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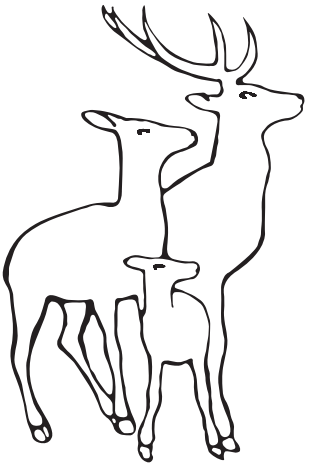

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Review

Local allergic rhinitis: a pediatric perspective

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ABSTRACT

Local allergic rhinitis (LAR) is a differentiated rhinitis phenotype defined by perennial or seasonal rhinitis symptoms without systemic atopy. The diagnosis can be made by a positive response to the nasal allergen challenge (NAC) (the gold standard for diagnosis) in the absence of skin prick test and/or serum allergen-specific immunoglobulin E.

Clinical and epidemiological studies have demonstrated that LAR affects individuals from different countries, races, and age ranges. Several studies have shown that the onset of nasal symptoms occurs during childhood in a significant proportion of LAR individuals. Evidence of LAR has been growing, especially in pediatric and Asian populations. A review of the literature reveals that most LAR studies of pediatric populations have appeared in the last three years. The prevalence of LAR in children ranges from 3.7% to 66.6%, and similar to what has been observed in adults, prevalence is higher in Western countries. Publications have shown that LAR in children can be either seasonal or perennial, and diagnosis of LAR confirmed by NAC have been reported with numerous allergens (house dust mites, pollens, molds, and dander).

These findings illustrate that LAR is an important differential diagnosis in children with presumed non-allergic rhinitis, and a through review of the very recent literature can contribute to the clinical identification and diagnosis of LAR in children with no evidence of systemic atopy, as well as update readers' knowledge of the topic.

Key words: childhood, local allergic rhinitis, nasal provocation test.

Rhinitis is an inflammation of the nasal mucosa. To manifest as chronic, two or more nasal symptoms, such as congestion, rhinorrhea, sneezing, and itching should persist for at least an hour a day for more than two weeks.[1] There are mainly two subgroups of chronic rhinitis: allergic rhinitis (AR), and non-allergic rhinitis (NAR).[2] Non- allergic rhinitis is a heterogenous group including occupational rhinitis, gustatory rhinitis, atrophic rhinitis, rhinitis of elderly, drug-induced rhinitis, hormonal rhinitis, cold-air induced rhinitis and idiopathic rhinitis.[2] Allergic rhinitis is a relatively homogeneus entity with nasal eosinophilia due to IgE-mediated inflammation.[3] Patients with allergic rhinitis have positivity for at least one of markers of atopy such as skin prick test (SPT) and/or serum allergen specific IgE (sIgE)[3], whereas NAR patients test negative for both.[4] However, this classification is very simplistic, and mixed phenotypes may exist in a subgroup of patients. Some authors argue that a new classification depending on the endotypes is needed.[5]

Studies from 1999 to 2004 by the International Study of Asthma and Allergies in Childhood (ISAAC) revealed prevalences of rhinitis at 8.5% in children aged 6-7 and 14.6% aged 13-14.[6] The 1989 Isle of Wight birth cohort of 1456 children reported prevalences of 2.8% and 11.8% in children aged 4 and 18, respectively, for rhinitis in individuals that have no allergic sensitization. The prevalences were reported as 3.4 % and 27.3%, respectively, for the same age groups with allergic sensitization.[7] Males are more susceptible to AR, and females

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more to NAR during adolescence. Allergic rhinitis in early childhood is a risk factor for asthma development in later childhood and adulthood.[8,9]

Classifying chronic rhinitis simply as allergic and non-allergic had some limitations since it did not take into account the form of rhinitis that exists with local allergen-specific immunoglobulin E (sIgE) in the nasal lavage fluid/ positive nasal allergen challenge (NAC) response without apparent specific systemic sensitization, otherwise known as "entopy".[10] Rondón et al.[11] alternatively suggested the term "local allergic rhinitis" (LAR) for conditions with nasal Th2 inflammatory responses with the potential local production of sIgE and positive NAC without evidence of systemic atopy. Local allergic rhinitis shares the same clinical symptoms with AR such as sneezing, itching, obstruction, and rhinorrhea and type 2 inflamation of nasal mucosa after an allergen exposure. The patients often have ocular symptoms and high frequency of asthma.[12,13]

Historical roots of the "local allergic rhinitis" concept

Initial studies on LAR date back to the 1940s. In 1947, Samter et al.[14] found a local reaction in non-allergic individuals after the passive transfer of nasal secretions of ragweed-allergic patients. Following the study by Tse et al.[15], which showed ragweed-specific IgE in nasal secretions of ragweed-allergic patients, several studies have revealed the presence of sIgE in nasal secretions.[6,16,17] Huggings and Brostoff[16] was the first to show local sIgE production after a NAC in rhinitis patients with a negative SPT. In 1979, Platts-Mills[6] measured the concentrations of allergen-specific immunoglobulin D (IgD), immunoglobulin A (IgA), and IgE against ryegrass pollen both in nasal secretions and serum of patients with ryegrass AR and concluded that more than 90% was produced locally. In 2003, the term "entopy" was suggested by Powe et al.[10] to differentiate local from systemic IgE production. Finally, in 2009, Rondón et al.[11] brought the definition "local allergic rhinitis" to the literature, still used today (Fig. 1).

Local Allergy: Pathophysiology

Local production of specific IgE and inflammatory mediators

Numerous studies have shown the local production of sIgE in the nasal mucosa of AR patients.[6,10,16,17] The expression of ε germline gene transcriptions and messenger RNA (mRNA) for the ε heavy chain in nasal B cells was shown by Durham et al.[18], and the class-switch recombination to IgE in the nasal mucosa of AR patients was also demonstrated.[19]

Rondón et al.[12,13] showed sIgE against perennial and seasonal allergens in the nasal secretions of LAR patients with a prevalence of 22% and 35%, respectively.

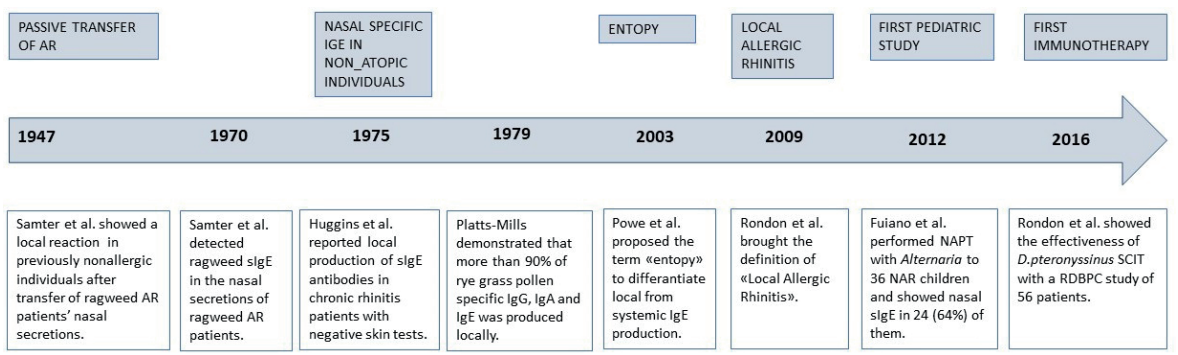

Fig. 1. Historical roots of local allergic rhinitis.

Th2 nasal inflammatory pattern

Non-allergic rhinitis is a heterogeneous group; several pathophysiological mechanisms for NAR have been proposed, including inflammatory and neurogenic mechanisms and also mucosal permeability changes.[20-24] Among non-atopic patients with rhinitis there are two types of rhinitis with Th2 type inflammation: LAR and non-allergic rhinitis with eosinophilia syndrome (NARES). LAR was previously mistakenly included in the NAR group. Recently, Th2-mediated inflammatory response was demonstrated in patients with LAR.[12,13] Flow-cytometric studies with nasal lavage fluids have demonstrated a similar leukocyte-lymphocyte phenotype with increased levels of eosinophils, basophils mast cells and CD4+ T cells, both in AR and LAR patients during natural aeroallergen exposure.[12,13]

Positive NAC responses

Previous studies have shown positive NAC results based on symptom scores plus objective parameters (acoustic rhinomanometry and anterior rhinomanometry) and sIgE/ inflammatory mediators in nasal secretions up to 66% of patients previously described as NAR.[12,13,25,26] An adult study from Turkey found a relatively small percentage such as 12.3% but they perfomed NAC only with *D pteronyssinus* in 65 patients with negative allergen skin prick tests, intradermal tests and serum sIgE.[25]

The activation of mast cells and eosinophils and an increase in nasal sIgE after aeroallergen stimulation have been shown by kinetic studies. Patients have immediate or dual responses to NAC in terms of tryptase, eosinophilic cationic protein (ECP), and sIgE release. Tryptase levels increase 15 minutes to 1 hour after allergen exposure in immediate responders and 15 minutes to 6 hours in dual responders, and a progressive increase in nasal sIgE from 1 to 24 hours has also been found in these studies.[11,27]

LAR evolution

It has been a point of interest as to whether LAR evolves towards AR or if it is a distinct entity. Although, there are no controlled prospective studies in the pediatric population, there is solid evidence in adults.[28,29] Rondon et al.[29] conducted a 10 year follow-up study including 197 LAR patients and 130 controls. Their 5 year and 10 year follow-ups showed similar systemic sensitizations in patient and control groups (6.8 vs 4.5 in 5 year follow-up and 9.7% vs 7.8% in 10 year follow-up).[28,29] After 10 years a significant proportion of LAR patiens (42%) self reported worsening of their symptoms and their quality of life.[29] A significant increase in rhinitis severity from 19 to 42% and 12% of new onset asthma as well as doubling asthma attacks were reported.[29] They also found a tendency towards polysensitization over time. The percentage of LAR patients with polysensitization was significantly higher in the 10 year follow-up compared with the baseline (52.8 % vs 36.4%, respectively) The results confirm LAR as a respiratory disease with chronic course along with worsening of symptoms, development of new nasal sensitizations and new-onset asthma, deterioration of asthma control and decrease in quality of life.[28]

In pediatric patients, three progression types were hypothesized by Arasi et al[30]:

Progression type 1: The nasal sIgE response and mild nasal symptoms start at preschool ages (LAR), then followed by systemic IgE production at school ages (AR).

Progression type 2: Children have nasal sensitization without any symptoms at preschool age but nasal symptoms start at school ages without any systemic sensitization (LAR).

Progression type 3: Local & systemic IgE sensitization start together without a prior 'LAR' stage.

Regarding the type 1 progression hypothesis, there is a pediatric study reporting patients with seasonal rhinitis symptoms without serum sIgE positivity but developing systemic sensitization to grass pollen during the following second or third pollen season.[31] There is not an existing study supporting the hypothesis on other progression types. With the current level of evidence we cannot go further in regards to the natural evolution of LAR in children.

Comorbidities

LAR and asthma

Current published studies suggest that asthma symptoms are reported by 20-47% of LAR patients[12,13] In a recent study asthma was confirmed by methacholine test in 50% of LAR patients.[32] This proportion was found to increase to 83.3% and 57.9% in AR and NAR individuals, respectively. On the other hand, 28.8% and 83.3% of LAR and AR patients, respectively experienced a positive response in the bronchial allergen challenge (BAC), and none of the NAR or healthy control subjects did.[32] Investigators also found a significant increase in airway hyperreactivity measured by metacholine test after allergen exposure. Moreover, allergen administration induced a significant increase in sputum eosinophils, monocytes and ECP in BAC+ patients regardless of their atopic status, with no changes in BAC-individuals.[32]

LAR and conjunctivitis

Patients with LAR occasionally suffer from ocular symptoms such as itching, reddness, burning and tearing during both natural exposure and NAC.[29] Pollen reactive patients are more prone to experience ocular symptoms compared to dust mite reactives.[33] A recent Japanese study suggests the existence of an ocular counterpart of LAR in non-atopic patients with conjunctivitis and detectable total IgE in tears.[34] Unfortunately, the specificity of IgE in tears was not defined, since conjunctival allergen challenge was not performed. There is still a lack of knowledge on the nature of ocular symptoms as to whether there is a real sensitization in conjunctiva or whether the symptoms occur as a result of nasal-ocular reflexes due to allergen exposure.

Evidence of LAR in children

There are a limited number of studies investigating LAR in the pediatric population (Table I).[35-43] The first pediatric study assessing nasal reactivity to allergens and nasal-specific IgE in non-atopic individuals was carried out by Fuiano et al.[35] in 2010. Two years later, the same study group found that 64% of children

Table I. Studies investigating LAR in children.

Author	Year	Country	Study group	Age (yrs)	Allergen	Positive response NAPT (n, %)
Fuiano et al[29]	2012	Italy	36 NAR (perennial)	4-18	Alternaria	23/36 (64%)
Buntarickporpan et al[30]	2015	Thailand	25 NAR (perennial)	8-18	DP	2/54 (3.7%)
Duman et al[31]	2016	Turkey	28 NAR (seasonal/perennial)	5-16	DP,DF, grass mix	7/28 (25%)
Zicari et al[32]	2016	Italy	18 NAR (perennial)	6-12	DP,DF, lolium	12/18 (66.7%)
Krajewska-Wojtys et al[33]	2016	Poland	121 NAR(seasonal)	12-18	Phleum, artemisia,birch	73/12 (52.5%)
Blanca-López et al[34]	2016	Spain	9 NAR (seasonal)	7-18	Phleum	4/9 (44.4%)
Ha EK et al[35]	2017	Korea	64 NAR (perennial)	1-18	DP	5/64 (7.8%)
Tsilochristou et al[36]	2019	Greece	24 NAR (seasonal/perennial)	6-18	Phleum, olea, alternaria, DP	7/24 (29%)

suffering from chronic rhinitis had negative SPTs but positive nasal-specific IgE against *Alternaria alternata*.[36] The prevalence of LAR in children ranges between 3.7% and 66.7%, with a lower prevalence in Asian countries (3.7-25%)[37,38,42] compared with European countries (44.4-66.7%).[39-41] The allergens involved in LAR are the house dust mite (*Dermatophagoides pteronyssinus*), mold (*Alternaria alternate*), grass, birch, and dog/cat epithelia. House dust mite is the most common allergen in all LAR children worldwide. Ha et al.[42] performed NAC with *Dermatophagoides pteronyssinus* on 145 children and diagnosed 5 of them as LAR. In a study from Turkey, Duman et al.[38] performed NAC with grass mix, animal dander, molds, and cockroaches and found positive responses in 7 out of 28 (25%) of patients based on a 40% decrease in nasal flow measured by anterior rhinomanometry or a 20% decrease in nasal flow with a total symptom score greater than two after allergen provocation. The largest study investigating LAR in children was performed by Krajewska-Wojtys et al.[40] in which NAC with *Phleum pratense, Artemisia vulgaris*, and birch pollens were performed on 121 patients, aged between 12 and 18 years with confirmed NAR but having typical seasonal nasal symptoms. LAR was confirmed in 73 (52.5%) patients against *Phleum pratense, Artemisia vulgaris*, and birch pollens in 17 (16.6%), 6 (5.9%), and 9 (8.9%) of patients, respectively. Zicari et al.[39] also found a high percentage (66.7%) of positivity on NAC with *Dermatophagoides spp*. and grass pollen. They stated that nasal sIgE levels for *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Lolium perenne* and also nasal IL-5 levels significantly increased after a positive NAC in 12 of 18 patients. The small percentage of LAR in Asian studies might be due to performing NAC with house dust mite only and differences in the objective evaluation of nasal obstruction in response to allergens.

Diagnostic approach for LAR

Since treatment varies, distinguishing between AR and NAR is important. Nasal allergological evaluation should be performed on patients with AR-like symptoms but a lack of SPT and/or serum sIgE positivity with aeroallergens (Fig. 2).

A positive NAC in the absence of systemic atopy is the basis for diagnosing LAR. Nasal allergen challenge, a highly sensitive diagnostic method, can be used on children. There are several standardized allergen solutions produced by different companies; some of them are ready-to-use solutions and some of them are sold as a

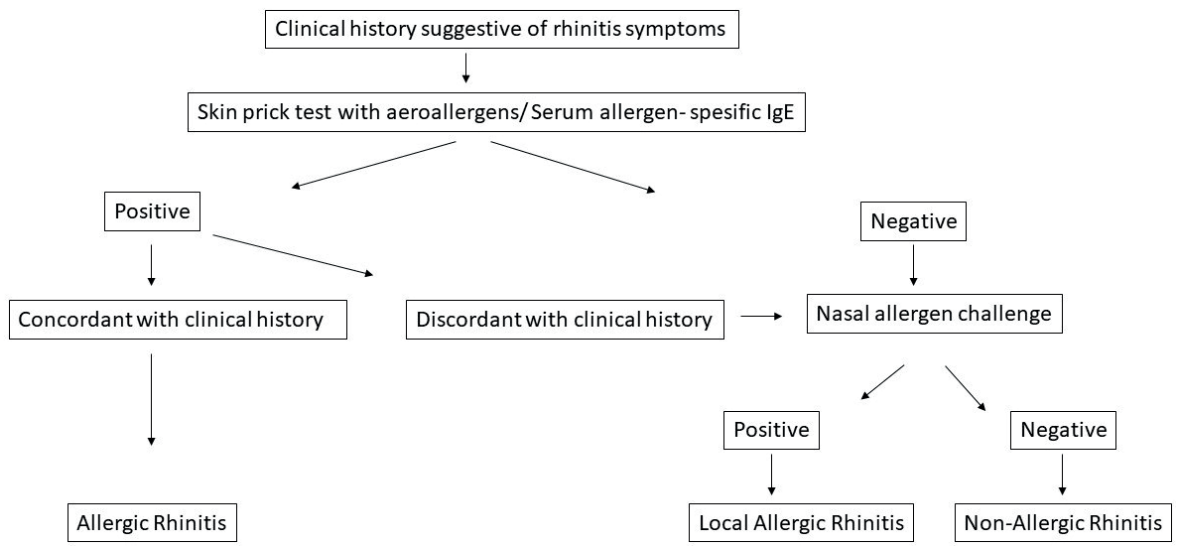

Fig. 2. Diagnostic approach for local allergic rhinitis.

freeze-dried lyophilizate.[44] Allergens should be chosen considering the clinical history such as sypmtomathic periods (perennial or seasonal) or having pets at home. *Dermatophagoides pteronyssinus, Alternaria alternata, Olea europaea, Phleum pratense*, cat/dog epithelia are some of the allergens that have been used for NAC in different studies.[26,38,41,45,46] Several methods have been used for applying allergens. Pump-aerosol spray has been suggested as the simplest and most reliable device.[47] It is adviced to apply 2 puffs (100µl) of allergen per nostril and evaluate the NAC response with symptom scores and objective assessment of nasal patency which can be measured via peak nasal inspiratory flow (PNIF), acoustic rhiometer, anterior rhinomanometry or 4-phase-rhinomanometry.[48] Nasal allergen challenge results should be accepted as positive in case of a strong increase of objective measurement or strong increase of symptoms or moderate increase of combined objective and symptom measurements.[48] Nasal allergen challenge is a time-consuming diagnostic prosedure that needs well trained staff. To facilitate the implementation of NAC in clinical practice, Rondon et al.[45] suggested a new sensitive and reproducible NAC protocol with a sequential application of multiple aeroallergens in one session (NAC-M). This protocol is suggested as 100% concordant with the NAC performed with single allergens (NAC-S) and helpful in reducing hospital admissions required to reach the diagnosis of NAR and LAR, respectively by 75% and 55% without inducing false positive results or irritant effects. Both NAC-S and NAC-M protocols have proven to be safe.[49]

Determining sIgE in nasal secretions is a non-invasive method and is highly specific; however, it has been found to have low sensitivity in most studies (22-40% of responses), a fact that can be attributed to the dilution effect, or other factors.[12,13] A recent study performed by Meng et al.[50] found a quite high diagnostic accuracy for nasal sIgE in LAR diagnosis. They evaluated 212 children with chronic rhinitis, 14 of them had nasal sIgE >0.35 kU/L. Twelve of these patients had significantly higher nasal sIgE levels compared to controls, and also positive response to NAC, so they were defined as LAR. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for local sIgE as a diagnostic tool for LAR was calculated as 91.7%, 95.1%, 78.6%, 98.3%, and 94.5%, respectively.

Several samples have been used to measure nasal sIgE (secretions, scraping, brushing, tissue homogenates, etc.)[51], none of them are validated for diagnosis of LAR. Recently a Spanish research group has described a minimally invasive and simple method using the solid phase of immunoCAP for *Dermatophagoides pteronyssinus*, which showed a sensitivity of 44 % for LAR diagnosis.[52]

The basophil activation test (BAT) has also been shown to be helpful in diagnosing LAR, with 50% sensitivity and specificity greater than 90% for *D. Pteronyssinus*[53]; it is also sensitive at 66% and specific greater than 90% for O. *Europea*[46], which means that, as an additional test, it is useful in LAR diagnosis.

The biomarkers such as eosinophilic cationic protein and tryptase have been studied in nasal secretions by several studies and found to increase approximately 50% after NAC, but have also not been validated.[11,27]

Therefore, both nasal sIgE, BAT and the other biomarkers should be regarded mostly as research tools which cannot be recommended for routine LAR diagnosis.[54,55]

Clinical relevance to differentiate LAR from AR and NAR

Although both subgroups of chronic rhinitis have the same clinical findings such as sneezing, rhinorrhea, nasal itching and nasal obstruction, each of them have some unique features to be differentiated. Comorbidities such as rhinosinusitis, sleep disturbances, learning impairment, otitis media with effusion and reduction in quality of life may occur due to

all chronic rhinitis subtypes.[56] However, allergic comorbidities such as asthma and conjunctivitis are commonly associated with AR and LAR. In a European survey, a strong association was found between asthma development and the presence of AR and chronic rhinosinusitis.[57] It is also known that presence of childhood AR is associated with an increased likelihood of childhood asthma.[58] Current published studies have reported an increase in bronchial symptoms and lower airway symptoms after 10 years of evolution of the disease in patients with LAR.[29] In addition to the aforementioned comorbidities some diagnostic methods can lead the physicians to correct diagnosis. Allergic rhinitis can easily be ruled out by SPT an/or sIgE. A further evaluation of the non-sensitized patients with NAC, nasal sIgE, and/or BAT is helpful to reveal patients with LAR.[59] (Table II).

Therapeutic options

Similar to AR patients, adults and adolescents suffering from LAR respond well to topical nasal corticosteroids and oral antihistamines.[12,13] Rondón et al.[60] demonstrated that a six-month pre-seasonal subcutaneous allergen immunotherapy (AIT) with grass pollen reduced both nasal and ocular symptoms, as well as rescue medication requirement. Additionally, the number of symptom-free days were increased. The clinical and immunologic effects of AIT in LAR were shown by a randomized double-blind placebo-controlled clinical trial with *D. pteronyssinus* subcutaneous immunotherapy (SCIT).[61] Randomized double-blind placebo-controlled studies have also been conducted with *Phleum Pratense*[62] and *Betula verrucosa*[63] SCIT, revealing a statistically significant improvement in rhinoconjunctivitis-affected quality of life with both allergens and a significant clinically important improvement with *Phleum pratense* SCIT alone. Allergen immunotherapy also increased the allergen dose tolerated in NAC.[62] Pediatric studies investigating treatment strategies, including AIT which is the sole disease-modifying treatment for IgE-mediated allergic diseases, are needed.

Local allergic rhinitis has gained more attention in pediatric circles in recent years with the introduction of diagnostic methods that can be easily applied to children, like the nasal sIgE and the BAT. Nasal allergen challenge is still the gold standard diagnostic method and performing NAC with multiple aeroallergens is both time-saving and safe. Longitudinal, prospective studies are needed to investigate the pathophysiology and evolution of LAR in terms of implementing intervention strategies from childhood.

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Table II. Symptoms and in vivo/in vitro markers for rhinitis phenotypes.

Rhinitis phenotype	Symptoms	In vivo/ In vitro markers				
		SPT	Serum sIgE	Nasal sIgE	BAT	NAC
Allergic rhinitis	Rhinorrhea	+	+/-	+/-	+	+
Local allergic rhinitis	Sneezing Nasal itching	-	-	+/-	+/-	+
Non-allergic rhinitis	Nasal obstruction	-	-	-	-	-

BAT: basophil activation test, NAC: nasal allergen challenge, sIgE: allergen-specific IgE, SPT: skin prick test.

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Original Article

SCN1A mutation spectrum in a cohort of Bulgarian patients with GEFS+ phenotype

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ABSTRACT

Background. Dravet syndrome (DS) is the most severe form of Generalized Epilepsy with Febrile Seizures plus (GEFS+) syndrome with a clear genetic component in 85% of the cases. It is characterized by fever-provoked seizure onset around six months of age and subsequent developmental deterioration later in life.

Methods. In the current study, 60 patients with fever-provoked seizures and suspicion either of GEFS+ (50 patients) or of DS (10 patients) were referred for *SCN1A* gene sequence analysis.

Results. *SCN1A* gene sequencing revealed clinically significant variants in 11 patients (18.3%); seven pathogenic (11.7%) and four likely pathogenic (6.7%). Five of these variants have not been reported previously. Among the preselected group of ten DS patients, five had pathogenic *SCN1A* variants which confirmed diagnosis of DS. In four patients with preliminary diagnosis GEFS+, the detected *SCN1A* variant enabled us to specify the diagnosis of DS in these patients. Thus, *SCN1A* sequencing led to confirmation of the genetic diagnosis in 50% (5/10) of DS patients, as well as clarification of the diagnosis of DS in 8% of GEFS+ patients (4/50).

In this study, four patients with truncating mutations had refractory seizures and additional psychomotor abnormalities. Additionally, pathogenic missense mutations were detected in three children with comparable phenotypes, which support the observations that missense mutations in critical channel function regions can cause a devastating epileptic condition.

Conclusions. This is the first systematic screening of *SCN1A* gene in our country, which expands the spectrum of *SCN1A* variants with five novel variants from Bulgaria and demonstrates the clinical utility of confirmatory *SCN1A* testing, which helps clinicians make early and precise diagnoses. It is important for a better follow-up, choice of proper treatment, avoidance of development of refractory seizures and neuropsychological complications. Identification of pathogenic variants in *SCN1A* in the milder GEFS+ and severe DS cases, will help to offer adequate prenatal diagnosis and improve the genetic counselling provided to affected families.

Key words: Dravet syndrome, *SCN1A* mutation, GEFS+, seizures.

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Dravet syndrome (DS) or Severe Myoclonic Epilepsy of Infancy (SMEI) is a well characterized epileptic encephalopathy in infants and has a clear genetic component in 85% of cases worldwide.[1] It is defined by seizure onset around six months of age, along with the appearance of recurrent febrile *status epilepticus* (SE). These are fever-provoked generalized or focal, clonic or tonic-clonic seizures. Other seizure types, including myoclonic, atonic and atypical absence generally appear around 1 and 4 years of age.[2] The seizures are often pharmaco-resistant and from their second year of life the affected children develop cognitive, motor and behaviour impairments.[3] Once appeared, the neuropsychological deficit is irreversible, does not respond to antiepileptic drugs (AEDs) and thus the DS prognosis is poor. DS is the most severe form of Generalized Epilepsy with Febrile Seizures plus (GEFS+) syndrome (OMIM 604233), which is a variable autosomal dominant epileptic condition that also associates with febrile and afebrile seizures, where the outcome is usually benign and the patients are sensitive to anti-epileptic treatment.

In the majority of DS cases (70-80%) *SCN1A* gene is affected.[4,5] *SCN1A* gene (OMIM 182389) encodes alpha subunit of voltage-gated sodium channel in GABAergic inhibitory interneurons, responsible for generation and propagation of action potentials in the brain.[6] The spectrum of *SCN1A* mutations includes single nucleotide variations (SNVs) in 70-80% of DS patients, while patients harbouring intragenic or whole *SCN1A* gene deletions are much less.[4,7] All of the mutations detected in milder GEFS+ cases are missense.[8] Almost all *SCN1A* mutations in DS patients are *de novo* (95%), although some of them are inherited from an affected parent, often with a milder phenotype.[9] Analysis of genotype-phenotype correlations show that truncating mutations, partial or whole gene deletions as well as missense mutations localized in pore-forming regions (S5-S6) and in voltage-gated S4 segment cause complete loss of function of the protein (haploinsufficiency) and as a result more severe phenotype[10,11], compared to missense mutations in the linker regions of the protein which cause milder GEFS+ phenotypes.[12,13] A recent study shows that truncating mutations cause earlier onset of seizures, appearance of other seizure types (myoclonic, atypical absence) and subsequent irreversible neuropsychological delay after seizure onset.[4]

In this sense, early and precise diagnosis of DS is critical, due to the high risk of SE and neuropsychological deficit, and that commonly prescribed antiepileptic sodium channel blockers may aggravate seizures in DS patients.[14,15] Herein, we report the results from a systematic screening of *SCN1A* gene in a cohort of Bulgarian patients with epilepsy in the GEFS+ spectrum. Amongst the detected *SCN1A* mutations five have not been previously reported and we attempt here to make genotype-phenotype correlations based on the predicted effect of the novel mutations on the encoded Nav1.1 protein structure and function.

Material and Methods

Subjects

In the current study a total of 60 patients were referred by clinicians from major neurology clinics in Bulgaria (n=60; 28 males, 32 females). Patients' clinical information, including type of seizures, the age at seizure onset, the frequency and duration of the convulsive seizures, as well as the response to administration of AEDs was gathered. The assessment of epileptic seizures was performed according to the revised diagnostic criteria of the International League Against Epilepsy classification.[16]

In the Dravet group, 10 patients were included, who had normal development before seizure onset, the occurrence of either fever-provoked or unprovoked partial, generalized seizures before the first year of life, appearance of other seizure types (myoclonic, atonic, absence), presence of intractable epilepsy, and a gradual psychomotor delay after the seizure onset, as well as presence of gait disturbances and other

pyramidal signs. The remaining 50 patients who were not fully consistent with the accepted diagnostic criteria or had milder forms of GEFS+ were also tested for *SCN1A* mutations.

All participants and/or their legal guardians gave written informed consent. The study was conducted with the approval by Ethics Committee of Medical University of Sofia (Ethical approval number: 2011-2655).

Genetic testing

DNA was extracted from peripheral blood lymphocytes of patients and their parents (where available) using automatic Chemagic Magnetic Separation Module I system (Perkin Elmer, Germany). The quality and quantity of the DNA samples were determined using the NanoDrop ND-2000 spectrophotometer.

