglycemia in the newborn is defined as a blood sugar value of >7 mmol/l, or >8,3mmol/l in premature babies and is a common problem in extremely immature babies.[1-3] While it is widely known that non-treated hyperglycemia leads to irreversible damage of the central nervous system, there is still a lack of knowledge about the short-term and long-term effects of hyperglycemia on the development of preterm infants.[3-5] The prevalence of hyperglycemia in preterm infants is variable (20-88%).[2] These numbers probably underestimate the actual prevalence, as approximately 50% of hyperglycemias are not being detected using standard sampling methods and different data on hyperglycemia in preterm infants are found in the literature.[1,2] There are many causes of hyperglycemia in neonates. The most common ones include excessive glucose intake, some

✉ Marina Stanojevic
marinastanojevic87@yahoo.com

Received 29th May 2020, revised 23rd August 2020, accepted 26th November 2020.

drugs (corticosteroids, diazoxide, phenytoin, theophylline), defective glucose metabolism and hyperosmolar milk formulas.[6] The main objective of this study was to examine the effect of early hyperglycemia on the morbidity/mortality of very low birth weight (VLBW) premature infants.

## Material and Methods

Data were prospectively collected from the Department of the Intensive Neonatal Care, Clinical Center Kragujevac during the 2017-2019 period. The analysis included all preterm children with a gestational age of ≤32 weeks.

Exclusion criteria were the following: Weight at birth of <400 gram, gestational age of >32 weeks, the presence of congenital malformations, chromosome aberrations, genetic metabolic diseases, moving to another hospital for further treatment and death within the first 24h of life.

Hyperglycemia was defined as a glucose level of ≥12 mmol/l in one measurement, or ≥10 mmol/l in two consecutive measurements, within the 2-4 h interval.[6] Glucose levels were measured from capillary blood, using a gas analyzer Gem Premier 3000. Continual intravenous insulin was used after the glucose restriction failed to lower glycemia, at blood sugar values of >14mmol/l. Data have been collected from the medical documentation, including routine diagnostic and therapeutic procedures conducted at the Department. All the information collected was coded in order to respect the right of anonymity of each respondent. Personal data was only available to authors for the purpose of obtaining the necessary results for scientific purposes. Ethical approval was obtained by the Faculty of Medical Science, University of Kragujevac on the scientific teaching council (number of decision IV-03-93/14 date 19.02.2020).

### *Statistical analysis*

The study data were analyzed with descriptive statistics methods and presented in tables. Mean or median was used as a measure of central tendency and standard deviation or interquartile range as a measure of dispersion for continuous variables. Categorical variables were presented as rates or percentages. After checking the normality of the data distribution for the continuous variables (Kolmogorov-Smirnov test), appropriate parametric or nonparametric tests were applied (Student's T-test for independent samples or Mann-Whitney U test). The significance of differences in the rates of categorical variables' values was tested by the Chi-square test. The null hypothesis was considered to be not true if the probability of difference was less than 0.05. The influence of hyperglycemia on the occurrence of diseases or death was evaluated by univariate and multivariate binary logistic regression analysis. The results were shown as crude and adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CI). All calculations were performed by statistical program for social sciences (SPSS version 18).

## Results

Of the total number of preterm infants 26/72 (36.11%) developed hyperglycemia >10mmol/l and 24/72 (33.33%) had glycemia>12mmol/l. Hypoglycaemia (<1.7mmol/l) developed in 4/72 (5.56%) preterm neonates. Of the babies with a birth weight <1500g 21/72 (29.17%) had normoglycemia. Infants with normal (41/72 (56.94%)) and elevated (31/72 (43.06%)) glucose levels did not differ in sex, gestational age, route of delivery, antenatal use of steroids, while the body weight at birth had a tendency to be lower in the hyperglycemic group but this did not reach statistical significance ($p$=0.052; Table I). The median of average blood glucose level during hospitalization in the hyperglycemic group was 11.8 (9.7-14.8) mmol/l, so these infants significantly more often required insulin ($p$<0.001) or inotropic ($p$=0.036) therapy, compared with normoglycemic infants (Table II). Four babies (4/21 (19.05%)) with normal glucose levels later developed hypoglycemia. Hyperglycemia correlated significantly with low APGAR score at the 5th minute

**Table I.** Baseline characteristics of newborns.

| Variable | Cases n=31 M (IQR) | Controls n=41 M (IQR) | Test value and significance of null hypothesis |
|---|---|---|---|
| Gestational age (weeks) | 29 (26-32) | 30 (29-32) | U=501.500 p=0.122 |
| Weight at birth (g) | 1180 (810-1930) | 1450 (1235-1895) | U=464.500 p=0.052 |
| APGAR score in fifth minute | 5 (3-8) | 7 (6-8) | U=464.000 p=0.048* |
| Mechanical ventilation (days) | 6 (4-11) | 11 (7.5-24) | U=395.000 p=0.006* |

p – statistical significance; * - statistically significant; M: median, IQR: interquartile range

**Table II.** Values of glucose and inflammation biomarkers.

| Variable | Cases n=31 M (IQR) | Controls n=41 M (IQR) | Test value and significance of null hypothesis |
|---|---|---|---|
| Average glucose (mmol/l) | 11.8 (9.7-14.8) | 4.7 (4.2-6.0) | U=19.000 p<0.001* |
| Average CRP (mg/l) | 8.3 (3.2-16.7) | 3.5 (1.4-6.2) | U=388.500 p=0.005* |
| Average PCT (ng/ml) | 4.68 (2.35-7.60) | 4.61 (1.92-10.70) | U=632.500 p=0.973 |

p – statistical significance; * - statistically significant; M: median, IQR: interquartile range, CRP: C reactive protein, PCT: procalcitonin

(*p*=0.048) and a shorter duration of mechanical ventilation (*p*=0.006) (Table I). Congruently, necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH) of the second and third-degree, and lethal outcomes were more frequent in hyperglycemic subjects. Normoglycemia was more frequently correlated with premature retinopathy (ROP) at site 2B, as well as IVH of the first degree (Table III) and significantly longer duration of mechanical ventilation (Table I). Table III also shows 13 deaths in the total group (two in the normoglycemic group). Seven (7/13 (53.8%)) among the 13 received intravenous insulin. Three infants with normoglycemia (3/21,(14.29%)) who later developed hyperglycemia were also treated with continuous intravenous insulin. The number of positive blood cultures taken during admission to the neonatal intensive care unit, as well as the values of inflammatory markers did not differ significantly in the observed groups.

The results of both univariate and multivariate binary logistic regression analysis of hyperglycemia association with investigated diseases or lethal outcome, with adjustment for potential confounders are shown in Table IV. Variables entered for multivariate analysis were: gender, gestational age, weight at birth, APGAR score in the fifth minute and mode of delivery, diseases NEC, bronchopulmonary dysplasia (BPD), ROP and IVH, and also lethal outcome. Statistically significant association with hyperglycemia was found for the following variables: NEC, IVH Grade 1, ROP at site 2B, and lethal outcome (death). After adjusting for confounding variables association

**Table III.** Clinical characteristics for cases and controls.

| Variable | Cases n (%) | Controls n (%) | Test value and significance of null hypothesis |
|---|---|---|---|
| Type of delivery | | | |
| Vaginal | 17 (54.8%) | 19 (46.3%) | $\chi2=0.510$ |
| Cesarean | 14 (45.2%) | 22 (53.7%) | p=0.475 |
| Antenatal steroid use | 9 (29.0%) | 16 (39.0%) | $\chi2=0.778$ p=0.378 |
| Dopamine use | 14 (45.2%) | 9 (22.0%) | $\chi2=4.374$ p=0.036* |
| Insulin use | 16 (51.6%) | 0 (0.0%) | $\chi2=27.207$ p<0.001* |
| Positive hemoculture | 10 (32.3%) | 9 (22.0%) | $\chi2=0.965$ p=0.326 |
| BPD | 4 (12.9%) | 10 (24.4%) | $\chi2=1.487$ p=0.223 |
| NEC | 8 (25.8%) | 2 (4.9%) | $\chi2=6.465$ p=0.011* |
| IVH | | | |
| None | 12 (38.7%) | 13 (31.7%) | |
| Grade 1 | 4 (12.9%) | 21 (51.2%) | $\chi2=14.040$ |
| Grade 2 | 8 (25.8%) | 3 (7.3%) | p=0.007* |
| Grade 3 | 5 (16.1%) | 2 (4.9%) | |
| Grade 4 | 2 (6.5%) | 2 (4.9%) | |
| ROP | | | |
| None | 12 (38.7%) | 6 (14.6%) | |
| 2B | 7 (22.6%) | 17 (41.5%) | $\chi2=6.095$ p=0.047* |
| 2A | 12 (38.7%) | 18 (43.9%) | |
| Death | 11 (35.5%) | 2 (4.9%) | $\chi2=11.176$ p=0.001* |

p – statistical significance; * - statistically significant; M: median, IQR: interquartile range, BPD: bronchopulmonary dysplasia, NEC: necrotic enterocolitis, IVH: intraventricular hemorrhage, ROP: retinopathy of prematurity

of hyperglycemia and ROP at site 2B lost its significance. ROP, BPD and sepsis did not correlate with hyperglycemia in our study.

## Discussion

The results of our study show that preterm infants with hyperglycemia have lower APGAR scores in the fifth minute and spend less time on mechanical ventilation because the number of days of mechanical ventilation was equal to the number of days until death in 1/3 of cases. The risk of hyperglycemia is inversely related to gestational age and birth weight and increases with the presence of comorbidities[1,2,6], similar to our results.

There is a higher risk for premature infants with VLBW and hyperglycemia to develop NEC and IVH grade ≥2, and eventually, die. These patients have significantly higher values of minimum, average, and maximum blood glucose levels and are more likely to require dopamine or insulin

**Table IV.** Logistic regression analysis of hyperglycemia as a risk factor for the occurrence of diseases or death.

| Disease/Death | Crude OR (95% CI) p | Adjusted# OR (95% CI) p |
|---|---|---|
| BPD | 0.459 (0.129-1.634) p=0.229 | 0.332 (0.084-1.317) p=0.117 |
| NEC | 6.783 (1.325-34.719) p=0.022* | 6.163 (1.043-36.427) p=0.045* |
| IVH | | |
| Grade 1 | 0.206 (0.055-0.777) p=0.020* | 0.166 (0.040-0.694) p=0.014* |
| Grade 2 | 2.889 (0.618-13.496) p=0.177 | 3.282 (0.566-19.023) p=0.185 |
| Grade 3 | 2.708 (0.440-16.680) p=0.283 | 1.916 (0.232-15.859) p=0.546 |
| Grade 4 | 1.083 (0.131-8.946) p=0.941 | 0.836 (0.079-8.888) p=0.882 |
| ROP | | |
| 2B | 0.206 (0.055-0.769) p=0.019* | 0.257 (0.055-1.200) p=0.084 |
| 2A | 0.333 (0.098-1.132) p=0.078 | 0.347 (0.095-1.267) p=0.109 |
| Death | 10.725 (2.165-53.130) p=0.004* | 7.576 (1.105-51.936) p=0.039* |

p – statistical significance; * - statistically significant; BPD: bronchopulmonary dysplasia, NEC: necrotic enterocolitis, IVH: intraventricular hemorrhage, ROP: retinopathy of prematurity
#- Adjusted for gender, gestational age, weight at birth, APGAR score in fifth minute and type of delivery

therapy. A study conducted in Iran in 2014 showed that hyperglycemia in preterm infants correlates with perinatal asphyxia, respiratory distress syndrome, and inotropic use, which is comparable to our results.[6] The reasons for the predisposition to hyperglycemia in VLBW infants are numerous and include all factors that interfere with insulin synthesis/secretion and/or cause insulin resistance. Both cases and controls in our study were of VLBW which did not differ significantly between these groups. This allows us to estimate the influence of other investigated factors adequately. First of all, the immature pancreas fails to synthesize enough proinsulin, with premature infants secreting proinsulin peptides that are recognized as "insulin" by standard tests but have only limited biological activity. It is generally accepted that preterm

infants have a limited ability to secrete insulin, which promotes hepatic glucose synthesis and activates gluconeogenesis. Immature liver in newborns has reduced sensitivity to insulin, so it continues to release glucose despite hyperglycemia. On the other hand, neonates with VLBW have increased insulin resistance in peripheral tissues due to poorer function of the immature insulin-receptor complex which is worsened during clinical stress.[7] Additional suppression of insulin secretion in preterm infants with temporary or permanent hypoxia is exacerbated by increased catecholamine tone (mainly norepinephrine), while other forms of clinical stress increase hepatic glucose synthesis through increased secretion of cortisol, glucagon, and growth hormone.[1,6] Treatment with catecholamines, corticosteroids, and some other drugs can also contribute to hyperglycemia. In studies in which corticosteroids (dexamethasone) were used postnatally to prevent chronic lung disease, the risk of hyperglycemia and glycosuria was increased. Furthermore, the absence, late-onset, or slow intake of parenteral nutrition limits the production of gastrointestinal incretins and their positive effects on insulin secretion.[8] Likewise, high-dose intravenous lipids directly reduce glucose oxidation, provide glycerol as a "fuel" for gluconeogenesis, and contribute to the formation of $\beta$-oxidation products in the liver that activates gluconeogenesis coenzymes, all contributing to hyperglycemia.[6] Similar to our results, previous publications state that hyperglycemia was a risk factor for NEC and sepsis, which can cause prolonged hospitalization.[1,6,9-11] Hyperglycemia usually occurs 2-3 days before the onset of clinical signs of infection and/or sepsis, more often in fungal than bacterial infections.[9] Contrary to our results, neonatal hyperglycemia may be correlated with an increased risk for retinopathy and BPD.[12-15] Our results showed that IVH grade 1 and ROP at site 2B were reported significantly more often in the group with normoglycemia ($p$=0.020 and $p$=0.019 respectively). The explanation may be a significantly longer duration of mechanical ventilation in these patients.[16,17]

However, the blood glucose threshold leading to such complications has not been established.[17,18] Most neonatologists would intervene at blood sugar values of 10.0-11.1 mmol/l.[6,18] Some studies[3,6,19-20] similar to our results, showed that more severe complications occur after prolonged hyperglycemia and it is often in correlation with the increased mortality rate. A study conducted in Jerusalem in 2013 showed that in patients with IVH diagnosed within the first 96 hours, prolonged hyperglycemia exacerbated existing intraventricular hemorrhage.[16] Thus, the duration of hyperglycemia is an important risk factor for the complex pathology of hyperglycemia.[21] During neonatal infections, high levels of inflammatory markers, cytokines, and catecholamines promote the development of insulin resistance, while glucose production in the liver, the central organ of glucose homeostasis, is not inhibited. Although the pancreas should produce more insulin to make up for this, relative beta deficiency develops due to the immaturity of the beta cells.[6]

Although continuous insulin infusion has been used for 25 years to treat clinically relevant neonatal hyperglycemia, in many institutions the first step in its treatment is limited glucose uptake.[19,22-24] A comparison between the two interventions proved the effectiveness of continuous insulin infusion in controlling glucose levels. Insulin-treated children had higher glucose intake, higher body weight, lower incidence of sepsis, and increased endogenous insulin secretion.[23] The advantage of early insulin therapy in the prevention and treatment of hyperglycemia has been demonstrated in critically ill adults, but remains questionable in preterm infants, especially those born highly immature because it increases mortality from hypoglycemia in the first 28 days of life.[6,24-26]

Measurements of blood glucose levels are clinically important, as the high blood glucose levels increase the risk for developing necrotizing enterocolitis and severe grades of intraventricular hemorrhages which lead to prolonged hospitalization or early mortality in VLBW premature children.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Aleksandra Simovic; data collection: Admir Kuc, Ema Jevtic; analysis and interpretation of results: Aleksandar Kocovic, Slavica Markovic, Marina Stanojevic; draft manuscript preparation: Maja Jakovcevski, Dejan Jeremic. All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

Ethical approval was obtained by the Faculty of Medical Science, University of Kragujevac on the scientific teaching council (number of decision IV-03-93/14 date 19.02.2020.).

## Source of funding

We don't have any sources of financial assistance for this research.

## Conflict of interest

All authors read and approved to submit the manuscript and there is no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 490-494
https://doi.org/10.24953/turkjped.2021.03.016

Case Report

# Neonatal diabetes mellitus due to a new *KCNJ11* mutation - 10 years of the patient's follow-up

Maja D Ješić[1,3], Helena Stock[1], Vera Zdravković[1,3], Smiljka Kovačević[1], Marko Savić[4], Miloš M Ješić[2,3]

[1]*Department of Pediatric Endocrinology,* [2]*Department of Neonatology, University Children's Hospital Tiršova, Belgrade,* [3]*Faculty of Medicine, University of Belgrade, Serbia and* [4]*Institute of Medical Statistics and Informatics, Faculty of Medicine, Belgrade, Serbia.*

## ABSTRACT

**Background.** Mutations in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the ATP-sensitive potassium channel, often result in neonatal diabetes.

**Case.** In this report, we describe a 10-year-old girl who is heterozygous for a new missense mutation in the *KCNJ11* gene and whose treatment was successfully switched from insulin to sulfonylurea (glibenclamide) therapy when she was one month old. 10-year data on a low-dose of glibenclamide monotherapy showed excellent glycaemic control with no reports of severe hypoglycaemia and microvascular complications.

**Conclusion.** An early genetic diagnosis of neonatal diabetes mellitus is highly beneficial because early switch from insulin to sulfonylurea is safe, avoids unnecessary insulin therapy and promotes sustained improvement of glycaemic control on long-term follow-up.

**Key words:** neonatal diabetes mellitus, new KCNJ11 mutation, sulfonylurea therapy.

Neonatal diabetes mellitus (NDM) is defined as the occurrence of diabetes mellitus within the first 6 months of life. It is a rare disease (1 in 400,000 live births) caused by genetic mutations that can occur spontaneously or be inherited from parents.[1,2] Affected infants frequently present with symptomatic hyperglycaemia and sometimes ketoacidosis.[2] The most common causes of permanent NDM are mutations in the *KCNJ11* and *ABCC8* genes which encode the two protein subunits (Kir6.2 and sulfonylurea receptor 1, SUR1, respectively) of the ATP-sensitive potassium ($K_{ATP}$) channel on pancreatic β-cells. The identification of the underlying genetic cause has led to improved treatment for patients with mutations in the *KCNJ11* and *ABCC8* genes.[2] Successful switch from insulin to oral sulfonylurea (SU) therapy with excellent initial glycaemic control has been reported in the majority of patients with *KCNJ11* mutations.[3-5] A key question is whether the excellent results in neonatal diabetes will be maintained or whether long-term therapy will cause SU failure or adverse effects. We report the 10-year effect of the switch from insulin to SU (glibenclamide) in a patient carrying the new missense mutation, V252L, in the *KCNJ11* gene.