All 26 exons including exon/intron boundaries of *SCN1A* gene were amplified by Polymerase Chain Reaction (PCR) in 28 separated reactions using previously reported primer pairs.[17] Sanger sequencing of the purified PCR products was conducted using ABI 3130xl (Applied Biosystems, Foster City, CA, USA). The sequences were analysed using ABI Sequencing Analysis v.5.3 and compared with reference sequence NG_011906.1. In the positive cases a segregation analysis was performed where parents' DNA was available. A total of 28 relatives were analysed (Table I).

Variant analysis

The candidate variants were examined using reference sequence NM_001165964 and public databases, including 1000G (http://www.1000genomes.org), ExAc (http://exac.broadinstitute.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), Human Gene Mutation Database - HGMD (www.hgmd.cf.ac.uk), as well as the database of Molecular genetics laboratory of the University of Antwerp (http://www.molgen.vib-ua.be/SCN1AMutations/). Missense variants were analysed by three bioinformatic tools - PROVEAN/SIFT, Polyphen2 and Mutation Taster. The pathogenicity of splice-site variants was assessed by NetGene2 and Human Splicing Finder software in order to evaluate their potential effects.[18-21] Variant interpretation and classification was carried out according to the criteria provided by American College of Medical Genetics and Genomics (ACMG) standards and guidelines.[22]

Results

Out of 60 patients, 22 carried sequence variations in *SCN1A* gene (36.6%) (Table I). Among them a total of 18 variants were found, five of which were novel. Using ACMG criteria for variant classification, variants with clinical significance were detected in 11 patients (18.3%) – seven pathogenic (11.7%) and four likely pathogenic variants (6.7%) (Table I). Five variants classified as likely benign, weak benign or benign were detected, among which three were recurrent (Table I). One benign variant, c.603-91G>A, represented a common polymorphism, associated with particular response to antiepileptic drug therapy. Last two of the detected variants were classified as variants of unknown significance (VUS) (c.4252-4A>G and p.Arg542Gln).

Among the preselected 10 patients with DS phenotype, five had *SCN1A* mutations (50%) (Patients2, 6, 7, 8, 10). Four additional patients referred with presumptive diagnosis of GEFS+ were found to harbour pathogenic mutations in *SCN1A* (Patients 1,4, 5 and 9; see Tables I, II). Their epileptic condition was afterwards clearly specified as DS. Thus, *SCN1A* sequencing led to the genetic clarification of the diagnosis of DS in 8% (4/50). In two milder GEFS+ cases variants with clinical significance were found in 2 patients (patients 3 and 11) – one novel splice-site (c.2383-1G>A) and one missense mutation (p.Met1654Ile).

Clinical summary of the patients with SCN1A mutations

In all 22 patients with detected mutations, the main clinical feature was fever-provoked or

Table I. Genotype characteristics of *SCN1A* gene alterations according to NM_001165964 reference sequence in the patients.

Patient, gender	Exon	Variant type	cDNA	Protein	Subunit location	Inheritance	Previous report	ACMG classification
1, M	24	Nonsense	c.4532C>A	p.Ser1505Ter	DIII -DIV	N/A	Yes[8,15,34,45,46]	(PVS1, PM2, PM4, PM1) Pathogenic
2, F	21	Missense	c.4135G>A	p.Val1379Met	DIIIS5-S6	*De novo*	Yes[40-42]	(PM2,PP2,PM1,PS1, PS2, PM5,PP3) Pathogenic
3, F	26	Missense	c.4980G>T	p.Met1654Ile	DIVS4-S5	N/A	Yes[8,20,42,47,48]	(PS3,PM2,PP2,PM1,PS1,PP3) Pathogenic
4, M	25	Nonsense	c.4744G>T	p.Glu1576Ter	DIVS2	N/A	Novel	(PVS1, PS1, PM2,PM1, PM4,PP3) Pathogenic
5, F	12	Frameshift	c.2136delC	p.Ser707AlafsTer3	DI-DII	N/A	Novel	(PM2, PM4,PM6,PVS1) Pathogenic
6, F	19	Frameshift	c.3837_3843delAATTGTT	p.Iso1280MetfsTer7	DIIIS2-S3	*De novo*	Novel	(PM2, PM4, PVS1, PS2) Pathogenic
7, M	20	Missense	c.3929T>C	p.Leu1310Pro	DIIIS4	*De novo*	Novel	(PS2,PM2,PP2,PM5,PP3) Pathogenic
8, F	IVS19	Splice-site	c.3864-1G>T (UTR)	-	-	Absent in healthy mother; father N/A	Yes[8]	(PM2,PVS1) Likely pathogenic
9, M	15	Missense	c.2804G>C	p.Arg935Pro	DIIS5-S6	Affected mother and sister heterozygous	Yes[42]	(PM2, PM5, PP1, PP3, PP4, PP5) Likely pathogenic
10, F	25	Missense	c.4854G>T	p.Arg1596Leu	DIVS2-S3	*De novo*	Yes[8,20,44]	(PS2, PM2,PP2,PP3) Likely pathogenic
11, M	IVS 13	Splice-site	c.2383-1G>A	-	-	*De novo*	Novel	(PVS1,PS2,PM2, PP3) Likely pathogenic
12, F	IVS 21	Splice- site	c.4252-4A>G	-	-	N/A	Yes	(PM2, PP5,BP4) VUS, Confliciting intepretation

N/A – parents were not available for segregation analysis; VUS: Variant of Unknown Significance;
PVS, PS, PM, PP - Evidence for pathogenicity according to Richards et al., 2015; PVS – very strong evidence; PS – strong evidence; PM – moderate evidence; PP – supporting evidence
BA, BS, BP – Evidence for benign impact according to Richards et al., 2015; BA – stand-alone evidence; BS - strong evidence; BP – supporting evidence.

Table I. Continued.

Patient, gender	Exon	Variant type	cDNA	Protein	Subunit location	Inheritance	Previous report	ACMG classification
13, M	10	Missense	c.1625G>A	p.Arg542Gln	DI-DII	Affected father heterozygous, absent in healthy mother	Yes	(PP2,PM1,PS1,BS4, BS2,BP4) VUS, Confliciting intepretation
14, F						Absent in affected mother, mother's first cousin and her two children		
15, M	11	Missense	c.806G>C	p.Arg27Thr	NTD	N/A	Yes	(BP7) Weak benign
16, M						N/A		
17, F						N/A		
18, F	IVS 4	Splice- site	c.603-91G>A	-	-	Brother – homozygous; parents- heterozygous	Yes	(PM2,BP4,BP7) Likely benign
19, F	17	Missense	c.3488C>G	p. Thr1174Ser	DII-DIII	N/A	Yes	(BS1,BS4,PP3,BP7) Likely benign
20, F						Absent in affected mother and sister	Yes	
21, F	11	Missense	c.1811G>A	p.Arg604His	DI-DII	Affected father heterozygous; absent in affected sister and healthy mother	Yes	(PM2,PP2, BS4) Likely benign
22, F	26	Missense	c.5749C>G	p.Arg1928Gly	CTD	Absent in healthy mother; father N/A	Yes	(BA1,BS2,PP3,BP7,BP6) Benign

N/A – parents were not available for segregation analysis; VUS: Variant of Unknown Significance;
PVS, PS, PM, PP - Evidence for pathogenicity according to Richards et al., 2015; PVS – very strong evidence; PS – strong evidence; PM – moderate evidence; PP – supporting evidence
BA, BS, BP – Evidence for benign impact according to Richards et al., 2015; BA – stand-alone evidence; BS - strong evidence; BP – supporting evidence.

unprovoked generalized tonic-clonic seizures (GTCS) (Table II). In three patients additional myoclonic (patients 8 and 9), atonic and absences seizures (patient 7) are observed. In eight of the patients GTCS were preceded by - focal unilateral seizures and subsequent second generalization. The seizure onset was between 3 months and 1 year and 8 months of age. Ten patients had positive family history and their relatives reported single febrile seizures (FS) in their childhood triggered by infectious disease and were remitted after 5 years of age. Eight patients exhibited at least one SE after the seizure onset; three patients referred as GEFS+ (Patients 1, 3, 9) and 5 patients as DS (Patients 2, 6, 7, 8, 10). In eight patients subsequent developmental and cognitive delay were observed - four DS patients (2, 6, 7 and 8) and four patients referred as GEFS+ (1, 4, 5, and 9). Additional dysmorphic features were observed in patients 7 and 8 (Table II).

Summary of the genotype information

Out of 11 clinically significant *SCN1A* mutations five were missense, two affected conservative splice sites and four were truncating mutations (2 nonsense and 2 frameshift deletions) (Table I). None of them were present in public variant databases, like ExAc or 1000 Genomes. Five of the identified pathogenic variants occurred *de novo* (3 missense, 1 splice site and 1 frameshift deletion) and one missense variant was inherited from the mother. In five patients segregation analysis was not performed because parents were not available (Table I). Five mutations have not been reported before – one nonsense (p.Glu1576Ter), two frameshift deletions (p.Ser707AlafsTer3, p.Iso1280MetfsTer7), one missense (p.Leu1310Pro) and one splice-site mutation (c.2383-1G>A) (Table I).

Genotype/phenotype correlations

Among the patients with detected clinically significant *SCN1A* variation, four (Patients 1, 4, 5, 6) were truncating mutations leading to premature stop of the synthesis of intact protein and are found in patients with severe epileptic phenotype (Fig. 1, Table II). Three of them were reported for the first time in the current study; p.Glu1576Ter, p.Ser707AlafsTer3 and p.Iso1280MetfsTer7 (Table I). All of the truncating mutations were found in children with earlier seizure onset (before age of one year), refractory epilepsy, no effect or partial response to the AED treatment and start of neuropsychological delay after seizure onset (Table II). Patients 1 and 5

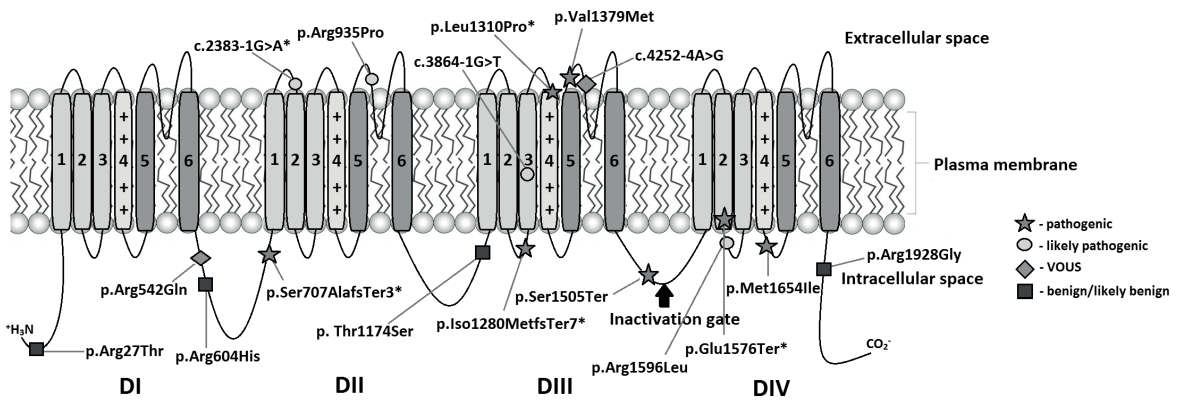

Fig. 1. Schematic representation Nav1.1 protein structure, showing four homologous domains (DI-DIV), each with six transmembrane segments (S1-S6). Segments five and six (S5, S6) form the ion channel pore and segment four is the voltage sensor. Segments S1-S3 have structural role for the protein. Segment five and six of each homologous domain and the re-entrant loops between them form the pore. The intracellular loop between DIII and DIV (indicated by an arrow) forms an inactivation gate, which is necessary for fast inactivation of the channel. On the diagram are illustrated the location and distribution of identified mutations (except the intronic variant c.603-91G>A) represented with different shapes.

Table II. Clinical characteristics of patients with clinically significant *SCN1A* mutations.

Patient	1	2	3	4	5
Age	3 years	2 years	3 years	4 years	9 years
Primary diagnosis	GEFS+*	DS	GEFS+	GEFS+*	GEFS+*
Age of onset	7 months	4 months	1 years and 8 months	7 months	5 months
Type of seizures	Febrile focal with evolution in GTCS	Febrile GTCS	Febrile GTCS	Febrile GTCS	Unprovoked GTCS
Seizure frequency	2-3 in a year	1-2 in a year	1 in 4 months	1-3 seizures in 3 months	1-2 in a month
SE	Yes	Yes	Yes	No	No
Antiepileptic treatment	VPA, LEV, TPM	VPA, LEV, CLB	VPA	VPA, - LEV, CLN	LTG, VPA, DZP, LEV
Response to AED	No effect	No effect	Good response	Partial effect	No effect
Developmental symptoms	Lower muscle tone, delayed motor development	Neuropsychological delay, motor delay, cognitive impairment	Normal to date	Neuropsychological delay, mild motor delay, lack of speech	Neuropsychological delay, hyperactive behaviour
Family history	No	Yes (FS)	Yes	No	Yes (FS)
Additional info	-	Does not segregate in the family	-	-	-
Mutation	p.Ser1505Ter	p.Val1379Met	p.Met1654Ile	p.Glu1576Ter	p.Ser707AlafsTer3

GEFS+* - the presumptive diagnosis has been changed to DS; FS- Febrile Seizures; LTG- lamotrigine; VPA- valproic acid; DZP- diazepam; LEV- levetiracetam; OXC – oxcarbazepine; CLN – clonazepam; CLB- clobazam; TPM – topiramate; STP – stiripentol; CBZ- carbamazepine; GTCS- Generalized Tonic Clonic Seizures; DS- Dravet syndrome; GEFS+ - Generalized epilepsy with febrile seizure plus; CMA- Chromosomal microarray.

Table II. Continued.

Patient	6	7	8	9	10	11
Age	4 years	2 years	4 years	4 years	4 years	2 years
Primary diagnosis	DS	DS	DS	GEFS+*	DS	GEFS+
Age of onset	7 months	9 months	1 year and 2 months	4 months	3 months	1 year and 5 months
Type of seizures	Febrile GTCS	Febrile GTCS, atonic, absence	Unprovoked myoclonic and GTCS	Myoclonic, GTCS	Febrile focal with evolution in GTCS	Febrile and afebrile GTCS
Seizure frequency	1 in a month	1-2 in a month	1-2 in a year	5-6 a day	3-4 in a month	1 in 2 months
SE	Yes	Yes	Yes	Yes	Yes	No
Antiepileptic treatment	VPA, LEV, CLB, STP	DZP, OXC, CLN, LEV	VPA, LEV, OXC	VPA, CLN, TPM	VPA, CLN, OXC, VPA, LEV	VPA, LEV, CBZ, TPM, CLB, STP
Response to AED	Partial effect	No effect	No effect	No effect	No effect	No effect
Developmental symptoms	Delayed speech, mild motor delay, cognitive impairment	Neuropsychological and motor delay after seizure onset	Neuropsychological delay after seizure onset, behaviour problems; lower muscle tone	Neuropsychological delay after seizure onset	Normal to date	Normal to date
Family history	No	No	No	Yes (epilepsy); segregates with the phenotype in the family	No	No
Additional information	-	Ataxia; hippocampal asymmetry, lymphadenopathy, dysmorphic features	Hypertelorism, low set ears, neck lymphadenopathy; normal CMA	Severe ID	-	-
Mutation	c.3837_3843delAATTGTT	p.Leu1310Pro	c.3879-1G>T	p.Arg935Pro	p.Arg1596Leu	c.2383-1G>A

GEFS+* - the presumptive diagnosis has been changed to DS; FS- Febrile Seizures; LTG- lamotrigine; VPA- valproic acid; DZP- diazepam; LEV- levetiracetam; OXC – oxcarbazepine; CLN – clonazepam; CLB- clobazam; TPM – topiramate; STP – stiripentol; CBZ- carbamazepine; GTCS- Generalized Tonic Clonic Seizures; DS- Dravet syndrome; GEFS+ - Generalized epilepsy with febrile seizure plus; CMA- Chromosomal microarray.

who carried nonsense p.Ser1505Ter mutations and frameshift p.Ser707AlafsTer3 respectively, did not respond to the administration of several AED (Table II). In the other two patients (4 and 6) with truncating mutations (Table I) a partial effect of AED treatment was observed – the seizures became less frequent with appearance of 1 or 2 convulsions annually. However, their neurological impairment persisted – both of them did not speak and exhibited cognitive and motor delay.

Two splice-site mutations detected in the current study are assessed by prediction tools as likely pathogenic mutations - c.3864-1G>T and c.2383-1G>A (Table I). The novel splice site variant c.2383-1G>A was detected in a child with pharmaco-resistant GTCS and occurred *de novo*. It is located in the canonical splice-acceptor site of intron 13 and according to NetGene2 and Human Splicing finder prediction software, there is high confidence for the use of the first alternative AG dinucleotide located 23 nucleotides downstream in exon 14. The *SCN1A* splice-site variant c.3864-1G>T has similar prediction parameters. It is located in the canonical splice-acceptor site of intron 19 (Table I) and was also detected in a 4-year-old child with refractory epilepsy, later seizure onset and presence of neuropsychological delay after seizure onset, as well as behaviour problems (Table II). According to prediction tools, there is high possibility for usage of alternative AG dinucleotide located downstream in exon 20.

Among the missense mutations, four were previously reported in other patients with comparable phenotype (Table I) and one is novel (p.Leu1310Pro in Patient 7). The p.Arg935Pro and p.Val1379Met mutations are located in highly conserved S5-S6 linker region, which is part of the pore forming region. The p.Leu1310Pro is located on voltage sensor segment of the third domain. All three missense mutations represent non-conservative AA substitutions with a potentially significant impact on physicochemical properties of the channel and are located in important domains for the channel function (Fig. 1). As a result, an early seizure onset and presence of additional to GTCS types of seizures (myoclonic, atonic, absence) were observed in patients 2, 7 and 9 (Table II). Moreover, these patients show refractory epilepsy to multiple AED applied and a neuropsychological delay after seizure onset. Patient 7 additionally exhibited ataxic gait and dysmorphic features (Table II). The p.Arg1596Leu (in Patient 10) and p.Met1654Ile (in Patient 3) mutations are located in the cytoplasm-facing loop, connecting S2-S3 segments and S4-S5 segments of the fourth domain, respectively (Fig. 1). Their localization outside of the pore-forming region probably results in a milder effect to the channel's function. In patient 10, who carried *de novo* p.Arg1596Leu a normal psychomotor development was observed on the day of examination. Patient 3 with detected p.Met1654Ile exhibited later seizure onset, as well as good response to valproic acid (VPA) and normal psychomotor development (Table II). For all clinically significant missense mutations the applied prediction tools showed that they had a demolishing effect to the function of the subunit.

All benign missense variants are situated in either linker regions or N- or C-terminal domains (NTD, CTD) (blue colored circles – Fig. 1). In these patients a later seizure onset is observed (median 2 years of age) and the mutations do not segregate with the phenotype in the family.

Discussion

This is the first large-scale screening of *SCN1A* in fever-provoked epilepsy in our country. To date, different type of *SCN1A* mutations in single cases have been reported.[23-25]

In the current study, a total of 7 pathogenic SNVs among 60 patients were detected (11.7%). Likely pathogenic mutations were found in four patients (6.7%), which makes an overall diagnostic yield of 18.3%. The observed diagnostic yield of pathogenic changes (11.7%) is in line with those recently reported cohorts of

patients with GEFS+ syndrome and pathogenic *SCN1A* mutations (10%).[4,5] As a result of our study, a pathogenic *SCN1A* variant was detected in five patients with presumptive diagnosis of DS. In four additional patients (1, 4, 5, 9) with preliminary diagnosis of GEFS+ mutational analysis revealed two nonsense mutations; p.Ser1505Ter, p.Glu1576Ter, of which the latter is novel (Table I), one novel frameshift deletion p.Ser707AlafsTer3, as well as one missense mutation p.Arg935Pro affecting the pore-forming linker S5-S6 segment from domain DIII (Table I). Thus, we were able to specify the genetic diagnosis in 50% of DS patients, as well as to clarify the diagnosis of DS in 8% of GEFS+ patients. The observed diagnostic rate is lower than those reported in the literature in similar cohorts of DS patients (70-80%).[4] This partly might be due to the small sample size of DS patients (n= 10), as well as comparatively loose selection criteria applied. Furthermore, *SCN1A* negative DS patients may have resulted from mutations in other epilepsy-associated genes, such as *SCN1B, HCN1, STXBP1, CHD2, GABRA1*, and *GABRG2*, that were not analyzed in the current study.[26-28] It is important to perform large-scale genomic approaches like clinical or whole exome sequencing in order to confirm this hypothesis.

The classification and interpretation of pathogenicity of the detected variants was carried out in accordance with the criteria provided by ACMG.[22] The authors provided two sets of criteria: one for classification of pathogenic and likely pathogenic variants and one for classification of benign and likely benign variants. Each of the criteria was divided in different categories – very strong (PVS1), strong (PS1-4), moderate (PM1-6) and supporting (PP1-5) for pathogenic criteria and stand-alone (BA1), strong (BS1-4) or supporting (BP1-6) for benign criteria.[22] The numbering within each category does not give any precedence of some criteria over others, but rather is used for differentiation. Given the available information for a particular variant, either taking into account previously published data, or data obtained from the current case under investigation, the combination of these criteria gives the final variant interpretation and classification. All criteria for variant assessment and the scoring rules for their combination are explained in detail by Richards et al., 2015.[22] The highly possible consequence of the novel c.2383-1G>A splice-site mutation detected in patient 11 might be a deletion of 23 nucleotides from exon 14 and formation of stop codon after four amino acids (AA) in downstream direction of the second domain from the Nav1.1 protein (Fig. 1) (c.2383_2406del; p.Val795LysfsTer4). The effect of the other splice site mutation c.3879-1G>T was also predicted to result in deletion of 68 nucleotides from exon 20 and frameshift deletion of 15 AA in S3 from the third domain (DIII) the Nav1.1 protein (p.Val1283AspfsTer15). Another possible consequence is the usage of the splice-acceptor site of some of the adjacent introns, subsequent exon skipping and activation of nonsense-mediated mRNA decay process with resultant non-productive translation. Both patient carriers 8 and 11 show severe refractory epilepsy and neuropsychological deterioration at age of examination (Table II). However, to clarify the potential pathogenicity of the splice *SCN1A* variants it is important to investigate their effect at the RNA level, as well as to perform a follow up of patients' development at a later stage.

In the current study all patients with truncating mutations (1, 4, 5, 6) had seizures refractory to AEDs and additional psychomotor abnormalities. Thus, we here confirm previously reported observations that truncating mutations (nonsense, frameshift, some splice site mutations) result in loss of function of the protein and are responsible for the occurrence of more severe epileptic phenotypes.[11,29-30] It is known that individuals harbouring missense mutations, in which the aberrant copy of the gene is expressed despite its altered function, show milder phenotypes of the GEFS+ spectrum.[29,34] Some missense mutations exclusively cause severe consequences, because they are localized in critical channel function

regions and/or the mutated AA has significantly different physicochemical properties, compared to the wild type AA.[35] Pathogenic changes in amino acid polarity have been detected in voltage sensor (S4) and ion-pore regions from Nav1.1 protein and are associated with the DS phenotype rather than milder GEFS+ syndrome.[11,13,35] In our study, the novel mutation p.Leu1310Pro is localized in α helix motif of voltage sensor S4 in domain DIII (Fig. 1). The wild type and mutant amino acids differ in structure and in this sense the substitution of leucine with proline leads to conformational change of the polypeptide chain, which might lead to disruption of the normal function of the voltage sensor segment. Patient 7 exhibited severe epileptic phenotype including refractory GTCS, ataxia and neuropsychological delay after seizure onset (Table II). The application of four antiepileptic drugs had no effect on the seizure frequency (Table II).

Additionally, one variant classified as likely pathogenic missense mutation (p.Arg935Pro) also changes the polarity of the AA residue in the pore forming S5-S6 DII segment of the Nav1.1 protein (Fig. 1). p.Arg935Pro segregates with the disease phenotype in the affected family – it is present in the sister with myoclonic seizures and moderate intellectual disability (ID), and in the mother, who has history of epilepsy in early childhood (Table I). The observed phenotypic heterogeneity is a characteristic phenomenon in dominant genetic epilepsies in general and might be due to individual epigenetic factors' influence or the presence of gene modifiers, which modulate the effect of the mutation via unrevealed pathomechanism.[36-38] Patient 9 showed classical manifestation of DS – refractory seizures, which evolve in neuropsychological delay and later in life in ID (Table II). This might be due to impaired channel function as a result of the different chemical nature and the size of proline, compared to arginine. Moreover, proline disrupts the interaction between the protein and the lipid phase of the membrane, which additionally may contribute to the abnormal function of the channel. To date, p.Arg935Pro was reported in a single Chinese patient with DS.[43] Mutations, defined as pathogenic and affecting the same AA, but different substitution (p.Arg935His, p.Arg935Cys), were reported in patients with DS and severe psychomotor delay, as well as in borderline DS cases.[8,39-41]

Patient 2, a 2-year-old boy also showed refractory seizures and severe psychomotor delay (Table II). He was found to have pathogenic missense mutation p.Val1379Met affecting the pore forming S5-S6 re-entrant loop of domain DIII with an important role for the proper function of the NaV1.1. subunit (Fig. 1). Similarly, in the other two cases with missense mutations (patients 7 and 9) the AA substitution was non-conservative. Leucine is hydrophobic AA, while methionine is sulfur-containing AA and has different spatial and physicochemical properties, compared to the wild type leucine. So far, this variant has been reported as pathogenic only in DS cases.[42-44] In all of them, including in our case the mutation appeared *de novo*. At first, patient 2 showed a temporarily good response to the administration of VPA, but subsequently his development gradually declined. The adds-on therapy with levetiracetam and clobazam failed to cease the seizures and at the moment the child exhibits fever-provoked GTCS, gait disturbances and cognitive delay (Table II).

The three cases described (2, 7 and 9) support the observations that missense mutations critical for the channel function regions can cause loss of function of the protein and as a result - devastating epileptic phenotype.[12,35] Patient 10, a 4-year-old girl was a carrier of *de novo* missense *SCN1A* variant (p.Arg1596Leu) classified according ACMG criteria as likely pathogenic. At the time of examination patient 10 exhibited refractory epilepsy to multiple AED and normal development (Table II). The mutation was reported in single DS patient[8], as well as in a case with milder GEFS+ phenotype.[11] A different AA substitution at the same position was described in patient with cryptogenic focal epilepsy, moderate ID and ataxia[45], as well as in a patient with classical GEFS+ features, normal

development and good response to AEDs.[46] Unfortunately, we cannot perform a follow-up of the patient's clinical outcome, because of lack of contact with the family.

In the current study, four patients did not have a family history of epilepsy and the segregation analysis revealed *de novo SCN1A* variant (patients 6, 7, 10, 11; see Tables I, II). In contrast, one *de novo SCN1A* pathogenic variant does not segregate with the epilepsy phenotype in the family (see Tables I, II, patient 2) – the parents experienced single FS in the past and did not carry the variant. This may be explained by the polygenic nature of FS. It is possible that the parents carry additional mutation in another epilepsy associated gene, which is the genetic cause or acts like a predisposition factor for the febrile seizures, observed in them. This factor might have also contributed in combination with the *SCN1A* mutation to their child's phenotype. Examples of specific genetic modifier genes, mutations in which modulate the effect of the corresponding *SCN1A* variant are *SCN8A, SCN9A, CACNB4*.[47-49] A missense SCN9A variant was found in DS patient from Caucasian origin inherited from the asymptomatic mother with history of FS. This patient also carried *de novo* frameshift mutation in *SCN1A* and the authors concluded, that *SCN9A* has a contributing role to the DS phenotype.[49] Similar findings were reported in cases with detected *SCN1A* mutation in combination with *CACNB4* mutation, the latter inherited from asymptomatic father with FS in the past. The *CACNB4* mutation resulted in increased neurotransmitter release in the excitatory neurons under insufficient inhibitory neurons caused by the nonfunctional Nav1.1 channel, caused by the *SCN1A* mutation.[48] However, to prove this hypothesis in our case, it is necessary to apply a wide-range of technology, such as next-generation sequencing, in order to identify this additional FS-related genetic modifier in family 2.

It is now known that in a family with a particular genetic disease the recurrence risk for a first degree relative to have the same disease caused by a *de novo* mutation is higher than that of the general population and is estimated at 1–4%.[50] Around 80% of the inherited *de novo* germline point mutations arise from the paternal allele and advanced paternal age at conception has been accepted as the major factor linked to the *de novo* mutations in the offspring, both at the population level and within the same family.[51,52] In particular, 10% of DS patients have inherited pathogenic *SCN1A* variant from an asymptomatic or mildly affected parent.[8,9] Furthermore, 7% of DS patients have seemingly healthy parents with germline mosaicism which increases the risk for passing the *SCN1A* mutation to the offspring.[53] This high incidence can increase the risk further and has an important value for the genetic counselling of the affected families. In order to prevent recurrence in such families it is recommended to perform prenatal diagnosis either by chorion villus sampling or amniocentesis, depending on the week of pregnancy.

The results of the current study are important in three aspects. Firstly, they broaden the *SCN1A* mutation spectrum with novel variants from Bulgaria. Secondly, they can guide the choice of medication, avoiding sodium channel blockers (lamotrigine, carbamazepine, phenytoin) which can provoke seizure aggravation in classical DS phenotypes. The danger of seizure aggravation is usually ignored in "milder" GEFS+ cases examined before age of 2 years with undetected loss-of-function mutations, because of the lack of knowledge for the evolution of the syndrome, based only on clinical examination. These cases may have the same genetic basis as the classical DS and may have similar AEDs response. In this sense, an early and precise genetic diagnosis is critical, because it will help the clinician to choose AEDs punctually and prevent the development of intractable epilepsy. Our results demonstrate the clinical utility of confirmatory *SCN1A* testing, which helps the clinician to make appropriate assessment about the diagnosis. And finally, identification of pathogenic *SCN1A* variants in severe DS, as well as the milder GEFS+ phenotypes, will

help to offer an adequate prenatal diagnosis and improve the genetic counselling offered to affected families.

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Original Article

The incidence and clinical effects of *Bordetella pertussis* in children hospitalized with acute bronchiolitis

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ABSTRACT

Background. Pertussis is a disease leading to high morbidity and mortality in neonates and infants. Bronchiolitis is the most common cause of hospitalization especially in children <2 year-old. Although the clinical findings are different in these two diseases, it is sometimes difficult to make this distinction in partially or fully vaccinated children. This study aimed to identify the incidence, clinical and laboratory effects of *B. pertussis* as a causative agent in hospitalized children with acute bronchiolitis.