✉ Miloš D Ješić
milos.jesic@udk.bg.ac.rs

Received 26th May 2020, revised 7th September 2020, 24th October 2020, accepted 26th November 2020.

## Case Report

A 10-year-old girl presented with marked hyperglycaemia ranging from 10 to 20 mmol/L and glucosuria without ketones on the fourth day of life. This patient had low birth weight (the birth weight of this full-term female neonate was 2780g, on the 5.32$^{th}$ percentile, -1.62 SDS). The patient was born to a 31-year-old mother whose pregnancy was uneventful. The physical examination was normal. There were no other factors (sepsis, infection, dextrose-containing intravenous fluids) that could account for the hyperglycaemia. In addition, there was no family

history of diabetes mellitus or hyperglycaemic disorders. On the fifth day of life, blood glucose was 18 mmol/L and urine contained 1+ glucose without ketones. The patient's C-peptide concentration was 0.18 nmol/L (normal 0.298-2.35) and the serum insulin level was 2.8 μIU/ml (normal 7-24), with a concomitant plasma glucose level of 21 mmol/L.

The initial management of the patient's hyperglycaemia included insulin therapy. At first the patient received infusions of regular insulin at a dose of 0.1-0.2 units/kg/h. On day 8 the infusions were discontinued, and the treatment was switched to a subcutaneous insulin regimen with short-acting human insulin (used to correct hyperglycaemia) and intermediate-acting insulin (administered three times a day to provide basal insulin needs). All in all, she received a daily dose of approximately 2 units/kg/day of insulin. During the neonatal period, genetic analysis was carried out at the Peninsula Medical School, Universities of Exeter & Plymouth. Subsequent testing for mutations associated with PNDM showed that the patient was heterozygous for a new missense mutation, V252L, in the *KCNJ11* gene.[6] The patient's mother was negative for the same mutation and the father was not the patient's biological father.

The patient was one month old when the transition from insulin to oral glibenclamide started at an initial dose of 0.12 mg/kg/day, twice daily. This initial dose was increased over the next 7 days to 0.2 mg/kg/day, while regular insulin was simultaneously tapered off. Her hyperglycaemia resolved completely once her glibenclamide dose was increased to 0.3 mg/kg/day, twice daily, and doses of regular insulin were no longer required. Over the following two weeks the need for glibenclamide slowly decreased and stabilized at 0.2 mg/kg/day and her blood sugar levels reached an average of around 10 mmol/L.

Five months after starting glibenclamide, the patient remained asymptomatic (HbA1c 5.8%); she was gaining weight and showing normal neurodevelopmental progress at follow-up. Her serum C-peptide level was 1.4 nmol/L, showing an eight-fold increase over that seen during insulin treatment alone when C-peptide was 0.18 nmol/L. Oral glucose tolerance test (glucose, 1.75 g/kg) was done one year after the sulfonylurea transition. The results showed the improvement of C-peptide secretory response during treatment. Glucose (0 min-120 min) and C-peptide (0 min-120 min) increased from basal glucose 5.5 mmol/L to 9 mmol/L and from basal C-peptide 0.4 nmol/L to 2.1 nmol/L. Subsequent clinical follow-up visits were at 6 to 12 month intervals. During the follow-up, height and weight were measured, self-monitored blood glucose levels were recorded, HbA1c was measured, and renal and liver function tests were performed.

The patient is now 10 years of age; her height is 140 cm (80$^{th}$ percentile), she weighs 30.5 kg (50$^{th}$ percentile) and shows signs of sexual development (Tanner stage 3). She has normal mental and social skills and strong motivation to learn at school. Recent glibenclamide dose was 0.3 mg/kg per day and her HbA1c was 6.1%. The dosage of glibenclamide was adjusted in accordance with the patient's blood glucose profile and median glibenclamide dose was 0.16 mg/kg/day (0.07-0.3 mg/kg/day) during the ten year of the follow-up period. Under stressful conditions, e.g. while suffering from a mild childhood infection, blood glucose levels remained within the range of 6-10 mmol/L on glibenclamide therapy. Excellent glycaemic control was maintained over the follow-up years and the median of HbA1c was 5.9% (5.6-6.4%; see Fig.1). The median of fasting C-peptide values was 1.8 nmol/L (1.4-2.0 nmol/L) based on 27 measurements over a 9-year period. No reports of severe hypoglycaemia and microvascular complications were recorded. No symptoms leading to suspicion of adverse effects caused by sulfonylurea, including the yellowing of the teeth, gastrointestinal adverse effects, renal and liver dysfunction have been noticed since the switch from insulin to sulfonylurea. A written consent was obtained from the parents for publication purposes.

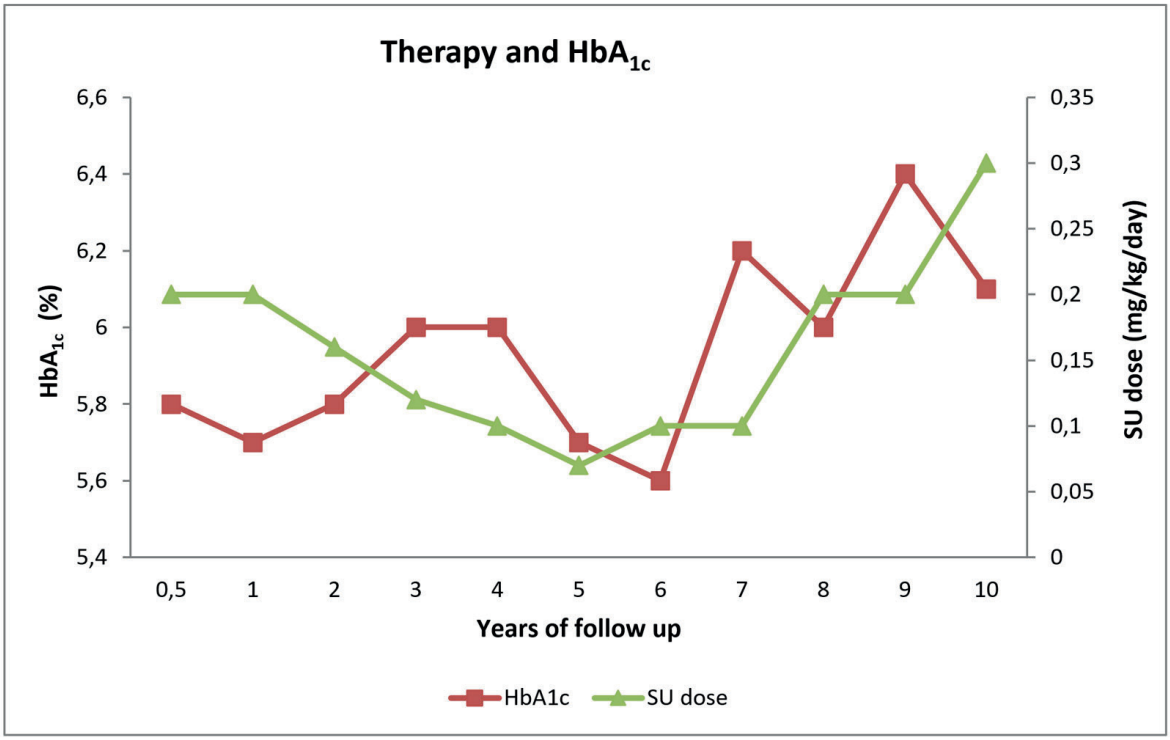


**Fig. 1.** Overview of the treatment and $HbA_{1c}$ levels during the follow-up of patient with permanent NDM attributable to a V252L in the *KCNJ11* gene. Treatment and $HbA_{1c}$ levels: the point of initial switch from insulin to sulfonylurea is 0 years. Green line with triangles shows sulfonylurea (glibenclamide) dose (mg/kg/day) and red line with squares shows $HbA_{1c}$ levels (%). The dose of sulfonylurea glibenclamide was calculated as the sum of the sulfonylurea doses (in mg) during a day divided by the weight in kg.

## Discussion

The number of genes that are found in children with neonatal diabetes continues to increase and there are more than 20 known genetic causes for NDM.[7-10] The various genes are associated with specific inheritance pattern, phenotype, and clinical features.[7] In a large series of 1020 patients diagnosed with NDM before 6 months of age, mutations in the potassium channel genes, *KCNJ11* and *ABCC8*, were found in 38.2% of neonatal diabetes but were identified less frequently in consanguineous families.[7] The clinical presentation varies from incidentally detected asymptomatic hyperglycaemia to severe dehydration and diabetic ketoacidosis (DKA).[11] A genetic diagnosis is crucial, because at least 90% of patients can transfer from insulin injections to oral SU.[12] After transferring to SU treatment, patients have improved glycaemic control at 1 year, without an increase in hypoglycaemia and with less glycaemic variability; however, the key question that remains unanswered is whether the excellent results in NDM will be maintained in the long term.[12-14]

Our patient is one of the youngest patients to commence oral SU therapy for the treatment of NDM because of a new *KCNJ11* mutation, and in a 10-year follow-up SU therapy has been proven safe and effective. The patient maintained excellent glycaemic control without the usual adverse effects of hypoglycaemia. In addition, our result is consistent with the findings of the first study of long-term efficacy and safety of SU that showed that SU failure is not a feature of *KCNJ11* permanent neonatal diabetes.[15] For the 81 patients included in the study which did not include our patient, the median age at diabetes diagnosis was 8.0 weeks, the median age at transfer from insulin to SU was 4.8 years

and 75 (93%) of 81 participants remained on SU therapy alone for the 10-year duration. In these patients, the response dose of SU (0.5 mg/kg/day vs 0.3 mg/kg/day in our patient) and the median maintenance dose of SU (0.23 mg/kg/day vs 0.16 mg/kg/day in our patient) was higher than in our patient whose treatment was successfully switched from insulin to glibenclamide therapy when she was only a month old. Earlier age at the initiation of SU treatment is associated with an improved response to SU therapy and could also lead to a lower maintenance dose which is in contrast with data from a large international cohort study.[15]

A few individuals who initially responded to sulfonylurea (6 of 81 patients) showed worsening glycaemic control on SU monotherapy.[15] The median age at sulfonylurea initiation was 7.4 years and the median age at insulin initiation was 15 years. This fact is important because puberty is associated with increased insulin resistance and suboptimal treatment adherence in diabetes. Patients requiring reintroduction of insulin were on a fairly modest SU dose (median 0.27 mg/kg/day, range 0.19-0.43), suggesting there was capacity to increase the dose further.[15] Taken together, their data suggest that factors other than sulfonylureas having stopped working at the level of the $K_{ATP}$ channel might have contributed to the need for the addition of insulin treatment in these patients. This finding contrasts with the SU monotherapy noted in our 10-year-old patient with signs of puberty (Tanner stage 3). The SU dose was increased at the beginning of puberty from 0.2 to 0.3 mg/kg per day in accordance with the patient's blood glucose profile with satisfactory glycaemic control.

In conclusion, we presented one of the youngest patients to commence oral glibenclamide therapy for the treatment of NDM because of a novel Kir6.2 mutation. An early genetic diagnosis of NDM is important because early switch from insulin to SU is safe, avoids unnecessary insulin therapy, and promotes sustained improvement of glycaemic control on long-term follow-up.

## Author contribution

Clinical data and preparation of the manuscript: Maja D Ješić; laboratory analysis: Helena Stock; clinical data: Vera Zdravković, Smiljka Kovačević; statistical analysis: Marko Savić; clinical advisor: Miloš Ješić.

## Conflict of interest

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 495-499
https://doi.org/10.24953/turkjped.2021.03.017

Case Report

# A rare case of juvenile amyotrophic lateral sclerosis

Muhittin Bodur[1], Rabia Tütüncü Toker[1], Ayşe Nazlı Başak[2], Mehmet Sait Okan[1]

*[1]Division of Pediatric Neurology, Department of Pediatrics, Uludag University Faculty of Medicine, Bursa; [2]Suna and İnan Kıraç Foundation, Neurodegeneration Research Laboratory, School of Medicine, Koç University Translational Medicine Research Center, İstanbul, Turkey.*

## ABSTRACT

**Background.** Amyotrophic lateral sclerosis (ALS) is a chronic motor neuron disease characterised by progressive weakness in striated muscles resulting from the destruction of neuronal cells. The term juvenile ALS (JALS) is used for patients whose symptoms start before 25 years of age. JALS may be sporadic or familial.

**Case.** Here, we present a sporadic case of JALS because of its rarity in children. The heterozygous p.Pro525Leu (c.1574C>T) variation was identified in the fused in sarcoma (FUS) gene.

**Conclusion.** The p.Pro525Leu mutation in the FUS gene has been detected in patients with ALS, characterised by early onset and a severely progressive course.

**Key words:** juvenile amyotrophic lateral sclerosis, FUS gene.

Amyotrophic lateral sclerosis (ALS) is a chronic motor neuron disease characterised by progressive weakness in striated muscles resulting from the destruction of neuronal cells.[1] It is a progressive disease characterised by the co-existence of upper and lower motor neuron findings.[1] The term juvenile ALS (JALS) is used for patients whose symptoms start before they reach 25 years of age.[1] These cases usually have prolonged survival. JALS may be sporadic or familial.[1] Here, we present a sporadic case of JALS because of its rarity in children.

## Case Report

A 17-year-old boy was admitted to our clinic with complaints of weakness and difficulty in speaking and swallowing. Weakness in the right foot had started approximately six months previously and had spread to the right arm, left arm and left leg in the three weeks before the admission. Before these complaints, he had no previous history of serious illness, muscle pain, trauma or adverse reaction to vaccinations, and he had no recent infections. His medical history revealed that he was born at term with a birthweight of 2,000g, and his neuromotor development was normal. There has been no consanguineous marriage in the family history, nor any disease similar to ALS. On physical and neurological examination, he had mild dysarthric speech and tongue fasciculation. His uvula was slightly deviated to the left, and his retching reflex was decreased. Muscle strength of right upper limb was 3/5 and left upper limb was 4/5 (in deltoids, biceps, triceps and hands). Muscle strength of right lower limb was 2/5 in the proximal muscles (in iliopsoas, hamstring, quadriceps femoris) and 1/5 in the distal muscles (in tibialis anterior, gastrocnemius) and left lower limb was 4/5. His tendon reflexes were hypoactive in the left lower and upper limbs and hyperactive in the right lower and upper limbs. His sensory examination was normal. There were atrophy findings in the right-upper and lower limbs. Laboratory findings established the following: hemogram and peripheral smear were normal;

✉ Muhittin Bodur
dr_muhittin@hotmail.com

Received 13th November 2019,
revised 22nd January 2020, 5th April 2020,
19th April 2020, 2nd June 2020, 15th July 2020,
12th August 2020, 7th September 2020,
14th September 2020, accepted 15th October 2020.

creatine kinase 179 (40–220 IU/L); sedimentation 6 mm/h; blood amino acids, tandem MS/MS and lactate were normal; vasculitis screening was found to be normal; and HIV serology was negative. Electromyography (EMG) showed a reduced amplitude of the motor potentials in the examined motor nerves. Fibrillation potential, sharp waves and prolonged polyphasic motor unit potentials (MUPs) were seen in bilateral tibialis anterior, bilateral gastrocnemius, right biceps, right deltoid, genioglossus and rectus abdominis muscles. The findings were evaluated as diffuse anterior horn involvement. Cranial MRI (Fig. 1) showed increased linear signal intensity on T2W images along both internal capsule hind legs to mesencephalon; the diffusion-based examination showed slight diffusion restriction in this area, and the change in intensity and diffusion restriction observed in the bilateral corticospinal tract was evaluated in terms of ALS with clinical findings. Cervical, thoracic and lumbar MRI were normal. Before genetic testing, the patient was diagnosed with JALS based on the clinical, laboratory, electrophysiological and MRI findings. The family was informed about ALS, treatment (riluzole) was commenced, and genetic examination was performed. Four months after diagnosis, the patient became bedridden. He received a tracheostomy and required mechanical ventilation. Eleven months after diagnosis, he died as a result of pneumonia and sepsis. The heterozygous p.Pro525Leu (c.1574C>T) mutation was identified in the fused in sarcoma (FUS) gene. Whole exome sequencing (WES) was confirmed by Sanger sequencing, and segregation analysis in the patient's parents showed that this variant was de novo. The parents were not carriers of the mutation. Informed consent was received from the family.