Methods. The study included patients diagnosed with acute bronchiolitis and admitted to the Division of Pediatric Infectious Diseases from January 2012 to December 2015, aged 24 months or younger, evaluated for viruses and bacteria with polymerase chain reaction in respiratory tract secretions.

Results. The study included 380 patients hospitalized with acute bronchiolitis. Of these patients, 85.8% were identified to be positive for at least one respiratory pathogen. The most commonly identified pathogens were respiratory syncytial virus (RSV) A/B, rhinovirus, parainfluenza virus, adenovirus, bocavirus and metapneumovirus A/B. *B. pertussis* was only detected in 5 patients (1.5%). In the patients with *B. pertussis* identified, coinfection with another virus was observed including rhinovirus (n= 2), influenza A virus (n= 1), coronavirus OC43 (n= 1) and RSV A/B (n= 1). The presence of *B. pertussis* did not appear to cause any significant clinical or laboratory differences in patients.

Conclusions. *B. pertussis* is a rare pathogen in patients admitted to hospital for acute bronchiolitis. However, in patients who do not respond to standard bronchiolitis treatment, *B. pertussis* should be considered as a causative agent. Early identification of this pathogen is important in terms of quarantining the patient, administering appropriate antimicrobial treatment, and prophylactic treatment to household and other close contacts.

Key words: acute bronchiolitis, *Bordetella pertussis*, hospitalized children, incidence.

Acute bronchiolitis is the most common lower respiratory tract disease which occurs due to inflammatory obstruction of the small airways in children. Generally, it is observed in the first 2 years of life.[1] Respiratory syncytial virus (RSV) is responsible for >80% of lower respiratory tract infections in children younger than 1 year.[2]

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Pertussis or whooping cough is an endemic disease with high morbidity and mortality in infants caused by *Bordetella pertussis*. Globally, nearly 16 million pertussis cases are observed each year and it causes 195,000 deaths. Pertussis typically causes a clinical picture characterized by three periods of catarrhal, paroxysmal and convalescent stages. Since the implementation of vaccinations, this classic clinical progression is observed less frequently.[3]

In patients presenting with bronchiolitis, coinfections caused by more than one

respiratory tract viruses may be seen. Similarly, *B. pertussis* can also be detected with respiratory tract viruses. However, there are different data in the literature about the frequency of pertussis in young children with bronchiolitis. Although the frequency is reported as high in some studies; it is detected too low in others.[4-9] This study aimed to identify the incidence of *B. pertussis* as pathogen in children with acute bronchiolitis, and to research the effect of its presence on clinical and laboratory features.

Material and Methods

The study included patients admitted to the Division of Pediatric Infectious Disease from January 2012 to December 2015 diagnosed with acute bronchiolitis, aged 24 months or younger, evaluated for both viruses and bacteria with polymerase chain reaction (PCR) in respiratory tract secretions. Within the first 48 hours of admission to hospital, patients had nasopharyngeal secretion samples taken with aspiration and were evaluated for the following viruses; RVS A/B, rhinovirus, adenovirus, bocavirus, metapneumovirus A/B, parainfluenza virus 1, 2, 3, 4, coronavirus 229E, NL63, OC43, HKU1, influenza virus A/B, H1N1, enterovirus and parechovirus) and bacteria; *B. pertussis, Mycoplasma pneumoniae, Legionella pneumophila, Chlamydophila pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae* with the PCR method.

Statistical assessment was completed with the Statistical Package for Social Sciences (SPSS) Windows 20 (IBM SPSS Inc., Chicago, IL). Normal distribution of data was analyzed with the Kolmogorov-Smirnov test. As numerical variables did not display normal distribution, they are shown as median (interval; min-max). Differences between numerical variables in two category groups were examined with the Mann-Whitney U test. For all analyses, $p<0.05$ was accepted as statistically significant.

Informed consent was obtained from the patients included in the study. The study was approved by the local ethics committee with number of 12-560-16 (27/06/2016).

Results

Of 847 patients admitted to hospital with acute bronchiolitis diagnosis, a total of 380 patients aged 24 months or younger with PCR investigation of respiratory tract secretions for viruses and bacteria were included in the study. Of the patients 161 (42.4%) were girls and 219 (57.6%) were boys. The median age of diagnosis was 4 months (range; 1-23 months).

Of the 380 patients, 326 patients (85.8%) were identified to be positive for at least one respiratory pathogen. *B. pertussis* was detected in 5 (1.5%) of these patients. Significant differences were not observed between *B. pertussis* positive and negative patients in terms of gender, age of diagnosis, history of prematurity, previous attendance at health organizations, presence of individuals with cough in the family and vaccination history ($p >0.05$) (Table I).

The most common agents were *S. pneumoniae* (66.3%), *H. influenzae* (43.9%), RVS A/B (53.7%) and rhinovirus (25.3%) in children admitted to the hospital for acute bronchiolitis. Coinfections with more than one virus were identified in 93 (24.5%) patients. The most common of these were rhinovirus and RSV A/B (4.5%) and rhinovirus and adenovirus (2.4%). Simultaneously detected pathogens in patients with *B. pertussis* were as follows; influenza A (n= 1), coronavirus OC43 (n= 1), RSV A/B (n= 1), rhinovirus (n= 2), *M. pneumoniae* (n= 1), *S. pneumoniae* (n= 2) and *H. influenzae* (n= 3). The demographic, clinical and laboratory characteristics of patients with *B. pertussis* are given in Table II.

Significant differences were not identified in terms of clinical, radiologic and laboratory findings of patients according to the presence of *B. pertussis* ($p >0.05$) (Table III). The proportion of patients positive for *B. pertussis* with white cell count above 10,000 was 60%, while this rate was 54.5% for *B. pertussis* negative patients ($p= 0.807$). For white cell counts above 15,000, the

Table I. Demographic findings of patients according to *Bordetella pertussis* positivity.

Variables	*Bordetella pertussis*		p
	Negative (n= 321)	Positive (n= 5)	
Gender*			
Female	137 (42.7)	2 (40.0)	0.904
Male	184 (57.3)	3 (60.0)	
Age of diagnosis (months)**	4 (1-23)	4 (1-13)	0.765
Prematurity*	26 (8.1)	-	-
Previous admission to another health center*	150 (46.7)	4 (80.0)	0.193
Household member with cough*			
None	47 (14.6)	-	0.574
Unknown	109 (34.0)	1 (20.0)	
Yes	165 (51.4)	4 (80.0)	
Mother	20 (12.1)	1 (25.0)	0.934
Father	9 (5.5)	-	
Siblings	60 (36.4)	1 (25.0)	
Others	9 (5.5)	-	
Several people	31 (18.8)	1 (25.0)	
Unknown	36 (21.8)	1 (25.0)	
Number of pertussis vaccines*			
None	74 (23.1)	1 (20.0)	0.512
1	75 (23.4)	1 (20.0)	
2	40 (12.5)	2 (40.0)	
3	112 (34.9)	1 (20.0)	
4	19 (5.9)	-	
Uncertain	1 (0.3)	-	

* Categoric variables shown as number (%)
** Numerical variables without normal distribution shown as median (min-max)

rate was 20% for patients positive for *B. pertussis* and 19.6% for *B. pertussis* negative patients (p= 0.993).

In this study, the most frequent admissions occurred in January and February (19.7%), followed by December (11.8%). Median hospital stay was 5 days (interval; 1-43 days). *B. pertussis* positive patients were admitted in February, March, May, August and October. Median hospital stay for *B. pertussis* patients positive was 4 days (interval: 2-10 days), while it was 5 days for *B. pertussis* negative patients (interval: 1-43 days) (p= 0.778).

Of patients admitted for acute bronchiolitis, 12.1% (n= 46) were hospitalized in intensive care units and median duration of hospitalization was 3 days (interval: 1-37 days). Of patients, 10.5% (n= 40) required mechanical ventilation. The rate of patients readmitted within one month after discharge was 7.6% (n= 29). None of the patients positive for *B. pertussis* was admitted to the intensive care unit. All *B. pertussis* positive patients received oxygen, oral salbutamol and oral clarithromycin therapy during hospitalization. The rate of use of clarithromycin among patients positive for *B. pertussis* was 100%, while it was 24.6% for *B. pertussis* negative patients (p=0.016). There were no significant differences for other administered treatments in terms of the presence of *B. pertussis*.

Table II. Demographic, clinical and laboratory characteristics of patients positive for *Bordetella pertussis*.

Variables	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (months)	13	1	5	4	3
Gender	Male	Female	Male	Female	Male
Year/month admitted to hospital	2015/August	2014/February	2013/March	2013/May	2014/October
Duration of complaint (days)	6	2	7	21	13
Dose of pertussis vaccination	3	0	2	2	1
Clinical findings	Fever, cough, rhinitis	Cough, rhinitis	Fever, cough, rhinitis, wheeze	Cough, wheeze	Cough, rhinitis, vomiting, facial flushing, wheeze
Physical examination findings	Tachypnea, retraction, prolonged expirium, rhonchi, rales	Prolonged expirium, rhonchi	Tachypnea, retraction, prolonged expirium, rhonchi, rales	Tachypnea, retraction, prolonged expirium, rhonchi, rales, wheezing	Tachypnea, prolonged expirium
Chest radiography findings	Infiltration	Hyperinflation	Infiltration + Hyperinflation	Infiltration + Hyperinflation	Hyperinflation
White cell/ lymphocyte count (/mm^3)	11100/3100	9300/6900	15300/6700	7300/2600	10600/6600
ESR/CRP values (mm/h, mg/L)	16/16.6	44/1	68/45.1	10/1	6/1
Coinfections	*Mycoplasma pneumoniae*	RSV, influenza A	Rhinovirus, *S.pneumoniae, H.influenzae*	Coronavirus OC43, *H. influenzae*	Rhinovirus, *S.pneumoniae, H.influenzae*
Bronchodilator / steroid treatment	+/+	+/-	+/+	+/+	+/+
Antibiotic treatment received	Clarithromycin	Clarithromycin	Clarithromycin, SAM	Clarithromycin	Clarithromycin

ESR: estimated sedimentation rate, CRP: C reactive protein

Discussion

Pertussis may result in severe progression in infants and children. Although the effective development of vaccination programs, globally each year it still causes millions of cases and thousands of deaths.[10] Acute bronchiolitis is the most common cause of hospitalization in children under 2 years of age.[11] Today, there is a great interest in the presence of pertussis in children hospitalized due to acute bronchiolitis and its effect on the clinical status. The detection of causative pathogens in respiratory tract infections by molecular methods and the life-threatening conditions of these two diseases in young children have aroused interest in the association of pertussis infection in hospitalized patients. In our study, *B. pertussis* was identified in only 5 patients; this suggests that it is a rare pathogen in patients with acute bronchiolitis. Many physicians prescribe macrolide treatment considering the possibility of pertussis in patients hospitalized. This in turn increases the use of macrolide antibiotics and contributes to

Table III. Clinical, physical examination, laboratory and imaging findings of patients according to *Bordetella pertussis*.

Variables	*Bordetella pertussis*		p
	Negative (n= 321)	Positive (n= 5)	
Clinical findings			
Cough*	321 (100)	5 (100)	-
Rhinitis*	240 (74.8)	4 (80.0)	0.789
Wheeze*	226 (70.4)	3 (60.0)	0.636
Fever*	153 (47.7)	2 (40.0)	0.733
Vomiting after cough*	27 (8.4)	1 (20.0)	0.364
Facial flushing during cough*	29 (9.0)	1 (20.0)	0.385
Conjunctival redness*	2 (0.6)	0 (0)	0.859
Facial swelling*	0 (0)	0 (0)	
Suspiration*	0 (0)	0 (0)	
Physical examination findings			
Prolonged expirium*	316 (98.4)	5 (100.0)	0.779
Rhonchi*	26 (83.5)	4 (80.0)	0.836
Rales*	247 (76.9)	3 (60.0)	0.331
Tachypnea*	235 (73.2)	3 (60.0)	0.614
Retraction	204 (63.6)	3 (60.0)	0.870
Wheezing*	46 (14.3)	1 (20.0)	0.543
Apnea*	6 (1.9)	0 (0)	0.758
Radiological findings			
Hyperinflation*	253 (79.1)	4 (80.0)	0.959
Infiltration*	158 (49.4)	3 (60.0)	0.683
Atelectasis*	35 (10.9)	0 (0)	0.434
Laboratory findings			
White cell (x103)**	10.6 (1.3-28.6)	10.6 (7.3-15.3)	0.819
Lymphocytes (x103)**	4.5 (0.9-16.5)	6.6 (2.6-6.9)	0.686
Platelets (x103)**	369 (39-896)	342 (269-764)	0.720
Eosinophil**	100 (0-1300)	300 (0-800)	0.194
Erythrocyte sedimentation rate**	20 (0-122)	16 (6-68)	0.493
C-reactive protein**	6.1 (0-117.2)	1 (1-45.1)	0.532

* Categorical variables shown as number (%)
** Numerical variables shown as median (min-mix)

the development of resistance. In our study, we have concluded that the frequency of pertussis is low in patients hospitalized due to acute bronchiolitis in the hospital and therefore macrolides should not be used unnecessarily.

Our findings are similar to Piedra et al.'s[4] study that identified only 4 cases (0.2%) among 2207 hospitalized children aged less than 2 year-old with acute bronchiolitis, whereas RSV was found in 72% of all children. In Korppi et al.'s[5] study respiratory viruses were responsible for 89% acute bronchiolitis in infants hospitalized aged under 6-months of age. RSV was found in 71% of cases, and *B. pertussis* was not detected in any patient. Similarly, Abu Raya et al.[6] analyzed 309 hospitalized children with bronchiolitis aged ≤2 years of age They found

that 7.7% of cases with *B. pertussis* and 67% of cases with RSV were hospitalized during the 2005-2006 peak acute bronchiolitis season. Just like the above studies Siberry et al.[7] identified only 1 of 166 patients admitted to the hospital with respiratory symptoms to have a positive *B. pertussis* PCR result during the RSV season. In contrast to these studies that found *B. pertussis* to be rarely detected in patients with acute bronchiolitis, in the literature, different data concerning the frequency is present. In Gökçe's et al.[8] study from Turkey, *B. pertussis* was identified 44 (25.6%) of 172 infants aged <6 months old and coinfection with other viruses was detected in 17 (38.6%) of 44 patients. Additionally, 51.1% of all infants had RSV which was a commonly isolated pathogen. The authors suggested that this high prevalence of *B. pertussis* was due to the fact that the patients had either not received any or only a single dose of the pertussis vaccine due to their small age. Another study in Finland reported that RSV was the most common causative pathogen and *B. pertussisis* was detected in 12 (8.5%) of 142 infants younger than 6 months hospitalized for acute bronchiolitis. In addition, coinfection with RSV was found in 8 of the patients with *B. pertussis*.[9]

Pertussis is commonly observed as coinfections with other respiratory tract pathogens. RSV with *B. pertussis* increases morbidity and mortality risk.[12] There are limited numbers of studies assessing the association of *B. pertussis* with RSV and coinfection varies from 0-78%.[4-6,13-16] The studies examining the relationship between RSV and *B. pertussis* coinfection in patients with acute bronchiolitis are summarized in Table IV.

Pertussis and acute bronchiolitis cause serious infections that require hospitalization especially in young children. In our study, the age interval for the whole population was 1.24 months, with 57.6% of patients being male. There were no significant differences identified between *B. pertussis* positive and negative patients related to gender and age.

In our research, cough was present in all patients. The rates of vomiting after coughing (8.4%) and facial flushing during coughing (8.9%) were low. Studies have identified the incidence of *B. pertussis* infection as 13-20% for patients admitted with long-term coughing complaints.[4] A study encompassing the 0-16-year age group in Turkey identified *B. pertussis* in 16.9% of patients with cough lasting more than two weeks.[5]

Leukocytosis along with lymphocyte dominance supports the diagnosis of pertussis. The increase in leukocyte count and degree of lymphocytosis is parallel to the severity of the disease. However, these findings are not unique to pertussis.[17,18] In our study, there were no significant differences observed in terms of laboratory findings between *B. pertussis* positive and negative patients.

Radiological investigation is not a necessity for pneumonia diagnosis in children; however, observation of infiltration on chest radiographs supports pneumonia diagnosis.[19] In our study, 49.4% of patients had infiltration and 79.1% had hyperinflation observed on chest radiographs, although there were no statistically significant radiological differences observed between *B. pertussis* positive and negative patients.

The information about the seasonality of pertussis is not clear. Epidemics are mainly observed in the winter and spring months.[4] Generally, the disease is endemic in the months of July to October.[20] Acute bronchiolitis peaks in the winter and spring months.[21] In our study, the majority of acute bronchiolitis patients were admitted to the hospital between December to May. Additionally, the majority of patients with *B. pertussis* identified were admitted from February to May. These findings lead to the consideration that admission for acute bronchiolitis accompanying *B. pertussis* increases during the winter and spring months. Though this hypothesis complies with the literature, there is a need for prospective studies with larger samples.

Table IV. *Bordetella pertussis* and RSV coinfection rates in patients with acute bronchiolitis.

Authors	Study years	Number of patients	Age	*B. pertussis* n (%)	BP-RSV Coinfection	*B. pertussis* Clinical findings
Frühwirth	1995-1998	183	< 18 years	71 (38.8%)	-	-
Greenberg	1998-2001	74	< 12 months	11 (15%)	6 (54%)	No
Crowcroft	1999-2000	142	< 5 months	33 (23%)	11 (33%)	No
Moore HC	2000-2005	1669	< 9 years	354 (21.2%)	-	-
Nuolivirta K	2001-2004	205	< 6 months	12 (8.5%)	8 (67%)	No
Guinto-Ocampo	2001-2005	141	< 12 months	18 (13%)	-	No
Cosnes-Lambe	2005-2006	126	< 4 months	19 (15%)	14 (73%)	No
Korppi M	2005-2006	117	< 6 months	9 (8%)	7 (78%)	No
Raya BA	2005-2006	309	< 24 months	24 (7.7%)	16 (67%)	43% prolonged coughing
Miron D	2005-2006	465	< 24 months	29 (6.2%)	-	-
Walsh PF	2005-2006	204	< 18 months	0	0	No
Pedro A	2007-2010	2207	< 24 months	4 (0.2%)	2 (50%)	2.4% prolonged coughing
Jolien T	2007-2010	3074	> 18 years	93 (3%)	-	37% prolonged coughing
Korppi M	2008-2010	408	< 24 months	0	0	No
Ivana PE	2009-2010	596	< 5 years	114 (19.2%)	15 (13.1%)	No
Gökçe Ş	2013-2016	172	< 6 months	44 (25.6%)	16 (36.4%)	9.1% prolonged coughing

Supportive care is the most important approach for pertussis treatment. Sufficient hydration and nutrition are important to reduce the frequency of coughing. Antibiotic treatment can only partly reduce symptoms and prevent infectiousness by eliminating microorganisms from the nasopharynx. Macrolide antibiotics are used for treatment.[22] In our study, all patients with *B. pertussis* used clarithromycin, and this was significantly high compared to the *B. pertussis* negative group.

There are some limitations of our research. Some patients did not have a viral or bacterial pathogen identified with the PCR method. These patients may have had non-infectious causes mimicking pulmonary infections like asthma or gastroesophageal reflux, or there may have been possible problems related to respiratory tract secretions not appropriately obtained, stored or studied, or infectious agents not yet identified or not included on the microbiology panel. Another limitation of the study was that we did not have data on whether patients received macrolide antibiotics before admission. PCR testing following antibiotic therapy also can result in false negative findings. Therefore, more extensive studies are needed.

In conclusion, *B. pertussis* is a rare pathogen in patients admitted to hospital for bronchiolitis. Clinical findings of pertussis may be atypical in partly-vaccinated infants and coinfection with a respiratory virus may create additional difficulties for pertussis diagnosis. For cases admitted to hospital who do not improve with conservative treatment, *B. pertussis* should be considered as a causative agent.

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Original Article

The experiences, perceptions and challenges of mothers managing asthma in their children: a qualitative study

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ABSTRACT

Background. Despite the fact that childhood asthma poses an important burden, especially on the families, little is known about the emotional experiences of mothers. This article describes the predominant emotional challenges and experiences reported by mothers of children with asthma.

Methods. Individual, semi-structured, in-depth interviews with a qualitative study method was used in collection of the data. Face-to-face interviews with mothers of children with asthma were conducted using socio-demographic data form and semi-structured interview forms developed by the investigators. The transcribed interview texts were analyzed according to a qualitative content analysis.

Results. A total of 20 mothers were interviewed. We found that the experience of mothers of the children with asthma was a strenuous journey of overriding an emotional rollercoaster, that is, from being thrown into a chaotic situation to later processing the difficult situation affected by the asthma. First moment at diagnosis, relaxation against uncertainties, anxiety, fear, truly acceptance, sadness were the predominant emotions. Mothers described experiences and challenges of frequent admission to emergency, administration of medication and treatments, school problems, limitations in physical activity and spouse relationship problems. Experiences of problems contributed to hopelessness, abandoned, angry and burnout. Mothers had concerns about the chronic nature of the disease, side effects of medications, complications that might develop, factors influencing the disease, and future plans.

Conclusions. Mothers of children with asthma described complex emotional journeys. This has implications for healthcare providers who need to be aware of the complexity of these emotional journeys to support parents more effectively, thereby helping improve patient outcomes. Parents should be trained for symptoms and disease management with a written action plan. It is imperative to realize multi-disciplinary team collaboration and to regularly review training and information. Future research should concentrate on promoting awareness, education, advocacy, and support for parents of asthmatic children.

Key words: asthma, caregivers, child, mothers, qualitative.

Asthma may decrease quality of life in children as it affects their physical, mental, social, and emotional development.[1,2] If asthma is uncontrolled, children may experience frequent emergency department admissions and hospitalizations along with many attrition from school and limitations in their daily activities such as playing, extracurricular activities, and doing sports. Parents have a critical role in the management of the disease, however, this situation may have a serious psychological effect on parents which, can lead to feelings of inadequacy, helplessness and depression.[3,4] Families' adaptation to the disease and difficulties in asthma management may influence asthma control. Previous studies in other countries have reported that parents may have insufficient knowledge on triggers, insufficient information for the care and difficulties on the recognition of asthma symptoms.[5-7] Parents' attitudes towards diseases are, to a certain extent, a

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reflection of the cultural characteristics and the health infrastructure of that society. Only few studies have been performed in Turkey for the perception of asthma on children[8], pharmacists[9], and physicians[10] but not on parents, hence this needs to be documented to improve asthma management. In our country, no study investigating the families' difficulties in managing asthma could be found.

As healthcare professionals, in order for us to help the child with asthma and the caregiver, it is very important to examine the real experiences of mothers during the caregiving process. Knowing the characteristics of caregiving mothers, having comprehensive knowledge about their experience, knowledge, and perceptions, determining the care to be given by healthcare institutions, improving this care, determining the sources for the service to be offered, determining the groups that are under risk of experiencing difficulty, establishing the groups according to these characteristics, and determining the need for education and knowledge are very important. For this reason, the qualitative studies on the difficulties, perceptions, and personal experiences of parents of children with asthma during the disease process are needed. The present study aims to document the experience, perceptions and challenges of mothers who have a child with asthma, therefore provide insights for better precautions to be taken in this regard.

Material and Methods

Study Design and Study Population

The present descriptive-qualitative study was carried out using the semi-structured interview method. A semi-structured survey form was used in in-depth interviews in order to determine the concerns of parents related to treatment and care.

The study population consisted of mothers of children who were followed up in the allergy outpatient department of Hacettepe University Ihsan Dogramacı Children's Hospital. At the beginning of the study, no specific number of study subjects was specified and it was planned to stop when adequate data was achieved. In other words, it was aimed to achieve data satisfaction in in-depth interviews.[11,12] By using purposeful sampling method, in-depth interviews were conducted with mothers.

The volunteer mothers, who had children diagnosed with at least 1 year and aged <5 years with a Turkish version of "Test for Respiratory and Asthma Control in Kids (TRACK)"[13,14] questionnaire total score of 80 and below (lack of good control over disease), no chronic disease other than asthma, and no communication or speaking disability, were involved. Since the group that could successfully manage the asthma process was fully controlled, the parents in this group have less experience on the difficulties in asthma management. Since the main target here is to examine thoroughly the families having difficulties during asthma management, the families that are under full-control according to TRACK test were not involved in the present study. The families in this group generally visit the departments for routine checks and have medication prescriptions. The present study was completed with 20 mothers having a child with asthma.

Data Collection Forms

Family and Child Information Form: This form included questions about the socio-demographic characteristics of children and their parents, information about the disease and treatment, and total score in TRACK.

The interview schedule

Using the semi-structured interview guideline prepared by the researchers, the open-ended questions about what they know about asthma, how it affects their lives, which difficulties they have had, what they have done during such difficulties, what they have experienced in this process, and which coping mechanisms they have used were asked. By asking questions such

as "Could you please provide more information about this subject" or "Could you please give more details", it was aimed to comprehensively examine the topic.

Test for Respiratory and Asthma Control in Kids: TRACK is a test that has proven reliability and validity.[13] The Turkish version of TRACK questionnaire, validity and reliability of which were shown before[14], was used. The TRACK questionnaire was developed for children aged <5 years in accordance with the guidelines of National Asthma Education and Prevention Program of the USA. It includes five items about the frequency of symptoms in the last four weeks, awakening at night and activity constraints due to symptoms, frequency of use of bronchodilator in the last three months, and use of oral corticosteroid within last year. Each of the items is rated between 0 and 20 points. Scores higher than 80 points indicate good control of the disease, scores between 60 and 80 points indicate partial control, and those lower than 60 points indicate an uncontrolled disease. Those children with a TRACK score ≤80 were involved in the present study

Data Collection Procedure

In the present study, the patients coming for examination in the department and meeting the inclusion criteria were examined by assistant researchers and the patients, who were "not controlled" according to TRACK test and who voluntarily participated, were referred to the primary investigator (PI). The PI met the patients in the outpatient environment and informed them about the objective and implementation steps of the research before every interview. The PI informed them that the patients would be asked questions by making use of a guideline and the patients would have an opportunity to talk as long as they want. In order to establish a warm connection with patients at the beginning of interviews, the descriptive patient information forms were filled first and, by making use of voice recording the families concerns, perceptions, and difficulties during the management of asthma were comprehensively analyzed.

The interview schedule was not strictly followed, instead it was adapted to mother' narratives and included in the process of reflecting and probing of what was important, for example, by responding: 'you said that ... tell more about that ..., what were your feelings.' The interviews lasted between 30 and 45 minutes and were conducted in a quiet room. The interviews were recorded using a voice recorder and were performed by the PI, who has been certified for this research method. The face-to-face interviews were continued until reaching the saturation and the data repeated themselves. Although the data saturation was achieved in some of questions, the data saturation was achieved in the 20th interview because different answers were given to the other questions and the process was stopped at the 20th interview. The data were collected between 1st November and 31st December 2019.

Data Analysis

The data were analyzed using SPSS 23 statistical software for descriptive statistics (percentage, mean values, standard deviation, and minimum–maximum values). The interviews were recorded digitally and transcribed verbatim. The first stage involved becoming familiar with the transcript and noting any essential aspects, observations, and preliminary interpretations. Then emerging themes were noted and transformed into more specific themes, which were clustered by connecting them, followed by capturing the main categories of meaning conveyed by the participants. At the end of the process, a summary of the higher- order themes were conducted. The first and the second author read the transcripts and developed the thematic framework independent of each other. The two authors also decided together which themes best described the parents' lived experiences. Finally, the third author read the themes and analyses to ensure that the meaning of the participants' narratives was significant.

Ethics and Informed Consent

The Non-Interventional Ethics Committee of Hacettepe University (08th January 2019, GO 19/36) approved the study. Written informed consents of the patients' legal guardians were obtained during diagnosis for use of patient data in scientific publications. The participant was not harmed in any physical or emotional manner. All possible or adequate information on the goal of the investigation; the procedures that were followed during the investigation; the possible advantages, disadvantages, and dangers to which the participant might have been exposed to; as well as the credibility of the researchers was disclosed to the participant. The participant was given information about what the study entails and what would be expected from the participant. This allowed her to make a voluntary decision to take part in the study. They were able to withdraw from the study at any time. The PI explained to the participant that emotions, such as uneasiness, might be experienced as she recalled previous unpleasant experiences. The participant's real names were not used. The participant had no concerns or reservations about the content of the interview. The transcripts therefore remained unchanged and did not influence the analysis and interpretation of the data.

Trustworthiness

To ensure trustworthiness, peer debriefing was used, where the research process and findings were reviewed and discussed with unbiased colleagues. Due to the fact that researchers possess specific knowledge on the research subject, it influenced the choice of design and interpretation and conclusion of results to some degree. They knew what they were looking for and were able to be more focused during the data collection process. The literature review allowed them to apply logical reasoning and interpretation of the data collected. Direct quotations from the interviews that were conducted were included.

Results

A total of 20 children (60% boys) with a mean age of 3.88 ± 0.80 years whose mothers were 36 (25-40) years old were included in the study. From these children 70% were on regular inhaled corticosteroid treatment and there were no reports of hospitalization, but there were on average 3 (1-7) emergency admittances during the past year. The mean (SD) TRACK score was 44±12.27. Table I presents the study sample characteristics.

The results are presented in five themes: feelings at diagnosis; feelings during the treatment; challenges, concerns und suggestions comprehended by the overall theme an emotional rollercoaster (Fig. 1). In the following quotations, the mother is denoted as M. The number denotes quotations cited from the 20 different interviews.

Emotional rollercoaster

Mothers reported that they were suddenly thrown into a chaotic situation, which often started with a sudden and unclear attacks, followed with enduring time of uncertainty of the process and, moreover, continued with learning successively to understand the circumstances they were set for and finally bringing the experience of having a child with asthma, putting into words how it all affected them.

The feelings of mothers at diagnosis

From the beginning, the parents found it very difficult to see their children constantly getting sick due to an unclear cause. The mothers experienced a relief when they heard the diagnosis because no exact diagnosis could be made in this process but the asthma diagnosis after the recurrent use of antibiotics removed the uncertainties, the anxiety and fear about the adverse effects of medications and potential permanency of disease, sadness because of the negative experience and speculations about the diagnosis, and true acceptance because sibling

Table I. Characteristics of study participants (n= 20).

Characteristics	Mean (SD)	Min-max
Mother's Age (years)	36 ± 1.80	25-40
Child's Age (years)	3.88 ± 0.80	2-5
Treatment duration (years)	2 ± 0.40	1-3
Emergency admittances within last year	3 ± 1.0	1-7
TRACK score	44 ± 12.27	5-60
Highest education level	n (%)	
High school	17 (85)	
University	3 (15)	
Current employment status		
Employed full-time	5 (25)	
At home full-time caregiver	15 (75)	
Family annual income		
Less than 3000 TL	1 (5)	
3001-5000 TL	14 (70)	
More than 5000 TL	5 (25)	
Child's gender		
Girl	8 (40)	
Boy	12 (60)	
Number of children		
1	12 (60)	
2 and more	8 (40)	
Regular inhaled corticosteroid treatment		
Yes	14 (70)	
No	6 (30)	

received the same diagnosis. Some of the mothers' own statements are presented below:

"The disease began with pneumonia, my child had many bronchiolitis, we stayed in the hospital and he used too much antibiotics. Then, they referred us to this unit. I was very sad and scared at the beginning and wondered if we could get rid of this disease. However, the medications resulted in relief and it brought a certain level of order to our lives (M2)"

"My child started taking Ventolin® when aged 3-4 months old, but no definitive diagnosis could be made. They told us that they couldn't diagnose the condition. My child had difficulties in breathing and then had cough and nausea. He was on antibiotics and Ventolin® and got better for one month but then became sick. When the asthma diagnosis was made, the uncertainty disappeared. Together with the treatment, we stopped using antibiotics and relaxed a little bit (M3)"

"The diagnosis was made 2 years ago. Since my mother had asthma, I felt very sorry because I knew that it was a very hard process. Thus, I was afraid. I worried about no cure being found (M11)."

Experience and Challenges

Most of the parents stated that their domestic and business lives were affected because of effects on the marriage, being unable to take care of other children and being frequently admitted to emergency services. There were most common challenges with the use of inhalers and medications, struggle with long-term treatments, the recurrent nature of the disease, school problems, limitations in physical activity, not being able to care for other children and spousal problems. Some of the mothers' statements are presented below;

"We had a minimum of 3 unplanned physician visits, and 3-4 planned physician visits last year. In attack

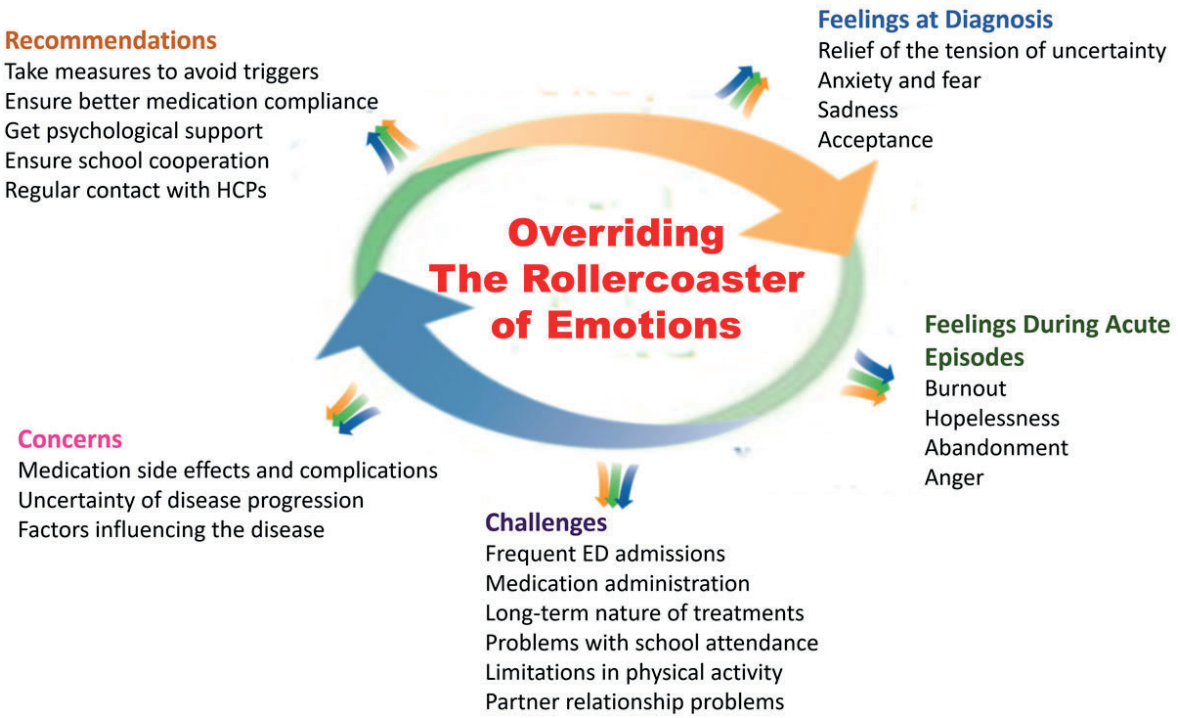

Fig. 1. Example of subthemes, theme and Main theme.

periods, we stop everything and take care of him. We cannot take care of our other child. We cannot pay attention to when he will come from school, what he will eat, or what he will do. His brother also feels sorry about this (M9)

"The antibiotics taste very bad, and children do not want to take them. She doesn't want to use the aero-chamber. She walks for 5 minutes and easily feels tired. These are all very difficult (M2)"

"I feel so weak. My husband helps more often. We use the inhaler but unintentionally because I am very tired. The repetition of this process is very tiring for me. Besides these problems, we are upset because our child cannot participate in activities (M3)"

"Our child adheres to medications and has no problem taking doses, but we have significant problems at school. Sometimes there may be family activities at school, including working with children. She runs for a very short time and gets tired very quickly. She is upset because she can't keep up with her friends, which affects us. If it takes too long or she sweats too much, problems such as coughing or wheezing begin (M10)"

"We spend all the time in the emergency unit in winter. I often argue with my husband in the attack periods. (M17)"

"We have to pay attention since our child should never sweat a lot. If there is a sick child at school, we worry so much because our child can easily get sick. I always put Ventolin® and an aero-chamber in his bag and remind his teachers of his medication. I tell them to give him the inhaler (M15)"

The feelings of mothers during the treatment

The mothers experienced a feeling of anger and abandonment when they described not being able to take care of their child themselves and when she is not helped by her husband. The mothers felt burnout and hopelessness, when frequently admitted to emergency services, the workload and caregiving burden increased significantly. Most of the parents stated that their domestic and business lives were affected because of influences on the marriage or being unable to take care of other children. Some of the mothers' own statements are presented below.

"I sometimes felt desperation and hopelessness. It had significant effects on my life. I feel abandoned. I'm very angry with my husband leaving all the responsibility to me (M1)."

"Our daily life is significantly affected. I always need to leave my job. Our lives are significantly affected. I'm burnt out. (M2)."

"My life is over. We almost cannot go out. I became obsessed with cleaning; I wipe everywhere using bleach and vinegar-water mixture. I have to shake the carpets every week, air the beds and sheets, change clothes every day, and clean everything using anti-allergic soap. I feel so tired. (M7).

"It tires me a lot because I don't know which factors trigger the attacks. I think that she is OK but, after only 2 weeks, she falls sick. Then, I start to think about what I have done wrong. I start blaming myself and feel hopelessness (M18)."

Concerns

Mothers have concerns about the chronic nature of the disease, side effects of medications, complications that might develop, factors influencing the disease, and future plans. Some of the mothers' own statements are presented below.

"I am really worried about whether the disease is permanent. I am also particularly concerned about complications and respiratory arrest as I am a healthcare professional. (M1)."

"We always think 'what if the disease was over? Will we always be able to cope with it? and will these medications always be administered?'. After all, every medication has effects and side effects. What will the side effects of these medications be? I am pregnant now; will my second baby have asthma? How will our lives change? Of course, we have concerns and fears about the future (M2)."

"The weather conditions worry me a lot. If the weather is bad, I worry about how my daughter will be affected. Bad weather conditions and air pollution immediately affect her. It worries me a lot (M6)."

"We heard that Ventolin® may cause sudden cardiac arrest. This worries me. We learned that using inhaler would permanently enlarge the lungs and I am also very anxious about this. Besides these issues, the side effects also worry me a lot. Her cough has recovered now, but what will happen in the future? She takes so much medication for a child her age that I'm very anxious about it.. It makes me very anxious to think that this disease will become chronic (M13)."

Coping and Recommendations

Mothers made several recommendations that they believed would assist families in overcoming the emotional rollercoaster they described. The suggestions of mothers for minimizing the difficulties are measures against the triggers, compliance with medications and treatment, psychological support, and cooperation with teachers and keeping in touch with health professionals. Some of the mothers' own statements are presented below.

"I recommend paying more attention to the protective measures. A friend of my husband recommended cow milk; we used it for 2 weeks, as well as carob extract and other similar herbal products together with the medications, but none of them produced an effect. One should never use them without physician recommendation. Compliance with the medication and treatment is very important (M1)."

"They must comply with the treatment. It may sometimes be very difficult to accustom the children. It is also very difficult for parents to adapt to the process. However, when complying with the treatment regularly, I benefited from the therapy. The number of attacks decreased and we had more comfortable periods. Psychological support can be considered for a child in need. If not financially affordable, it is very difficult to outsource the psychological support. However, if the hospital offers such an option, they might be more comfortable (M2)."

"In case of an intense cough, they should stay away from pets and take measures against the triggers because staying in the same environment with a pet for only one hour may cause an increase in the

attacks. Thus, it is very important to take measures against the triggers and allergies (M5)."

"A good treatment process and education play an important role. I wish we had more information about when and what to do. Mothers should never quit the therapy and keep in touch with medical staff. They should keep their eyes on their children. These children are very vulnerable. I love perfumes but I cannot use any perfume because of my child's hypersensitivity. I clean every day. The most important point I want to emphasize is that they should keep their eyes on their children and do their best to overcome this disease (M6)."

"I believe that teachers should also be informed. I think that the teachers do not know how to approach the children and which games they should play, which activities they should do. They do not know how to behave during the attack periods. The families should establish closer communication with and inform the teachers (M10)."

Discussion

The present study aimed to document the experience, perceptions and challenges of parents who have a child with asthma. The first theme was mother's feelings at disease diagnosis. Mothers emphasized certain specific points such as uncertainties until diagnosis, very frequent use of antibiotics, problems arising from frequently going to the emergency unit because of recurring symptoms, and relaxation because of the elimination of uncertainties after the diagnosis was made. In a systematic review examining the experiences of families having child with asthma, it was found that the parents experienced uncertainty and fear because of the lack of an exact diagnosis, and they had similar relaxation after the diagnosis.[5,15,16] Taking care of a child with asthma is very difficult for the primary caregivers. Uncontrolled asthma causes a decrease in quality of life and is related with negative physical and psychological effects and four times higher costs.[15,16]

The second and third prominent theme concerned challenges and feelings during treatment. Experiences of problems contributed to hopelessness, feelings of abandonment, anger and burnout. The asthma-related difficulties of families were found to be frequent admissions to the hospital, increased workload and caregiving burden at home, problems with other family members, and effects on professional life. Similarly, in previous studies, it was reported that the workload of mothers increased, some mothers had to quit their jobs, they had to ignore their own needs, and the activities performed together with and the care for the spouse and healthy child(ren) were significantly reduced.[17-19] It was emphasized that mothers clean the house almost every day and they change bedsheets every week. Additionally, they do not know what to do, feel desperate, constantly making an effort in order to prevent their children from getting sick, sleep beside their children at night and constantly keep their eyes on their children, and always take leave from their jobs during disease periods, which affects their professional life.[20,21] Challenges were feelings of guilt and blame, concerns about reproduction, feeling helpless and alone, feeling worried about the future, the burden and problems during the diagnostic process. The interpersonal challenges that were identified were the feeling of isolation, negative impacts on relationships, financial impacts, lack of knowledge and understanding among their community, lack of support, and lack of help from the health professionals.[22]

Mothers stressed the difficulties in administering medications. It was also determined that they tend to use complementary-alternative treatments in order to prevent the attacks. In a systematic review on the difficulties of families having child with asthma, it was reported that the families had difficulties in compliance with medications, which affects the control of asthma. The complexity of medications and the inability to understand asthma control causes asthma management driven by the beliefs of parents.[23] Similarly, in previous studies, the causes of noncompliance with medications were as follows: the children not liking the

taste and smell of medications, the emotional discomfort, the use of complementary-alternative treatments, the cease of use of medications in the asymptomatic period, and the intermittent use of medications.[24,25] Some parents have difficulties in distinguishing the reliever medications from the prophylactic ones. Even the parents, who have child with a long history of asthma, may lack in knowledge or be confused. How to use the inhaler and the correct dosage and timing sometimes may not be understood accurately. They may employ different strategies in managing asthma depending on the perceived benefits of the use of medication. Some of the parents try to detect asthma before the intense symptoms, whereas others wait until the first asthmatic attack. The families stated that they tried different medications with an experimental approach, and they administered these medications based on their own decisions.[23] The compliance with medications and treatment is vital for the control of asthma. For this reason, it is very important to raise parents' awareness as well as their compliance with therapy.[25-27] Family's compliance with medication is vital for ensuring and maintaining routines at home.[23]

The fourth theme was concerns of mothers. The most important concerns of mothers about the caregiving process were about the permanence of the disease, side effects of the medications, and potential complications that might develop. Similarly, in previous studies, the families had concerns about the course of the disease and complications. Long-term use of medications also worried the families.[23,28,29] Moreover, families also stated worrying about the long-term effects of oral and inhaled steroids. Some of the participants stated that they worried about future steroid addiction of their children, as well as the caries and the effects on growth and development of organs.[17,23] The families emphasized that asthma medications cause hyperactivity, concentration problems at school, sleep disorders, restlessness, and anxiety among the children.[30-32] Families are the key actors playing a key role in children's compliance with medications.[17,27,33] For this reason, they should be informed and trained by healthcare professionals. This training should be on time, consistent, updated, evidence-based, and specific to the individual.[18] In several studies, it was emphasized that the families overlooked or underestimated the severity of their child(ren)'s disease, and this constituted a major obstacle for asthma management.[32] This result suggests that there may be a deficiency in the knowledge level of families. In the present study, several families could accurately specify the causes and triggers of asthma (environmental factors, genetics, smoking, air pollution, allergy, infection, etc.). However, some of the mothers stated that they had no knowledge of asthma triggers. This result is in line with those obtained in previous studies. In a previous study, the families stated that they did not have sufficient knowledge, and this increased their concerns about the use of medication and disease management.[23]

The final theme was recommendations of mothers. In order to minimize problems, the mothers suggested taking measures against triggers, complying with medications and treatment, and cooperating with teachers, as well as receiving training and psychological support from their spouse and healthcare professionals. It was observed that the parents took measures against asthma attacks, especially for the triggers of attacks. The practices of families are as follows: frequently replacing bed sheets, ventilating the room, and doing cleaning on a regular basis. Similarly, in previous studies, the measures most frequently taken by families in order to cope with the disease were determined to be as follows: keeping the room moist and ventilated, not smoking at home, frequently changing bed sheets, using hypoallergenic beds, and limiting physical activity.[20,21,25]

In studies of the experiences of families, the families having child with asthma, emphasized the lack of training and psychological support provided by healthcare professionals.[19,33-35] Moreover, in previous studies, it was reported that families had problems with school personnel concerning managing asthma and

that many families did not send their children to school during periods of exacerbations.[36] The mothers are generally the primary caregivers of children, and many mothers feel alone in managing asthma.

Family members are confident with the health service quality and their support positively affect the asthma management.[23] In a systematic review on the obstacles and difficulties in asthma control, the most important factors were reported to be the families' knowledge level, beliefs, access to healthcare services, and trust in emergency services.[24] Bellin et al.[20] declared that the mothers felt that they were not understood by healthcare professionals in the hospital environment. Parents having a child with asthma generally need high-quality care and to be listened to, understood, and respected by healthcare personnel, as well as to be given training offering simple and understandable information. Health care professionals will be able to more effectively anticipate and address the needs of caregivers.[23,37] Illness perceptions were associated with asthma control and emotional problems.[38] Previously it was emphasized that establishing asthma action plans are necessary in order to minimize the problems of families.[24,39-43]

In conclusion, this is the first study that examines in detail the experience of mothers with children with asthma and the findings of the current study demonstrated the significant impact mothers emotional problems have on the parents' everyday life and the importance of health professionals' knowledge to identify and support mothers with these conditions. Acknowledging these experiences and their implications will improve interventions and support by health professionals as they assist families. For this purpose, it is recommended to establish asthma action plans in hospitals, offer written and verbal information to the families about diagnosis of disease, recognition, and treatment of symptoms, and use of medications by the healthcare professionals; prepare training guidelines, offer a supportive approach and regular follow-up, and meet psychosocial needs. Future research should examine the role of nurses and their experiences of supporting parents having children with asthma. Future research should concentrate on promoting awareness, education, advocacy, and support for such parents. It would be useful to compare this data with different societies and to examine the changes that will occur in our society over time.

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Original Article

Clinical characteristics of children with congenital anomalies of the kidney and urinary tract and predictive factors of chronic kidney disease

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ABSTRACT

Background. Congenital anomalies of kidney and urinary tract (CAKUT) are the leading causes of chronic kidney disease (CKD) in childhood. Determining the clinical course, outcome, and prognostic factors of this heterogeneous disease group is important to provide appropriate management and follow-up. Therefore, we aimed to identify the risk factors of CKD in CAKUT and the differences in clinical courses between disease subgroups.

Methods. Three hundred patients (M/F: 203/97) divided into 16 CAKUT categories were enrolled in the study. Logistic regression and survival analyses were performed to determine the risk factors for CKD that is defined as estimated GFR (eGFR) lower than 90 ml/min/1.73 m^2 for at least 6 months.

Results. The median age of the study population at the time of the diagnosis was 0.6 years (IQR; 0.1-4.0 years). Among available prenatal diagnoses (n= 138), hydronephrosis (HN) (n= 83; 60.1%) and multicystic dysplastic kidney (MCDK) (n= 39; 28.2%) were the most frequently encountered ones. A total of 24 patients had CKD, and 13 of them (54.1%) progressed to end stage renal disease (ESRD). Patients with posterior urethral valve (PUV) had CKD and ESRD more frequently when compared to the other diagnostic groups ($p < 0.001$ for CKD, and $p < 0.001$ for ESRD). Furthermore, the PUV subgroup progressed to ESRD (median 3.63 years) earlier than the other subgroups. The diagnosis of PUV, proteinuria on the first admission, vesicoureteral reflux, and oligohydramnios were identified as independent predictors for CKD in the multivariate logistic regression analysis.

Conclusions. Knowing predictive factors for CKD in patients with CAKUT is valuable for physicians in order to determine appropriate treatment strategies and prognosis.

Key words: CAKUT, children, chronic kidney disease, risk factors.

Congenital anomalies of kidney and urinary tract (CAKUT) are one of the most common causes of chronic kidney disease (CKD), accounting for 30% of the pediatric CKD population. CAKUT occurs in approximately 3-6 per 1,000 live births,[1] presents an increased risk for CKD, and thus constitutes the most frequent cause of the end stage renal disease (ESRD) requiring renal replacement therapy for survival in children.[2] CAKUT can be diagnosed as early as 18-20 gestational weeks, with the most common prenatal finding being oligohydramnios.[1] It has a broad phenotypic spectrum that consists of many subgroups with different clinical severity and prognosis. It is important to know the clinical presentations and differences in the clinical courses of CAKUT subgroups and to identify the predisposing factors to CKD in order to initiate appropriate treatment in a timely manner. We aimed to determine the risk

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factors for CKD in patients with CAKUT, the differences in respect to the clinical courses, the frequencies of subgroups, and any additional unfavorable impact of CAKUT on the patients.

Material and Methods

This retrospective study consisted 300 patients with the diagnosis of ''Congenital anomalies of the kidney and urinary tract (CAKUT)'' between years 2000-2016. All patients with CAKUT, except for isolated vesicoureteral reflux (VUR), were enrolled in the study. The diagnosis of CAKUT was based on renal ultrasonography, 99m-dimercaptosuccinic acid (DMSA), 99m-diethylenetriamine pentaacetic acid (DTPA) or Tc-99m-mercaptoacetyltriglycine (MAG3) radionuclide scanning and/or voiding cystourethrography (VCUG). Of the patients, 149 had DMSA, 101 had MAG3, 46 had DTPA scanning, and 67 had VCUG either in our hospital or elsewhere. VUR was grouped into low (i.e. grades 1, 2, and 3) or high (i.e. grades 4 and 5). The study population was grouped into 16 categories (Table I). The diagnosis groups of posterior urethral valve (PUV), multicystic dysplastic kidney (MCDK), renal agenesis (RA), hydronephrosis (HN), ureteropelvic junction obstruction (UPJO), and the others which are composed of other diagnosis subgroups of CAKUT, were included in the survival analysis and risk factor analysis for CKD. Patients with hydronephrosis determined by antenatal ultrasonography were defined as antenatal hydronephrosis (antenatal HN).[3] Antenatal HN and HN corresponded to non-obstructive and non-refluxing renal pelvis dilatation. All available data including renal ultrasonography, DMSA, DTPA/MAG3 scanning, VCUG, serum biochemistry, history of urinary tract infections (UTI), antibiotic prophylaxis, urological interventions, proteinuria on the first admission, and the follow-up periods were obtained from the hospital records. Estimated glomerular filtration rate (eGFR) was calculated with the Schwartz formula (k= 0.33 for premature infants <1 year old; k=0.45 for full term infants <1 year old; k= 0.55 for all boys and girls aged 2-12 years and girls aged 13-21 years , ; k= 0.70 for boys aged 13-21 years).[4] CKD was defined as eGFR below 90 ml/min/1.73 m^2 for at least 6 months. Staging of CKD was based on "KDIGO criteria"[5] according to eGFR values as stage 2 (89-60 ml/min/1.73 m^2), stage 3 (59-30 ml/min/1.73 m^2), stage 4 (29-15 ml/min/1.73 m^2), and stage 5 (ESRD, <15 ml/min/1.73 m^2). Specific diagnostic criteria[6,7] were used for the children younger than 2 years and neonates for CKD diagnosis during enrollment. All the patients were evaluated for prematurity, CKD, proteinuria, and UTI. Midstream urine samples after appropriate perineal cleaning were tested, and urine culture was performed to diagnose UTI in the patients with urinary tract symptoms for older children. Urine was collected by urethral catheterization or suprapubic aspiration in infants and neonates. Proteinuria was defined as >0.2 mg/mg.creatinine of a urinary protein/creatinine ratio (Up/Uc) for children older than 2 years[8] and >0.5 mg/mg for children younger than 2 years.[9] Children who did not attend the follow-up visits regularly, who did not have long term follow-up periods or who we were not able to contact by telephone were excluded from the survival analyses.

All procedures in this retrospective study were performed in accordance with the ethical standards of the institutional ethics committee (GO 13/166-27) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all individual participants included in the study.

Statistical Analysis

Statistical Package for the Social Science (SPSS) version 18.0 was used for data analysis. A Chi-square test was applied for assessing the demographic features of the patients. Mean values, medians, interquartile ranges (IQR), and standard deviations (SD) were calculated based on the clinical and laboratory results. Chi-square, Fishers exact, and Yates correction tests were used to analyze the differences between groups. Mann-Whitney U and Kruskal Wallis

tests for continuing variables and the Wilcoxon Signed Rank test for dependent variables were applied. Possible risk factors for CKD identified with univariate analyses were included in the logistic regression analysis to determine the independent predictors of the patient result. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. Survival analysis of diagnosis groups during the follow-up period regarding the progression to CKD was assessed by Kaplan Meier analysis. A p-value less than 0.05 was considered statistically significant.

Results

A total of 300 children (M/F: 203/97) with the diagnosis of CAKUT were included (Table I). The median age at the first admission was 0.6 years (IQR; 0.1-4.0 years), the mean duration of the follow-up was 5.7 ± 3.9 years, and the mean age at the last visit was 8.7 ± 6.1 years. Of the patients, 138 (46%) were diagnosed in the antenatal period at a mean gestational week of 25.5 ± 5.8. Among this group, HN (n= 83; 60.1%) and MCDK (n= 39; 28.2%) were the two leading diagnoses. While the diagnosis of antenatal HN was confirmed in 67 out of 83 patients with postnatal USG, initial prenatal diagnosis was changed in 16 patients after the delivery as follows: UPJO in seven, normal in six, MCDK in two patients, and renal agenesis in one patient (Fig. 1).

Of 67 patients who had VCUG, 24 had no reflux, 43 had reflux (30 low grade, 13 high grade). Twelve out of 43 patients with VUR (28%) also had diagnosis of PUV. Of 20 patients with PUV, 7 had high grade VUR (35%), 5 had low grade VUR (25%). UTI was frequent in the high grade VUR group compared to the low grade VUR group (p= 0.031). Rate of proteinuria at the first admission was similar in patients with VUR (7/43, 16.3%) and without (6/24, 25%) (p= 0.527). Similarly, rate of proteinuria at the first admission was also comparable in patients with high and low grade VUR (15 .4% vs 16.7%, respectively, p= 0.648). The frequency of CKD was 33.3% (10/30) in low grade VUR and 38.5% (5/13) in high grade VUR patients (p= 0.742). Diagnoses were as follows in patients with low grade VUR and CKD: PUV (n= 5), MCDK (n= 1), RA (n= 1), HN (n= 1), UPJO (n= 1), horse shoe kidney (n= 1) and in patients with high grade VUR and CKD: PUV (n= 4), RA (n= 1). A total of 149 patients (49.7%) who underwent 99mTc DMSA; 49 (32.8%) had unilateral, 4 (2.6%) had bilateral renal scarring.

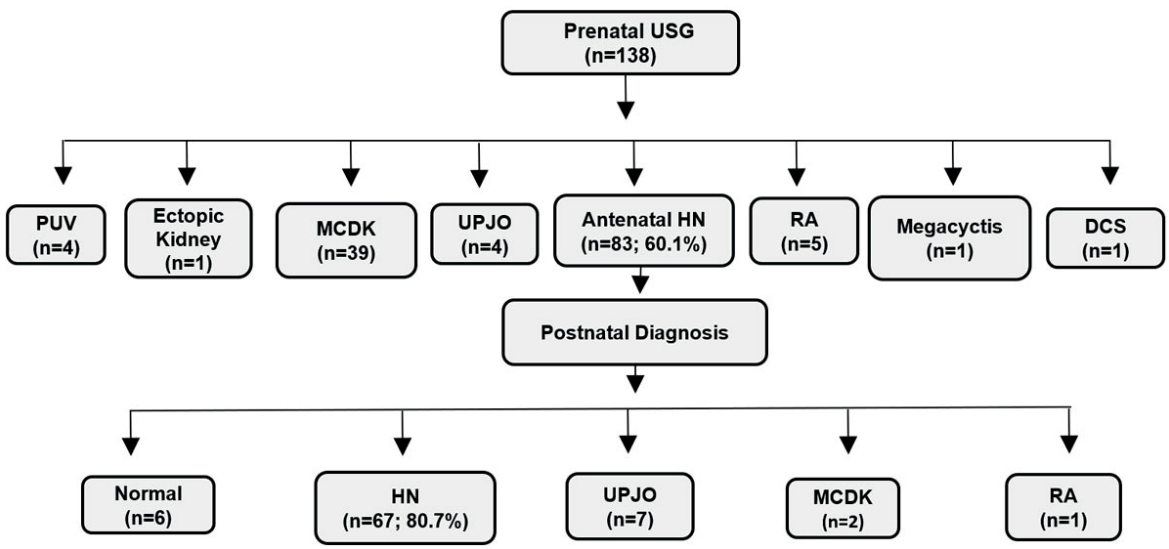

Fig. 1. Change of Prenatal Diagnosis of the Study Group in Postnatal Period

USG: ultrasonography, PUV: posterior urethral valve, MCDK: multicystic dysplastic kidney, HN: hydronephrosis, UPJO: obstruction of ureteropelvic junction, RA: renal agenesis, DCS: double collecting system

Table I. Distribution of congenital anomalies of the kidney and urinary tract (CAKUT) subgroups in the study.

Diagnosis	n (%)	F (n/%)	M (n/%)	UTI		Operation		CKD		Oligohydramniosis		Low grade VUR	High grade VUR	Prematurity (n)
				No	Yes	No	Yes	No	Yes	No	Yes			
Antenatal HN	67 (22.3)	21 (31)	46 (69)	53	14	60	7	66	1	64	3	3	3	14
UPJO	33 (11)	8 (24)	25 (76)	26	7	18	15	31	2	31	2	1	0	0
MCDK	55 (18.3)	21 (38)	34 (62)	52	3	47	8	54	1	51	4	7	0	2
Unilateral renal agenesis	38 (12.6)	16 (42)	22 (58)	35	3	38	0	36	2	38	0	2	1	1
Ectopic kidney	24 (8)	8 (33)	16 (67)	23	1	22	2	22	2	24	0	2	0	1
HN	22 (7.3)	9 (41)	13 (59)	16	6	18	4	20	2	22	0	2	1	2
PUV	20 (6.6)	0 (0)	20 (100)	13	7	0	20	8	12	17	3	5	7	3
Horseshoe kidney	14 (4.6)	7 (50)	7 (50)	14	0	11	3	13	1	14	0	3	0	1
Hypoplasic kidney	14 (4.6)	3 (22.5)	11 (78.5)	10	4	12	2	14	0	13	1	3	0	0
Extra-renal pelvis	4 (1.3)	2 (50)	2 (50)	3	1	3	1	4	0	4	0	0	1	0
DCS	2 (0.6)	1 (50)	1 (50)	1	1	2	0	2	0	1	1	1	0	0
Solitary kidney in pelvic location	3 (1)	0 (0)	3 (100)	2	1	3	0	3	0	3	0	0	0	1
Renomegaly	1 (0.3)	0 (0)	1 (100)	1	0	1	0	1	0	1	0	0	0	1
Megacytis	1 (0.3)	0 (0)	1 (100)	1	0	0	1	0	1	1	0	0	0	0
UVJO	1 (0.3)	0 (0)	1 (100)	0	1	1	0	1	0	1	0	0	0	0
Ectopic ureter	1 (0.3)	1 (100)	0 (0)	0	1	0	1	1	0	1	0	1	0	0
Total	300	97 (32.4)	203 (67.6)	250	50	236	64	276	24	286	14	30	13	26

HN: hydronephrosis, MCKD: multicystic dysplastic kidney, UPJO: obstruction of ureteropelvic junction, PUV: posterior urethral valve, DCS: double collecting system, UVJO: obstruction of ureterovesical junction, CRF: chronic renal failure, F: female, M: male, VUR: vesicoureteral reflux, UTI: urinary tract infection, CKD: chronic kidney disease

We evaluated the relationship between VUR, UTI and CKD. Of 43 patients with VUR, 15 had CKD (35%), and 10 of these CKD patients had ESRD (66.6%). Patients with VUR experienced CKD and ESRD more significantly than the patients without VUR (p <0.001 for CKD, p <0.001 for ESRD). VUR was found to be an independent risk factor for CKD in multivariate logistic regression analysis [OR 3.642; 95% CI 1.073-12.355, p= 0.038]. Although CKD occurred more in patients with UTI (p= 0.043), albeit the ratio of ESRD was not different between the patients with or without UTI (p= 0.373), UTI was not found an independent risk factor for CKD. Patients with bilateral renal scarring progressed to CKD more frequently than patients with unilateral renal scarring (p= 0.001). However, having bilateral renal parenchymal scarring was not found an independent risk factor for CKD in multivariate regression analysis.