## Discussion

ALS is a neurodegenerative disease, which usually starts in adulthood and is characterised

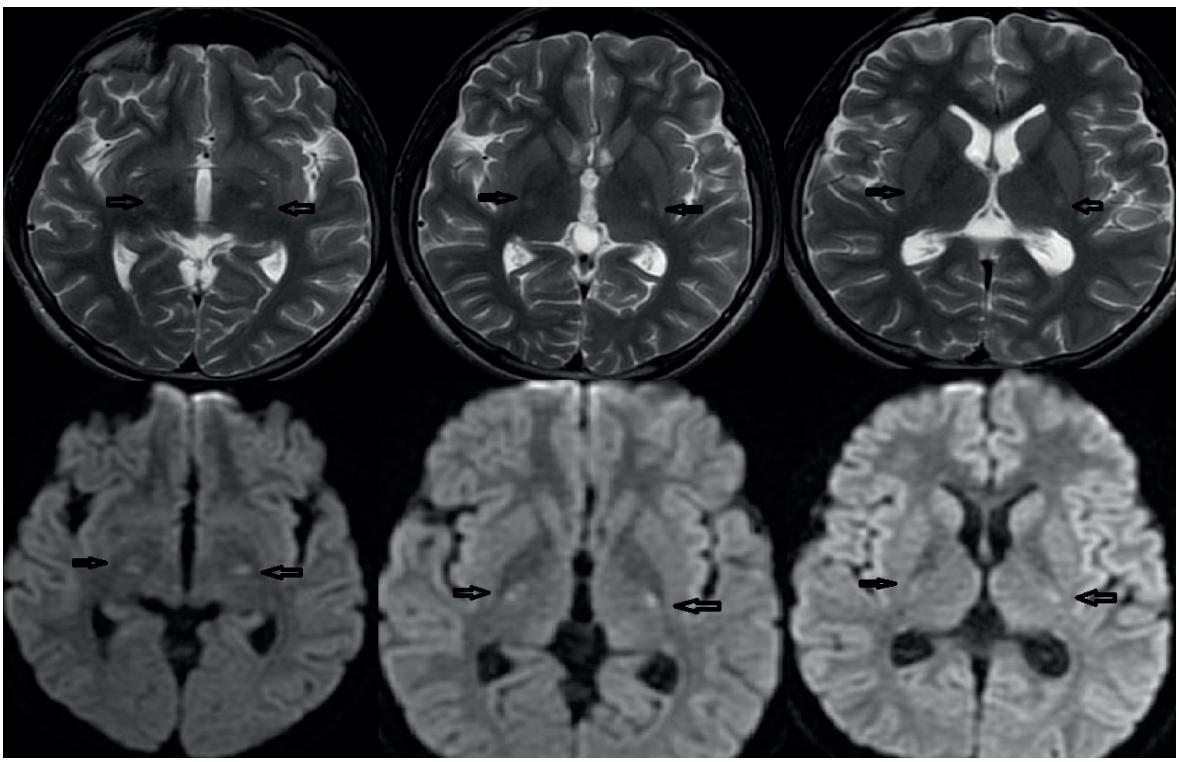

**Fig. 1.** MRI images of the case: increased linear signal intensity on T2W images along both internal capsule hind legs to the mesencephalon; the diffusion-based examination: slight diffusion restriction in this area.

by progressive muscle weakness and muscle atrophy.[2] Approximately 10% of ALS cases are familial, and 90% are sporadic, but the majority of JALS cases have a genetic origin.[3] ALS is characterised by progressive damage to motor neurons responsible for the control of striated muscle groups in the cortex, brain stem and spinal cord. ALS progresses with loss of upper and lower motor neurons. Progressive muscle atrophy and diffuse paralysis develop over time. Death from ALS is due to respiratory failure[4] and occurs within an average of 3–5 years from the onset of the disease. Although the genetic background of the disease is well understood in familial cases, the underlying pathology is still not clear, and there is no effective treatment.[5] ALS is diagnosed clinically. Muscular atrophy and vigorous deep tendon reflexes with focal-onset progressive course and weakness spreading to other extremities over time suggest ALS as the underlying cause.[6] Pyramidal pathway involvement may be seen in ischemic events, demyelinating disorders or infections. However, the change in intensity observed in our patient's bilateral corticospinal tract on cranial MRI has been reported among the characteristic clinical findings in the diagnosis of ALS. The World Federation of Neurology identified El Escorial criteria (1994) for use in the diagnosis of ALS,[7] and these were revised in 2000.[8] These criteria divide the motor system into 4 anatomic regions: bulbar, cervical, thoracic, and lumbosacral. Clinical evidence of upper motor neuron (UMN) and lower motor neuron (LMN) pathology is sought within each region; the certainty of diagnosis depends on how many regions reveal UMN and/or LMN pathology.[9] Patients with signs of LMN degeneration (weakness, atrophy and clinical fasciculations) and UMN degeneration (spasticity, pathologic reflexes, etc.) may be suspected as having ALS. Careful history, physical and neurological examination must search for further clinical evidence of LMN and UMN signs in four regions of the central nervous system.[7] According to the El Escorial criteria, upper and lower motor neuron degeneration findings should be present at the same site for ALS diagnosis. The clinical picture should progressively spread from one side to the other. Other factors that may explain lower or upper motor neuron findings should be excluded in the light of laboratory, electrophysiological or neuroimaging data.[7] In the examination of our patient, there were bulbar involvement (dysarthric speech, fasciculation in the tongue, difficulty in swallowing) and weakness that started from the lower right limb and spread to other limbs. In addition, there were findings of lower motor neuron involvement (weakness, muscle atrophy, DTR hypoactivity) in the left lower limb (lumbosacral) and left upper limb (cervical), and findings related to the involvement of upper motor neurons (weakness, DTR hyperactivity) in the right lower limb (lumbosacral) and right upper limb (cervical) were detected. Electromyography (EMG) is the most important component of the electrodiagnostic evaluation in ALS. To support a diagnosis of ALS, the needle electrode examination should reveal decreased motor unit recruitment with rapid firing of a reduced number of motor units, and/or large amplitude, long duration MUP with or without evidence of remodelling (increased number of phases) in combination with abnormal spontaneous activity including positive sharp waves (PSWs), fibrillations, and/or fasciculation potentials (FP).[9] In this case, a reduced amplitude was observed in the examined motor nerves; intense denervation findings (fibrillation potentials and sharp waves) were obtained in the extremity muscles (cervical, lumbosacral) and midline muscles tract (bulbar, thoracic); and prolonged polyphasic motor unit potentials (MUPs) were observed. These findings were consistent with the EMG findings of ALS, evidence of chronic neurogenic change and evidence of acute denervation. In this case according to the examination and EMG findings, the findings of lower motor neuron in the bulbar and thoracic region, and both upper and lower motor neuron in the cervical and lumbosacral region were detected. As a result of these findings, this case was evaluated as clinical probable ALS. In this case, clinical, laboratory, electrophysiological

and imaging findings were consistent with the El Escorial criteria, and no other cause of upper and lower motor neuron destruction could be detected. To date, there are 29 genes that are considered to be causes of or highly correlated with ALS.[10] FUS mutations have been observed in patients with JALS, where symptoms start before the age of 25 years.[11] The FUS gene is located on chromosome 16p11.2 and consists of 14 introns and 15 exons. It encodes the FUS protein of 526 amino acid residues that belong to the multifunctional DNA/RNA-binding proteins. The FUS protein is ubiquitously expressed in the cell nucleus and cytoplasm and continuously moves between the two areas. It is involved in cell proliferation, DNA repair, transcription regulation, RNA transport and microRNA processing.[12] FUS mutations are reported to be responsible for 4% of familial ALS[13] and less than 1% of sporadic ALS cases,[14] which illustrates their rarity. Among these mutations, p.P525L FUS mutation has been consistently associated with young-onset, rapid disease course and high proportions of de novo mutations in sporadic cases.[15] In this case, the heterozygous p.Pro525Leu (c.1574C>T) mutation was identified in the FUS gene, and analysis in the patient's parents showed that this variant was de novo. In reviewing the literature, 6 cases with P525L mutation were observed in pediatric ALS cases (Table I). In accordance with the literature our case showed early onset and rapid progression, and the patient died 11 months after diagnosis. There is no effective treatment for the disease. Riluzole, which has been used since 1996, is a glutamate antagonist and is the only drug that prolongs survival. A daily dose of 100 mg Riluzole prolongs survival in ALS patients by an average of 3-5 months.[16] There is a need for new treatment methods that will increase the survival rate for patients with the disease. Although JALS is rare in children, it should be kept in mind, especially in cases with upper motor and lower motor neuron findings.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Muhittin Bodur , Rabia Tütüncü Toker , Ayşe Nazlı Başak , Mehmet Sait Okan; data collection: Muhittin Bodur , Rabia Tütüncü Toker , Ayşe Nazlı Başak, Mehmet Sait Okan; analysis and interpretation of results: Muhittin Bodur, Rabia Tütüncü Toker, Ayşe Nazlı Başak, Mehmet Sait Okan; draft manuscript preparation: Muhittin Bodur, Rabia Tütüncü Toker, Ayşe Nazlı Başak, Mehmet Sait Okan

All authors reviewed the results and approved the final version of the manuscript.

## Conflicts of interest

The authors declare no conflict of interest.

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**Table I.** P525L FUS mutations in pediatric ALS cases.

| Age at onset, year | Gender | Sporadic/Famialial | Site of onset | Disease duration (months) | Reference |
|---|---|---|---|---|---|
| 11 | F | S | Limb | 14 | 16 |
| 13 | F | S | Limb | 20 | 17 |
| 13 | M | N/A | Limb | 23 | 18 |
| 16 | M | F | Bulbar | 12 | 19 |
| 17 | F | S | Bulbar | 24 | 20 |
| 17 | M | S | Limb | 15 | 20 |
| 17 | M | S | Limb and Bulbar | 11 | This report |

F: female, M: male, S: sporadic, F: famialial, N/A: not available.

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The Turkish Journal of Pediatrics 2021; 63: 500-505
https://doi.org/10.24953/turkjped.2021.03.018

Case Report

# Sertoli-Leydig cell tumor, thyroid follicular carcinoma and rhabdomyosarcoma of the uterine cervix in a prepubertal girl with pathogenic germline variant in *DICER1* gene

İbrahim Karnak[1], Ahmet Cevdet Ceylan[2], Mithat Haliloğlu[3], Alev Özön[4], Diclehan Orhan[5], Tezer Kutluk[6]

*Departments of [1]Pediatric Surgery, [3]Radiology, [4]Pediatric Endocrinology, [5]Pathology, [6]Pediatric Oncology, Hacettepe University Faculty of Medicine, Ankara; Department of [2]Medical Genetics, Ankara City Hospital, Ankara, Turkey.*

## ABSTRACT

**Background.** DICER1 syndrome is a hereditary cancer predisposition syndrome which is related *DICER1* gene and may present a variety of manifestations.

**Case.** A prepubertal girl with ovarian Sertoli-Leydig cell tumor, thyroid follicular carcinoma, embryonal rhabdomyosarcoma of the cervix and lung cyst is presented. Genetic analysis demonstrated mutation (c.3377delC, c.71delC) in 14q32.13 loci and confirmed the diagnosis of DICER1 syndrome.

**Conclusion.** The case is presented to emphasize the importance of early diagnosis of alterations in *DICER1* gene and close follow-up for the development of DICER1 syndrome related pathologies, and necessity for genetic evaluation of the family.

**Key words:** Sertoli-Leydig cell tumor, differentiated thyroid carcinoma, rhabdomyosarcoma, DICER1 syndrome, lung cyst.

The human DICER1 gene contains 27 exons, encodes 1922 amino-acid long protein and it is located on chromosome 14q32.13. It is a ribonuclease (RNase) III endoribonuclease and a key component of the RNA interference pathway.[1]

Pathogenic germline *DICER1* variants cause a hereditary cancer predisposition syndrome, DICER1 syndrome, with a variety of manifestations including pleuropulmonary blastoma (PPB), ovarian sex-cord stromal tumors, particularly Sertoli-Leydig cell tumor (SLCT), lung cysts, cystic nephroma (CN), renal sarcoma and Wilms' tumor, nodular hyperplasia of the thyroid, nasal chondromesenchymal hamartoma, ciliary body medulloepithelioma, genitourinary embryonal rhabdomyosarcoma (ERMS) and brain tumors such as pinealoblastoma and pituitary blastoma.[1-5]

This case is a prepubertal girl initially presented with macrocephaly, SLCT, thyroid nodule and lung cyst. Extremely rare coexistence of two rare pathologies raised the possibility of common pathogenetic pathway, possibly *DICER1* related pathogenesis. The authors share their experience on this illustrative case of DICER1 syndrome to stress the necessity of intensive work to investigate the components of this syndrome and close follow up of the child for the development of other DICER1 associated pathologies.

## Case Report

A 5-year 10-month-old girl was admitted for right ovarian mass. The mass was diagnosed by ultrasonography (US) examination during

✉ İbrahim Karnak
ikarnak@hacettepe.edu.tr

Received 7th May 2020, accepted 28th September 2020.

evaluation for urinary tract infection. Physical examination revealed a fullness on palpation of the suprapubic region, and a mobile mass in the Douglas's pouch on rectal touch. An asymptomatic thyroid nodule (2 x 1.5cm) in the right thyroid lobe was also diagnosed incidentally during palpation of the neck. The family medical history revealed Hashimoto's thyroiditis in maternal aunt, and goiter with benign nodule in maternal grandmother.

Magnetic resonance (MR) imaging showed lobular, solid-cystic mass of 7.2 x 6.3 x 5.5 cm size in the rectouterine pouch and normal ovaries. Ultrasound of the neck revealed a solid nodule (2 x 1 cm) in the right thyroid lobe, a cystic nodule (0.4 x 0.4 cm) in the left thyroid lobe and reactive lymph nodes.

Alpha-feto protein (2.72 ng/ml; N: 0-9), beta-HCG (<0.5 mIU/ml), total testosterone (<0.02; N: 0.06-0.82 ng/ml), FSH (1.48 mIU/ml, N), LH (<0.1 mIU/ml; N), estradiol (<5 pg/ml; N: <20), free-T4 (14.23 pmol/L; N: 7.86-14.41), TSH (2.01 μIU/ml; N: 0.34-5.6), thyroglobulin (35.5 ng/ml; N: 1.15-50) levels, complete blood count and blood biochemistry were within normal ranges.

At operation, a Pfannenstiel incision was used. Intraperitoneal fluid was sampled for cytology, a solid mass (8 x 8 cm) originating from right ovary was excised completely with a rim of normal ovarian tissue. Left ovary was normal in appearance and normal consistency on palpation. Omentum was excised and peritoneum was sampled. Fine needle aspiration and tru-cut biopsies were taken from the nodule in the right thyroid lobe. Postoperative course was uneventful.

Pathology report confirmed moderately differentiated Sertoli-Leydig cell tumor of the ovary with clear surgical margins (Fig. 1). Peritoneal fluid, omentum and peritoneum were tumor free. The thyroid nodule was a benign follicular lesion. No further treatment was given due to the stage I disease.

Postoperative computerized tomography of the thorax revealed an air cyst (25 x 20 x 20 mm) in the left lower lobe of the lung (Fig. 2). Postoperative control MR images showed no residual or recurrent tumor in the abdomen, and normal appearing ovaries bilaterally.

The patient was under close follow-up and control thyroid ultrasound revealed a solid nodule (17 x 18 x 27 mm) in the right lobe and 2 nodules (5 x 5.5 x 10 mm solid and 15 x 7 x 9 mm cystic) in the left lobe of the thyroid. The patient underwent a right thyroid lobectomy and excision of both nodules from left lobe of the thyroid. Frozen section of all tissues was reported as free of malignant tissue. However permanent sections showed a differentiated thyroid carcinoma area in the right lobe nodule and a complementary thyroidectomy was performed later. Histopathology of the remaining thyroid demonstrated follicular carcinoma in the left lobe nodule with capsular invasion (Fig. 3). Postoperative thyroid scan revealed no residual tissue or pathological

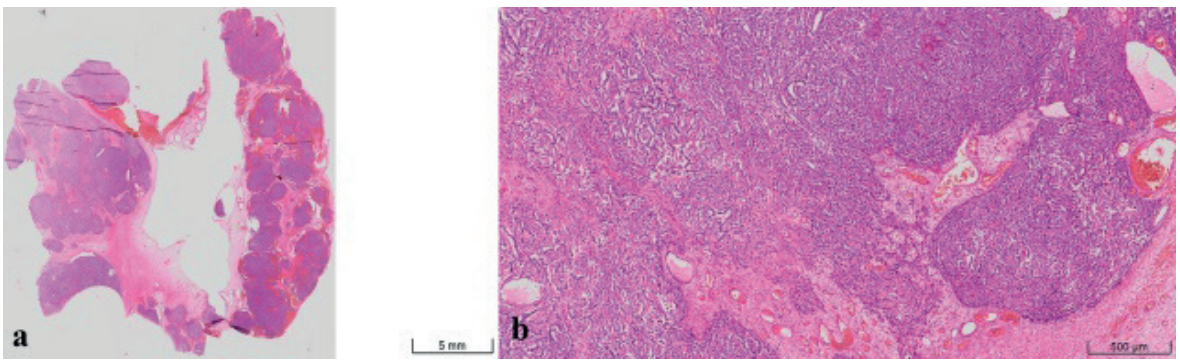


**Fig. 1.** Moderately differentiated Sertoli-Leydig cell tumor showing a lobulated pattern **(a)**. Sertoli cells forming tubules and cords admixed with small clusters of Leydig cells **(b)**.

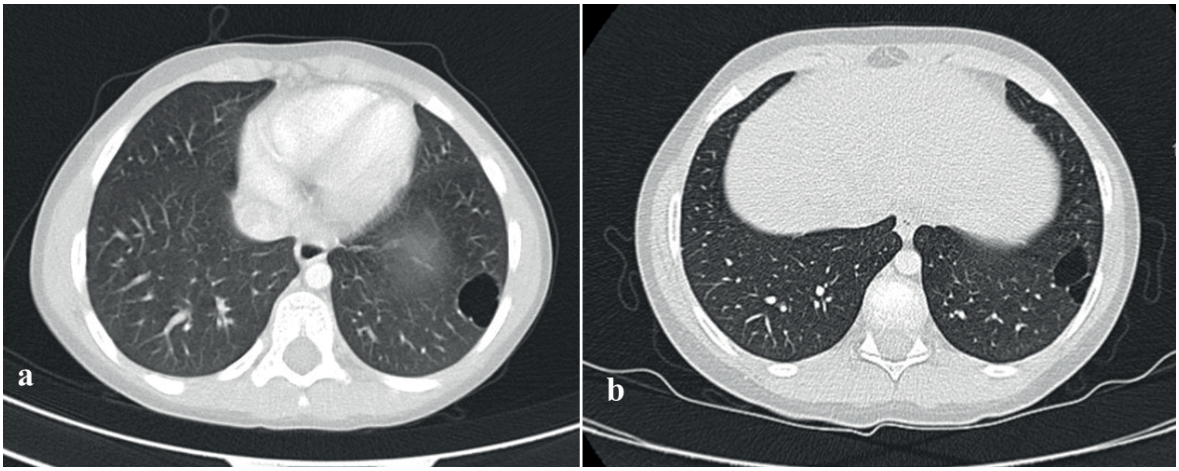


**Fig. 2.** Thorax CT revealed stabile air cyst in the left lower lobe of the lung, at the time of diagnosis **(a)** and after 2 years **(b)**.

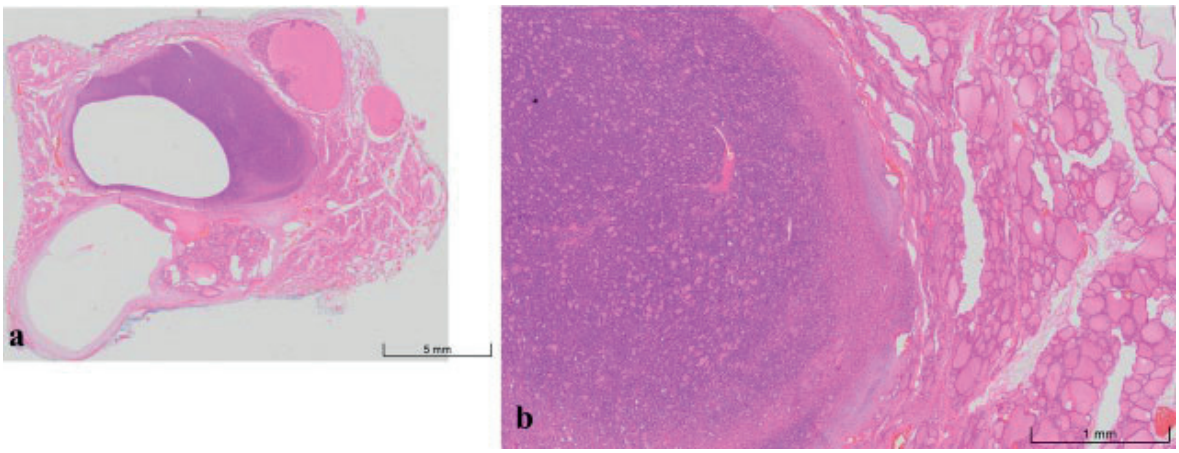


**Fig. 3.** Follicular carcinoma with microfollicules **(a)** and capsular invasion **(b)**.

lymph node and radioactive iodine-131 ablation was performed.