Sixty-four patients (21.3%) had at least one urological operation, 39 of which occurred in the first year of life. Valve ablation was performed in all patients with PUV (n= 20). Of the CKD group, 18 patients (75%) underwent urological operations. Diagnoses were as follows: PUV (n= 12), UPJO (n= 2), horseshoe kidney with neurogenic bladder (n= 1), megacyctis (n= 1), HN (n= 1), and ectopic kidney with neurogenic bladder (n= 1). A nephrectomy was performed to the non-functional kidney in 15 patients [MCDK (n= 6), HN (n= 2), ectopic kidney (n= 2), PUV (n= 1), ectopic ureter (n= 1), UPJO (n= 1), antenatal HN (n= 1), and extrarenal pelvis (n= 1)] due to recurrent UTI despite antibiotic prophylaxis, recurrent urolithiasis, or treatment-resistant hypertension. Patients with PUV and UPJO more significantly underwent urological intervention in the first year of life (PUV *vs* other subgroups, p= 0.002, UPJO *vs* other subgroups; p= 0.001). Patients with oligohydramnios had more urological interventions compared to the subjects without (p <0.001). Patients who underwent surgical operations had an increased incidence of UTI (71.9%) and received more frequent antibiotic prophylaxis (57.9%) compared to the non-operated patients (UTI; p= 0.006 and antibiotic prophylaxis; p <0.001).

The diagnosis of PUV, oligohydramnios, VUR, and proteinuria on the first admission were the independent risk factors for CKD in multivariate logistic regression analysis (Table II). Among the other disease subgroups, the diagnosis of PUV independently conferred a worse prognosis in survival analysis as well (Fig. 2). There were 24 patients with the diagnosis of CKD [stage 2 (n=6), stage 3 (n= 3), stage 4 (n= 2), stage 5 (n= 13) (ESRD)]. Both CKD and ESRD were observed more frequently in the PUV subgroup than the other diagnosis groups (p <0.001). PUV was an independent predictor for CKD [OR 6.518; 95% CI 1.573-27.003, p= 0.01)]. Furthermore, patients with PUV progressed to ESRD (median 3.63 years) earlier than the other subgroups. Eleven patients [PUV (n= 7), unilateral renal agenesis (n= 2), MCDK (n= 1), and horseshoe kidney with neurogenic bladder (n= 1)] received dialysis treatment, and 7 of them [PUV (n= 5), renal agenesis (n=1), and horseshoe kidney with neurogenic bladder (n= 1)] underwent renal transplantation. The median eGFR values at the last visit and follow-

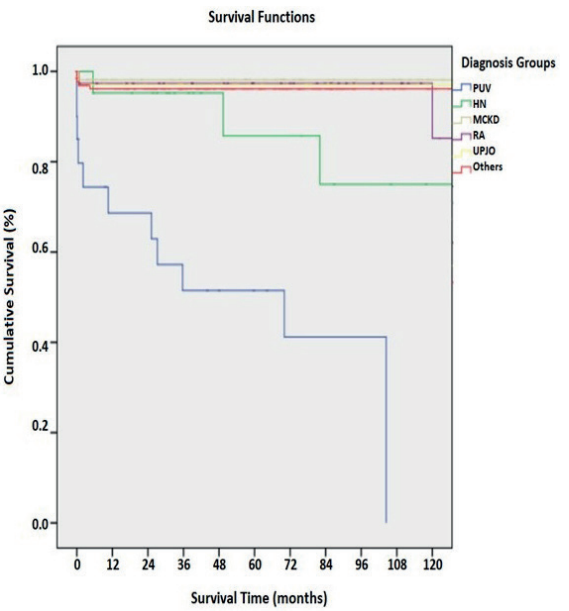

Fig. 2. The Follow Up Period up to the Time of CKD in Months Based on Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) Phenotypes

PUV: posterior urethral valve, MCKD: multicystic dysplastic kidney, HN: hydronephrosis, UPJO: obstruction of ureteropelvic junction

Table II. Predictive factors of chronic kidney disease in CAKUT.

Predictive Factors for CKD	Univariate			Multivariate		
	OR	95 % CI	p	OR	95 %CI	p
VUR	13.192	5.128-33.940	*0.001*	3.642	1.073-12.355	*0.038*
Oligohidramnios	17.933	5.568-57.757	*0.001*	11.672	2.380-57.242	*0.002*
UTI	2.222	0.869-5.678	*0.095*			
Prematurity	4.518	1.603-12.730	*0.004*			
Diagnosis of PUV	25.103	8.854-71.169	*<0.001*	6.518	1.573-27.003	*0.010*
Gender	3.674	1.069-12.632	*0.039*			
Bilateral paranchymal scar	5.186	1.980-13.578	*<0.001*			
Proteinuria at first admission	4.518	1.603-12.730	*0.004*	11.193	2.310-54.235	*0.003*

CKD: chronic kidney disease, OR: odds ratio, CI: confidential interval, VUR: vesicoureteral reflux, UTI: urinary tract infection, PUV: posterior urethral valve, HN: hydronephrosis.

up period after renal transplantation of these seven patients were 89.5 ml/min/1.73 m^2 (IQR: 22.1-101.0 ml/min/1.73 m^2) and 116.0 months (101.0-136.0 months), respectively. One patient with PUV and the patient with renal agenesis progressed to CKD after renal transplantation, whereas other five patients had normal graft functions at the time of writing of this report. Those patients who progressed to CKD after kidney transplantation received deceased donor kidneys. Graft losses were due to non-compliance to the immunosuppressive medications in the PUV patient and BK nephropathy in the other. Patients with oligohydramnios (n= 14) had bilateral renal and urinary tract anomalies. These patients progressed to CKD more frequently (n= 7/14, 50% *vs* n= 17/286) and earlier [9.62 (IQR 2.32-59.63) months *vs* 59.72 (IQR 6.80-120.01) months] than the patients without oligohydramnios (p <0.001 for both). Similarly, ESRD observed earlier in patients with oligohydramnios than those without. The median age of the patients with ESRD and oligohydramnios (n= 5) was 3.31 months (IQR; 0.00-55.37 months) whereas the median age of the patients with ESRD without oligohydramnios (n= 8) was 56.82 months (IQR; 0.10-83.79 months). In multivariate analysis, oligohydramnios was found as an independent risk factor for CKD [OR 11.672; 95% CI 2.380-57.242, p= 0.002)]. There were 26 patients who were born prematurely (8.6%) with a median gestational age of 35 weeks (IQR: 33-36 weeks). There was no difference between the patients with or without prematurity in terms of eGFR values on the first admission and at the last visit (p= 0.577 on the first admission, p= 0.252 at the last visit). Prematurity was significantly observed in patients with oligohydramnios (p= 0.025) and CKD (p= 0.002), but not in patients with ESRD (p= 0.318). Prematurity was not found an independent predictive factor for CKD. Fifteen patients had proteinuria on the first admission. The diagnosis of these patients were as follows; PUV (n= 6), UPJO (n= 3), RA (n= 2), MCDK (n= 1), DCS (n= 1), AHN (n= 1), and HN (n= 1). Of these patients, eight had CKD and five of them progressed to ESRD. Additionally, having proteinuria on the first admission was an independent risk factor for CKD regardless of CAKUT subtype [OR 11.193; 95% CI 2.310-54.235, p= 0.003)].

Discussion

CAKUT consists of a heterogeneous group of disorders, and the clinical courses of these subgroups are extremely different.[10] Most of the patients with CAKUT often progress to ESRD at a slower rate; however, certain subgroups do progress more rapidly.[10] In this study, we focused on the differences regarding the prognosis of each subgroup and the factors influencing the progression to CKD. The current study indicates that the prognosis is highly dependent on the CAKUT phenotype.

In different CAKUT subgroups, PUV was found to be an independent risk factor for CKD as underlined in previous reports.[11] Besides PUV, VUR, proteinuria on the first admission, and oligohydramnios were also found to be associated with CKD in our study.

PUV leads to bilateral renal obstruction and renal dysplasia in addition to bladder dysfunction.[12] It confers a poorer prognosis than the other frequently seen obstructive uropathy, UPJO, which tends to occur unilaterally and have a milder phenotype.[13] PUV is the leading cause of CKD in newborn males,[14] and ESRD occurs in a wide range between 5% and 64% in the reports.[15] We found that CKD and ESRD were observed in 55% and 40% of the patients with PUV, respectively. A possible reason for these high ratios is likely to be due to the longer follow-up period of our patients, and the nature of our hospital (i.e. a tertiary referral clinical center). In our study, 40% of our patients with PUV progressed to ESRD despite relatively early surgical ablation This indicates that CKD in this group of patients is not solely due to mechanical obstruction itself but accompanying renal dysplasia, which is expected in this patient population.[12]

In the current study, the patients with oligohydramnios progressed to CKD in a shorter follow-up period than those who do not have. Oligohydramnios indicates severe abnormality in the urinary system in the antenatal period.[16,17] Therefore, oligohydramnios is mostly associated with renal impairment, poor prognosis, and ESRD.[16,17] Klaassen et al.[17] revealed that all infants with oligohydramnios developed CKD, and 40% of these individuals progressed to ESRD in the first 3 months of life. In our study group, patients with oligohydramnios progressed to CKD and ESRD at a ratio of 50% and 36%, respectively. Additionally, those patients progressed to CKD at a mean age of 9.62 months. As we could not reach the prenatal information of all patients, the proportion of CKD and ESRD in patients with oligohydramnios would most likely be higher. In agreement with previous reports,[16,17] our patients with oligohydramnios experienced ESRD and underwent surgical interventions more frequently than the patients without. In addition, having oligohydramnios was identified as an independent risk factor for CKD in our study. Taken together, oligohydramnios should be considered as one of the earliest markers of CKD in patients with CAKUT.

In our study, although we did not find statistically significant difference between low and high grade VUR in terms of CKD, presence of VUR has been identified as an important risk factor in the course of CKD as shown previously.[18] The impact of VUR on renal survival is controversial. In some of the studies,[11] VUR has been associated with poor renal survival, but on the contrary, the others have established better results.[19] In the literature, it has been shown that VUR accompanies with PUV at a ratio of 50%.[20] Similar to existing literature data, in our study, 60% of PUV patients had VUR, and 35% of them were high grade. The relation between VUR and CKD identified in the current study may be explained by the frequent association of VUR with PUV, which was established as main cause of CKD in CAKUT. This may also explain no difference between low and high grade VUR in terms of CKD as most of the CKD patients had PUV diagnosis, which was considered the main cause for CKD. In the previous reports, UTI has been frequently observed in patients with CAKUT.[21] Recurrent UTI rarely leads to CKD without any underlying renal abnormalities[22] and has been shown to worsen the prognosis of the patients with congenital kidney diseases and bilateral renal impairment.[23] As noted in previous studies, CKD was significantly observed in patients who had recurrent UTI. However, we did not find UTI as a risk factor for the development of CKD in the current study. In contrast to published data,[24] UTI did not influence the course of CKD or ESRD in our study population. Another important parameter of the progression to CKD is the presence of parenchymal scarring in patients with VUR. The existing UTI and inflammatory process are well-known reasons for renal scarring.[25] The

relevance of renal scarring in CKD has been shown, and the renal parenchymal scarring has been reported as a risk factor for CKD.[18,26] Studies have investigated the effect of the scarring on renal survival;[18,26] however, few studies have analyzed whether this scarring is unilateral or bilateral.[27] In a detailed research,[27] patients with bilateral renal scarring have been reported to have more pronounced creatinine elevation and lower GFR values. In the present study, we also showed that the bilateral renal scarring had a significant association with CKD in patients with CAKUT.

Proteinuria is considered an important predictor of CKD in CAKUT[28] and it is prominent during the follow-up period in patients with chronic renal disease.[29] In our study, proteinuria at the referral time was marked in patients with CKD. Proteinuria has also been shown as an unfavorable factor in renal survival in patients with CAKUT.[11] In agreement with existing literature data, we demonstrated that proteinuria on the first admission was an independent predictor for CKD, and therefore should be monitored during follow-up.

Prematurity was another parameter that also significantly associated with patients who had CKD. In preterm infants, the size and the number of the glomeruli and GFR levels are significantly diminished compared to full-term babies.[30] Having a baseline reduced number of nephrons in premature infants may contribute to the ongoing programmed cell-death in CAKUT, and this may facilitate the progression to CKD. There are conflicting data in the literature regarding prematurity and CKD. It has been reported that, preterm infants have normal renal functions.[31] However, Melo et al.[32] suggested that prematurity was an independent predictor for renal failure in CAKUT. In our study we did not find prematurity as a risk factor for CKD, but this would be related to the lack of full antenatal information of the study patients.

Our study has some limitations that usually occur in retrospective studies. We had missing data regarding prenatal ultrasonographic assessments. In addition, the number of patients in some subgroups was too small, and subgroups were extremely heterogeneous. In the current study, VCUG was not performed in all patients. Therefore, actual VUR ratio might be different than we found. We did not find bilateral renal scarring as an independent risk factor for CKD. This could be an artifact as we had very small number of patients having bilateral renal scarring (n=4). Additional limitation of our study would be an unequal follow-up durations of the patients with different CAKUT subgroups. All of these factors restrict to make a clear comparison between the subgroups. Patient registries would overcome this inability as these registries yield more patients with rare CAKUT subgroups and sufficient follow-ups. On the other hand, our study was conducted in a broad pediatric CAKUT population that was monitored during a fairly long period.

In conclusion, the current study gives a general perspective about children with the diagnosis of CAKUT who applied to a tertiary clinical center in Turkey. Diagnosis of PUV, VUR, proteinuria on the first admission, and oligohydramnios have been identified as predictive factors for CKD. Renal prognosis of the patients highly depends on the CAKUT phenotype. PUV is associated with the poorest prognosis among the CAKUT subgroups. Surgical interventions seemed not to prevent but only postpone the process of CKD in the obstructive phenotypes of CAKUT. These factors may have clinical utility during management and estimate a long-term prognosis in patients with CAKUT.

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Original Article

Investigation of the relationship between cord clamping time and risk of hyperbilirubinemia

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ABSTRACT

Background. Although the relationship between umbilical cord clamping time and various parameters such as hemoglobin (Hb) levels, iron deficiency, and risk of neonatal jaundice has previously been studied, to the best of our knowleadge there have been no studies investigating the relationship between cord clamping time and the risk of significant hyperbilirubinemia. We aimed to investigate the relationship between the time of umbilical cord clamping and transcutaneous bilirubin (TcB) measurements made on various postnatal hours, Hb and serum total bilirubin (STB) levels measured on postnatal 4th day, and the risk of development of significant hyperbilirubinemia requiring phototherapy treatment.

Methods. Eligible newborns were divided into two groups on the basis of the time of cord clamping: those clamped late (60 seconds or more; Group I) and those clamped early (less than 60 seconds; Group II). Groups were compared with respect to the parameters of cord Hb, postnatal TcB measurements at 6th, 48th, 96th and 168th hours, and 96th hour Hb, STB and direct bilirubin levels.

Results. TcB levels at the 96th and 168th hour were significantly higher in Group I when compared to Group II ($p<0.001$ and $p<0.001$, respectively). The 96th hour STB level was significantly higher in Group I when compared to Group II ($p<0.001$). The need of phototherapy requirement was higher in Group I when compared to Group II ($p=0.001$). Increase in cord blood Hb for each 1 gr/dl caused a 3.94-fold increased risk in the requirement of phototherapy treatment. Cord clamping time showed statistically significant positive correlations with both cord blood and 96th hour venous Hb levels, with both 96th hour and 168th hour TcB levels, and with 96th hour STB levels.

Conclusions. Newborns whose cords are clamped late should be followed up closely with respect to high postnatal bilirubin levels and other risks associated with significant hyperbilirubinemia requiring phototherapy treatment.

Key words: hyperbilirubinemia; risk management; transcutaneous bilirubin measurements.

One of the most serious issues concerning the risk of the development of significant hyperbilirubinemia is the timing of umbilical cord clamping. International Liaison Committee On Resuscitation–Consensus on Science with Treatment Recommendations (ILCOR-CoSTR) recommends delaying cord clamping by at least 60 seconds in newborns who do not require resuscitation.[1] Such a delayed cord clamping provides the newborn an additional blood volume of at least 30% and an additional erythrocyte mass up to 60%.[2-4] On the other hand higher umbilical cord hemoglobin (Hb) levels due to delayed cord clamping have caused an increased incidence of significant hyperbilirubinemia requiring phototherapy,

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and each 1 gram increase in cord Hb levels has increased phototherapy requirement by 1.7 times in a study conducted in healthy newborns.[5]

Although there is only one study investigating the relationship between cord blood parameters (Hb level) and predicting the risk of later developing significant hyperbilirubinemia in the era of early discharge from the hospital,[5] there is, to our knowledge, no study investigating the relationship between the time of umbilical cord clamping and the risk of significant hyperbilirubinemia development especially with both serum total bilirubin (STB) and transcutaneous bilirubin (TcB) measurements. We, thus, aimed to investigate the relationship between the time of umbilical cord clamping and TcB measurements made on various postnatal hours, Hb and STB levels measured on postnatal 4th day, and the risk of development of significant hyperbilirubinemia requiring phototherapy treatment.

Material and Methods

The study was conducted at the Division of Neonatology, Department of Pediatrics of Ufuk University Faculty of Medicine between May 2016 and May 2017. Cord blood samples were obtained from all term and late preterm newborns born during the study period.

Umbilical cords of newborns who late clamping was performed were clamped and cut holding the cord on the level with placenta for 60-90 seconds after birth, and those who were clamped early were clamped and cut at a distance of 2-3 cm near to umbilicus within 60 seconds. Milking was not performed in early cord clamping. Complete blood count, blood group and direct antiglobulin (Coombs) tests were studied from the samples taken from the cord blood. TcB measurements were made at the 6th, 48th, 96th and 168th hours, and venous Hb, STB and direct bilirubin levels were measured at the 96th hour in order to determine the risk of significant hyperbilirubinemia and investigate the relationship between umbilical cord Hb and postnatal bilirubin levels. Patients were divided into two groups on the basis of the time of cord clamping: those clamped late (60 seconds or more; Group I) and those clamped early (Less than 60 seconds; Group II). Groups were compared with respect to the parameters of cord Hb, postnatal TcB measurements at 6th, 48th, 96th and 168th hours, 96th hour Hb, STB and direct bilirubin levels, gender, gestational age, route of delivery, birth weight, Apgar scores at 1st and 5th minutes, maternal age, number and ratio of late preterm newborns and weight loss until postnatal 4th day as grams and per cent. Correlation analysis between the time of cord clamping and all the other parameters were performed for all cases included (Group I+Group II).

Newborns with a gestational age of ≥35 weeks were included in the study. As prematurity itself is a major risk factor in the etiology of hyperbilirubinemia, preterm newborns with a gestational age of <35 weeks were not included in the study. Other exclusion criteria were newborns with isoimmunization (ABO, Rh or subgroup incompatibilities), direct Coombs test positivity, findings of hemolysis on blood smear (anisocytosis, spherocytosis, polychromasia, poikilocytosis), anemia, reticulocytosis and/or glucose-6-phosphate dehydrogenase deficiency, the presence of any major congenital anomaly, respiratory distress, pathologic weight loss of >10%, the need of resuscitation at birth and clinical or culture-proven sepsis.

In all cases in the study, gender, birth weight, route of delivery, maternal age, gestational age, Apgar scores at 1st and 5th minutes, blood groups and Rh types in mother-infant pairs, umbilical cord Hb level, postnatal 96th hour Hb, serum total and direct bilirubin levels measured with the colorimetric method (diazotized sulfanilic acid reaction, Roche Diagnostics GmbH, Mannheim, Germany), postnatal TcB measurements at 6th, 48th, 96th and 168th hours, and the presence of phototherapy requirement were recorded. The diagnosis of significant hyperbilirubinemia was made considering

gestational and postnatal age, and risk factors of the newborns[6,7], and these cases were put on phototherapy. Phototherapy treatment was performed with high-intensity special blue light LED devices emitting ≥40 μW/cm²/nm irradiance from a 45-cm distance for 24 hours.

TcB measurements were made with a trancutaneous bilirubinometer (Bilichek, Respironics Inc, Monroeville, PA, USA). To take a measurement with this trancutaneous bilirubinometer, the probe was positioned on the infant's skin, and five individual scans were taken to produce one measurement that was displayed in mg/dL. If an erroneous measurement is taken, an error message is displayed, and the scan should be repeated. The TcB measurements were performed on the sternum/thoracic region. The sternum/thoracic region was not exposed to direct sunlight, and environmental lighting was constant during the study period.

The study was conducted according to clinical practice guidelines and approved by the local ethics committee (IRB number: 18052016-4). Informed consent was obtained from either parent of each patient.

For statistical analysis of the data Statistical Package for Social Sciences (SPSS) 18.0 program was used. Shapiro-Wilk test was used in determining normal distribution. Fisher's Exact x^2 test was used to compare the nominal values such as gender, route of delivery, weight loss, number of late preterm and term newborns and whether there was a need of phototherapy treatment between the groups. Of the continuous variable parameters, gestational age and serum direct bilirubin levels were compared with T test, and maternal age, birth weight, STB, Hb and weight loss were compared with Independent Sample T test. Pearson's test was used in analysis of the correlation between the time of cord clamping and the other parameters. Values were given as mean±standard deviation and median (25-75%) range. A p value of ≤0.05 was considered as statistically significant.

A power analysis revealed that to achieve 80% power to detect a 2 mg/dl difference in transcutaneous bilirubin levels between groups using a p-value of 0.05, 80 patients would be needed in each group.

Results

The study was completed with a total of 172 newborns (82 and 90 in Group I and Group II, respectively). Demographic data of the study groups, and comparison of the cord clamping time, cord Hb, postnatal TcB measurements at 6th, 48th, 96th and 168th hours, 96th hour Hb,

Table I. Demographic features of the patients.

Parameter	Group I (n= 82)	Group II (n= 90)	p value
Birth weight (gram)*	3264 ± 420 (2420-4295)	3282 ± 416 (2140-4190)	0.771
Gestational age (weeks)*	38.23 ± 1.05 (36-40)	38.37 ± 1.17 (35-41)	0.429
Number of late preterm/term newborns	15/67	16/74	0.9301
Apgar score at 1st minute**	9 (8-9)	9 (8-9)	0.438
Apgar score at 5th minute**	9 (8-9)	9 (8-9)	0.438
Maternal age (years)*	29.4 ± 4.83 (19-40)	31.1 ± 4.2 (21-44)	0.18
Route of delivery (cesarean/vaginal)	71/11	70/20	0.133
Gender (male/female)	46/36	39/51	0.94
Need of a phototherapy treatment (yes/no)	25/57	9/81	0.001
Weight loss until postnatal 4th day (<5%/5-9.99%)	69/21	61/21	0.728
Weight loss until postnatal 4th day (gram)*	160 ± 14 (112-201)	164 ± 15 (113-207)	0.073

*: Values are given as mean±standart deviation (minimum-maximum)
**: Values are given as median (Range 25th-75th centile)

Table II. Cord clamping time and transcutaneous and serum total bilirubin values of the patients.

Parameter	Group I (n=82)	Group II (n=90)	p value
Cord clamping time (seconds)**	67 (63-69.1)	11 (9-14)	<0.001
Cord blood hemoglobin (g/dl)*	17.17 ± 1.59 (12.6-22.2)	14.57 ± 1.83 (8.7-20.1)	<0.001
Transcutaneous bilirubin at 6th hour (mg/dl)*	4.43 ± 1.01 (2-6.3)	4.19 ± 1.15 (1.7-6.9)	0.151
Transcutaneous bilirubin at 48th hour (mg/dl)*	9.73 ± 2.30 (4.9-15.6)	9.04 ± 2.52 (3-14)	0.065
Transcutaneous bilirubin at 96th hour (mg/dl)*	14.71 ± 2.89 (6.7-25.0)	11.21 ± 3.14 (3.5-18.1)	<0.001
Transcutaneous bilirubin at 168th hour (mg/dl)*	11.68 ± 3.35 (5.5-22.1)	9.52 ± 3.27 (2.8-19.9)	<0.001
Venous hemoglobin at 96th hour (g/dl)*	17.89 ± 1.90 (12.3-23.2)	16.78 ± 1.99 (13.9-22.9)	<0.001
Serum total bilirubin at 96th hour (mg/dl)*	14.49 ± 3.39 (2.2-25.0)	10.34 ± 3.78 (1.8-20.3)	<0.001
Serum direct bilirubin at 96th hour (mg/dl)*	0.36 ± 0.1 (0.1-0.6)	0.32 ± 0.1 (0.2-0.7)	0.271

*: Values are given as mean±standart deviation (minimum-maximum)
**: Values are given as median (Range 25th-75th centile)

STB and direct bilirubin levels between the study groups are given in Table I and Table II.

There were no statistically significant differences between the study groups regarding route of delivery, gender, gestational age, birth weight, maternal age, Apgar scores at 1st and 5th minutes, weight loss, 6th hour and 48th hour TcB measurements (Table I). However, there were statistically significant differences between the study groups regarding cord clamping time, cord blood Hb, TcB measurements at 96th and 168th hours, and 96th hour venous Hb and STB levels. The need of phototherapy requirement was higher in Group I (n=25) when compared to Group II (n=9), and thus the incidence of significant hyperbilirubinemia was higher in newborns whose cord was clamped late (p= 0.001) (Table II).

Cord blood Hb level was significantly higher in Group I when compared to Group II (17.17 ± 1.59 g/dl vs 14.57 ± 1.83 g/dl, p <0.001) (Table II) (Fig. 1). Analysis of the relationship between cord blood Hb level and phototherapy treatment revealed that increase in cord blood Hb for each 1 gr/dl caused a 3.94-fold increased risk in the requirement of phototherapy treatment (Odds ratio= 3.94, p= 0.0012, 95% confidence interval (CI)=1.71-9.08) (Fig. 1).

Regarding postnatal TcB values, there were no statistically significant differences between the groups on the basis of 6th and 48th hour TcB values, however 96th and 168th hour TcB levels were significantly higher in Group I when compared to Group II (p<0.001 and p <0.001, respectively) (Table II) (Fig. 2).

96th hour STB level was significantly higher in Group I when compared to Group II (p <0.001) (Table II) (Fig. 3).

In all of the study cases (n= 172) correlation analysis revealed that cord clamping time showed a statistically significant postitive correlation with both cord blood and 96th hour venous Hb levels (p <0.0001, r= 0.426, 95% CI= 0.291-0.544 and p= 0.0002, r= 0.291, 95% CI= 0.144-0.426, respectively). Cord clamping time also showed a statistically significant postitive correlation with both 96th hour and 168th hour TcB levels (p <0.0001, r= 0.307, 95% CI= 0.161-0.440 and p= 0.0009, r= 0.257, 95% CI= 0.108-0.395). There was a statistically significant postitive correlation between cord clamping

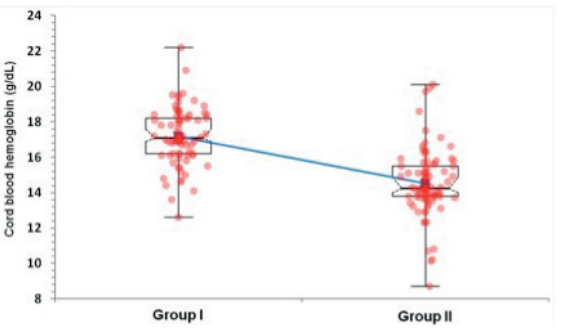

Fig. 1. Comparison of the cord blood hemoglobin levels between the groups.

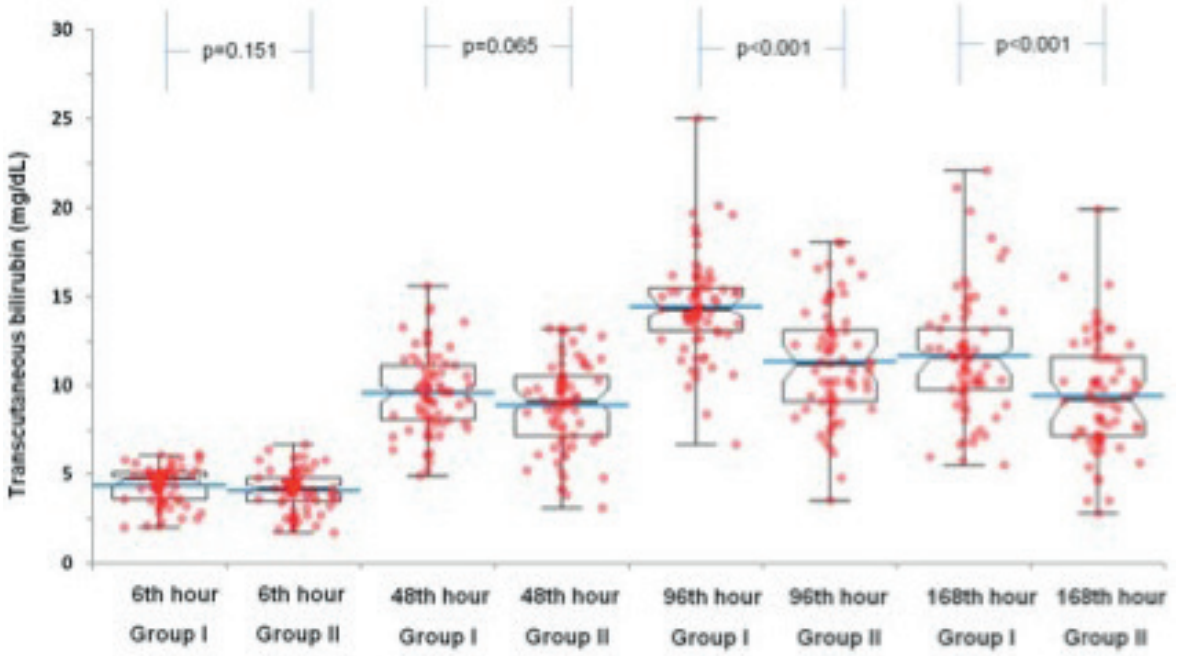

Fig. 2. Comparison of the 6th, 48th, 96th and 168th hour transcutaneous bilirubin values between the groups.

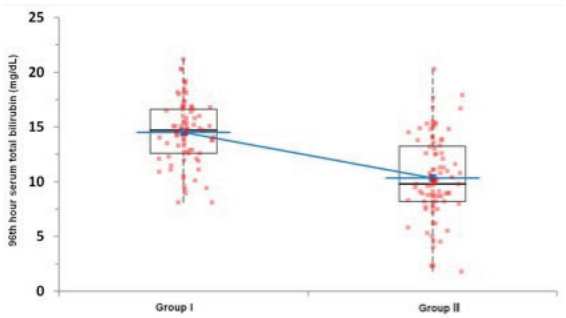

Fig. 3. Comparison of the 96th hour serum total bilirubin levels between the study groups.

time and 96th hour STB levels (p <0.0001, r= 0.310, 95% CI= 0.165-0.443).

Discussion

Although the relationship between umbilical cord clamping time and various parameters such as postnatal and early infancy Hb levels, iron deficiency, iron deficiency anemia and risk of neonatal jaundice requiring phototherapy treatment has previously been studied[5,8-11], to the best of our knowledge, there have been no studies investigating the relationship between cord clamping time and the risk of significant hyperbilirubinemia by serial longitudinal TcB measurements, and early postnatal Hb and STB measurements. This study is the first in this field in this regard.

Early cord clamping is defined as clamping the cord in 60 seconds after delivery of the baby, and late cord clamping is defined as clamping the cord when the pulsation stops or at least 60 seconds or more after delivery of the baby in the metaanalytic Cochrane study.[12] Mercer et al.[9] have reported that late cord clamping increases neonatal Hb levels at birth and gains time for transfer of blood from placenta to newborn. This placental transfusion provides the newborn an additional 30% blood volume and an additional erythrocyte volume up to 60%.[2-4] This increased placental transfusion provides a higher neonatal Hb concentration and an increased erythrocyte flux to vital organs, a better neonatal cardiopulmonary adaptation, a higher incidence of successful breast-feeding, and better body iron stores and fewer iron deficiency anemia.[3,4] On the other hand early cord clamping increases feto-maternal transfusion and a great amount of blood remains in placenta.[12] Nesheli et al.[10]

compared early versus late cord clamping in 66 newborns, and reported higher neonatal Hb and hematocrit levels and better serum iron status at 6 months of age in newborns whose cord was clamped late. They defined high neonatal Hb levels as a predisposing risk factor for polycytemia and hyperbilirubinemia. Mercer et al.[9] also compared early versus late (>5 minutes) cord clamping with respect to venous Hb and STB levels at 24th and 48th hours of life, and reported no statistically significant difference in STB levels between the two groups despite higher Hb levels in those whose cord is clamped early.

In the single study investigating the relationship between umbilical cord Hb level and the risk of neonatal hyperbilirubinemia, higher cord Hb levels were observed with increasing cord clamping time, and both were associated with an increased risk of significant hyperbilirubinemia requiring phototherapy treatment.[5] However umbilical cords of all the cases were clamped in the first 15 seconds after delivery without constituting early or late clamping groups in that study.[5] In the cases receiving and not receiving phototherapy, mean Hb level was 17.4 g/dl and 15.8 g/dl, respectively, and the authors did not recommend late cord clamping for their country.[5] In our study the two groups were composed according to the criteria of early and late clamping, and mean cord Hb levels were 14.57 g/dl and 17.17 g/dl in the groups of early and late clamping, respectively. In the study by Nakagawa et al.[5] cord blood Hb level determined the phototherapy requirement with an Odds ratio of 1.74. Odds ratio in our study was 3.94 indicating that late clamping of the cord increased the risk of significant hyperbilirubinemia by 3.94-fold.

Chien et al.[13] compared early (<60 sec) versus late (>180 sec) cord clamping in 105 newborns. They followed the newborns with respect to significant hyperbilirubinemia postnatally for 4 to 7 days after hospital discharge, and no significant difference was observed between the early and late clamping groups. Yang et al.[14] compared two eras of a delayed cord-clamping protocol (before and after) for term neonates and reported significantly higher transcutaneous bilirubin levels, an increased number of serum blood draws, and more clinical diagnoses of jaundice. However, they reported no increase in the incidence of phototherapy. In our study both 96th hour STB and TcB levels and 168th hour TcB levels were significantly higher in newborns whose cord was clamped late. Higher bilirubin levels on day 4 and after and significantly higher phototherapy requirement in the end in these newborns may be explained by both the combined effects of the degradation of high Hb load taken over from the umbilical cord (Late clamping) and transformation of Hb to Heme in these days (Physiologic jaundice).

In the Cochrane meta-analysis of 15 different studies, the ratio of newborns receiving phototherapy was higher (4.36% vs 2.74%) and mean Hb concentration at 24th-48th hours of life was higher in newborns whose cord was clamped late.[12] Mercer et al.[9] reported higher Hb levels at 24th-48th hours of life in newborns whose cord was clamped late (>5 min) when compared to those whose cord was clamped early (19.4 g/dl vs 17.8 g/dl). In our study mean 96th hour Hb level was statistically significantly higher in cases whose cord was clamped late in comparison to those whose cord was clamped early (17.8 g/dl vs 16.7 g/dl). According to the Cochrane meta-analysis and recommendations of the American College of Obstetricians and Gynecologists, potential harms of increased Hb should be evaluated by clinicians considering the region they worked and the accessibility to facilities of jaundice diagnosis and treatment.[12,15] For example late cord clamping should be less preferred in regions where the treatment of neonatal jaundice is not easily accessible and untreated significant hyperbilirubinemia has the risks of long-term complications.[12,15]

In this study, we investigated the relationship between umbilical cord clamping time, and transcutaneous bilirubin levels and the risk of neonatal significant hyperbilirubinemia, and demonstrated that late cord clamping caused higher postnatal Hb and bilirubin (TcB and

STB) levels, and a higher incidence of significant hyperbilirubinemia requiring phototherapy treatment in comparison to early cord clamping. Although positive effects of late cord clamping on neonatal Hb levels and body store iron status in late infancy have been demonstrated in previous studies, newborns whose cord are clamped late should be followed up closely with respect to high postnatal bilirubin levels and other risks associated with significant hyperbilirubinemia requiring phototherapy treatment.

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Original Article

Gender-related differences in etiology of organic central precocious puberty

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ABSTRACT

Background. Central precocious puberty (CPP) is idiopathic in 90% of girls and 60% of boys, while some cases are caused by lesions of central nervous system (CNS), a condition often referred to as organic CPP. We aimed to analyze the etiology of organic CPP in a large cohort of girls and boys and determine gender-related differences.

Methods. Medical files of 256 girls and 120 boys diagnosed and treated for CPP in a single center in the last two decades were reviewed. Patients were classified into four groups with respect to previous history and MRI findings: (1) previously established CNS pathology at the time of diagnosis, (2) novel CNS pathology previously asymptomatic, (3) incidentalomas considered to be unrelated to CPP, and (4) completely normal MRI. Group 1 and 2 were considered as organic CPP whereas group 3 and 4 were considered as idiopathic CPP.

Results. Prevalence of CNS pathology was significantly higher in boys than girls (21.7% vs 6.2%). Previous CNS pathologies such as developmental anomaly of CNS, parenchymal injury, necrotic lesions and hydrocephalus were present in 3.5% of girls and 8.3% of boys. Prevalence of novel CNS pathology as determined by imaging among neurologically asymptomatic patients was 2.8% in girls and 14.5% in boys. The most common novel CNS pathologies in boys were hamartomas (5%) and suprasellar arachnoid cysts (3.3%); which were significantly lower in girls (0.8 and 0.8% respectively). Onset of organic CPP was before six years in girls, and seven years in boys.

Conclusions. Organic CPP was 3.5 times more common in boys compared to girls. It is possible to detect an underlying CNS pathology in one out of every five boys with CPP. Frequency and distribution of organic etiology also differ between girls and boys, hypothalamic hamartomas and suprasellar arachnoid cysts being more common in boys than girls. The likelihood of novel intracranial pathology associated with CPP is quite low in girls with an onset after six years of age and in boys with an onset after seven years of age.

Key words: central precocious puberty, cranial MRI, etiology, pituitary MRI, precocious puberty.

Central precocious puberty (CPP) is idiopathic in up to 90% of girls, and 60% of boys, however, some cases are due to lesions of the central nervous system (CNS), a condition often referred to as organic CPP.[1-5] We have recently shown that majority of CPP in boys were idiopathic rather than organic, however 26% of CPP are still caused by organic lesions of CNS.[6] Nevertheless, boys are more likely to have organic lesions than girls. Hamartomas of the tuber cinereum are the most frequent type of CNS tumor that causes CPP in very young children. Other CNS tumors associated with CPP include astrocytomas, ependymomas, optic and hypothalamic gliomas, and pinealomas. It's yet unclear why organic lesions are more common in boys with CPP. To our knowledge, there is as yet no study comparing the sex distribution of underlying etiology of organic CPP, in order to determine gender-related differences. In this study, we aimed to analyze the etiology of

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organic CPP in a large cohort of girls and boys, and determine gender related differences.

Material and Methods

Medical files of 256 girls and 120 boys diagnosed and treated for CPP in a single center in the last two decades were reviewed. Chronological and bone age, height, pubertal stage, hormone levels as well as findings of CNS imaging at the time of diagnosis were analyzed. Diagnosis of CPP was made in girls clinically by breast development (Tanner stage two or higher) before eight years of age, and biochemically using an elevated serum estradiol (≥ 10 pg/ml), as well as an elevated peak luteinizing hormone (LH) (≥ 5 IU/L) during GnRH test. In boys diagnosis was based on testicular enlargement (≥ 4 ml) before nine years of age with a pubertal elevation of testosterone level (≥30 ng/dl), as well as an elevated peak LH (≥ 5 IU/L) during GnRH stimulation test.[7-9] GnRH test was performed as previously described.[10] Tanner staging was used to determine pubertal stages.[11] Prader orchidometer was used to measure the volume of the testes. All patients except those having organic pathology and those having pubertal stage four or above were followed for three to six months before the treatment decision. GnRHa treatment was given to the cases with progressive CPP which was determined according to the following criteria; a. Growth velocity above six cm/year, b. Advanced bone age (bone age - chronological age ≥ 2 years), c. Rapid progression of pubertal stages (progression of puberty from one stage to another in less than six months), d. Deficit in predicted adult height compared to target height.[12] Those patients with progressive CPP who received GnRHa treatment were included in the study.

Body weight was measured using a digital body weight scale, and height was measured in the standing position with a wall-mounted stadiometer. Bone age was assigned by a pediatric endocrinologist using the method of Greulich and Pyle.[13] The percentile curves of the Centers for Disease Control and Prevention (CDC) were used to interpret the growth data. Height standard deviation scores (SDS) for both chronological and bone age were calculated using CDC charts.

FSH, LH, estradiol (ARCHITECT System, Abbott Laboratory Diagnostics, USA), and testosterone (IMMULITE 2000 System, Siemens, UK) levels were measured using immunochemiluminometric assay (ICMA). Lowest measurable levels of FSH, LH, estradiol and testosterone assays were 0.3 and 0.07 IU/l, 10 pg/ml and 20 ng/dl, respectively.

All patients had an imaging study designed for precocious puberty at 1.5 Tesla MR scanners: axial T2-weighted imaging (WI) (TR/TE; 3000-3800/90-100 ms), diffusion WI (TR/TE; 3500-3800/90-96 ms, applied maximum b value of 1,000 s/mm^2) covering the whole brain in addition to a standard pituitary imaging protocol which included sagittal and coronal T1WI (TR/TE; 530-600/15-20 ms) and coronal T2WI (TR/TE; 3300-3600/85-95 ms), and dynamic T1 coronal and repeat sagittal T1WI imaging following intravenous Gadolinium (Gd)-based contrast material injection.

All MRI studies were evaluated by neuroradiologists for presence of any lesion in the hypothalamic-pituitary region and other CNS lesions located in the brain parenchyma or extraaxial spaces. In cases with an abnormality, a tailored MRI examination was performed where needed. Some of these patients with CNS abnormalities were already being followed-up for their disease when they were studied for precocious puberty and these were recorded. Patients were classified into four groups with respect to previous history and MRI findings: (1) previously established CNS pathology at the time of diagnosis, (2) novel CNS pathology previously asymptomatic, (3) incidentalomas considered to be unrelated to CPP, and (4) completely normal MRI. Group 1 and 2 were considered as organic CPP whereas group 3 and 4 were considered as idiopathic CPP. This study was approved by the Ethics Committee

of Hacettepe University (Approval number: GO 19/452-42). The requirement for informed consent was waived due to the retrospective nature of the study.

Statistical Analyses

Statistical analyses were performed using the Statistical Package for Social Sciences software for Windows (version 19.0; SPSS Inc., Chicago, IL, USA). Continuous variables were reported as the mean ± standard deviation, and categorical variables were shown as numbers and percentage. Student's t-test and one-way ANOVA with post hoc Tukey's HSD test were used to analyze differences between independent groups. Categorical variables were analyzed using Pearson's chi-square, Fisher's exact chi-square or likelihood ratio test. A p value of less than 0.05 was considered statistically significant.

Results

CNS pathology was significantly more prevalent in boys (21.7%) in comparison to girls (6.2%) (p <0.001) (Table I). 3.5% (9/256) of girls and 8.3% (10/120) of boys had previously established CNS pathologies such as developmental anomalies of CNS, parenchymal lesions and hydrocephalus detected by cranial MRIs. All the remaining cases were neurologically asymptomatic. In this group, a novel CNS pathology was identified in 2.8% (7/247) of girls and in 14.5% (16/110) of boys on MRI. The most common novel CNS pathologies in boys were hypothalamic hamartomas (5%) and suprasellar arachnoid cysts (3.3%). The frequency of suprasellar arachnoid cysts (0.8%) and hypothalamic hamartomas (0.8%) were significantly lower in girls (p <0.001) (Table II). Suprasellar arachnoid cysts were > 5 cm in size causing hydrocephalus in all cases. The prevalence of incidentalomas (microadenomas and millimetric pars intermedia cysts) were similar in both sexes (8.6% vs. 9.2%) (Table I).

Puberty started before six years in girls and seven years in boys with CNS pathology. All girls diagnosed with CPP younger than two years as well as boys younger than three years had an underlying hypothalamic hamartoma (Table III). The remaining two boys with hamartomas were diagnosed at four and five years of age. Age at diagnosis was smaller, bone age was greater, height SDS adjusted for bone age was lower, and sex steroid levels and peak LH during GnRH test were higher in patients with a CNS pathology in comparison to those with idiopathic CPP (Table IV).

Discussion

The prevalence of idiopathic CPP in boys is increasing in recent studies, however, organic CPP is still more common in boys than in girls.[6] The prevalence of CNS pathology in CPP, excluding incidentalomas, is 3.3-15.8% in girls, and 26-40% in boys in recent reports.[6,12,14] However, one recent study reported no demonstrable change in the epidemiology of organic CPP in boys.[15] Neurofibromatosis and optic glioma were more common in that study in comparison to ours, while prevalence of hamartomas were similar (6%). Although idiopathic CPP was more common than organic CPP in boys in our study, still, organic CPP was 3.5 times more common in boys compared to

Table I. Cranial and/or pituitary MRI findings of the patients.

	Idiopathic		Organic	
	Normal n (%)	Incidentaloma n (%)	Known CNS pathology* n (%)	Novel CNS pathology** n (%)
Girls	218 (85.2)	22 (8.6)	9 (3.5)	7 (2.7)
Boys	83 (69.2)	11 (9.2)	10 (8.3)	16 (13.3)

* Previously established CNS pathology with neurological symptoms
** Novel CNS pathology without neurological symptoms and signs

Table II. Frequency of CNS pathologies in cases without neurological findings.

	Boys n(%)**	Girls n(%)***	Boys/Girls ratio	p value
Hypothalamic hamartoma	6 (5)	2 (0.8)	6	<0.001
Suprasellar arachnoid cyst	4 (3.3)	2 (0.8)	4	<0.001
Hemorrhagic macroadenoma*	1	2	N/A	N/A
Optic glioma*	2	1	N/A	N/A
Craniopharyngioma*	1	0	N/A	N/A
Pineal germinoma*	1	0	N/A	N/A
Pinealoblastoma*	1	0	N/A	N/A
Total	16(13.3)	7(2.7)	5	<0.001

* Statistical analysis could not be performed due to the small number of cases
** Percent in total number of boys
*** Percent in total number of girls

Table III. CNS findings with respect to age groups as determined by MRI.

	Pituitary or cranial MRI	Age at onset of pubertal findings					
		Girls n=256			Boys n=120		
		0-2 yrs n (%)	2-6 yrs n (%)	6-8 yrs n (%)	0-3 yrs n (%)	3-7 yrs n (%)	7-9 yrs n (%)
Idiopathic CPP	Normal		30 (11.7)	188 (73.4)		12 (10.0)	71 (59.2)
	Incidentaloma		4 (1.6)	18 (7.0)		2 (1.7)	9 (7.5)
Organic CPP	Novel CNS pathology previously asymptomatic	2 (0.8)	5 (2.0)		4 (3.3)	12 (10.0)	
	Previously established CNS pathology (previously symptomatic)		9 (3.5)			10 (8.3)	
Total		2 (0.8)	48 (18.8)	206 (80.4)	4 (3.3)	36 (30)	80 (66.7)

girls (21.7% vs 6.2%). Hypothalamic hamartomas and suprasellar arachnoid cysts were the most common lesions in boys, and hamartomas were six times, arachnoid cysts were four times more frequent in boys than in girls. Interestingly, we did not observe any predominance of a specific lesion in girls. Novel organic CNS lesions in girls included hamartomas, arachnoid cysts, hemorrhagic macroadenoma and glioma, none of which had any predominance over another.

In the literature, lesions associated with CPP are hamartomas, pituitary, pineal or suprasellar arachnoid cysts, hypothalamic pilocytic astrocytomas, pineal tumors or cysts, hypothalamic teratomas, and gliomas.[1,3,16-19] Hypothalamic hamartomas (HH) are rare, tumor-like malformations formed during fetal development. They are present at birth, however, become symptomatic during childhood. Two clinical phenotypes are described. They can either present with CPP or epilepsy and additional neurobehavioral symptoms. For those that present with CPP, symptoms usually start as early as 1-3 years of age, whereas neurological symptoms such as epilepsy present later.[17,19-23] In a study including one boy and 20 girls with CPP, hypothalamic hamartomas were present in 14%, and all showed pubertal signs in the first two years of life.[20] In the current study, pubertal signs were observed before two years of age in two girls with hamartoma, whereas onset of puberty was before three years of age in four boys, and between three and seven years of age in the remaining two. MRI of patients with CPP typically shows HH in the anterior

Table IV. Clinical and hormonal characteristics of patients with organic CPP vs idiopathic.

	Girls			Boys		
	Organic (n=16)	Idiopathic (n=240)	P value	Organic (n:26)	Idiopathic (n=94)	P value
Chronological age at diagnosis (CA) (yrs)	4.6 ± 1.1	7.8 ± 0.8	<0.001	5.0 ± 1.4	8.4 ± 1.1	<0.001
Age at initiation of symptoms (yrs)	4.1 ± 1.1	6.8 ± 0.8	<0.001	4.4 ± 1.4	7.4 ± 0.9	<0.001
Bone age (BA) (yrs)	8.2 ± 0.9	10.0 ± 0.5	<0.001	8.5 ± 1.3	10.2 ± 1.2	<0.001
BA advancement (BA-CA) (yrs)	3.6 ± 1.5	2.2 ± 0.9	<0.001	3.5 ± 0.9	1.8 ± 0.5	<0.001
Height-SDS	1.8 ± 1.0	1.5 ± 0.8	0.355	1.8 ± 1.0	1.1 ± 0.9	<0.001
Height-SDS for BA	-2.3 ± 0.8	-0.7 ± 0.7	<0.001	-1.9 ± 0.9	-0.6 ± 0.7	<0.001
Pubertal stage			0.565			0.456
T2	5 (31.2%)	85 (35.4%)		9 (34.6%)	34 (36.2%)	
T3	9 (56.3%)	125 (52.1%)		13 (50%)	46 (48.9%)	
T4	2 (12.5%)	30 (12.5%)		4 (15.4%)	14 (14.9%)	
Basal FSH (IU/L)	3.8 ± 1.0	4.5 ± 1.5	0.405	3.7 ± 1.4	3.5 ± 1.6	0.505
Basal LH (IU/L)	1.6 ± 0.9	1.3 ± 0.7	0.386	1.5 ± 0.9	1.3 ± 0.9	0.705
Basal E2 (pg/ml)	64.8 ± 21.4	30.6 ± 12.6	<0.001			
Basal testosterone (ng/dl)				94.6 ± 34.0	20.2 ± 13.5	<0.001
Peak stimulated LH (IU/L)	17.1 ± 3.5	12.3 ± 4.1	<0.001	26.2 ± 4.8	13.1 ± 5.0	<0.001

hypothalamus, tuber cinereum and pituitary stalk. For those that present with epilepsy, gelastic (laughing) seizure is usually the first symptom during infancy.

Some brain lesions detected on MRI while investigating the etiology of CPP may indeed be incidentalomas. Incidentaloma, can be defined as a lesion detected through imaging, performed for other reasons rather than to identify an excess or lack of pituitary hormones.[24] In the current study, the prevalence of incidentaloma was similar in both sexes, as well as similar to that reported in the literature.[1,18,25] Suprasellar arachnoid cysts may not always be associated with precocious puberty. Adan et al.[26] reported that only 1/3 of suprasellar arachnoid cysts were associated with precocious puberty. In the current series, all the suprasellar arachnoid cysts were large in size (>5 cm) causing hydrocephalus. Therefore, they were included in the organic group of CPP.

The prevalence of organic lesions is high in early onset CPP. Three recent studies analyzing girls with CPP classified cases with respect to age as younger than 6 years and ≥ 6 years of age. CNS lesions were more prevalent in those with early onset CPP (17.1-26.9%) in comparison to those with later onset CPP (0-1.9%).[3,16,25] In the current study, all cases with an onset before two years of age in girls and three years of age in boys had an underlying organic lesion. None of the girls with an onset of CPP after six years and boys after seven years of age had any organic lesion.

A number of studies analyzed various clinical and biochemical features that may predict intracranial pathology in girls with CPP, however similar studies in boys are scarce. Clinically, the probability of a CNS lesion underlying CPP is higher in girls before five years of age, with rapid pubertal development and significantly advanced bone age.[20,27,28] Studies comparing biochemical features of idiopathic vs organic CPP have conflicting results. In some, higher basal gonadotropin levels, stimulated LH and FSH peaks, as well as basal serum estradiol levels were associated with organic lesions, whereas in others such an association could not be shown.[2,16,17,29-32] In the current study, patients with organic CPP had an earlier onset with advanced bone age, and higher sex steroid levels as well as higher peak stimulated LH.

Magnetic resonance imaging is an expensive, as well as invasive technique requiring intravenous gadolinium injection, and even sedation in some cases. Therefore, it is practical to define criteria to differentiate those with a likelihood of organic lesion in order to use imaging selectively in CPP. There is a common agreement that all boys with CPP should undergo cranial and pituitary MRI. In the current study a novel CNS pathology was detected in 14.5 % of boys, all younger than seven years of age. Likelihood of a novel CNS pathology was five times more frequent in boys compared to girls (14.5% vs 2.8%). Also it is possible to detect a CNS lesion in approximately one out of every five boys with CPP (21.7%). Thus, we also recommend neuroimaging in all boys with CPP.

Since girls are more likely to have idiopathic CPP, there are conflicting opinions on CNS imaging in girls especially in those aged 6-8 years. There are studies that suggest routine brain and pituitary MRI should not be carried out on girls who are neurologically normal and whose puberty started after six years of age since likelihood of underlying intracranial pathology and tumor in girls at this age group is quite low.[25,33] However, other studies recommend MRI in all girls with CPP regardless of age in order to rule out an underlying CNS lesion even if the risk is low.[1,27,30] Based on our data, we recommend a cranial and pituitary imaging to all girls younger than six years of age. We believe that routine MRI is unnecessary beyond six years of age in girls unless there are neurological findings.

Organic CPP is 3.5 times more common in boys compared to girls. It is possible to detect an underlying CNS pathology in one out of every five boys with CPP. Frequency and distribution of organic etiology also differ between girls and boys. In boys, organic causes are more frequent, and hypothalamic hamartomas and suprasellar arachnoid cysts are more common than girls. Organic cause underlying CPP is quite rare in girls older than six years, and boys older than seven years. We recommend pituitary and cranial MRI for all boys with CPP regardless of age since there is a risk of CNS lesion in one out of every five boys with CPP. The likelihood of novel intracranial pathology associated with CPP is quite small in girls with an onset after six years of age. We recommend pituitary and cranial MRI for all girls with a pubertal onset younger than six years, as well as those with accompanying neurological findings suggestive of intracranial lesion in those with an onset of puberty after six years of age.

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Original Article

Point-of-care ultrasound use in pediatric intensive care units in Turkey

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ABSTRACT

Background. Point-of-care ultrasound is commonly used in pediatric intensive care units in recent years. The aim of this study was to find an answer to the question "where are we now in Turkish pediatric intensive care units for point-of-care ultrasound use?".

Methods. This was a multicenter, descriptive study in which we developed an online survey. We asked 45 questions about point-of-care ultrasound using fields and training status of clinicians in pediatric intensive care units.

Results. A total of 29 units responded to the questions completely. Of all included units 41.4% were in public hospitals, 6.9% in city hospitals (public-private partnership) and 51.7% in university hospitals. The most common use of point-of-care ultrasound was central venous catheter insertion. Lung ultrasound use rates for detection of pleural effusion, evaluation of pneumothorax, and diagnosis of pneumonia were 93.1%, 86.2%, and 34.5%, respectively. Critical care echocardiography use rate was 79.3%. In 89.7% of the units, intensive care specialists had been specifically trained for the use of point-of-care ultrasound.

Conclusions. Our study showed that point-of-care ultrasound was not only used for central venous catheterization but also for widespread fields in pediatric intensive care units. With an experienced team, it is possible to perform rapid, noninvasive and repeatable ultrasonographic assessment of patients. In our view point-of-care ultrasound is the new stethoscope of critical care physicians.

Key words: central venous catheter, echocardiography, intensive care unit, lung, point-of-care ultrasound.

Point-of-care ultrasound (POCUS) or critical care ultrasound is a bedside ultrasonographic assessment and is applied to patients by the clinician in charge.[1] It provides rapid and real time answers about the clinical problems of patients. Use of bedside ultrasonography by clinicians other than radiologists is gradually becoming common.[2] Pediatricians commonly use POCUS, especially in emergency and intensive care departments. In fact, POCUS has become a part of the physical examination of critically ill children in pediatric intensive care units (PICUs) in recent years.[3,4] Patients in PICUs frequently have critical problems and need quick assessments due to their hemodynamical instability.[5] Through POCUS results, clinicians can manage treatment approaches. The most important advantages of this technique are that it is easy to use, repeatable, noninvasive, cheap, painless and radiation-free.[6]

Adult studies have shown improved clinical outcomes in patients with the use of POCUS.[7] Clinicians can use POCUS with many aspects, such as critical care echocardiography to evaluate myocardial contractility and cardiac index measurements or to detect pericardial tamponade[8], lung ultrasound to evaluate pneumothorax, pleural effusion and pneumonia[9], ophthalmic ultrasound for the

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clinical follow-up of increased intracranial pressure with optic nerve sheath diameter (ONSD) measurements[10], fast intraabdominal assessment to detect perihepatic or perisplenic hemorrhage[11], vascular ultrasound to insert central venous catheter or invasive arterial catheter[12], to measure vena cava inferior maximum and minimum diameters to evaluate the volume status of patients with vena cava inferior collapsibility index[13] (spontaneous breathing patients) and vena cava inferior distensibility index (mechanically ventilated patients).[14]

In the world and in our country, most PICUs have POCUS technology, and the frequency of training courses intended for pediatric intensive care and emergency care specialists are increasing.[15] There are many adult studies on POCUS use in intensive care units; however, in the pediatric field, the reports of pediatric emergency departments stand out.[16] There is limited data available in the literature describing the status and fields of use of POCUS in PICUs.

The main purpose of our study was to analyze the use of POCUS in Turkish PICUs. We aimed to detect the common clinical applications of POCUS and the training status of pediatric intensive care specialists in tertiary PICUs at university and state hospitals in Turkey.

Material and Methods

We planned a multicenter, cross-sectional, descriptive study. We developed an online survey using the SurveyMonkey online platform, including 45 questions. The main topics of the survey were the descriptive characteristics of the hospital and the physician responding to the survey, features of the ultrasound machine, POCUS application fields and frequencies in the PICU and training status of the clinical staff including clinicians and nurses. The survey was distributed by the Turkish Society of Pediatric Emergency and Intensive Care to the clinical chiefs of PICUs via e-mail. The study protocol was approved by the local Ethical Committee of Çukurova University Medical Faculty (March 2019; 86).

Results

Characteristics of chief in charge for the survey and PICU's

The survey reached 36 units, and 29 of them responded to the questions completely. Each survey was answered only once by each PICU and by the chief clinician of the department. The pediatric intensive care experience of the participant chief of the department was below 5 years in 48.3% (n = 14), 6-10 years in 31.0% (n = 9), 10-15 years in 17.2% (n = 5) and above 15 years in 3.4% (n = 1). Of all included PICUs, 41.4% (n = 12) were in public hospitals, 6.9% (n = 2) in city hospitals (public-private partnership) and 51.7% (n = 15) in university hospitals. All participating PICUs only provided medical and post-surgery care for pediatric patients. The types of patients followed in PICUs are shown in Table I.

Of all units, 27.6% (n = 8) had fewer than 10 beds, 51.7% (n = 15) had 11-20 beds, 13.8% (n = 4) had 21-40 beds, and 6.9% (n = 2) had more than forty

Table I. Types of patients followed in 29 pediatric intensive care units.

Type of patients	Number (%) of PICUs involved
Multiple trauma	22 (75.9)
Medical	
Neuromuscular	29 (100.0)
Metabolic disorders	28 (96.6)
Gastroenterology	25 (86.2)
Endocrinology	29 (100.0)
Nephrology	28 (96.6)
Infectious diseases	29 (100.0)
Cardiac surgery	16 (55.2)
Neurosurgery	24 (82.8)
Extracorporeal membrane oxygenation	14 (48.3)
Transplant	8 (27.6)
Postoperative pediatric surgery	26 (89.7)

PICUs: pediatric intensive care units

beds. As for the number of physicians on staff, 10.3% (n = 3) of the units had more than eight physicians, 24.1% (n = 7) had between five and eight and 65.5% (n = 19) had fewer than five.

Status of ultrasound machine in PICU

Of all the PICUs, 96.6% (n = 28) had a dedicated ultrasound machine, and 41.4% (n = 12) of those shared the ultrasound machine with other units in the hospital. Only one (3.4%) of the units that responded to our survey had no ultrasound machine. Of the PICUs with ultrasound machines, 72.4% (n = 21) had portable systems, while 27.6% (n = 8) had non-portable ones.