Two years later from ovary preserving surgery, the patient admitted with vaginal bleeding of one-month duration. Cysto-vaginoscopy revealed a polypoid lesion (2.5 x 1.5 cm) protruding from cervical ostium into the vagina and this polypoid lesion was excised totally. Histopathological evaluation reported as botryoid rhabdomyosarcoma (Fig. 4). She received VAC (ARST0331) regimen for 24 weeks. She is currently free of disease and under follow up with stable pulmonary air cyst.

The next generation sequencing revealed c.3377delC (p.Thr1126fs*18) at *DICER1* (NM_177438.2) classified as pathogenic germline variant. The parents and sister of the index patient were recommended for detailed genetic analyses for DICER1 status.

The parents of the patient were informed about the preparation of an article and an informed consent was obtained from parents.

## Discussion

DICER1 syndrome is inherited as an autosomal dominant disease which is associated with germline mutations in *DICER1*. This syndrome consists of PPB, SLCT of the ovary, genitourinary embryonal RMS, lung cyst, thyroid nodule or differentiated thyroid carcinoma, pituitary

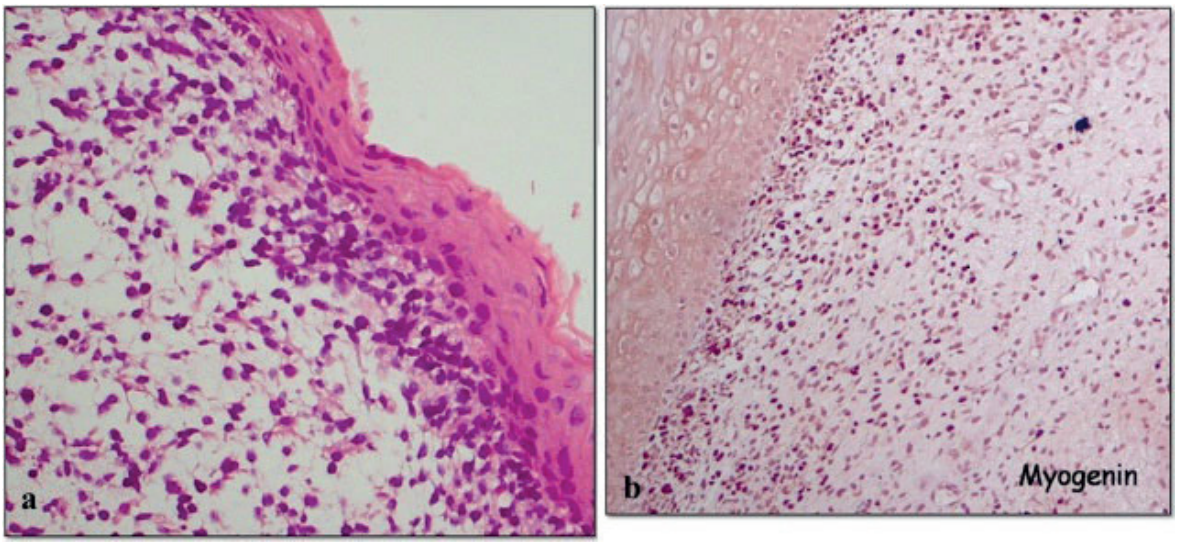


**Fig. 4.** Botryoid rhabdomyosarcoma with cambium layer beneath intact epithelium and rhabdomyoblasts on a loose myxoid stroma **(a)**. Myogenin positive tumor cells in cambium layer **(b)**.

blastoma or pinealoblastoma. Macrocephaly may be seen as a non-tumoral expression in DICER1 syndrome patients.[1]

Sertoli-Leydig cell tumor of the ovary is a rare tumor in children and coexisting thyroid nodule is highly unusual even in a high pediatric surgical and oncological patient volume hospital. The history of thyroid disease in relatives prompted us for close follow up of the patient and was considered highly suggestive for DICER1 syndrome. Sertoli-Leydig cell tumor of the ovary was treated by ovary-sparing surgery. Intermediate differentiation of tumor, clear margins and tumor free peritoneum and as well as peritoneal fluid suggested low stage disease requiring no additional treatment for SLCT. The diagnosis of thyroid malignancy necessitated complementary thyroidectomy and radioactive iodine-131 ablation therapy.

Pleuropulmonary blastoma is a rare pathology, in spite of being the most common primary lung malignancy in children. It can progress from a multicystic lesion (Type I) to mixed solid-cystic (Type II) and solid stage (Type III) tumors. A fourth type of PPB, Type Ir "regressed" is not malignant. Therefore, it should be kept in mind that pulmonary cystic lesion in a child with pathogenic DICER1 variant can be Type I PPB until proven otherwise. The lung cyst has been asymptomatic and stable radiologically for 4 years in our patient. Since we do not have histological evaluation of the lung cyst in our patient, the lesion is under regular follow up by chest X-rays and CTs.

Only a few children with SLCT and thyroid pathology, with or without associating genitourinary ERMS, has been reported to date.[6-11] Our patient developed embryonal RMS of the cervix and was treated by endoscopic surgery and chemotherapy.

The genetic evaluation for *DICER1* status is required for every child with SLCT, PPB or CN and/or accompanying thyroid pathology.[12] It has been suggested that *DICER1* mutation poses a low-risk of malignancy and *DICER* sequencing and gene dosage determination is recommended in molecular analysis of pediatric thyroid specimens.[13] Additionally, *DICER1* sequencing has been recommended in children with multinodular goiter, multiple multinodular goiter cases within the same family, or its association with benign or malignant tumors.[14]

Once a pathogenic variant is diagnosed in *DICER1*, follow up process of the patients is

quite stressful for the clinician and the family members. The family members should be informed about the syndrome as well as the signs and symptoms of possible pathologies, and the clinician should be alert during surveillance and follow up.

A chest X-ray at birth, every 4-6 months until 8 years of age and every 12 months for 8-12 years of age and a chest CT at 3-6 months of age has been recommended for investigation of lung pathologies in a child with *DICER1* syndrome.[2] The recommendations for screening thyroid pathology is baseline thyroid US by 8 years of age then every 3 years or with suspicious symptoms/findings on physical examination. If the child receives chemotherapy or radiotherapy, a baseline US of the neck, and then annual US for 5 years, followed by US every 2 to 3 years is recommended if no nodules are detected initially.[2]

It has been recommended that female reproductive tract should be checked by pelvic and abdominal US every 6-12 months beginning at 8 - 10 years of age until at least 40 years, by keeping the information in mind that the current oldest patient with *DICER1*-associated SLCT is 61 years old. Our case demonstrates that *DICER1*-associated SLCT can be encountered in younger ages and baseline US examinations should be performed before 8 years of age. Our case will be followed up by US for the development of metachronous or recurrent genitourinary neoplasms till the recommended ages.[2] *DICER1*-associated renal pathologies can be investigated by abdominal US every 6 months until 8 years of age, then every 12 months until 12 years of age.[2]

The patient should be checked by physical examination as well as detailed ophthalmologic examinations from 3 years of age through to at least 10 years of age. Urgent MRI has been recommended for any symptom of intracranial pathology.[2] Bueno et al.[15] suggested annual whole-body MR, chest X-ray and US examination of the target body system for at-risk pediatric patients with *DICER1* syndrome.

The pediatric oncological and pediatric surgical professionals should have information about *DICER1*-related pathologies. *DICER1* status should be determined in every child with SLCT, PPB or CN, and/or accompanying thyroid pathology. The parents of children with *DICER1* syndrome should be aware of signs and symptoms suggestive for *DICER1*-related pathologies.

## Author contribution

İbrahim Karnak, surgical treatment, writing, editing, orginizations for genetical analysis Ahmet Cevdet Ceylan, genetical analysis, genetic counsulling, writing Mithat Haliloğlu, detailed evaluation of radiological examinations, writing, editing Alev Özön, detailed endocrinological evaluation and follow up, writing, editing Diclehan Orhan, histopathological evaluation, writing, editing Tezer Kutluk, oncological treatment, writing, editing, follow up, mentorship.

## Conflict of interest

The authors declare no conflict of interest.

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The Turkish Journal of Pediatrics 2021; 63: 506-509
https://doi.org/10.24953/turkjped.2021.03.019

Case Report

# Anaphylaxis to levetiracetam in an adolescent: a very rare occurence

Şeyma Kahraman[1], Şeyda Değermenci[1], Mehmet Ali Oktay[1], Deniz Menderes[2], Okşan Derinöz Güleryüz[3], Ebru Arhan[2], Arzu Bakırtaş[4], Hacer İlbilge Ertoy Karagöl[4]

*Divisions of [2]Pediatric Neurology, [3]Pediatric Emergency and [4]Pediatric Allergy, [1]Department of Pediatrics, Gazi University Faculty of Medicine, Ankara, Turkey.*

## ABSTRACT

**Background.** Antiepileptic drugs (AEDs) are among the most common causes of severe delayed-type hypersensitivity reactions such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and Drug Reaction with Eosinophilia and Systemic Symptoms(DRESS) in children. These reactions are more commonly seen with aromatic AEDs such as phenytoin and carbamazepine than the non-aromatic or new generation AEDs. However immediate-type hypersensitivity reactions such as urticaria/angioedema, anaphylaxis are very rare with AEDs.

**Case.** Levetiracetam is an increasingly used new non-aromatic antiepileptic drug and reported to have a better safety profile in daily practice. We present the first adolescent case who developed an anaphylactic reaction with intravenous levetiracetam, not reported in this age group before in the literature.

**Conclusion.** Hypersensitivity reactions in the form of anaphylaxis can be rarely observed with new generation AEDs. Therefore, when any antiepileptic drug is started on any patient, immediate type serious reactions such as anaphylaxis should be kept in mind, not only focusing on delayed reactions such as SJS, TEN,or DRESS.

**Key words:** adolescent, anaphylaxis, antiepileptic drugs, levetiracetam.

Antiepileptic drug (AED)- induced hypersensitivity reactions(HSR) which are reported to be more common in children, present with a variety of clinical manifestations ranging from benign maculopapular exanthems to severe delayed reactions such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), Drug Reaction with Eosinophilia and Systemic Symptoms(DRESS) and also organ-specific disease such as agranulocytosis and drug-induced liver injury.[1] Immediate-type HSRs such as urticaria/angioedema or anaphylaxis are very rare with AEDs. Delayed reactions are more commonly seen with aromatic AEDs like phenytoin and carbamazepine than the new generation or non-aromatic AEDs.[1] Levetiracetam is an increasingly used new non-aromatic AEDs and reported to have a safer tolerability profile in daily practice.[2] Here, we report an adolescent case of anaphylaxis due to levetiracetam, in order to point out that new AEDs can result in HSRs and also, severe immediate reactions should be expected

## Case Report

A 15-year-old girl with no previous diagnosis of epilepsy presented to the pediatric emergency department with a history of two seizures in the same day. Later in the emergency department, the patient experienced another seizure with jaw-locking, nystagmus, spasms in legs and arms

✉ Hacer İlbilge Ertoy Karagöl
theilbilge@yahoo.com.au

Received 24th May 2020, revised 17th August 2020, accepted 27th August 2020.

for 15 to 20 seconds. This seizure was treated with a rapid IV administration of midazolam. Investigations for seizure revealed a normal full blood count, normal urea and electrolytes, glucose, liver functions, arterial blood gas parameters. In addition, the patient's cranial computed tomography and electrocardiography were normal. Upon consultation with the Pediatric Neurology Department, 20 mg/kg levetiracetam IV infusion was started 2 hours after midazolam administration. Five minutes after the start of infusion flushing, cough, stridor, and vomiting was observed, respectively. Hypotension was not observed but the oxygen saturation of the patient dropped to 92%. The infusion was interrupted immediately and 0.01 mg/kg intramuscular adrenaline, 1 mg/kg IV pheniramine and 1 mg/kg IV methylprednisolone were administered. Symptoms improved within half an hour. Serum tryptase could not be tested at the time of reaction. The patient was referred to our allergy clinic for further evaluation. It was learned that the patient had no previous diagnosis of a drug allergy nor was she using any other drugs at the time of reaction. Patient history revealed a previous atopic eczema and cow's milk-induced allergic proctocolitis in infancy diagnosed in another medical center that developed remission and tolerance 14 years ago. Family history was negative for allergic diseases including drug allergy. Six weeks later, we carried out skin prick tests (10mg/mL, 100mg/mL) and intradermal skin tests (1mg/mL, 10mg/mL, 100mg/mL) with levetiracetam, but all the results were negative. Skin tests with midazolom (5mg/ml prick and 0.05 mg/ml intradermal) and latex (500 μg/ml with the standart extract ALK Abello, Madrid) were performed and found negative. In addition, the patient had a lumbar puncture the day after anaphylaxis which was performed with 5 mg midazolom intravenously for sedation as preparation for the procedure which acted as a provocation test with midazolom and found negative. The patient was also tested for other well-known food and aero-allergens by skin prick tests and found negative. Drug provocation test (DPT) was planned with levetiracetam to confirm the diagnosis but the relatives of the patient did not accept DPT. In patient follow-up, no organic pathology explaining the seizures was found. The patient is still being followed with an epilepsy diagnosis and asymptomatic under topiramate treatment. The mother of the patient has given her written consent to publish the report.

## Discussion

Herein, we present a case of anaphylactic reaction to levetirecetam. The reports on levetirecetam hypersensitivity include a limited number of adult and child cases.[3,4] These reactions are mostly delayed-type reactions as SJS, TEN, and acute generalized exanthematous pustulosis (AGEP) in adults and as DRESS in a child.[1,3,4-6] A turkish child who developed an immediate-type reaction due to levetiracetam use was reported before our case.[7] This case was a newborn and anaphylactic shock was reported when levetiracetam was administered due to treatment-resistant seizures.[7] However, it has not been stated which antiepileptics were applied for seizure control before levetiracetam and if applied, the time interval between them. It is also unclear whether another antiepileptic drug and / or medication was used during or before levetiracetam infusion.[7]

Immediate-type reactions such as urticaria, angioedema and anaphylaxis due to AEDs are very rare compared to delayed-type reactions.[1,8-11] Standardised diagnosis of these reactions includes first skin prick and intrardermal tests, respectively, and if negative, DPT is recommended.[1,12] We performed prick-intradermal tests, respectively, with the same levetiracetam commercial drug (Keppra® 500mg/5ml) administered to our patient at the time of reaction, and all tests were negative. Drug provocation test was neccessary to confirm diagnosis except in severe anaphylaxis[12] however it could not be done due to nonapproval by the patient and the parents. Therefore, an IgE mediated reaction could not

be confirmed or excluded in our patient, since DPT could not be performed.

An IgE-mediated early type HSR could not be excluded just by history of the first exposure to the drug as in the examples of taxanes and cetuximab.[13,14] In addition, there is insufficient data related with pathogenesis of immediate type HSRs with anti-epileptic medications compared to severe type 4 cutaneous HSRs[1] Therefore we performed skin tests with levetiracetam to our patient although it was the first application of this antiepileptic to the patient.

Sometimes the excipients of the drugs may be the main trigger of the HSRs.[15] Therefore we cannot exclude this possibility for the excipients of levetiracetam (Sodium acetate, Glacial acetic acid, Sodium chloride).

Clinical cross-reactivity is reported within traditional aromatic AEDs such as carbamazepine, phenytoin and phenobarbital, especially due to their structural similarity.[16] Since cross-reactivity has not been reported between non-aromatic AEDs so far[1], we continued with topiramate for the treatment of epilepsy in our patient and was tolerated well. Again, some risk factors such as age, presence of co-morbid disease and the presence of other concomitant medications, have been reported, especially for delayed-type reactions occurring with AEDs.[1] When we evaluated our patient in this regard, we did not detect any of those risk factors. Traditionally atopic status is not accepted as a risk factor for drug HSRs however it may increase the severity of the reaction.[17] Therefore we thought that the history of atopic eczema and allergic proctocolitis in infancy in our patient as risk factors for the severity of the reaction.

In conclusion, HSRs can be observed also with new generation AEDs, although infrequent, and even immediate type anaphylactic reactions may occur, more rarely. However, the exact mechanisms are not clear. Therefore, when any antiepileptic drug is started on any patient, all types of mild and serious reactions including anaphylaxis should be kept in mind, not only focusing on severe cutaneous adverse drug reactions such as SJS, TEN, DRESS or AGEP.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: Hacer İlbilge Ertoy Karagöl, Arzu Bakırtaş; data collection: Şeyma Kahraman, Şeyda Değermenci, Mehmet Ali Oktay, Deniz Menderes, Okşan Derinöz Güleryüz, Ebru Arhan; analysis and interpretation of results: Hacer İlbilge Ertoy Karagöl, Şeyma Kahraman; draft manuscript preparation: Hacer İlbilge Ertoy Karagöl, Arzu Bakırtaş, Şeyma Kahraman. All authors reviewed the results and approved the final version of the manuscript.

## Conflict of interest

The authors have no report conflict of interest to declare.

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The Turkish Journal of Pediatrics 2021; 63: 510-515
https://doi.org/10.24953/turkjped.2021.03.020

Case Report

# Infantile tremor syndrome secondary to peroral vitamin B12 replacement therapy: a report of two cases with myoclonus

Beril Dilber[1], Gökçe Pınar Reis[2]

*Departments of [1]Pediatric Neurology and [2]Pediatric Hematology-Oncology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey.*

## ABSTRACT

**Background.** Abnormal movements such as tremors and myoclonus may be observed during both treatment and deficiency of vitamin B12, particularly in infants. Infantile tremor syndrome (ITS) is defined by the tetrad of pallor, developmental delay/regression, skin pigmentation, and brown scanty scalp hair.

**Case.** In this report, two cases with ITS aged less than one year who had myoclonic movements during vitamin peroral B12 treatment are discussed based on hematologic, neurological, and magnetic resonance images (MRI) findings, one of whom developed a whole-body tremor and rhythmic myoclonic movements, titubation, and restlessness in the hands and feet as well as diffuse cerebral atrophy on brain MRI.