Clinical use of POCUS

A total of 55.2% (n = 16) units reported clinical using of POCUS daily, 41.4% (n = 12) of them every two or three days, 3.4% (n = 1) weekly. In 72.4% (n = 21) of the PICUs, more than 75% of the pediatric intensive care specialists regularly used POCUS for clinical assessment, and this frequency range changed between 50% to %75 in 14.2%, 25% to 50% in 3.5% and fell below 25% in 10.7%. In 89.7% (n = 26) of the PICUs, intensive care specialists had been specifically trained for the use of POCUS. All participants agreed on the clinical benefits of the use of POCUS for the assessment of critically ill children in PICUs. Table II shows the purposes and frequencies of use of POCUS. The most common use of POCUS was central venous catheter insertion (n = 16; 55.2%). A total of 7 (24.1%) units never used anatomical land-marks for catheterization and always inserted central venous catheter with real-time POCUS (internal jugular vein in 100%, femoral vein in 62.1%, subclavian vein in 13.8%). Twelve units (41.4%) used POCUS for arterial catheterization and two units always inserted the arterial line with POCUS. Fourteen units (48.3%) reported that they never used POCUS for arterial line insertion. Critical care echocardiography use rate in PICUs was 79.3% (n = 23) in our study (assessment of myocardial contractility in 59%, cardiac output and cardiac index in 31%, pericardial effusion in 93%). The results of our survey showed that 31% of the units in our country used POCUS for ONSD measurements in patients with increased intracranial pressure. Twenty-four (82.8%) of our participating specialists were trained by the Turkish Society of Pediatric Emergency and Intensive Care for POCUS use. In addition; the nurses in one of the participating units had training about ultrasound use for peripheral venous catheter insertion. Sixteen units (55.2%) agreed on the necessity of nurse training.

Table II. Purposes of point-of-care ultrasonography in pediatric intensive care units.

Purposes	Number (%) of PICUs involved
Critical care echocardiography	23 (79.3)
Assessment of cardiac contractility	17 (58.6)
Assessment of cardiac index	9 (31.0)
Management of cardiorespiratory arrest	16 (55.2)
Assessment of airway	3 (10.3)
Assessment of nasogastric tube	2 (6.9)
Assessment of intravascular blood volume	
Vena cava inferior collapsibility index	12 (41.4)
Vena cava inferior distensibility index	8 (27.6)
Central venous catheter insertion	16 (55.2)
Arterial catheterization	12 (41.4)
Diagnosis of pneumothorax	25 (86.2)
Diagnosis of pneumonia	10 (34.5)
Diagnosis and intervene of pleural effusion	27 (93.1)
Assessment of free intraperitoneal fluid	21 (72.4)
Assessment of diaphragm paralysis	13 (44.8)
Assessment of optic nerve	9 (31.0)

PICUs: pediatric intensive care units

Discussion

Use of POCUS has led to great advances in rapid and repeated evaluation and intervention of critically ill pediatric patients in PICUs. Day by day, the use of POCUS becomes an important skill for pediatric critical care medicine providers.[17] Most adult studies have shown that POCUS use improves the clinical

outcomes of critically ill patients and decreases mortality and morbidity.[18] In recent years, most PICUs have acquired POCUS technology, and the training courses for pediatric intensive care specialists have become popular.[15] The aim of our study was to detect the frequency of use and availability of POCUS and the specific training status of pediatric intensive care specialists in Turkish PICUs.

Echocardiography is becoming a standard of critical care in many intensive care units, and more clinicians are learning how to perform bedside critical care echocardiography techniques as more pediatric intensivist are becoming familiar with POCUS in PICUs.[19,20] The echocardiography type referred to as critical-care echocardiography has become a part of the routine evaluation of patients in the PICU by pediatric intensivists.[20,21] This noninvasive technique allows the intensivist to measure ejection fraction, cardiac output and cardiac index and to assess pericardial effusion and becomes a guide for the management of treatment and ensuring the hemodynamic stability of critically ill patients.[21] The importance of cardiac index in guiding fluid and inotropic management in septic shock was emphasized in the recent clinical practice parameters published in 2017, which highlighted the significance of cardiac index measurement in the PICU.[22] In another survey similar to our study, the results showed a 72.7% rate of POCUS use for the assessment of cardiac function. In the first national survey from the USA, Lambert et al.[23] reported a 37.5% rate of POCUS use for myocardial function. In our survey, the rate of critical care echocardiography use in Turkish PICUs was found to be 79.3% (assessment of myocardial contractility in 58.6%, cardiac output and cardiac index in 31%, pericardial effusion in 93.1%).

Community-acquired and ventilator-associated pneumonia are common and important problems in PICUs.[24,25] In addition, chest X-ray is still a widespread tool for the diagnosis of pneumonia. In the last years, most studies have shown that bedside lung ultrasound performed by pediatric critical care providers was highly accurate for the diagnosis of pediatric pneumonia.[25] Lung ultrasound has proven useful for detecting lung abnormalities in adults, and recent studies have reported the usefulness of lung ultrasound in children with pneumonia and bronchiolitis.[24,25] Furthermore, if you can use POCUS for the diagnosis of pneumothorax, intervention can be easy and rapid and can manage pleural effusion drainage with less complication and greater success.[26-28] Besides, lung ultrasound allows the evaluation and drainage of pleural effusion, which is another useful aspect of lung ultrasound. The most important advantage of lung ultrasound is that it is a radiation-free technique.[1,28] Cortes et al.[29] reported POCUS use rates for pleural effusion, pneumothorax and other lung diseases such as pneumonia as 73.3%, 50% and 46.7%, respectively.[30] Our survey results showed that 93.1% of the units used POCUS to detect pleural effusion, 86.2% for the evaluation and intervention of pneumothorax, 34.5% for pneumonia diagnosis, 72.4% for the drainage of pleural effusion and 31% for the clinical follow-up of pneumonia in our country.

The use of POCUS decreases the risk of complication and the number of attempts in some procedures such as central-peripheral venous catheterization and arterial catheterization. It has been reported that the use of US guidance significantly improves the rate of successful peripheral intravenous access, especially in patients who are difficult to access and decreases the amount of time to perform the procedure, the number of percutaneous punctures and needle redirections compared to traditional approaches such as palpation and landmark guidance.[30] Pediatric critical care nurses had training for ultrasound use for peripheric venous catheter insertion only in one of our participating units. We thought that this was a low ratio for our study, and when we asked about the necessity of POCUS training for nurses, nearly half of our participating units stated that they found this use of POCUS redundant.

In pediatric emergency and intensive care departments, central venous catheter placement may be required in life-threatening conditions where fluid and drug resuscitation are needed or in complex patients with poor vascular access. Ultrasound-guided pediatric central venous catheterization has been shown to be superior to traditional landmark and palpation techniques.[31] A survey study reflecting the results of 128 PICUs in the USA showed that using POCUS for central venous catheterization was more common than peripheral or arterial catheter insertion and the internal jugular vein was the preferred initial site in their survey, similar to our results.[23] The most common use of POCUS in our survey was central venous catheter insertion. Furthermore, 24.1% of the units never used anatomical land-marks for catheterization and always inserted central venous catheter with real-time POCUS.

Arterial catheterization, commonly used in infants and small children in intensive care units and operating rooms, can be technically challenging.[32] Nevertheless, the ultrasound-guided technique improves the first-attempt success rate in both adults and children.[33] The author of another survey study from Spain reported a POCUS use rate of 75% for arterial catheterization.[29] The survey results showed that 41.4% of the units in our country used POCUS for arterial catheterization and 16.6% of them always inserted the arterial line with POCUS.

One of the most important parts of POCUS for noninvasive and rapid assessment of fluid status in critically ill children is the measurements of vena cava inferior diameters.[13] Vena cava inferior is a vessel that is highly sensitive to fluid changes, and the collapsible vessel varies in size with respiratory changes in intra-thoracic pressure.[34] There is a vena cava collapsibility index for children with spontaneous breathing and a distensibility index for mechanically ventilated children. These features of inferior vena cava have allowed pioneering clinicians to measure both collapsibility indexes.[35] We asked about vena cava inferior maximum and minimum diameter measurements and vena cava inferior collapsibility index (children with spontaneous breathing) and vena cava inferior distensibility index (mechanically ventilated children) use for the management of fluid treatment, and the responses of participants showed that 43.4% of the units commonly used the collapsibility index and 27.6% commonly used the distensibility index in their units.

One such noninvasive modality includes the estimation of ONSD use in bedside ultrasounds.[10] The optic nerve sheath is an anatomical extension of the dura mater, and the subarachnoid space around the optic nerve is continuous with the subarachnoid space.[36] Due to this direct communication, pressure changes in the intracranial compartment are transmitted to the intra-orbital subarachnoid space around the optic nerve. Dilatation of the optic nerve sheath has been shown to be a much earlier manifestation of intracranial pressure rise.[37] It is not easy to take computed tomography or magnetic resonance images in a hemodynamically unstable patient. Ultrasound technology allows to take repeated and radiation-free images of the optic nerve sheath in patients with increased intracranial pressure at the bedside.[38] The results of our survey showed that 31% of the units in our country used POCUS for ONSD measurements in patients with increased intracranial pressure.

The current cardiopulmonary resuscitation guidelines recommend performing POCUS when a reversible cause of cardiopulmonary arrest is suspected, although it is stated that improvement of outcomes with the use of POCUS in cardiopulmonary arrest has not been yet demonstrated. POCUS is helpful in cardiopulmonary resuscitation in diagnosing reversible causes of cardiac arrest.[39] The rate of bedside ultrasound use for the efficacy of cardiopulmonary resuscitation was found to be 55.2% in our study.

Point-of-care ultrasound has been reported as a diagnostic tool for the confirmation of nasogastric tube placement for neonates and adults in the literature.[40-42] The results of these

studies showed a decreased need for abdominal radiography for nasogastric tube placement. Our results showed that 6.9% of our units sometimes use POCUS to verify nasogastric tube location.

In recent years, the training curricula for pediatric emergency care and critical care providers have gradually increased. The results of one study showed a high concordance between echocardiographic image quality and trained pediatric critical care providers and cardiologists. In the same study, Conlon et al.[42] detected significantly increased image interpretation ability in trained clinicians compared to the others. Most of the participants in our survey were trained in the course organized by the Turkish Society of Pediatric Emergency and Intensive Care. This provided a homogeneity for the fields of use of POCUS. We think that the rise of training courses is important for the common use of POCUS in PICUs.

Our study had some limitations. As with the other voluntary surveys, our results could not directly show the real frequency of use and likely only showed the views of those surveyed.

In conclusion, POCUS is an important technology frequently used in most PICUs in Turkey. In our view, POCUS is the new stethoscope of critical care physicians. If you have POCUS and have an experienced team, you can perform fast, noninvasive and repeatable assessments with clinical changes of patients without the need for an external consultant. Our study shows that POCUS was not only used for central venous catheterization. The results revealed the most important clinical benefits of POCUS use by pediatric intensive care providers. Our results confirm the widespread use of POCUS in PICUs. We believe that our study is important in terms of the training status of clinicians for POCUS use. Despite the good training level and homogeneity of training status in our country, we believe that the training courses should continue to increase.

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Original Article

Carbapenem and colistin resistance in children with *Enterobacteriaceae* infections

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ABSTRACT

Background. Carbapenem-resistant *Enterobacteriaceae* (CRE) are an emerging global public health threat. As a reserve agent, colistin has been the drug of choice for the treatment of infections caused by CRE. The aim of this study was to determine the risk factors of carbapenem and colistin-resistant *Enterobacteriaceae* infections and to investigate the outcomes.

Methods. We conducted a retrospective study in a single university hospital between the years 2013 and 2017 including 150 patients with *Enterobacteriaceae* infections.

Results. Of 150 *Enterobacteriaceae* infections, 62 (41%) were carbapenem and 23 (15%) were colistin-resistant. Colistin resistance rates among *Enterobacteriaceae* species increased from 4% in 2014 to 25% in 2017. The in-hospital mortality of the patients with colistin-resistant and with carbapenem-resistant infections were 39% (9/23) and 45% (28/62), respectively. Prior exposure to polyantibiotic therapy for Gram negative bacteria was found as a predictor of CRE (OR = 6.4; 95% CI 3.07-13.6; p = 0.001) infections. The median length of hospital stay prior to positive culture (OR = 1.02; 95%CI, 1.0-1.04; p = 0.003) and history of surgery during the admission (OR = 2.46; 95% CI 1.2-5.1; p = 0.005) were found as the predictors of CRE infections. Underlying necrotizing enterocolitis and/or short-bowel syndrome (OR=6.38; 95%CI 1.16-35; p = 0.033) and mechanical ventilation prior to index culture were found as predictors of colistin resistance (OR = 9.4; 95% CI 2-40.4; p = 0.004).

Conclusions. Recognizing the risk factors of carbapenem and colistin resistant *Enterobacteriaceae* infections is essential in order to conserve carbapenem and colistin since there are no new antibiotics to treat multidrug-resistant *Enterobacteriaceae* infections.

Key words: carbapenem-resistance, colistin-resistance, Enterobacteriaceae, outcome, risk factors.

Enterobacteriaceae species are common cause of infections in both community and healthcare settings worldwide. Carbapenems are the mainstay of therapy for infections caused by *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs). The emergence and dissemination of carbapenem resistance among *Enterobacteriaceae* in all over the world represents a serious threat to public health and significantly limits the treatment options for life threatening infections.[1] Although colistin is currently considered as a last-resort treatment for infections caused by multi-drug resistant (MDR) bacteria, colistin resistance in *Enterobacteriaceae* species has been reported in several countries around the world and its prevalence has continued to increase, thus becoming a great healthcare concern.[2-5] It is important for healthcare facilities to understand how common CRE are in their institutions. Recognizing the risk factors of carbapenem and colistin-resistant *Enterobacteriaceae* infections is essential in order to conserve carbapenem and colistin, since there are no new antibiotics to treat

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MDR *Enterobacteriaceae* infections. Interventions to control CRE are evolving as more data and experience become available. Although several factors that increase the risk of infection with CRE have been reported, there is limited data on the epidemiology, risk factors, treatment, and outcomes in pediatric populations with colistin-resistant *Enterobacteriaceae* infections.[6-8]

In this study it was aimed to characterize the clinical features of patients with carbapenem and colistin-resistant *Enterobacteriaceae* infections. The other goals were to identify the risk factors, investigate the outcomes and determine the frequency of colistin and carbapenem resistance in clinical isolates of *Enterobacteriaceae* species.

Material and Methods

The clinical and microbiological data of all patients with isolation of *Enterobacteriaceae* from different specimens between December 2013 and December 2017 were retrospectively evaluated in our university hospital. Only patients determined to have an active infection were included in the study. Patient demographics, comorbidities, dates of admission, outcomes, medications, history of surgery, use of mechanical ventilation, and procedures applied during the hospitalization were included in the study. To identify CRE definition CDC surveillance reports in 2015 were used. According to CDC reports CRE was identified in two situations; 1-Resistant to any carbapenem antimicrobial (i.e. minimum inhibitory concentrations of ≥4 mcg/ml for doripenem, meropenem, or imipenem or ≥2 mcg/ml for ertapenem) 2- For bacteria that have intrinsic imipenem non-susceptibility (i.e. *Morganella morganii, Proteus spp., Providencia spp.*), resistance to carbapenems other than imipenem.[9] Identification and susceptibility testing of clinical isolates were performed using automated laboratory system; Vitek-2 (bioMérieux). Minimum inhibitory concentrations (MICs) were interpreted according to European Committee on Antimicrobial Susceptibility testing (EUCAST) breakpoints.[10] Empiric antimicrobial therapy was identified as the antibiotics those used before the index blood culture results and those used at least 48 hours. Healthcare-associated infections were diagnosed according to Centers for Disease Control and Prevention definitions.[11] This study was approved by the Institutional Ethics Committee (5th October 2018; report number 81/5). All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Statistical analysis

An X^2 test or Fisher exact test was used to compare the categorical variables, while Student t-test or Mann-Whitney U test was used to compare the continuous variables, as appropriate. Univariate and multivariate logistic regression analyses were used to assess factors associated with in-hospital mortality and CRE infections. In-hospital mortality was compared between the CRE group and CSE group by using Kaplan-Meier estimation and compared using the long-rank test. A p-value <0.05 was considered statistically significant for all tests. All analyses were performed with SPSS Statistics (version 21.0. IBM Corp, Armonk, New York).

Results

Demographics and clinical history of the patients with CRE

One-hundred-fifty patients with *Enterobacteriaceae* infections were included in the study; 76 were male (50.7%), 74 were female (49.3%). In total 62 patients (41.3%) were infected with CRE. Twenty-eight (45.2%) of the CRE isolates were in an ICU ward (neonatal and pediatric). The wards in which patients with CRE infections were isolated are shown in Table I.

Table I. Hospital location of patients with *Enterobactericeae*.

Department	CRE (n= 62)	CSE (n= 88)	Total (n= 150)
Pediatric intensive care unit, n (%)	14 (22.6)	17 (19.3)	31 (20.7)
Neonatal intensive care unit, n (%)	14 (22.6)	48 (54.5)	62 (41.3)
Pediatric hematology and oncology, n (%)	15 (24.2)	17 (19.3)	32 (21.3)
Pediatric cardiovascular surgery, n (%)	7 (11.3)	2 (2.3)	9 (6)
Burn unit, n (%)	2 (3.2)	0 (0)	2 (1.3)
Pediatric surgery, n (%)	1 (1.6)	1 (1.1)	2 (1.3)
Other pediatric wards, n (%)	9 (14.5)	3 (3.4)	12 (8)

CRE: carbapenem-resistant *Enterobactericeae*, CSE: carbapenem-sensitive *Enterobactericeae*

Carbapenem resistance was observed in 47 of 98 (47.9%) *Klebsiella species*, in eight of 23 (34.7%) *E.coli* species, in three of ten (30%) *Enterobacter* species, in one of two (50%) *Citrobacter* species and in three of eight (37.5%) *Stenotrophomonas* species. CRE was isolated from blood in 51 (82.3%) patients, from respiratory tract in eight (12.9%), from urine in one patient (1.6%) and from wound specimens in two patients (3.2%). Ventilator-associated pneumonia (VAP) was observed in 22 patients (35.5%) and pneumonia other than VAP was observed in six patients (9.7%).

All patients were hospitalized for >48 hours before their CRE infection. Underlying medical conditions were reported in 54 (87.1%) of 62 patients with CRE infection. The most common condition was cardiac disease (17 patients, 27.4%), 14 (22.5%) had oncologic processes, nine (14.5%) had pulmonary disease, nine (14.5%) had a history of prematurity, four (6.4%) patients had necrotizing enterocolitis and/or short-bowel syndrome. A history of surgery was reported in 31 (50%) of 62 patients; 15 (48.3%) of the procedures were gastrointestinal, and 10 (32.2%) were cardiac procedures. An indwelling device was reported in 60 (96.8%) of 62 children. Of 62 patients with CRE infections, bacteremia was observed in 51 patients (82.2%). There was no statistical difference in terms of gender between CRE and carbapenem-susceptible *Enterobacteriaceae* (CSE) groups (p = 0.25). The median age of patients in CRE group was 29 days (range: 4 days – 2.71 years) and in the CSE group was 1.03 year (range: 5 days - 22.7 years). Patients in the CRE group were younger than the patients in the CSE group (p = 0.004). Demographics and clinical findings of patients with CRE are shown in Table II.

Risk factors of CRE infections

Although patients with CRE infections were younger and had higher rates of central catheter compared to the CSE group, in regression analysis neither age nor central line was associated with the risk of CRE infections. Risk factors of CRE infections are demonstrated in Table III. Prior to the index culture, empiric polyantibiotic therapy for Gram negative bacteria was detected in 31 (50%) patients with CRE vs. 16 (18.2%) patients with CSE. Prior exposure to polyantibiotic therapy for Gram negative bacteria was found as a risk factor of CRE (p = 0.001). The median length of hospital stay prior to positive cultures with CRE infections was longer than the length of hospital stay in patients with CSE infections (p = 0.003).

Logistic regression analysis revealed that three variables were independently associated with the isolation of CRE strains: length of hospitalization prior to index culture (OR, 1.02; 95% CI, 1.01 -1.04, p=0.003), previous polyantibiotic therapy (OR, 6.4; 95% CI, 3.07-13.6, p=0.001) and history of surgical intervention during admission (OR, 2.46; 95% CI, 1.2-5.1, p=0.005).

Table II. Demographics and clinical findings of patients with and without carbapenem-resistant *Enterobacteriaceae*.

Demographics and clinical findings	CRE (n = 62)	CSE (n = 88)	p
Age, median (range)	29 days (4 days - 2.7 years)	1.03 year (5 days - 22.7 years)	0.004*
Male, n (%)	28 (45.1)	48 (54.5)	0.25
Mechanical ventilation prior to index culture, n (%)	36 (58)	53 (60.2)	0.79
Central venous catheter prior to index culture, n (%)	60 (96.8)	77 (87.5)	0.047*
Empiric antibiotic exposure prior to index culture, n (%)			
- Piperacillin/tazobactam	4 (6.4)	4 (4.5)	0.39
- Carbapenem	12 (19.3)	19 (21.6)	0.51
- 3rd or 4th generation cephalosporins	4 (6.5)	13 (14.8)	0.87
- Aminoglycoside	6 (9.7)	28 (31.8)	0.75
- Flouroquinolone	4 (6.5)	3 (3.4)	0.21
- Carbapenem + aminoglycoside	12 (19.3)	4 (4.5)	0.001*
- Carbapenem + except aminoglycoside	8 (12.9)	7 (7.9)	0.072
- Aminoglycoside + except carbapenem	11 (17.7)	5 (5.7)	0.004*
- No empiric therapy	1 (1.6)	5 (5.7)	
Length of hospital stay prior to index culture (days), median (IQR); [min-max]	26 (12-42.5); [1-150]	18 (12-31.5); [4-100]	
Operation: yes, n (%)	31 (50)	26 (29.5)	0.015

*: p <0.05
CRE: carbapenem-resistant *Enterobacteriaceae*, CSE: carbapenem-susceptible *Enterobacteriaceae*

Table III. Predictors of carbapenem-resistant *Enterobacteriaceae* and colistin-resistant *Enterobacteriaceae*.

Predictors for resistance	OR (95% CI)	P
Carbapenem resistance		
- Length of hospitalization prior to index culture	1.02 (1.01-1.04)	0.003*
- Polyantibiotic therapy prior to index culture	6.4 (3.07-13.6)	0.001*
- History of surgical intervention during admission	2.46 (1.2-5.1)	0.005*
Colistin resistance		
- Urinary catheter	0. 32 (0.18-0.38)	0.012*
- Mechanical ventilation prior to index culture	9.4 (2.0-40.4)	0.004*
- Underlying necrotizing enterocolitis and/or short-bowel syndrome	6.38 (1.16-35)	0.033*

*: p <0.05

Demographics and clinical history of the patients with colistin-resistant Enterobacteriaceae

Twenty-three patients had colistin-resistant *Enterobacteriaceae* infections. The median age of the children with colistin-resistant *Enterobacteriaceae* infections was 150 days (range, 5 days -11.8 years). Nine (39%) patients were male and 14 (61%) patients were female. The median length of hospital stay prior to positive culture results of colistin-resistant species was 26 days (range, 5-150 days; interquartile range, 11-46 days). The rate of colistin resistance in *Enterobacteriaceae* species increased markedly from 4.9% in 2014 to 25% in 2017. In 2014 two out of 41 *Enterobacteriaceae* infections were colistin-resistant (4.9%). In 2015 eight out of 37 infections (17.8%), in 2016 six (18.2%) were colistin-resistant and in 2017 seven (25%) were colistin-resistant. Nineteen (82%) patients were in an ICU ward (neonatal and pediatric). Underlying medical conditions were

reported in all patients with colistin-resistant *Enterobacteriaceae* infections. The most common condition was cardiac disease (nine patients, 39%), six (26%) had pulmonary disease, five (21.7%) had a history of prematurity, three patients (13%) had necrotizing enterocolitis and/or short-bowel syndrome. A history of surgery was reported in 11 (47.8%) out of 23 patients. Central line was reported in 22 (95.7%) patients. Mechanical ventilation prior to index culture was reported in 21 (91.3%) patients. Urinary catheter was reported in 15 (65.2%) patients. Source of isolates were blood in 20 (87%) patients. Sixteen (69.6%) colistin-resistant strains were *Klebsiella* species, six (26.1%) were *Serratia* species, one (4.3%) was *E.coli* species. Demographics and clinical findings of patients with CRE are shown in Table IV.

Risk factors of colistin-resistant Enterobacteriaceae infections

Mechanical ventilation prior to index culture were more common in patients with colistin-resistant group (21/23) than colistin-susceptible group (68/127) which was 91.3% vs. 53.5% respectively. Risk factors of colistin resistance are shown in Table III. In the regression analysis mechanical ventilation prior to index culture was determined as a risk factor of colistin resistance (OR, 9.4; 95% CI, 2-40.4; p = 0.004).

Urinary catheter was reported higher in the colistin-resistant group (15/23) than the colistin sensitive group (46/127) which was 65.2% vs. 36.2%, respectively. In logistic regression analysis urinary catheter was the risk factor of colistin resistance (OR, 0.32; 95% CI, 0.18-0.38; p = 0.012). The other independent risk factor of colistin resistance was underlying necrotizing enterocolitis and/or short-bowel syndrome (OR, 6.38; 95% CI, 1.16-35; p = 0.033).

Outcome in CRE and colistin-resistant Enterobacteriaceae infections

The in-hospital mortality of the CRE group was (28/62) 45.2%. There were no statistically significant difference in mortality rates between CRE and CSE groups which was 45.2% vs. 36.7% (p = 0.071). The median length of hospital stay after the index culture in CRE group was 21 days (range, 0-96 days) vs. 19 days (range, 0-160 days) in CSE group (p = 0.12) (Fig. 1). The in-hospital mortality rates of patients infected by colistin-susceptible *Enterobacteriaceae* was 36.2% (46/127) and colistin-resistant *Enterobacteriaceae* was 39.1% (9/23). In Kaplan-Meier analysis there were no statistically significant difference

Table IV. Demographics and clinical findings of patients with and without colistin resistance *Enterobacteriaceae*.

Demographics and clinical findings	Colistin resistant (n = 23)	Colistin sensitive (n = 127)	P
Age, median (range)	150 days (5 days – 11.8 years)	165 days (4 days - 22.7 years)	0.56
Male, n (%)	9 (39.1)	67 (52.8)	0.22
Urinary catheter, n (%)	15 (65.2)	46 (36.2)	0.009*
Underlying necrotizing enterocolitis, n (%)	3 (13.1)	4 (3.1)	0.038*
Mechanical ventilation prior to index culture, n (%)	21 (91.3)	68 (53.5)	0.001*
Piperacilin/tazobactam, n (%)	1 (4.3)	7 (5.5)	1.0
Carbapenem, n (%)	5 (21.7)	26 (20.5)	1.0
3rd or 4th generation cephalosporin, n (%)	2 (8.7)	15 (11.8)	0.66
Aminoglycoside, n (%)	3 (13)	31 (24.4)	0.23
Carbapenem + aminoglycoside, n (%)	3 (13)	13 (10.2)	0.36
Carbapenem + except aminoglycoside (%)	4 (17.4)	11 (8.7)	0.74
Aminoglycoside + except carbapenem, n (%)	5 (21.7)	11 (8.7)	0.035*

*: p <0.05

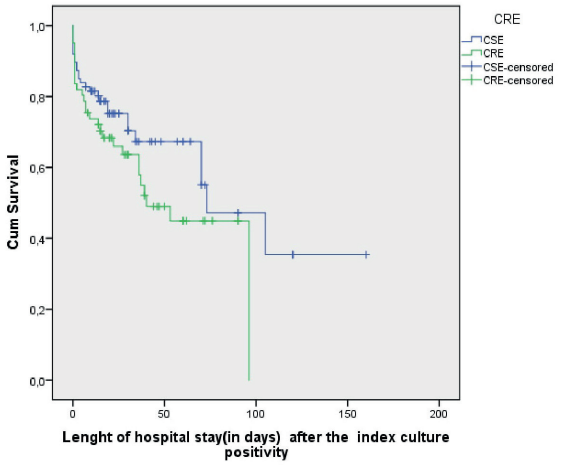

Fig. 1. Kaplan-Maier analysis comparing hospital survival of the patients with and without carbapenem resistance. (CRE: carbapenem-resistant *Enterobactericeae*, CSE: carbapenem-sensitive *Enterobactericeae*).

in in-hospital mortality rates between the colistin-susceptible and colistin-resistant *Enterobacteriaceae* infections (p = 0.9). The median length of hospital stay after the index culture in colistin-resistant group was 20 days (range, 0 - 76 days) vs. 19 days (range, 0 - 160 days) in colistin-susceptible group (p = 0.80).

Discussion

Our 4-year surveillance clearly confirmed that infections caused by *Enterobacteriaceae* isolates are an important clinical problem and high rates of carbapenem and/or colistin resistance can be encountered with these infections. We also found a high rate of colistin resistance that emerged over a period of four years.

The inability to recognize CRE infections when they first occur in a health care facility leads to the loss of the chance of early treatment choices before these infections are transmitted more widely. For this reason it is important to be aware of the risk factors for CRE infections.[6] In our study empiric polyantibiotic therapy for Gram negative bacteria, long hospital stay before index culture and history of surgical intervention were found as risk factors of CRE infections. Mechanical ventilation prior to index culture, urinary catheter exposure, underlying necrotizing enterocolitis and/or short-bowel syndrome was found as risk factors of colistin resistance.

One of the most concerning risk factors of CRE infections found in our study was the empiric polyantibiotic therapy for Gram negative bacteria. Use of antimicrobials including carbapenems, cephalosporins, and fluoroquinolones associated with CRE infections or carriage of CRE has been reported in the literature.[12-14]

In a 4-year case-control study involving 102 patients, the only common variable associated with CRE infections was the cumulative number of antibiotic exposures prior to CRE infections.[8] In another case-control study from Greece both prior exposure to antibiotics and duration of the prior antibiotic treatment were identified as the risk factors of CRE.[15]

Injudicious use of broad-spectrum agents may lead to the development of clinical resistance during therapy.[16] Broad spectrum antibiotics can destroy the sensitive flora, and lead to the colonization and proliferation of the resistant mutant strains.[16]

There is an evolving body of medical literature suggesting an important relationship between prior antimicrobial therapy and the subsequent identification of carbapenemase-producing bacteria. Our data was consistent with previous reports that empiric polyantibiotherapy which was mentioned in our study carbapenem plus aminoglycoside and aminoglycoside combination therapy other than carbapenem were consistent with higher rates of CRE infections. In order to get rid of the "broad-spectrum is best" approaches, we have to find novel ways to detect pathogens early.