**Conclusion.** The infants of mothers with nutritional vitamin B12 deficiency may develop sudden abnormal movements following peroral vitamin B12 therapy and that the differential diagnosis of these disorders is highly important for the prevention of long-term neurological sequela by treatment.

**Key words:** infantile tremor syndrome, myocolonic movement disorders, cerebral atrophy, peroral vitamin B12 therapy.

In Turkey, infantile vitamin B12 deficiency is mostly caused by a low intake of vitamin B12 in the diet. Vegetarian diet, pernicious anemia, and low socioeconomic level are common etiological factors associated with vitamin B12 deficiency in breastfeeding mothers and their children.[1] Vitamin B12 begins accumulating in the infant within the first six months after birth while mothers often remain asymptomatic and the deficiency in the infant may not be noticed until the onset of neurological effects.[2] Infantile symptoms including restlessness, nutritional problems, and neuromotor growth retardation become pronounced over time. Abnormal movements such as tremor, myoclonus, and choreoathetosis that are observed before or during the B12 vitamin injection treatment or due to cobalamin (Cbl) deficiency are rare but have been previously reported, particularly in infants.[3-8]

In a recently published study, nutritional Cbl deficiency, particularly during infancy, was shown to cause a wide variety of abnormal movements such as involuntary eye deviations, eyelid twitching, and contraction in the whole or part of the body despite normal serum vitamin B12 levels and/or neurological development.[9] In this report, we present two cases with Infantile tremor syndrome (ITS) aged less than one year old who developed myoclonic movements following peroral vitamin B12 replacement therapy.

## Case 1

An eight-month-old girl with thin brown hair was admitted with hypotonia. Her parents were distant relatives and she had a low socioeconomic status. The patient was

✉ Beril Dilber
beriltem@gmail.com

Received 10th June 2020, revised 14th July 2020, 8th August 2020, 13th September 2020, accepted 26th November 2020.

being breastfed and she had been born at term weighing 3.3 kg after an uncomplicated pregnancy and delivery. She started to smile at 3 months and had gained head control at 4 months of age (head circumference: 40 cm [25 p]). By the age of 6 months, she gradually became less active. On examination, she looked pale and hypotonic. Her head circumference was 41 cm (<3p), body weight was 8 kg (10 p), and body height was 68 cm (25p). Cranial nerve examination was normal. Although she was generally hypotonic, she had normal strength and brisk reflexes. A complete blood count (CBC) indicated hemoglobin 8.9 (range, 12-17) g/dL, hematocrit 27.2% (range, 42-52%), mean corpuscular volume (MCV) 85.1 (range, 80-94) fL, ferritin 100 (range, 23.9-336.2) μg/L, folic acid 10.56 (range, 3.1-19.9) μg/L, serum homocysteine 45 (range, 5-15) μmol/L, serum methylmalonic acid 4.85 (range, 0.0-3.60) μmol/L, and serum vitamin B12 46 (range, 126.5-505) ng/L. On the other hand, the mother's vitamin B12 level was also low (98 ng/L [range, 126.5-505] ng/L). Peripheral blood analysis indicated anisocytosis and hypersegmented neutrophils. Coronal T2-weighted images showed abnormal brain MRI findings including enlargement of frontotemporal subarachnoid spaces, enlargement of the cerebral sulci, and thinning of the cortex by age (Fig 1). Electroencephalogram (EEG) showed no seizure activity. Peroral cyanocobalamin 1,000 mcg/day therapy due to vitamin B12 deficiency was initiated. Twelve hours after the initiation of the therapy, the patient developed a whole-body tremor and rhythmic myoclonic movements, titubation, and restlessness in the hands and feet. She was conscious of her environment despite being restless and uneasy. However, these symptoms disappeared during sleep. The following day, clonazepam 0.1 mg/kg/day was initiated due to the persistence of the tremor and then the complaints decreased gradually.

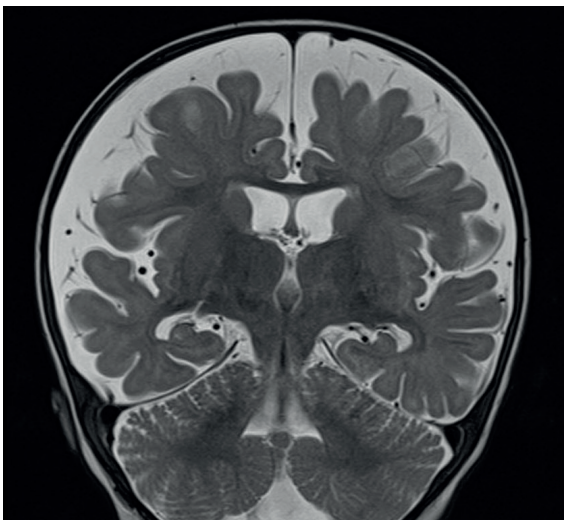

**Fig. 1.** Coronal T2-weighted image showing abnormal brain MRI findings including enlargement of frontotemporal subarachnoid spaces.

**Case 2**

A ten-month-old boy was referred to our clinic with an initial diagnosis of pancytopenia. This patient was also being breastfed. Although he was conscious of his environment, he was apathetic. He had been born at term with a birth weight of 3.2 kg after an uncomplicated pregnancy and delivery. He started to smile and control his head at 2 months of age. However, although he could hold his head, he could not sit without support. His head circumference was 43 cm (<3p), body weight was 9 kg (10 p), and body height was 70 cm (25p). He had several hyperpigmented lesions on the arm, back, sacral region and he had weak and sparse hair (Fig 2 a, b, c). Cranial nerve examination was normal. All deep tendon reflexes were present and brisk. CBC parameters included hemoglobin 7.6 g/dL, hematocrit 26.2%, MCV 82.1 fL, thrombocyte $132x10^3$/μL, ferritin 200 μg/L, folic acid 8.56 μg/L, serum homocysteine91 μmol/L, and serum methylmalonic acid 3.85μmol/L. Both his and his mother's serum B12 vitamin levels were low (38 and 115 ng/L, respectively). Peripheral blood analysis indicated hypersegmented neutrophils, anisocytosis, and poikilocytosis. A peroral cyanocobalamin 1000 mcg/day supplementation therapy was initiated. Two days later, the tremor and rhythmic myoclonic movements in his feet were initially considered as seizures and antiepileptic treatment was started. Cranial magnetic resonance imaging

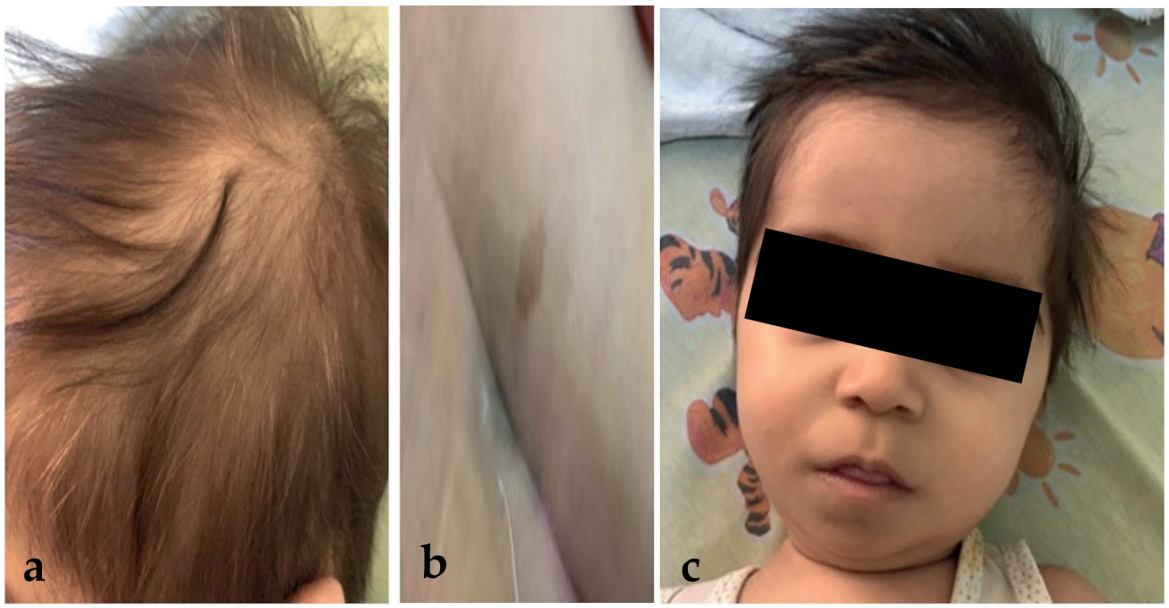


**Fig. 2.** Clinical image showing hypopigmented sparse hair, knuckle pigmentation, and apathy.

(MRI) visualized cerebral atrophy while EEG was normal. On the third day, a clonazepam therapy was added to the ongoing oral cyanocobalamin 1000 mcg/day replacement therapy. Two days later, the complaint of tremor decreased gradually and the myoclonus disappeared completely.

## Discussion

Vitamin B12 is an essential vitamin mostly ingested via animal products. Vitamin B12 deficiency can be seen at any age throughout life and remains a serious health problem both in Turkey and other developing countries. A recent study conducted in Turkey reported that the rates of maternal and infantile vitamin B12 deficiency were 76.7% and 60.8% during the first month post-delivery, respectively.[1] On the other hand, although vitamin B12 deficiency has been reported extensively, the clinical spectrum of abnormal movements due to infantile B12 deficiency has been shown to be remarkably wide.[9,10] ITS is characterized by paleness, growth retardation, tremor, hyperpigmentation, movement disorders, and sparse brown hair.[11] Common neurological complications associated with vitamin B12 deficiency include hypotonia, optic atrophy, myelopathy, and lethargy. Tremor and myoclonus can be associated with vitamin B12 deficiency and may recur suddenly and worryingly after the initiation of vitamin B12 supplementation therapy.[4,11-16] Nevertheless, the primary cause of abnormal movement that occurs after treatment is not fully known.

Chalouhi et al.[16] suggested that overstimulation of the cobalamin-folate interaction leads to a metabolic disequilibrium. Emery et al.[17] proposed that persistent hyperglycemia results in movement disorders during vitamin B12 treatment, and de Souza et al.[18] reported that there are multiple factors associated with the pathophysiology of the hypersensitization effect of vitamin B12 deficiency, primarily including endothelial dysfunction induced by excitatory amino acids and increased methyltetrahydrofolate and homocysteine levels.[16-18] Elevated homocysteine shows a similar effect at the N-methyl-Daspartate type of glutamate receptor and also activates the basal ganglia.[19,20] Following vitamin B12 therapy, infants may present with tremor, myoclonus, and even seizures in addition to neurological symptoms.[5] In such patients, EEG is often normal and is a significant source of information

in the differential diagnosis of seizure-like symptoms.[2,4,5] Various neuroradiological findings may be observed in cranial imaging in infants with vitamin B12 deficiency, namely including cortical atrophy, thinning of the corpus callosum, structural abnormalities, and retardation in myelination. Taşkesen et al.[21] showed that thinning of the corpus callosum was detected in 6 (40%), cortical atrophy in 5 (33.3%), ventricular dilatation in 3 (20%), large sylvian fissures in 5 (33.3%), hydrocephalus in 3 (20%), asymmetric large lateral ventricle in 2 (13.3%), and retardation and myelination in 2 (13.3%) patients while 4 infants had normal MRI findings. On the other hand, cranial imaging findings are highly useful in the differential diagnosis of patients that develop ITS following the initiation of vitamin B12 replacement therapy, particularly due to the risk of thromboembolism.[22] In such patients, myelination problems and basal ganglia lesions are commonly seen and cerebral atrophy is the most common and reversible problem.[15] In our report, both patients presented with hypotonia, neuromotor and growth retardation, cerebral atrophy, and microcephaly. Moreover, abnormal movements such as myoclonic and tremors were also observed in both cases following peroral vitamin B12 replacement therapy. These findings were initially confused with seizure-like activity although EEG examinations were normal in both patients. Moreover, both patients were detected with bilateral cerebral frontotemporal atrophy and enlarged subarachnoid spaces on cranial MRI.

Nutritional vitamin B12 deficiency is effectively treated with peroral cyanocobalamin 1000 mcg/mL (Dodex®).[23-25] Bahadir et al.[25] noted that the vitamin B12 levels did not decrease in children aged less than two years during the long term after treatment. On the other hand, Sezer et al.[23,24] found that the intramuscular and oral administration of cyanocobalamin had a similar effect, and this finding has been reported in several book chapters as well. In our clinic, vitamin B12 replacement therapy is routinely administered peroral due to the effectiveness and practicality of this approach.

Unless treated, infantile vitamin B12 deficiency may lead to irreversible problems. Almost 30% of cases of ITS may be accompanied by microcephaly and 75% of them may present with changes of pigmentation and sparse hair.[11] In addition to these symptoms, abnormal movement may also be seen following vitamin B12 replacement therapy, as seen in both of our patients. Although a consensus on the role of Vitamin B12 deficiency in children with ITS is likely to be developed, it is still debatable. Moreover, it remains unclear as to why these abnormal movements often manifest as tremor. In such cases, myoclonus may also occur as a result of increased receptor synthesis. To our knowledge, all the ITS cases reported in the literature occurred after intramuscular administration of drugs while only one case in the series of Tunçer et al.[12] occurred following peroral treatment.

In 2011, the Turkish Society of Hematology reported a guideline for B12 replacement therapy regardless of age and recommended an initial dose of 250-1000 μg/day B12 for the first week. The dose range is remarkably wide and the initial usage doses proposed in children vary across the studies in the literature. Sezer et al.[24] demonstrated the effectiveness of the oral dose in their study, in which the treatment of 10 out of 142 children started at a dose of 100 mcg/day and no side effect was observed. Yılmaz et al.[26] indicated that they converted their treatments to hydroxocobalamin as a result of the oral cyanocobalamin treatment which was initiated at a dose of 250 mcg with 24 infants aged 2-18 months. Although the reason for the impairment in the initial treatment of cases may be caused by the use of high-dose cyanocobalamin, Bahadir et al.[25] started a treatment of 1000 mcg cyanocobalamin in patients with a B12 level of <50 pg/ml and observed no side effects in any patient. Although it is recommended that low-dose treatment initiation is a controlled condition to prevent the possible side effects such as movement disorders and other possible side effects that may occur after B12 deficiency treatment, there is a need for studies to show

the likelihood of dose-related movement disorders in children. On the other hand, there is not a definite recommended dose for oral B12 replacement therapy particularly for infants.

In both of our patients, abnormal movements occurred following peroral vitamin B12 replacement therapy. Meaningfully, abnormal movements such as tremor and myoclonus may be observed during the B12 vitamin treatment or particularly due to vitamin B12 deficiency. We consider that more care should be taken for various movement disorders that may occur after the replacement therapy initiated in infants and their mothers with vitamin B12 deficiency. Knowledge of treatment options in such patients is of paramount importance since these cases are commonly encountered in clinical practice. Clonazepam 0.1 mg/kg/day is the mainstay treatment of ITS while propranolol 2 mg/kg/day, piracetam 4.8 g/day, and carbamazepine 10 mg/kg have been shown to be effective in ITS patients.[8,27]

Early diagnosis and treatment of vitamin B12 deficiency accompanied by growth retardation and macrocytic anemia are highly important for the prevention of infantile tremor. It should be noted that the infants of mothers with nutritional vitamin B12 deficiency may develop sudden abnormal movements following peroral vitamin B12 therapy and that the differential diagnosis of these disorders is highly important for the prevention of long-term neurological sequela by treatment. Moreover, extensive knowledge of the medications to be used in such patients is of paramount importance for the prevention of unnecessary drug use. Assessment and treatment of maternal vitamin B12 levels, especially in the last three months of pregnancy or after childbirth, is of vital importance for preventing myelination in the infants.

All the authors declare that they have no conflict of interests in this study. The study was not funded by any institution or establishment. A written consent was obtained from the parents of both patients.

**Author contribution**

Beril Dilber, and Gökçe Pınar Reis contributed to the medical care and diagnose for this study.

**Conflict of interest**

All the authors declare that they have no conflict of interests in this study.

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The Turkish Journal of Pediatrics 2021; 63: 516-521
https://doi.org/10.24953/turkjped.2021.03.021

Case Report

# Mild encephalopathy with reversible extensive white matter lesions in a child with acute adenoviral infection and a literature review

Mustafa Emre Akın[1], Ayşegül Neşe Çıtak Kurt[2], Gülsüm İclal Bayhan[3], Zeynep Dinçer Ezgü[4], Sevtap Şimşek Bulut[1]

*[1]Department of Radiology and Divisions of [2]Pediatric Neurology and [3]Pediatric Infection, [4]Department of Pediatrics, Ankara, Yıldırım Beyazıt University Faculty of Medicine, Yenimahalle Training and Research Hospital, Ankara, Turkey.*

## ABSTRACT

**Background.** Mild encephalopathy with a reversible splenial lesion (MERS) is a known clinical-radiological description. However, MERS with extensive lesions (MERS type-2) is rarely associated with adenovirus. There are only three published cases of MERS type-2 associated with adenovirus infection.

**Case.** We present a 10-year-old previously healthy girl who presented with speech difficulty and mild encephalopathy after three days of prodromal illness. The magnetic resonance imaging (MRI) revealed bilateral diffusion restriction in the parietal white matter, splenium and genu of the corpus callosum without mass effect and slight thickening at the splenium of corpus callosum with no contrast enhancement. With empirical and support treatment, her neurological examination was completely normal by the 18th hour. The nasopharyngeal respiratory adenoviral PCR resulted positive. She was discharged with total clinical and radiological resolution on the 10th day of admission. The case was diagnosed with MERS type-2 which is rarely associated with adenoviral infection.

**Conclusion.** This report is the first case of adenovirus related MERS type-2 in a Turkish child. Pediatricians, child neurologists, child infection specialists and radiologists should recognize this condition to ensure appropriate diagnosis.

**Key words:** encephalopathy, corpus callosum, adenovirus, child.

Mild encephalopathy with a reversible splenial lesion (MERS) is a clinic-radiological syndrome characterized by transient mild encephalopathy and various magnetic resonance imaging (MRI) findings including transient lesions in the splenium of the corpus callosum.[1] MERS has two radiological subtypes based on imaging findings. In MERS type-1 reversible lesions are limited to the splenium of corpus callosum whereas, in type-2, lesions extend to the splenium of the corpus callosum and might involve the entire corpus callosum, subcortical white matter near central sulcus.[2] The syndrome is characterized by a prodromal period of 1-7 days, followed by encephalopathy which may be accompanied by behavioral change, seizures and hallucinations. The syndrome mainly affects children and young adults and the prognosis is generally good with complete or nearly complete clinical resolution over days without treatment. Radiological recovery can last days to weeks.[3] The exact pathophysiology is unknown. Viral infections are found to be the most common associated etiological factor in childhood.[4] To the best of our knowledge, there are only three published cases of MERS type-2 associated with adenovirus infection.[5,6] We present here the first case of adenovirus related MERS type-2 in a Turkish child and a literature review on adenovirus related MERS.

✉ Mustafa Emre Akın
dremreakin@gmail.com

Received 15th April 2020, revised 8th July 2020, 26th July 2020, accepted 7th September 2020.

**Case Report**

A previously healthy 10-year-old girl was admitted to our hospital with slurred speech and confusion. She had had a fever for the last three days. She had not received any medication aside from acetaminophen and cefaclor for the last three days. She had no history of neurologic disorders or developmental delays. The family history was unremarkable and there was no consanguinity between parents. On admission, she had hyperemia in the oropharynx and neurological examination revealed lethargy in the following hours. There were no signs of focal neurologic deficit or meningeal irritation. Laboratory investigations revealed: hemoglobin 14.3 mg/dl, white blood cell count 6510/mm$^3$ and platelets 179000/mm$^3$; C reactive protein (CRP) 64,10 mg/L (0-5 mg/L), serum glucose level 98 mg/dl, Na 136 mmol/L, and K 3.4 mmol/L. Serum biochemical investigations were within normal range. There were no leukocytes on the microscopic examination of the cerebrospinal fluid (CSF). The concentration of CSF protein and glucose levels were 16.4 mg/dl (15-45 mg/dl) and 55.4 mg/dl respectively. On the day of admission, an emergency cranial computer tomography evaluation was unremarkable. The brain MRI revealed bilateral diffusion restriction in parietal white matter, splenium and genu of corpus callosum without mass effect and slight thickening at the splenium of the corpus callosum with no contrast enhancement (Fig. 1). The patient was hospitalized and given empirical oseltamivir and acyclovir treatment. The electroencephalography (EEG) was consistent with generalized intermittent slow wave activity. At the 18$^{th}$ hour of admission, the neurological exam was normal. The CSF bacterial culture and CSF herpes simplex virus-1 (HSV-1) and HSV-2 viral polymerase chain reaction (PCR) were negative. Tandem MS, serum and urine amino acid and organic acids, lactate and pyruvate levels were normal. The nasopharyngeal respiratory viral PCR resulted positive for adenovirus and negative for respiratory syncytial virus-A (RSV-A), RSV-B, rhinovirus, parainfluenza-1, parainfluenza-2, parainfluenza-3, parainfluenza-4 viruses, coronavirus, influenza A, influenza B, human metapneumovirus, human bocavirus, enterovirus, and parechovirus. On the 10$^{th}$ day of admission, the neurological examination, brain MRI examination (Fig. 2) and EEG were completely normal. The child was discharged with no sequela on the 10$^{th}$ day of admission. The clinical and radiological course of this case was discussed by a team composed of radiologists, pediatrician, child neurologist, and child infectious disease specialist and revealed the diagnosis of MERS type-2. The family provided informed consent for this report.

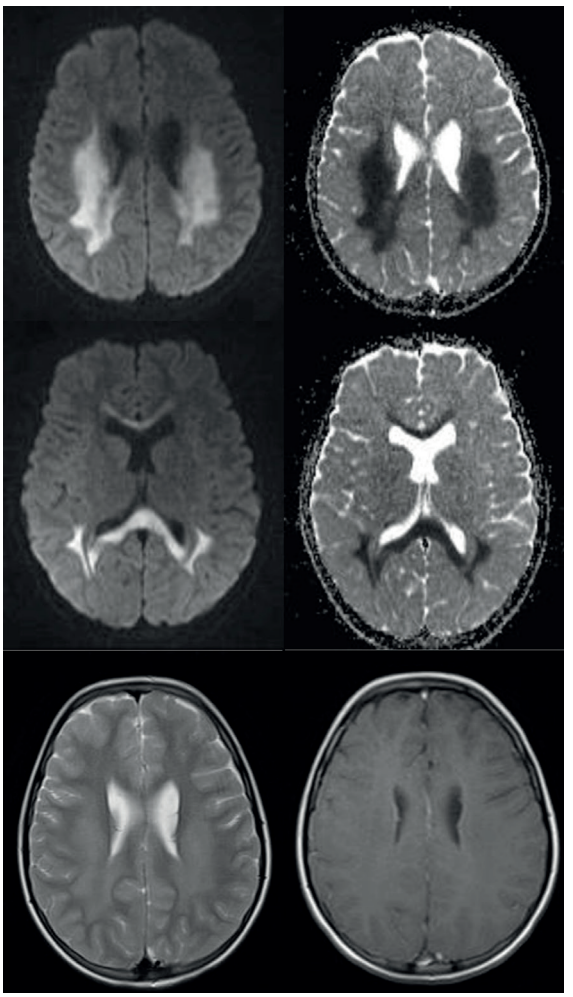

**Fig. 1.** Bilateral diffusion restriction in parietal white matter, splenium and genu of the corpus callosum without mass effect and slight thickening at the splenium of the corpus callosum at the admission.

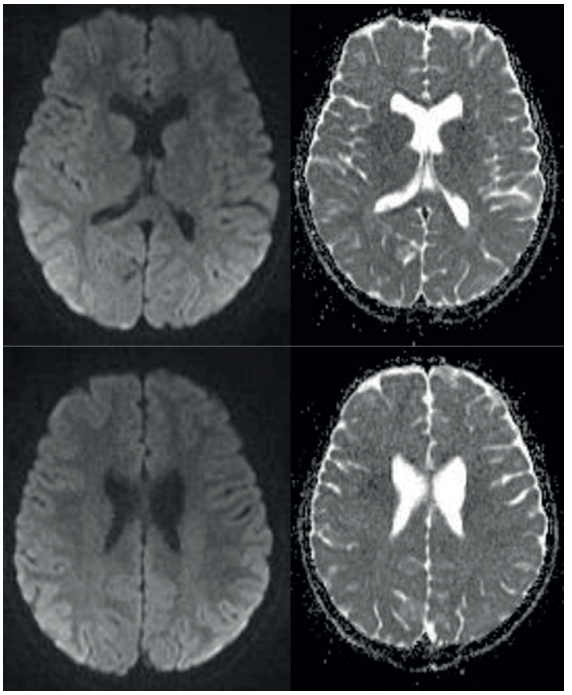

**Fig. 2.** Normal diffusion magnetic resonance imaging examination findings on the 10th day of admission.

## Discussion

This report is the first case of adenovirus related MERS type-2 in a Turkish child who had extensive symmetrical white matter involvement on MRI. Clinical findings had resolved in a matter of hours and were followed by radiological findings on the 10th day of admission.

In patients presenting with reversible splenial lesions, the diagnosis of MERS is established if the patient has an acute onset of impaired consciousness lasting for more than 12 hours, with no evidence of inflammatory changes in the cerebrospinal fluid.[7] Acute disseminated encephalomyelitis (ADEM) which is a demyelinating disease of the central nervous system that typically presents as a monophasic disorder associated with multifocal neurologic symptoms and encephalopathy, should be considered in the differential diagnosis.[8] Encephalopathy often develops rapidly and almost simultaneously with multifocal neurologic deficits after the onset of viral infections in matter of days to weeks while CSF analysis reveals mild pleocytosis. Although the involvement of the corpus callosum is frequent in ADEM, the distribution of the lesions is asymmetric contrary to the symmetric involvement seen in MERS. Resolution of white matter lesions may take weeks to months and require treatment with corticosteroids. The severe phase of ADEM typically lasts from two to four weeks. The patients' clinical condition may deteriorate and patients may also develop new neurologic signs during hospital admission.[8] Diffuse axonal injury (DAI) is an another condition in which the corpus callosum involvement is frequent and should be considered in the differential diagnosis of MERS. DAI describes a process of widespread axonal damage that is observed after traumatic brain injury.[9] The patients are usually comatosed at admission. DAI is pathologically defined by axonal damage in multiple regions of the white matter that often causes multiple neurological impairments. Brainstem, corpus callosum and subcortical white matter are the most common affected white matter regions.[9] The patient in this report developed symptoms soon after the onset of adenovirus with no trauma history and also quickly recovered completely without corticosteroids. CSF analysis revealed normal cell count. Therefore these findings are unlikely to be manifestations of ADEM or DAI. Other differential diagnoses of the white matter lesions including splenium of the corpus callosum involve, posterior reversible encephalopathy syndrome, ischemia, multiple sclerosis and lymphoma are excluded clinically and radiologically.[10]

The exact pathophysiology of MERS is unknown. Proposed mechanisms of MERS include intramyelinic axonal edema,[3] oxidative stress,[12] immune system activation,[11] elevated levels of IL-6 in which leads to antidiuretic hormone release, hyponatremia, systemic inflammation[13] and inflammatory infiltrates.[1] The etiology of MERS is summarized in Table I. In our case, there was no hypoglycemia and hyponatremia. The metabolic tests excluded most of the inherited metabolic diseases. Our

**Table I.** Etiology of mild encephalopathy with a reversible splenial lesion (MERS).

| Etiology of MERS* | |
|---|---|
| Infectious etiology | Non-infectious etiology |
| Viral | Metabolic diseases |
| Influenza virus (most common) | Hypoglycemia |
| Rotavirus | Hyponatremia |
| Measles | Antiepileptics |
| Herpesvirus-6 | Antiepileptic drug withdrawal |
| Epstein-Barr virus | High attitude cerebral edema |
| Varicella zoster virus | Malnutrition |
| Mumps | Anorexia nervosa |
| Cytomegalovirus | Charcot-Marie-Tooth disease |
| Adenovirus | Marchiafava-Bignami disease |
| Bacterial | B12 deficiency |
| Salmonella enteritidis | Systemic lupus eritematozus |
| Legionella pneumophila | Side effect of chemotherapeutics |
| Escherichia coli | Methyl bromide exposure |
| | Diet pills |

*Tada et al (2004), Garcia-Monco JC et al (2011), Karampatsas K et al (2015), Chen WX et al (2016), Feraco P et al (2018)

case had neither history of drug use other than antibiotics and acetaminophen nor a history of being at high altitude. Clinical and laboratory findings suggested that our patient has encephalopathy rather than encephalitis as the patient's CSF examination revealed no signs of inflammation. Hence the diagnosis of adenovirus associated MERS with extensive white matter involvement was established and supported by the resolution of clinical and radiological findings on the 10$^{th}$ day of admission. Although our case and most cases reported in the literature with viral etiology depends on nasopharyngeal swabs,[5,6,14] CSF adenoviral PCR would be very beneficial for our case to prove the etiology.

Human adenovirus is a ubiquitous, non-enveloped virus with linear double-stranded DNA and a known cause of febrile syndromes, mainly in early childhood.[17] Adenovirus infection rarely involves the central nervous system in immunocompetent children.[18]

We performed a PubMed and a Google search with the following terms: "mild encephalopathy with reversible splenial lesion", "reversible splenial lesion", "adenovirus" "child" and "children". A total of 4 publications, from 2010 to 2016, were included in the review. Chen et al reported 15 MERS cases, one of the cases was a child with adenovirus related MERS type-1.[14] Fang et al.[19] reported 29 children with MERS from China. This report included 2 children with adenovirus-related MERS. One of these children was diagnosed as MERS type-1 and positive for adenovirus, echovirus and coxachie virus. The second child was positive for only adenovirus and diagnosed as MERS type-2.[19] Imamura et al.[5] reported 2- and 6-year-old sisters with MERS type-2 from Japan, in one of the patients, adenovirus was isolated from a nasopharyngeal swab. Although the other patient's viral etiology could not be determined, the authors diagnosed clinically both patients as adenovirus related MERS type-2. Ka et al.[6] reported 7 children with MERS from Australia, of these 4 were MERS type-2 and one was associated with adenovirus. In total there have been only 3 reports identified which included adenovirus related MERS type-2 cases in the literature.[5,6,19]

The mechanism of the radiological findings in MERS is not established clearly. MERS

frequently involves splenium of the corpus callosum and seldomly extends to other corpus callosum parts and periventricular white matter which predicate subtype.[7,8,20] Restricted diffusion in white matter and corpus callosum is thought to be the results of intramyelinic edema, interstitial edema in tightly packed fibers and transient inflammatory infiltrate.[3,11] These lesions frequently involve only the splenium of the corpus callosum in reported cases and rarely periventricular white matter and other parts of the corpus callosum. In our case the MRI findings were consistent with type 2 MERS and the degree of white matter involvement was extensive even among the other reported MERS type-2 cases with various etiologies.[6,7,14] Ka et al.[6] reported no correlation between the extension of MRI findings and clinical symptoms or prognosis. Similarly, in our case, there was extensive white matter involvement in the MRI whereas, the child only had speech difficulties and lethargy which resolved in 18 hours.

In conclusion, MERS should be considered in the differential diagnosis of children presenting with neurological findings and reversible splenial lesions. Child neurologists, pediatricians, radiologists and child infectious disease specialists should keep in mind that MERS type-2 can be associated with different infectious agents including adenovirus.

### Author contribution

Authors confirm contribution to the paper as follows:

Mustafa Emre Akın analyzed and interpreted the patient data regarding the disease and drafted the manuscript. Ayşegül Neşe Çıtak Kurt performed the neurological examination, Gülsüm İclal Bayhan and Zeynep Dinçer Ezgü interpreted the infection markers, Mustafa Emre Akın and Sevtap Şimşek Bulut evaluated the MRI examination of the case, and all authors had major contributions in writing the manuscript. All authors read and approved the final manuscript.

### Conflict of interest

The authors declare no conflict of interests.

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The Turkish Journal of Pediatrics 2021; 63: 522-526
https://doi.org/10.24953/turkjped.2021.03.022

Case Report

# A case report of intracranial hypertension and aseptic meningitis: anti-tumor necrosis factor associated or juvenile idiopathic arthritis related

Ümmüşen Kaya Akça[1], Okan Sökmen[2], Ertuğrul Çağrı Bölek[3], Selcan Demir[1], Levent Kılıç[3], Işın Ünal Çevik[2], Yelda Bilginer[1]

*[1]Division of Pediatric Rheumatology,Department of Pediatrics, Departments of [2]Neurology and [3]Rheumatology, Hacettepe University Faculty of Medicine, Ankara, Turkey.*

## ABSTRACT

**Background.** The adverse effects of tumor necrosis factor alpha inhibitors (TNFi) are well characterized but rare adverse events are increasing day by day.

**Case.** We presented an 18-year-old girl with rheumatoid factor positive polyarticular juvenile idiopathic arthritis (JIA) who developed fever, headache, and nausea after the second dose of adalimumab. In addition to her suspicious complaints for meningitis, she had bilateral papilledema and partial abducens nerve palsy. Leptomeningeal contrast enhancement was noted in magnetic resonance imaging (MRI) of the brain. Brain MRI venography was normal. The cerebrospinal fluid (CSF) opening pressure was high but CSF analysis was normal. She was diagnosed with non-infectious subacute meningitis. Since brain biopsy was not performed, no definite distinction could be made between TNFi related aseptic meningitis or cerebral involvement of JIA. Due to the onset of neurological complaints after initiation of adalimumab treatment and rare cerebral involvement in JIA, the drug-associated aseptic meningitis was likely to be responsible in our patient. Adalimumab was discontinued and methylprednisolone followed by methotrexate treatment were initiated. Her symptoms resolved and control brain MRI was normal.

**Conclusion.** Pediatric rheumatologists should be aware of this potentially severe side effect of anti-TNF treatment.

**Key words:** aseptic meningitis, intracranial hypertension, tumor necrosis factor alpha inhibitors.

Tumor necrosis factor alpha inhibitors (TNFi) are used in many inflammatory diseases including juvenile idiopathic arthritis (JIA), rheumatoid arthritis, inflammatory bowel diseases, and psoriasis. Despite their clinical effectiveness, various adverse events have been reported with their increasing use. While demyelination is a well-known neurological adverse effects of TNFi, TNFi-associated aseptic meningitis (TAAM) is a rare and serious complication.[1,2] Booker et al.[3] reported a patient who was treated with etanercept for rheumatoid arthritis and presented with aseptic meningitis. A literature review reported 10 adult patients who used anti-TNF with different diagnoses and developed aseptic meningitis. Despite reported cases in adults, to our knowledge, aseptic meningitis has not been reported in children using anti-TNF therapy in the literature.

The mechanisms underlying TAAM have not been fully elucidated. Since the anti-TNF agents cannot cross the blood-brain barrier, the drug-related direct toxic effect is not considered.[4] It may be due to a delayed hypersensitivity reaction or peripheral inhibition of TNF alpha which may lead to upregulation of proinflammatory pathways in the brain.

✉ Yelda Bilginer
yeldabilginer@yahoo.com

Received 13th January 2020, revised 2nd June 2020, accepted 12th September 2020.

In our study, we presented a patient with rheumatoid factor positive JIA who most likely developed TNFi-related aseptic meningitis.

## Case Report

A 18-year-old girl has been followed up for 4 years with the diagnosis of rheumatoid factor positive polyarticular JIA. In the first 6 months of the disease, she had active arthritis on bilateral elbow, wrists, and ankle joints. She used non-steroidal anti-inflammatory drugs and methotrexate for ten months. Etanercept treatment was started due to disease activity (JADAS: 10) and was used for 1.5 years. In her follow-up, etanercept was switched to adalimumab (40 mg every 2 weeks) due to persistent disease activity (JADAS: 16). After the second dose of adalimumab, she complained of progressive dull, throbbing type headache, aggravated by Valsalva maneuvers, lying down, worse at night, accompanied by photophobia, phonophobia, and blurred vision. Headaches had become constant for the last 10 days and headache severity was 9/10 (VRS: verbal rating scale). She also reported fever which started after second dose of adalimumab, lasted for one day, and reached to 38.7°C. She had arthralgia and swelling in the right knee and left ankle joint. She did not use any other medication except adalimumab. On physical examination, her blood pressure was 110/70 mmHg (<50th percentile) and fever was 37.8°C. On neurological examination, there were no signs of meningeal irritation or nuchal rigidity. Bilateral papilledema and partial abducens palsy were noted. Motor, sensory, reflex, cerebellar, and extrapyramidal system examinations were normal. She had active arthritis in the right knee and left ankle joint. Examination of other systems were unremarkable. Laboratory test results revealed normal leukocyte count (7,100/mm$^3$ [4.1-11.2]) and blood biochemistry, increased erythrocyte sedimentation rate (73 mm/hour [0-25]) and C-reactive protein levels (12.3 mg/dl [0-0.8]). In contrast enhanced brain magnetic resonance imaging (MRI), leptomeningeal enhancement was identified (Fig. 1). The cerebral venous sinuses were normal on brain MRI venography. Lumbar puncture was performed. The cerebrospinal fluid (CSF) appearance was clear however the opening pressure was high (250 mm). The

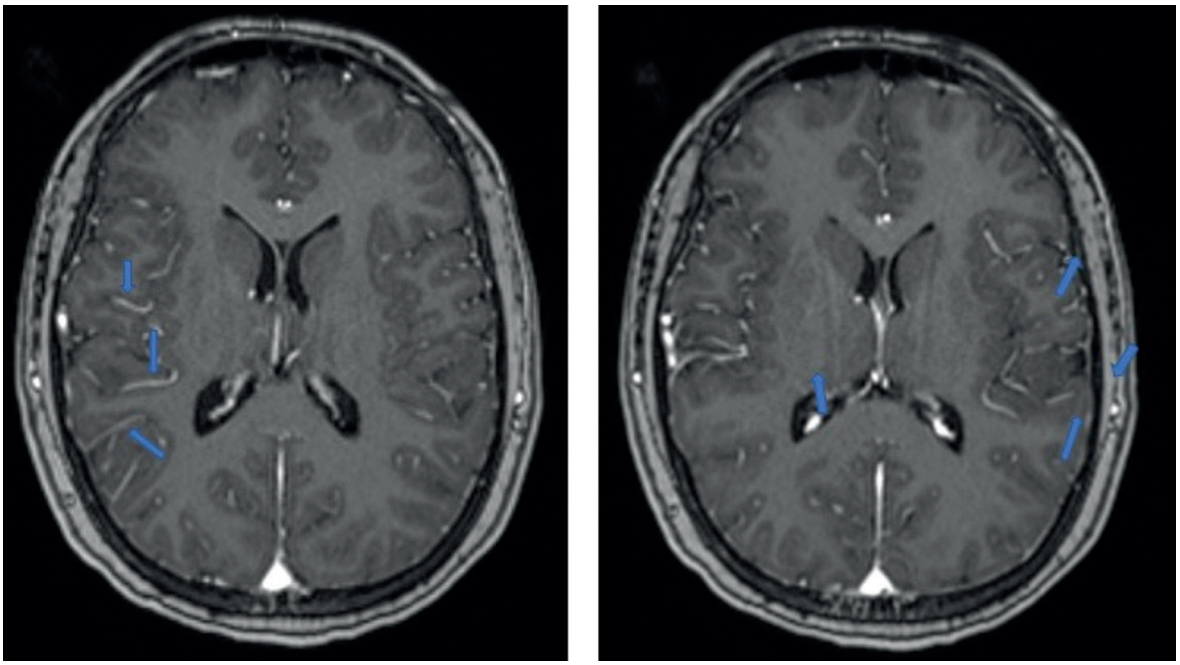

**Fig. 1.** Leptomeningeal enhancement on contrast enhanced brain magnetic resonance imaging (arrows).

CSF protein level was normal (20 mg/dl). The CSF glucose level was 70 mg/dl and blood glucose level was 91 mg/dl. On microscopic examination, few white blood cells were noted. CSF cultures were negative. CSF antibodies against infectious agents (measles, toxoplasma, rubella, cytomegalovirus, Epstein-Barr virus, mumps virus, herpes simplex virus, borrelia, brucella, varicella zoster virus, cryptococcus, and Treponema pallidum) were all negative. She was diagnosed with non-infectious subacute aseptic meningitis. Adalimumab treatment was discontinued and methylprednisolone (48 mg/day) treatment was started. Acetazolamide (1000 mg/day) and topiramate (100 mg/day) for intracranial hypertension and methotrexate (15 mg/week) for polyarticular JIA were initiated in the follow up. Clinical signs of arthritis improved on the 6th day of treatment. At the end of the first month, acute phase reactants were completely normalized. One month after discharge, the patient was free of headache.

Informed consent was received from the patient and her parents for this report.

## Discussion

To our knowledge, this is the first reported case of a child with rheumatologic disease with possibly anti-TNF related aseptic meningitis and intracranial hypertension. Aseptic meningitis has been reported in adult patients receiving TNFi as an uncommon adverse reaction. Case reports of aseptic meningitis associated with adalimumab, etanercept and infliximab treatment in patients with rheumatoid arthritis have been published.[3,6-8] In addition to rheumatoid arthritis, TAAM has also been reported in patients with psoriatic arthritis, inflammatory bowel disease and psoriasis.[9-11] Simultaneously, our patient had intracranial hypertension. The clinical characteristics of intracranial hypertension were the presence of headache, pulsatile tinnitus, transient visual obscurations, 6th cranial palsy and papilledema. The three main principles of treatment were to treat the underlying disease, to protect the vision and to minimize the headache.[12] Acetazolamide and topiramate treatments were initiated to resolve headaches and to reduce intracranial pressure.

Patients taking biologic agents are at higher risk for infections. Meningitis especially related to *Listeria monocytogenes* infection have been reported in patients treated with anti-TNF drugs.[13] However, it is not easy to distinguish whether it is drug-related or co-incidental. Aseptic meningitis should be considered in patients with meningeal symptoms when CSF culture results are negative. Our patient was also diagnosed with aseptic meningitis due to meningeal symptoms, leptomeningeal enhancement, and lack of identifiable microorganism in the CSF. Although high leukocyte count, normal glucose, and elevated protein CSF levels are usually observed in patients with aseptic meningitis, Matsuura-Otsuki et al.[14] reported normal CSF findings of a patient with psoriatic arthritis who developed infliximab-associated aseptic meningitis.[15] Pashankar et al.[16] also found normal CSF results in a child that presented with aseptic meningitis after co-trimoxazole treatment. It has been reported that many drugs lead to aseptic meningitis. Nonsteroidal anti-inflammatory drugs, antimicrobials, intravenous immunoglobulin, intrathecal agents and vaccines are the biggest culprit agents.[17]

There have been rare case reports of cerebral involvement in juvenile idiopathic arthritis. Jan et al.[18] reported in 1972 that 13 of 170 patients with juvenile idiopathic arthritis had signs of CNS dysfunction. They found EEG abnormalities during the acute toxic stages in many cases and other manifestations of toxic encephalopathy such as irritability, drowsiness, stupor, convulsions, and marked meningismus in severe cases. However, they ruled out meningitis with normal CSF findings. In adults, rheumatoid meningitis, a form of aseptic meningitis, is a rare well known extraarticular manifestations of rheumatoid arthritis. Rheumatoid meningitis has been reported in patients with a long-standing history

of seropositive RA, albeit rare.[19] It usually develops in long-term follow-up. Rheumatoid meningitis has been proven with brain biopsy in some cases. A necrotizing granulomatous meningitis which is identical for rheumatoid nodules has also been shown.[20] Our patient had rheumatoid factor positive JIA which has been considered as the early onset of adult RA. Since the onset of neurological complaints occurred after adalimumab treatment, drug induced aseptic meningitis was likely to be responsible for our case. Biopsy which was the diagnostic limitation in our patient was necessary for the precise separation of these two diagnoses.

Discontinuation of the drug responsible for drug-related aseptic meningitis is the basis of treatment. It was reported that systemic steroid treatment can be used in drug induced aseptic meningitis.[21] The time to resolution might take place until 21 days after discontinuation of the drug.[4] We also discontinued adalimumab treatment and began methylprednisolone treatment. Clinical recovery occurred within two weeks after drug cessation.

In conclusion, we presented a child with intracranial hypertension and aseptic meningitis possibly induced by adalimumab treatment. It is important for pediatric rheumatologists to be aware of this potentially severe side effect of TNFi treatment. It may be necessary to perform a more detailed examination in patients using biological agents who present with neurological symptoms.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Ümmüşen Kaya Akça, Yelda Bilginer; data collection: Ümmüşen Kaya Akça, Okan Sökmen, Ertuğrul Çağrı Bölek, Selcan Demir; analysis and interpretation of results: Levent Kılıç, Yelda Bilginer, Işıl Ünal Çevik; draft manuscript preparation: Yelda Bilginer. 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 regarding the publication of this article.

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The Turkish Journal of Pediatrics 2021; 63: 527-532
https://doi.org/10.24953/turkjped.2021.03.023

Case Report

# Vogt Koyanagi Harada syndrome in a 15-year-old girl, steroids side effects and recurrences

Gamze Yıldırım, Cemal Çavdarlı, Emine Yıldız Özdemir, Mehmet Numan Alp

*Department of Ophthalmology, Niğde Ömer Halisdemir Education and Research Hospital, Niğde, Turkey.*

## ABSTRACT

**Background.** Vogt-Koyanagi-Harada Syndrome is rare in childhood and is usually seen between the 2nd and 5th decades. We present a 15-year-old girl with findings of incomplete Vogt-Koyanagi-Harada Syndrome.

**Case.** In the first visit, anterior chamber inflammation, vitritis, serous retinal detachment and papillitis were observed in her both eyes. She also had neurological symptoms such as a headache. During the systemic treatment period, some of the side effects related to steroids emerged. Additionally, the symptoms and findings of the disease relapsed while the steroid dose was reduced.

**Conclusion.** Early diagnosis and selection of an individualized appropriate treatment provided good clinical and visual results without any serious complications in our case.

**Key words:** Vogt-Koyanagi-Harada syndrome, steroid side effects, panuveitis, immunosuppressive agents.

Vogt-Koyanagi-Harada Syndrome (VKHS) is an autoimmune disease characterized by idiopathic, chronic and diffuse granulomatous uveitis. The syndrome also includes neurological, auditory and dermatological symptoms. The most common ocular findings of VKHS are iridocyclitis, vitritis, optic disc edema, and exudative retinal detachments.[1] Although VKHS is rare in childhood and usually emerges between 20 and 50 years; the disease can be a potential cause of blindness as a result of uveitis in children. Major sequelae that led to visual impairment include cataracts, glaucoma, retinal pigment epithelial changes, choroid neovascular membranes, and the scars of retina-choroiditis.[1,2] The ocular complications are more severe in children than in adults. Thus, early and aggressive therapy with corticosteroids and / or other immunosuppressive agents have been involved in the treatment of children with VKHS.[3] Clinical features of VKH disease have been identified well in adults, however, only case reports exist for children (Table I). We report a 15-year-old girl who presented with relapsed VKHS while the systemic steroid therapy was reduced for sparing from the side effects of steroids.

✉ Gamze Yıldırım
gmz_y_06@hotmail.com

Received 8th April 2020, revised 7th July 2020, 19th July 2020, 6th September 2020, accepted 16th October 2020.

## Case Report

A 15-year-old girl presented to our clinic with decreased vision in both eyes. In her anamnesis, she had a headache that had not responded to analgesics (paracetamol and ibuprofen, respectively) and continued for two days before one week of admission, and additionally, her vision decreased progressively two days before the admission. The patient underwent complete ophthalmologic examination including biomicroscopy, tonometry, and indirect ophthalmoscopy. Fundus fluorescein angiography (FFA) and ultrasonography were also performed. In the patient's examination, the best-corrected visual acuity (BCVA) was 20/100, bilaterally, according to the Snellen chart and intraocular pressure was 14mmHg in the right and 16 mmHg in the left eye, respectively.

**Table I.** Summary of the previous published reports of Vogt-Koyanagi-Harada syndrome (VKHS) in children in the last 10 years.

| Reference | Age | Sex | Clinical findings | Treatment |
|---|---|---|---|---|
| AlQahtani et al.[4] | 4 | M | Band keratopathy, anterior uveitis, posterior synechiae, iris atrophy, cataract, vitiligo and polisosis | Corticosteroid Cyclosporine Adalimumab Methotrexate |
| Katsuyama et al.[5] | 3 | F | Posterior synechiae, optic disk swelling, serous retinal detachment | Corticosteroid |
| Khan et al.[6] | 16 | F | Serous retinal detachment | Corticosteroid |
| Takada et al.[7] | 3 | M | Ciliary hyperemia, slight corneal oedema, keratic precipitates, serous retinal detachment, aseptic meningitis | Corticosteroid |
| Bilgiç et al.[8] | 14 | M | Pale optic disc, retinal pigment atrophy, vitiligo | - |
| Vergaro et al.[9] | 12 | M | Retinal vasculitis, retrocochlear hearing loss, aseptic meningitis, cerebral ischemia | Corticosteroid |
| Bušányová et al.[10] | 11 | M | Granulomatous panuveitis, exsudative retinal detachment | Corticosteroid |
| Hernandez et al.[11] | 6 | M | Cataract, panuveitis, optic nerve head edema, exudative retinal detachment, vitiligo | Methotrexate |
| Venaille et al.[12] | 10 | M | Anterior uveitis, optic disk swelling, serous retinal detachment, aseptic meningitis alopecia, vitiligo | Corticosteroid Cyclosporine Mycophenolate mofetil |
| Benfdil et al.[13] | 14 | F | Uveitis, serous retinal detachment, choroidal depigmentation, poliosis | Corticosteroid |

Biomicroscopic examination revealed granulomatous anterior chamber reaction and vitritis in both eyes (Fig. 1). Additionally, the fundus examination revealed bilateral exudative retinal detachment in the posterior pole and hyperemic optic discs (Fig. 2). After the complementation of the clinical examination, multimodal-imaging was performed. Bilateral serous retinal detachment was also observed in the enhanced depth imaging of spectral domain optical coherence tomography (EDI-SD-OCT) scans (Fig. 3a). Subfoveal choroidal thickness of both eyes was measured in the first visit (401 μ, 412 μ in the right eye and the left eye, respectively). FFA showed a starry sky appearance which is specific for VKHS (Fig. 4). Ocular B-scan ultrasonography showed serous retinal detachment and no scleritis (Fig. 2). Body temperature was 36.6 ° C. Hemogram, sedimentation rate, C- Reactive Protein (CRP), and all of the routine biochemical values were within normal limits. The rheumatoid factor (RF), antinuclear antibody (ANA), anti-ds-DNA, Anti Smooth Muscle Antibody (ASMA) and Extractable Nuclear Antigen (ENA) profiles were negative. Treponema pallidum hemagglutination test and serous immunoglobulin M of varicella-zoster, herpes simplex and cytomegalovirus viruses were all negative. Lyme antibody test and brucella agglutination test were negative. Tuberculin skin test was negative, and her Enzyme-Linked

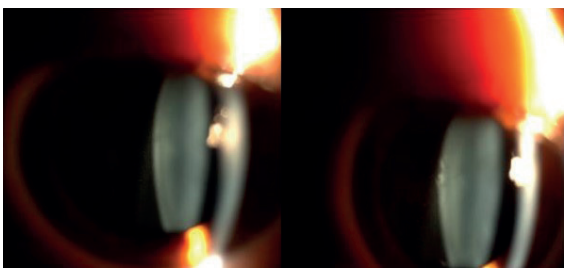

**Fig. 1.** Anterior segment images at the first visit: inflammatory cells in the anterior vitreous were observed in both eyes.

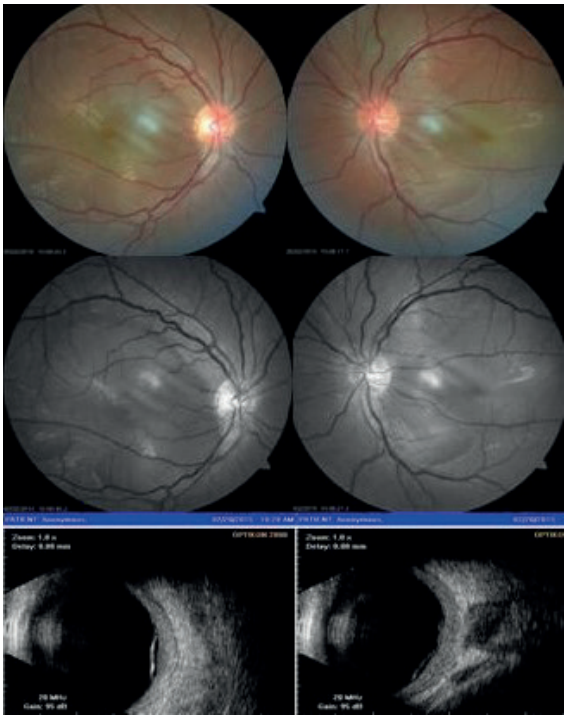

**Fig. 2.** Color photographs of the fundus and ocular B-scan ultrasonography images at the first visit.

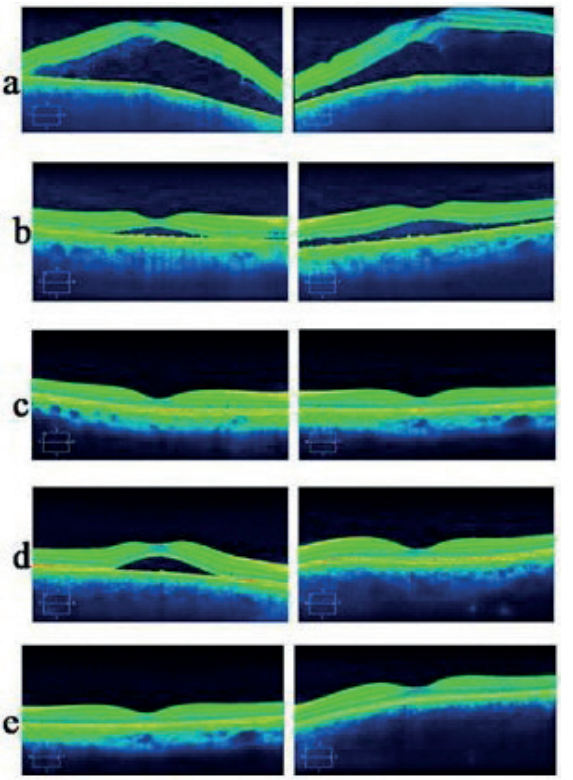


**Fig. 3.** a-OCT images at the first visit: Serous retinal detachment in both eyes. b-OCT images at the seventh day of steroid treatment: OCT images showed decreased subretinal fluid in both eyes. c-OCT images at first month follow-up visit: Subretinal fluid was lost in OCT images of both eyes. d-OCT images at second month follow-up visit: Subretinal fluid was seen again in OCT image of the right eye. e-OCT images at follow-up visit two weeks after low dose azathioprine treatment: Subretinal fluid was lost in the right eye.

Immunosorbent Assay (ELISA) of HIV was negative. Serum angiotensin-converting enzyme level was normal. Pathergy test was negative. Aseptic meningitis was reported as a result of neurology consultation. The patient had no tinnitus or hearing loss, and all audiology tests were normal. There was no pathological finding in the dermatology consultation. No pathology was observed on the chest X-ray.

The patient was hospitalized with a systemic and topical (for the inflammatory findings in the anterior chamber and vitreous) steroid treatment plan. After intravenous steroid treatment (methylprednisolone 1,000 mg for 3 days), oral steroid (prednisolone 1 mg / kg) was continued. On the seventh day of the treatment, BCVA increased to 20/40 bilaterally, and her intraocular pressures were within normal limits (14 to 20 mmHg). Bilateral OCT images revealed decreased subretinal fluid (Fig. 3b). Bilateral BCVA of the patient was 20/20 at the control visit of the first month while she was under a 0.5 mg/kg oral prednisolone treatment. Furthermore, the SD-OCT image showed the disappearance of the subretinal fluid in both of the eyes (Fig. 3c). However, the patient had complaints and findings of weight gain and the onset of Cushingoid-like fat tissue distribution, thus, she was consulted to the department of endocrinology. The endocrinology department planned a gradual reduction and termination of the oral steroid treatment. The steroid treatment was gradually decreased. While she was at the dose of 8 mg/day at the follow-up visit in the second month, the patient's visual complaints emerged again, and her BCVA was measured as 20/25 in the right, and 20/20 in the left eye. SD-OCT image of the right eye

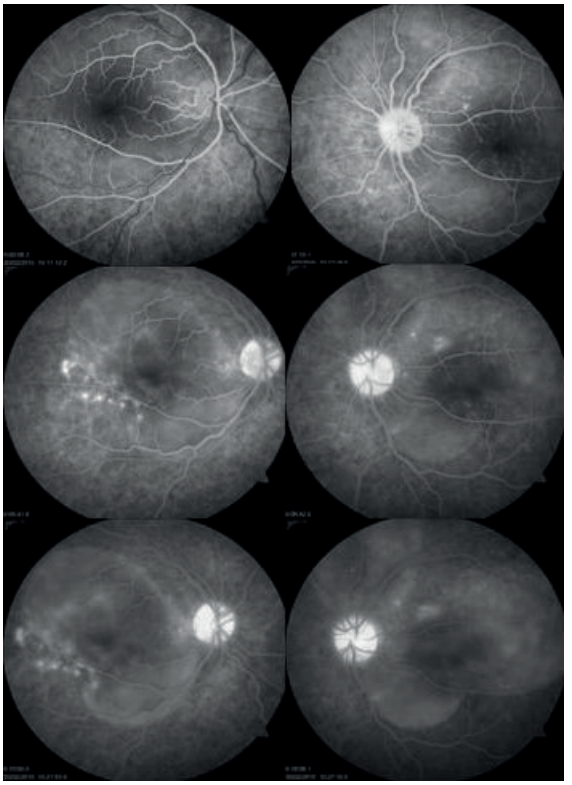

**Fig. 4.** Fundus fluorescein angiography (FFA) images at the first visit: FFA demonstrated serous retinal detachment of posterior pole and papillitis in both eyes.

showed subretinal fluid again (Fig. 3d). The patient was re-consulted to the department of pediatrics, and after monitoring her hemogram and biochemistry results, the current treatment was continued with a low-dose steroid (prednisolone 4 mg/day) and 50 mg (1mg/kg/day) azathioprine. At the follow-up visit, which was two weeks after the last visit, bilateral BCVA was 20/20, and the subretinal fluid disappeared again on the SD-OCT image of the right eye (Fig. 3e). Subfoveal choroidal thickness of both eyes was measured again and there was thinning in the choroidal thickness of both eyes according to the first visit (315 μ, 311 μ in the right eye and the left eye, respectively). Her oral steroid dose was completely stopped and she had no complaints and residual findings of the side effects of steroids. The patient was treated with only low-dose azathioprine (50mg/day) during the rest of the first year and no recurrence was observed in the last 3 years.

An informed consent form was obtained from the patient and her family for the publication of this case.

## Discussion

The etiology of VKHS is still unknown, although an immune response against melanocytes has been held responsible for the pathogenesis of the disease. The immune response to melanocytes in the uveal system results in bilateral panuveitis, optic disc hyperemia and serous retinal detachment.[14] There is no specific diagnostic method for VKH disease. The diagnosis of the disease is made after the exclusion of other causes of uveitis. Therefore, VKHS is diagnosed using established diagnostic criteria, based on clinical findings.[2]

The differential diagnosis includes infectious agents such as syphilis, tuberculosis, toxoplasma, AIDS; systemic inflammatory diseases such as Behçet's disease, systemic lupus erythematosus, rheumatoid arthritis, Wegener's granulomatosis, ulcerative colitis and sarcoidosis; ocular diseases such as sympathetic ophthalmia, intraocular lymphoma, central serous chorioretinopathy, posterior scleritis, uveal effusion syndrome, malignant hypertensive retinopathy.[15] Additionally, starry sky appearance is one of the characteristic features of VKHS, however starry sky appearance on fluorescein angiography can be seen in numerous conditions, including sympathetic ophthalmia, central serous chorioretinopathy, posterior scleritis, leukemia, DIC, malignant hypertension. Juvenile rheumatoid arthritis is the most common etiology of pediatric anterior uveitis and disease associated with ANA.[16] Clinical presentation of VKH may mimic sympathetic ophthalmia but there is no history of ocular trauma or ocular surgery in patients with VKHS.[17] Serum angiotensin converting enzyme level, a chest radiograph can aid in the differential diagnosis for sarcoidosis. In addition, retinal vascular findings in Behçet's disease and sarcoidosis are more evident.[15] The RPE sub-infiltrates

seen in B cell lymphoma are much larger and fewer than the yellow infiltrates seen in VKHS.[15] Ultrasonography can help differentiate VKHS from posterior scleritis.[18] Patients with idiopathic uveal effusion syndrome do not have intraocular inflammation; and exudative retinal detachments tend to be chronic and they are resistant to medical therapy such as corticosteroids or antimetabolites.[19]

Our patient had no history of penetrating ocular trauma or surgery before the onset of the first uveitis. In our patient's laboratory tests and imaging methods, no findings or test results were found for diseases above stated for differential diagnosis. In our case, early phase findings of the disease were bilaterally present and also had neurological findings. Therefore, according to the revised diagnostic criteria of VKHS; our patient was defined as incomplete VKHS.[20] If our patient had symptoms of alopecia, poliosis or vitiligo, we could describe the disease as complete, but these findings were not observed in the last 3 years. We can associate the absence of these symptoms with early diagnosis and correct treatment.

Multiple treatment methods have been tried for VKHS, including corticosteroid, cyclosporine, antimetabolites, and alkylating agents.[21] Insufficient immunosuppressive therapy will cause relapses in the disease and the disease will tend to become chronic. Early and high-dose systemic corticosteroids are used to suppress inflammation in the acute stage of the disease. High-dose corticosteroids are associated with side effects such as hyperglycemia, hypertension, immunodeficiency, weight gain, cushing syndrome and secondary glaucoma.[22] In the literature; due to side effects or in resistant cases, agents such as cyclosporine, azathioprine, adalimumab, methotrexate, mycophenolate have been added to the treatment (Table I).

Azathioprine, is usually used in the treatment of immunological diseases such as inflammatory bowel disease (IBD) and autoimmune hepatitis in children.[23] In the literature; it has been reported that azathioprine is combined with steroids in the treatment of children with VKHS.[24] We used low dose steroids (4 mg/day) and systemic azathioprine (50 mg/day) to overcome side effects and relapses. Two weeks after the indicated treatment, steroid treatment was discontinued. Azathioprine has been shown to be effective by some authors in patients with corticosteroid intolerance.[25] Azathioprine can cause some serious side effects, such as myelosuppression and gastrointestinal disorders. However, these side effects are unusual with low dose use (1mg/kg/day) compared to high doses.[22] During the first 3 years of follow-up, we did not observe any recurrence, sunset glow fundus appearance and dermatological problems. These findings are consistent with previous literature, which concluded that VHS patients who were diagnosed and treated immediately did not develop dermatologic lesions.[26]

Finally, VKHS is a disease rarely presented in young patients, and it can be misdiagnosed due to its rarity. Because of the major complications of the disease that threaten the ocular vision, patients should be treated as soon as possible with corticosteroids and/or immunosuppressive drugs. In conclusion, early diagnosis and selection of timely and appropriate treatment protocol provided a good visual prognosis without serious complications in the patient. Further case series and randomized-controlled studies with large sample sizes are needed to enlighten the other probable effective therapies or their side effects also in the young VKHSs.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

## Author contribution

Study conception and design: Gamze Yıldırım, Cemal Çavdarlı; data collection: Mehmet Numan Alp; analysis and interpretation of results: Emine Yıldız Özdemir; draft manuscript preparation: Gamze Yıldırım.

All authors reviewed the results and approved the final version of the manuscript.

## Conflicts of interest

There are no conflicts of interest.

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The Turkish Journal of Pediatrics 2021; 63: 533-537
https://doi.org/10.24953/turkjped.2021.03.024

Short Communication

# The role of culture on parenting boys as a potential risk factor in the development of male eating disorders during adolescence in Turkey

Nuray Kanbur[1], Sinem Akgül[1], Devrim Akdemir[2]

*[1]Division of Adolescent Medicine, Department of Pediatrics and [2]Department of Child and Adolescent Psychiatry, Hacettepe University Faculty of Medicine, Ankara, Turkey.*

The eating disorder (ED) field has made significant progress in successfully translating research on risk factors for the development of EDs into preventive interventions with documented efficacy in reducing ED symptomatology, as well as the future onset of EDs.[1] However, as the etiologic underpinnings of ED vary in male adolescents as compared to female adolescents this remains an area to be investigated and has been a hot topic in the recent literature.[2] This paper provides an international perspective for the role of culture on parenting boys as an etiological factor for the development of male EDs during adolescence.

Despite the fact that of all psychiatric illnesses eating disorders are the most gendered; recent evidence suggests that males comprise approximately 1 in 4 presentations of bulimia nervosa (BN) and anorexia nervosa (AN).[3] It is likely that due to a female-oriented conceptual and diagnostic framework being used, the prevalence rates of traditional male EDs in adolescents cited in most previous research is underestimated.[3] However, consideration of the age of ED onset among males is important for assessing the etiology and type of ED. The body type that is mostly idealized and internalized among young adult males centers on muscularity. The widespread screening and assessment strategy for thinness-oriented ED does not take into account differences in body image among males.[4] On the other hand, in younger patients with the inclusion of avoidant/restrictive food intake disorder (ARFID) as an ED in the DSM-5[5] specialty ED clinics have reported preadolescent male presentation of ARFID to comprise more than one-third of ARFID cases. Across all ages, sexual minority status may be a contributing risk factor for male ED. Further, particularly in adolescent males, the rates of psychiatric and/or medical co-morbidity were reported very high.[6,7]

Given the multiple clinical presentations of male EDs, it is inevitable that any single risk factor is likely to have a complex relationship with eventual ED presentation, in interaction with multiple other factors. However, at the Adolescent Medicine and Adolescent Psychiatry Clinics of Hacettepe University Children's Hospital in Turkey, an increasing number of male adolescents with EDs were noted to have mothers with specific parenting behaviors.[7] While the role of parenting style in the development of disordered eating behaviors and EDs has been reported[8,9], little information about the impact of culture on parenting behaviors is available and the mother-son relationship represents a potentially promising area for further ED research, especially when mothers favor sons over daughters as a cultural phenomenon.

During the past year (January 1st-December 31st, 2020), the number of newly diagnosed eating disorder cases that have applied to the Adolescent Medicine Clinic was 42 (39 female and 3 male adolescents). The male/female ratio was 1/13. Although it would be very

✉ Nuray Kanbur
nuraykanbur@hotmail.com

Received 29th December 2020, revised 12th January 2021, 21st January 2021, accepted 21st January 2021.

informative to conduct studies looking at the impact of culture on parenting behaviors as a risk factor for EDs among adolescent males, the small number of male cases seen in our clinics has made this unachievable.

Studies have shown that the male child has a unique status for Turkish families. Recently, it was reported that most adult Turkish children perceived that their mothers favored sons because of higher filial expectations from sons.[10] Norms of filial responsibilities have been very prominent in Turkish society and expectations are also gendered that there is an understanding of sons as the "old-age insurance" in Turkish society.[11] Expectancies concerning economic assistance and security and the fact that sons have 'the privilege' of carrying on the family name has led to a preference for sons over daughters. Accordingly, it is very common that mothers favor boys over girls in Turkey and mothers are more responsible in the upbringing of a child than fathers.[10]

In recent years, we have followed a small handful, albeit dramatic cases of male EDs with a multidisciplinary team of Adolescent Medicine and Child and Adolescent Psychiatry. Some of the ED cases of male adolescents in our clinic were evaluated to be the results of certain parenting styles of their mothers in our culture such as over-valuing the male child and being more controlling over them.[7] In adolescence, the adolescent-parent relationship needs to change with the young individual's efforts to be more independent, but on the other hand, the supportive emotional bond with the parents is also important.[12] However, the aforementioned parenting style restrains the psychological autonomy of male adolescents, leading to problems with separation and individuation as in most female ED cases.[13] Our clinical implication is that an important factor for developing an ED specific to the adolescent age group in males could be the cultural differences in parenting boys in different populations. While sexual minority status may be the foremost risk factor for male ED during adolescence in most western cultures,[14] it does not seem to be the most frequent risk factor in our clinic though we still have cases of sexual minority individuals.

The psychometric analyses of Parental Bonding Instrument (PBI) with discrepant results in different cultures provide evidence that parenting styles vary grossly in Western and Eastern cultures.[15] The PBI[16] is a widely-used assessment tool for measuring parental characteristics that affect the parent-child relationship and was initially developed for Western populations where it is best represented either by a two-factor model (care and overprotection) or a three-factor model (care, overprotection, and autonomy).[17] Studies with PBI from Eastern cultures (i.e. Japanese, Chinese) supported a four-factor model that included an "indifference factor" which was very different from those among Western populations. The indifference factor reflected aspects of parenting specific to Eastern cultures, which tend to value group cohesion over individualization and independence.[15,18] The four-factor model may apply to most Eastern cultures, but yet more research is needed to generalize.

The relationship between culture and parenting is very interesting. Child-rearing behaviors are culturally determined to an extent. Cultural values can affect parenting behaviors which alter the quality of the relationship between mother and child. Although the maternal sensitivity is crucial for the development of a good relationship, expression of this sensitivity in different cultures may vary according to the emphasis on individuation or dependency in that culture.[12] Thus, discrepancies in parenting behaviors across Western and Eastern cultures are not necessarily surprising. While Western cultures traditionally highlight individuality and privacy, Eastern cultures tend to foster collectiveness and view the family as a singular unit.[16] Eastern cultures may view parental control as caring, whereas Western populations would likely view this negatively. An item classified as reflecting overprotection in one culture may be interpreted as reflecting care

in another. E.g. in the Turkish validation study of PBI, the items related to the controlling behaviors are loaded on the care factor instead of the overprotection by a two-factor model ("care/control" and overprotection subscales).[19] Autonomy is not seen as a strong construct in parenting in Eastern cultures as it is in Western ones. However, although authors claim that Eastern and Western cultural differences clearly exist, there may also be other factors specific to various cultures and even transitioning between western and eastern cultures in regards to parenting. With this point of view, it may be more accurate to call some cultures like Turkey as "non-western" instead of eastern.

In Turkey, maternal overprotection of sons and mothers favoring sons over daughters are not new concepts though these greatly differ from urban to rural areas or depending on the region of the family descent. In Turkey, it was reported that disordered eating behaviors increased among adolescent males perceiving parental lack of concern, overprotectiveness or prohibitive control of autonomy.[20] We do not have enough evidence to discuss this as a cause of ED but the clinical implication we do have is that parenting styles of mothers favoring sons over daughters and mother–son relationships are strong correlates of the symptoms of male ED in this age group in our clinical sample though not large. A striking example of this was a 14 years old male patient with AN-restrictive type who was the fourth child to be born after three girls, he proudly explained to us that 'to compensate for this', his parents gave him three names instead of one. Even though he was a teenager, he still co-slept with his mother. When his mother was questioned as to why, she stated that 'she did not want him to get cold at night' and would awake to wrap him up. When the decision was made to admit this patient to the hospital his mother plead with us to help her "only child" ignoring the fact that she had three elder daughters.[7]

Another phenomenon is that the overprotection of mothers might have contributed to enmeshment and restricted boys' needs for being autonomous and developing a healthy sense of self.[21] A typical example of this was a 17 years old male patient with AN- restrictive type at presentation who had a transition from thinness-oriented to muscularity-oriented ED. This patient became extremely angry and bitter towards his mother during the course of the treatment and openly stated that a majority of his behavior was to punish his mother for her over-controlling behavior. Although he had an older sister, his mother did not act this way towards her and she refused to change this behavior repeating that he had the utmost importance for her.[7] Therefore, parents should contribute to their children's psychological autonomy and healthy separation-individuation process by supporting their independent decisions and expression of themselves while both providing appropriate acceptance/interest/affection/involvement and control/supervision to their children.

We believe an important point to investigate is why some mothers in Turkey develop parenting styles mentioned above. One hypothesis is that in some cultures, women can gain considerable power in their old age by having sons who support their mother's voice in the household. Therefore, with little need for direct reinforcement from men, women continue to be vulnerable by reinforcing a vicious twist termed the 'patriarchal bargain'.[22] In fact, this term was initially defined by a Turkish author as a tactic in which a woman chooses to accommodate and uphold patriarchal norms, accepting gender roles that disadvantage women overall but maximizing her own power, safety and options. In the end, both girls and boys are harmed from the vulnerability of these mothers and it is crucial to foster change to make daughters and sons more equally valuable to their parents in every culture.

It is also important to point out that mothers are not the sole care takers and paternal parenting styles may also have an impact on ED development in boys. However, in Turkey, the primary care taker who has the most influential caregiving role in the family during

childhood has been shown to be mothers.[23] Thus, we cannot say that mothers are the only ones causing the consequences of this cultural phenomenon because of what they have done. Fathers are also responsible for what they have not done. As a result, we present these clinical observations of certain parenting styles without making a distinction solely for mothers.

In conclusion, we suggest that specific designs for future investigations of risk factors of EDs among adolescent males should include cultural sensitivity that distinguishes western and non-western cultures, as well as the parenting styles for boys, which may act as a risk factor for the development of EDs in males within varying societies.

**Key words:** eating disorder, male, culture, parenting.

### Author contribution

The authors confirm contribution to the paper as follows: The initial draft of the manuscript was prepared by Nuray Kanbur, Devrim Akdemir and Sinem Akgül contributed equally. Language editing was conducted by Sinem Akgül. All authors approved the final version of the manuscript.

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