Regarding the other risk factors other than antibiotic exposure, Patel et al.[8] found that invasive infections with carbapenem-resistant *K. pneumoniae* were independently associated with longer length of stay when compared with patients with carbapenem-susceptible *K.*

pneumoniae. Also in our study long hospital stay before index culture was also found as a risk factor of CRE. During long hospital stay patients can be exposed to multiple invasive procedures.

Surgical intervention particularly gastrointestinal procedures were found as high-risk procedures for CRE infections.[17,18] Since most, ESBL-producing *Enterobacteriaceae* reside in the gastrointestinal tract it was not surprising to find this relationship. Although this relationship raises questions such as whether patients should be screened for ESBL-producing *Enterobacteriaceae* prior to surgery or whether modified surgical antibiotic prophylaxis in areas with high ESBL-producing *Enterobacteriaceae* prevalence is needed.[19] However this topic was beyond the scope of our study. Also there is no known answer for these situations. Invasive CRE infections are associated with worse outcomes as compared to CSE infections. CRE isolation has been associated with all-cause hospital mortality ranging from 29% to 52%.[20]

In our study the rate was 45.2%. Although CRE infections are known to be associated with high mortality, we did not find any difference between the mortality rates of CRE and CSE groups. Also in the study by Bhargava et al.[21] and Candevir Ulu et al.[22] mortality was not statistically different between carbapenem-resistant and susceptiblestrains, which was similar to our study.

In the present study, the ratio of colistin-resistant infections in *Enterobacteriaceae* infections (32.3%) was similar to what was previous reported by Zarkotou et al.[23] and Capone et al.[3] which demonstrated 25% to 37 % of resistance to colistin.

Although, colistin resistance has been associated with high mortality, there is controversy about the impact of resistance to colistin on prognosis.[3,5,23-26] In our study mortality rates with colistin-resistant *Enterobacteriaceae* infections was not different from colistin-susceptible *Enterobacteriaceae* infections which was 45.2% and 45% respectively.

Our study has several limitations. Firstly, clinical data in this study were the single center experiences obtained from medical records of the patients retrospectively. Secondly, we used Vitex 2 for colistin MIC determination. Although, recently, a joint recommendation by CLSI and EUCAST released in 2016 recommended broth microdilution as a standard method for MIC testing of colistin, the use of broth microdilution methods for susceptibility may not be practical in laboratories depending on individual workloads and is rarely used in routine microbiology laboratories.[27] Also Vitex 2 was previously reported as a good testing method for colistin MIC determination.[28,29] In the literature there are evolving studies evaluating the commercial testing methods comparing to broth microdilution for colistin MIC determination.

Further investigations will increase our understanding of these serious infections and give us an opportunity to find practices for reducing the frequency and the mortality of these infections.

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Original Article

A national survey on use of less invasive surfactant administration in Turkey

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ABSTRACT

Background. The aim of the study was to assess the rate of utilization, policy of premedication, technique, equipment, experience on safety and efficacy for less invasive surfactant administration or minimally invasive surfactant therapy (LISA/MIST) use in Turkey.

Methods. An online survey was designed and distributed via Google Forms tool to 350 neonatologists from 173 units through NICU-Turk mailing list of the Turkish Neonatal Society. Participants were asked to answer the survey for their own neonatal intensive care unit (NICU).

Results. LISA/MIST use rate was 81.6% among 87 NICUs which responded (response rate was 50.2%). LISA was used regularly in 23 of the units (26.4%), occasionally in 35 (40.2%), rarely in 12 (13.8%), and only for clinical trials in 1 (1.1%). LISA/MIST has been never applied in 16 units (18.4%).

Conclusions. LISA/MIST is widely used in Turkey similar to several regions in Europe but unlike the USA. Future studies are expected to further clarify some questions about LISA/MIST procedure, especially on its efficacy and safety.

Key words: less invasive surfactant administration, minimally invasive surfactant therapy, respiratory distress syndrome, surfactant, survey.

Preterm infants requiring surfactant replacement are typically treated by using the INSURE (INtubation SURfactant Extubation) technique. Recently, less invasive surfactant administration or minimally invasive surfactant therapy (LISA/MIST) has come into use in neonatal practice.[1,2] Encouraging results from the initial small feasibility studies were followed by larger randomized controlled trials (RCTs). The first RCT including 12 German neonatal intensive care units (NICU) found that surfactant administration via a thin catheter reduced the need for intubation and mechanical ventilation (MV).[1] Later on, our group compared the LISA technique to INSURE and reported lower rates of both MV and bronchopulmonary dysplasia (BPD) in preterm infants who received surfactant by LISA method.[2] A systematic review of 30 trials (5598 neonates) examining ventilation strategies demonstrated that use of LISA was associated with lower odds of the composite outcome of death or BPD and severe intraventricular hemorrhage (IVH) in preterm infants. Calculation of the ranking possibilities found LISA to be the best strategy in this group of infants.[3] Similarly, another systematic review of six RCTs demonstrated that LISA use in infants with respiratory distress syndrome (RDS) was associated with reduced incidences for need for MV and BPD/death at 36 weeks of gestation.[4] LISA/MIST has been recommended for the administration of surfactant in addition to INSURE technique, with an advantage not needing intubation. LISA has started to come to the forefront with low BPD and/or mortality rates in recent years.[3,4]

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A survey of LISA use across Europe showed variations in equipment used and techniques applied for LISA as well as different views on the indications and perceived efficacy of this intervention.[5] Only five NICUs from Turkey participated in this survey, therefore we did not have any idea on the current practice regarding LISA/MIST in Turkey.

The aim of our survey trial was to assess the rate of utilization, policy of premedication, perceptions of indication and feasibility in regard to gestational age, technique, and equipment as well as experience of safety and efficacy used for LISA/MIST.

Material and Methods

An online survey was designed by using the Google Forms tool. We contacted all NICUs within the Turkish Neonatal Society database (NICU-Turk) and requested that the questionnaire be completed by a single representative from each NICU. Representatives were neonatologist who regularly worked as attendings in the NICU. The study was approved by the local ethics committee (2018/16-4). Informed consent was obtained from the participants.

The first ten questions focused on general information regarding participants' demographic data, knowledge and used LISA/MIST in their NICUs. Subsequently, participants who used LISA/MIST were routed to additional usage-specific questions (seventeen) about LISA/MIST. Similarly, the questionnaire was completed by asking 3 questions for those who did not use LISA/MIST.

Statistical Analyses

Data analysis was descriptive. Categorical variables are presented in absolute numbers and percentages, and quantitative data are reported as median and minimum-maximum. Data analysis was performed using SPSS Version 21 (IBM Corp, Armonk, New York).

Results

Our survey was sent to all neonatologists (n= 350) who are included in the NICU-Turk mailing list of Turkish Neonatal Society. One responsible person from each unit was asked to complete the questionnaire. Ninety-seven neonatologists answered the survey, but since two people from 10 centers were from the same center, the answers of the senior neonatologists were accepted and included in the analysis. Of the 173 NICUs 87 (50.2%) answered the survey, which represented 195 out of 350 neonatologists (55.7%).

Participation rate of academic and public (University Hospitals, Training and Research Hospitals) institutions (61/82, 70.1%) was quite high whereas participation from private hospitals was low (11/91, 12.1%). The median number of incubators in these centers was 27 (6-120) and median number of neonatologists was 2 (1-12). The median number of newborns under 1500 g infants per year in participating NICUs was 82 (2-1000).

Seventy percent of the responding neonatologists were working in an academic (University Hospital and Training and Research Hospital) setting. The huge majority (90.8%) was from level III–IV NICUs, and 77% of the respondents were administering surfactant at least 50 times per year (Table I). According to the survey results, LISA/MIST was used by 71 of the responding neonatologists (81.6%). Figure 1 shows that the frequency of LISA/MIST use.

Among those who were using LISA/MIST, 52.1% had a protocol for its use. The vast majority (95.8%) did not use sedation/premedication with LISA/MIST. The majority (59 NICUs, 83.1%) used the feeding tube, six (8.45%) an angiocath, five (7%) a vascular catheter and one (8.6%) suction catheter for the application. Almost all neonatologists (97.2%) inserted the catheter orally and only 12.7% used Magill forceps. Seventy four percent of respondents gave 200 mg/kg of surfactant and the majority (54.9%) gave it over 30 sec-1 min, followed

Table I. Basic questions and answers within the survey.

Questions and answers	Number of respondents (n= 87)
Type of hospital	
University Hospital	38 (43.7)
Training and Research Hospital	23 (26.4)
Public Hospital	15 (17.2)
Private Hospital	11 (12.6)
Level of your center	
Level II	8 (9.2)
Level III	63 (72.4)
Level IV	16 (18.4)
How many times per year is surfactant administered in your center?	
<20	4 (4.6)
20-50	16 (18.4)
50-100	32 (36.8)
>100	35 (40.2)
Do you use any non-invasive surfactant administration (LISA/MIST) techniques in your center?	
Yes	71 (81.6)
No	16 (18.4)

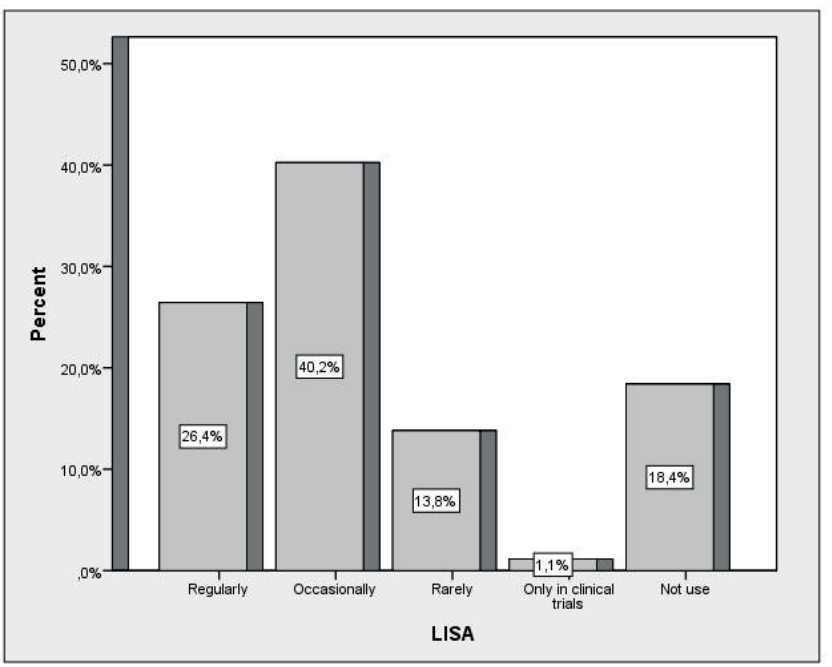

Fig. 1. LISA/MIST use frequencies in Turkey.

by 21.1% in <30 sec. The most preferred non-invasive ventilation mode during LISA/MIST was nasal intermittent positive pressure ventilation (NIPPV) (57.7%) (Table II).

Adverse events of LISA/MIST were experienced by 84.5% of neonatologists. Tracheal surfactant reflux (76.1%), bradycardia (26.8%), hypoxia (22.5%) and need for intubation (19.7%) among others were observed. Perceived efficacy and safety of LISA/MIST among participating neonatologists were high as 52.1% and 81.7%, respectively. Twenty nine percent of the respondents believed that the current literature on LISA/MIST is strong enough to recommend it as standard care, 14% of respondents felt that the literature is not strong enough, 47.9% believed more evidence is needed and the remaining 8.5% were not sure (Table III).

LISA/MIST has never been applied in 16 NICUs (18.4%). Leading reasons for not using LISA/MIST were lack of experience (50%) and inconclusive evidence for the use of LISA/MIST (25%). INSURE (75%) or intubation (25%) was used for surfactant therapy instead in these NICUs. Majority of neonatologists not applying LISA/MIST (87.5%) reported that they would consider utilizing it in the future (Table IV).

Discussion

Recently, LISA has been promoted as an alternative method for surfactant administration. Several reports have shown that LISA is becoming established in daily practice.[1,4] Many guidelines have accepted LISA as standard care in a selected group of premature infants. The recent European Consensus Guidelines and Turkish Guidelines on surfactant use recommended LISA as the preferred mode of surfactant administration for spontaneously breathing babies on continuous positive airway pressure (CPAP), providing that clinicians are experienced with this technique (level of evidence: B2).[6,7] However, there is still a wide variation regarding many aspects of the LISA/MIST method.[4] We aimed to investigate the neonatologists' knowledge, attitude and practices regarding the LISA/MIST approach. In addition to general information on this topic, our survey was carefully structured to examine LISA/MIST usage specific questions for those who do not use LISA/MIST.

Seventy percent of the respondents came from academic and public institutions with the vast majority practicing in level III and IV NICUs that commonly use surfactant in their practice. The utilization rate of LISA (81.6%) in this survey was one of the highest reported to date in the literature, just after the rate reported from Spain (89%).[8] Indeed, the rate of regular use of LISA in our survey was only 26.4% but the high rate was due to additional use in NICUs beside INSURE technique. In a recent large scale survey of 37 European countries, the mean utilization rate was 52%,[5] and previous studies reported even lower use. A national statistical analysis in Poland in 2015 reported that LISA was used in only 1% of hospitals.[9] Nordic hospitals had a utilization rate of 32% in a survey conducted in 2016.[10] Recent surveys from European countries such as Spain[8] and Turkey (our study) show that LISA use rates have increased since 2015. Thus, we believe that within a few years the technique will be widely used in other NICUs. On the contrary, LISA usage in the USA and UK have been found to be 15% and 18.7% in new surveys.[11,12] The authors thought that the reason for the low rate of LISA use in these countries was due to the lack of structured training in the LISA method.

LISA/MIST usage specific questions revealed that fifty percent of the respondents have written a protocol for LISA use. Intrestingly, the majority of neonatologists consider LISA even in extremely immature infants. This may be in line with results of a recent study in infants at 23–26 weeks gestational age, where increased survival without major complications was seen in the group of infants treated with LISA. However, subgroup analysis from this study revealed the most benefit in the more mature infants of 25 and 26 weeks.[13] According to the results of our survey, although 33.8% of NICUs

Table II. LISA/MIST usage-specific questions and answers within the survey.

Questions and answers	Number of respondents (n=71)
If you are using LISA/MIST, do you have a protocol for its use?	
Yes	37 (52.1)
No	34 (47.9)
What is the preferred catheter in LISA/MIST?	
Feeding tube	59 (83.1)
Vascular catheter	5 (7)
Angiocath (Hobart method)	6 (8.45)
Suction catheter	1 (1.4)
Do you shorten the catheter in LISA/MIST?	
Yes	43 (60.6)
No	28 (39.4)
Which intubation method do you use in LISA/MIST?	
Oral	69 (97.2)
Nasal	2 (2.8)
Do you use Magill forceps in LISA/MIST?	
Yes	9 (12.7)
No	62 (87.3)
Do you use any sedation/premedication when using LISA/MIST?	
Yes	3 (4.2)
Benzodiazepines	3
Atropine	-
Opioids	-
No	68 (95.8)
How much time do you allow for surfactant delivery when using LISA/MIST?	
<30 sec	15 (21.1)
30 sec – 1 min	39 (54.9)
1 – 2 min	7 (9.9)
2 – 3 min	10 (14.1)
>3 min	-
Which is the most preferred gestational age to use LISA/MIST?	
All gestational age	24 (33.8)
<24 w	-
24 – 26 w	6 (8.5)
26 – 28 w	15 (21.1)
28 – 32 w	21 (29.6)
>32 w	5 (7)
Which is the most preferred time interval to use LISA/MIST?	
Every time	24 (33.8)
0 – 6 h	44 (62)
6 – 12 h	2 (2.8)
12 – 24 h	1 (1.4)
>24 h	-

LISA/MIST: less invasive surfactant administration or minimally invasive surfactant therapy, CPAP: continuous positive airway pressure; NIPPV: nasal intermittent positive pressure ventilation; BIPAP: bilevel positive airway pressure

Table II. Continued.

Questions and answers	Number of respondents (n=71)
Which surfactant do you prefer to use for LISA/MIST?	
Poractant alfa	67 (94.4)
Beractant	3 (4.2)
Calfactant	1 (1.4)
Which dose of surfactant do you prefer to use for LISA/MIST?	
100 mg/kg	11 (15.5)
200 mg/kg	53 (74.6)
Other	7 (9.8)
Would you consider LISA/MIST again if an infant needs a repeat dose of surfactant after 6-12 h?	
Yes	44 (62)
No	27 (38)
Which non-invasive ventilation method do you prefer during LISA/MIST?	
Ventilator-derived nasal CPAP	26 (36.6)
Bubble CPAP	3 (4.2)
NIPPV	41 (57.7)
BIPAP	1 (1.4)

LISA/MIST: less invasive surfactant administration or minimally invasive surfactant therapy, CPAP: continuous positive airway pressure; NIPPV: nasal intermittent positive pressure ventilation; BIPAP: bilevel positive airway pressure

Table III. Adverse events, perceived efficacy and safety during LISA/MIST.

Parameters	n (%)
Adverse events	
None	11 (15.5)
Tracheal surfactant reflux	54 (76.1)
Bradycardia	19 (26.8)
Hypoxia	16 (22.5)
Need for intubation	14 (19.7)
Unilateral surfactant administration	8 (11.3)
Other (gastric deposition, airway obstruction)	2 (2.8)
Perceived efficacy	
Very high	8 (11.3)
High	37 (52.1)
Medium	24 (33.8)
Low	2 (2.8)
Very low	-
Perceived safety	
Yes	58 (81.7)
No	1 (1.4)
Not sure	12 (16.9)
Do you believe that the current literature on LISA/MIST is strong enough to recommend it as standard care?	
Yes	21 (29.6)
No	10 (14.1)
More evidence is needed	34 (47.9)
Not sure	6 (8.5)

Table IV. LISA/MIST non usage-specific questions and answers within the survey.

Questions and answers	Number of respondents (n=16)
If you do not use the LISA/MIST method, which method do you prefer more?	
Intubated	4 (25)
INSURE	12 (75)
Why do you not use the LISA/MIST method?	
Lack of experience	8 (50)
Inconclusive evidence for the use of LISA	4 (25)
Other (missing consensus, low efficacy, adverse events, congestion)	4 (25)
Do you consider utilizing the LISA/MIST method in the future?	
Yes	14 (87.5)
No	2 (12.5)

LISA/MIST: less invasive surfactant administration or minimally invasive surfactant therapy, INSURE: INtubation SURfactant Extubation

preferred LISA/MIST irrespective of gestational age, generally mentioned preferred weeks were 28-32 (29.6%) and 26-28 (21.1%). The LISA/MIST usage rate for 24-26 weeks was only 8.5%.

Sedation or premedication use rates vary widely among NICUs. For example, 52% of neonatologists did not use any premedication for performing LISA in the European survey.[5] Unexpectedly, very few babies (4.2%) received sedation before the procedure in our survey results. Refrainment from sedative use may be due to the possible complications. We think that compared to invasive intubation some neonatologists perceive LISA/MIST as being less traumatic or maintenance of spontaneous breathing being superior to analgesia or sedation. Most physicians seem to use a feeding tube (83.1%) in our survey. Compared to the European survey, the rate of use of a feeding tube seems to be quite high in our study. In both European and USA surveys, angiocath (34% and 20.3%) was the second most frequently used method after the feeding tube (56% and 46.3%).[5,11] Magill forceps for placement use rate was 65% in the European survey[5] whereas only a minority of neonatologists (12.7%) used Magill forceps to facilitate endotracheal placement of the catheter in our survey. Overall in the literature, the majority of neonatologists distribute the surfactant fairly slowly, over 1 min or more.[5,11] On the contrary, the more preferred time in our survey was 30 sec-1 min, which may explain higher tracheal surfactant reflux rate. Other major adverse events included bradycardia and hypoxia. The most preferred non-invasive method seems to be NIPPV as unexpected when we compare with the nasal CPAP rate in other surveys. NIPPV has been shown to reduce the need for intubation recently, which may be a reason for its preference in our survey.[14]

There are some limitations in our survey. First, the response rate was 50.2%. Although, participation rate of academic and public institutions was quite high. Most responses came from level III-IV NICUs with high patient loads. Second, we contacted one person from each unit, so the answers might have reflected unit policy rather than personal views. Third, majority of the responders said that they used LISA/MIST occasionally. Although the frequency of LISA/MIST use in the survey could be answered as "regularly, occasionally, rarely, only in clinical trials and not use", "regularly, occasionally and rarely" words might have different meanings for each respondent or unit because each NICU had a different number of incubator and different criteria for LISA/MIST use.

In conclusion, LISA/MIST has become an increasingly common practice in recent years. This technique is widely applied in European

NICUs including Turkey, unlike the USA. Future studies are expected to further clarify some questions regarding patient selection, the type of thin catheter use, time of administration, non-invasive ventilation method during LISA, sedation/premedication, procedural efficacy and safety of LISA. We think that each country should evaluate its own data according to their settings.

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Original Article

Assesment of colistin related side effects in premature neonates

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ABSTRACT

Background. Colistin is an antibiotic in the polymyxin group and is especially important in the elimination of multi-drug resistant gram negative bacteria. To date, there are many studies investigating colistin related side effects, especially nephrotoxicity. However, there are few studies involving premature neonates, and this study aimed to investigate the side effects of colistin in this particular patient group.

Methods. Between January 2016 and May 2019, the medical records of premature neonates treated with colistin were retrospectively reviewed. The diagnosis of acute kidney injury (AKI) was performed according to the modified neonatal KDIGO criteria. Serum electrolyte levels were recorded at the initiation of colistin treatment and 4-7 days after.

Results. A total of 47 premature neonates; with a median gestational age of 27 weeks and median weight of 970 g at birth were included in the study. The median postnatal day of colistin initiation was 24 days and mean duration of colistin therapy was 15.95 ± 3.70 days. Colistin was combined with aminoglycosides in 44.6% of the patients. Acute kidney injury was documented in 17.0% of premature neonates. (n = 6 for stage 1, n = 2 for stage 2, none of the patients had stage 3). In univariate analysis, gestational age and concomitant aminoglycoside use were associated with AKI development (OR, 0.446; 95% CI 0.238-0.832; p = 0.011 and OR, 1.324; 95% CI 1.023-7.584; p = 0.024). Mean magnesium level significantly decreased after colistin treatment (1.70 ± 0.84 vs. 1.57 ± 0.29, p = 0.017) and the frequency of hypomagnesemia increased after colistin use (78.7% vs. 91.5%, p = 0.031). Frequency of elevated AST increased from 23.4% to 44.7% following colistin use (p = 0.031).

Conclusions. Colistin-related side effects observed in premature neonates are not as common as in pediatric patients. Electrolyte imbalance is observed more frequently in this age group following colistin use. We suggest strict serum electrolyte level monitoring, especially magnesium, in premature neonates that are receiving colistin.

Key words: colistin, side effect, premature neonates, hypocalcemia, hypomagnesemia, acute kidney injury.

Nosocomial infections in neonatal intensive care units (NICUs) are among the most important factors that affect the survival of neonates.[1] In particular, infections caused by multi-drug-resistant (MDR) bacteria have a more pronounced effect on mortality, and it is well known that these bacteria are becoming increasingly common in NICUs worldwide.[2,3] In a study examining neonates with bacteremia, the frequency of MDR bacteremia was found to be 18.6% among all neonatal gram negative bacteremia cases.[4]

Colistin (colistimethate sodium) is a cyclopeptide antibiotic in the polymyxin group.[5] Although many years have passed since its discovery, it is still being frequently investigated because of its potent effect on MDR gram negative bacteria. Colistin is especially recommended for the elimination of carbapenem-resistant isolates of

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Acinetobacter baumannii, Klebsiella pneumoniae and *Pseudomonas aeruginosa*.[6] Colistin-related side effects were reported in the literature before the 1970s.[7] In adult studies, the incidence of adverse events was close to 50%, while the two most common side effects were nephrotoxicity and neurotoxicity.[8]

Colistin is attached to megaline in the proximal tubule because of its cationic structure, and accumulates in the proximal tubule with long-term use.[9] Colistin is thought to increase cytoplasmic membrane permeability in proximal tubular cells, and cause an overflow of anion, cation and water into the cell and severely damaging them.[8,10] There are a number of studies evaluating colistin-related nephrotoxicity in the pediatric age group.[11,12] However, such studies focusing on premature children are quite few.[13]

The aim of this study was to investigate the incidence of colistin-related side effects in premature neonates and to determine risk factors.

Material and Methods

Study design and data collection

Between January 2016 and May 2019, the medical records of premature neonates treated with colistin in the NICU of University of Health Sciences Antalya Training and Research Hospital were retrospectively reviewed using electronic health records and archived patient files. Prematurity was defined as birth before completing 37 weeks of gestation. Demographic characteristics, Apgar score at 5th minute, comorbid conditions [low birth weight, respiratory support, umbilical/central venous catheter application, respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), etc.] and all antimicrobial drugs used prior to colistin were recorded. Study exclusion criteria: a) neonates who received colistin treatment for less than 6 doses, b) those with kidney injury before starting colistin, c) neonates with congenital anomalies of the kidney and urinary system on ultrasonographic imaging. Permission for this study was officially granted by the Ethical Board of the University of Health Sciences, Antalya Training and Research Hospital [decision number: 194/May 20, 2019].

Definitions

Small for gestational age (SGA) was defined as birth weight <10th percentile for the gestational age.[14] The diagnosis of PDA was confirmed by the presence of left-to-right shunt in echocardiographic examination in patients with PDA clinical symptoms. The diagnosis of NEC was made using Modified Bell´s Staging Criteria.[15]

The diagnosis of acute kidney injury (AKI) was performed according to the modified neonatal KDIGO criteria (stage 0: no change in serum creatinine; stage 1: >0.3 mg/dl increase in serum creatinine value in the last 48 hours or 150 to 200% increase in serum creatinine level compared to baseline; stage 2: an increase in serum creatinine level between 200 and 300% compared to baseline; stage 3: an increase in serum creatinine level of >300% compared to baseline, or a serum creatinine value >2.5 mg/dl or renal replacement therapy).[16]

Serum creatinine, sodium, potassium, calcium, magnesium, alanine transaminase (ALT) and aspartate transaminase (AST) levels were recorded at the initiation of colistin treatment and 4 to 7 days after. Hyponatremia was defined as serum sodium levels ≤135 mmol/L, hypokalemia ≤3.5 mmol/L, hypocalcemia ≤8.5 mg/dl, hypomagnesemia ≤1.9 mg/dl, hypertransaminasemia ALT level ≥35 U/L and/or AST level ≥50 U/L.

Colistin dosage

Colistin was started either in neonates with MDR gram negative bacteremia in blood, urine or tracheal aspirate culture results or in neonates with clinical signs of sepsis despite the use of broad spectrum antibiotics. Multi-drug-resistance was considered as the identification

of a gram negative bacteria that was resistant to at least three different antibacterial groups.[17] Colistin treatment was administered at 5 mg/kg/day given in 3 doses dissolved in 5 ml saline, for a minimum infusion duration of 30 minutes. The colistin formulation contained 150 mg of colistin base-equivalent colistimethate sodium (Colimycin; Kocak Farma, Istanbul, Turkey and Kolistate; Biem Farma, Ankara, Turkey). Postnatal age at the time of treatment with colistin and treatment duration were recorded. Colistin can be used as monotherapy or combined therapy with aminoglycoside or carbapenem in premature neonates.

Statistical analysis

Descriptive statistics were presented as frequency, percentage, mean, standard deviation (SD), median and interquartile range (IQR). The Shapiro-Wilk test, Kolmogorov-Smirnov test, histogram and Q-Q graphics were used for the evaluation of normality of distribution. The McNemar test was used in the analysis of relationships between categorical variables. For the comparison of continuous variables, the paired-samples t-test was used for variables that showed normal distribution, while the Wilcoxon test was used in those with non-normal distributions. Univariate logistic regression analyses were applied to identify risk factors for AKI. Odds ratios (ORs) were calculated with 95% confidence intervals (CI). Statistical analyses were performed by using the SPSS version 21.0 package program for Windows (IBM, Armonk, NY). P values of <0.05 were accepted to show statistical significance.

Results

A total of 47 premature neonates (27 males, 57.4%) were included in the study. Clinical features are presented in Table I. All patients required parenteral nutrition, and median parenteral nutrition duration was 27 days (range, 10-161 days; IQR, 23-37).

The median postnatal day of colistin initiation was 24 days (range, 9-74 days; IQR, 14-35) and mean duration of colistin therapy was 15.95 ± 3.70 days. Culture positivity was present in 9 (19.1%) of the neonates treated with colistin. In blood culture samples, *Enterobacter cloacae* was isolated in four, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in one patient each, while in tracheal aspirate samples, *Acinetobacter baumannii* in one and *Klebsiella pneumoniae* in two patients. The antimicrobial drugs utilized before colistin treatment and combined with colistin are summarized in Table I.

The serum creatinine, serum electrolytes and transaminase levels evaluated before and during colistin treatment are presented in Table II. Acute kidney injury was documented in 17.0% of premature neonates. (n = 6 for stage 1, n = 2 for stage 2, none of the patients had stage 3). The median gestational age was 25 weeks (range from 24-27 weeks) in neonates with AKI and 27 weeks (range from 23-34 weeks) in neonates without AKI (p <0.001). Also, the median birth weight was 760 g (range from 650-1100g) in neonates with AKI and 1040 g (range from 610-2100 g) in neonates without AKI (p= 0.041). The incidence of AKI was higher among those in which aminoglycoside and colistin were combined compared to non-combination (23.8% vs 11.5%; p= 0.031).

The risk factors for AKI in premature neonates were analyzed by univariate regression analysis. Gestational age and concomitant aminoglycoside use were associated with AKI development. On the other hand, Apgar score at 5th minute, birth weight, presence of PDA or NEC, and starting day of colistin were not significantly related with AKI (Table III).

Mean serum calcium and magnesium levels were significantly decreased after colistin treatment (9.59 ± 0.84 vs. 9.20 ± 1.04, p = 0.048; 1.70 ± 0.84 vs. 1.57 ± 0.29, p = 0.017, respectively). The number of patients with electrolyte imbalance before and during colistin treatment show that the frequency of hypomagnesemia and hypokalemia were increased after colistin use (78.7% vs. 91.5%, p = 0.031; 4.3% vs. 27.7%, p = 0.007, respectively) (Table II).

Table I. Characteristics and outcomes of the study population (n = 47).

Variables	Values
Sex (male), n (%)	27 (57.4)
Median gestational age (weeks)	27 (range 3-34; IQR 26-28)
Median birth weight (g)	970 (range 610-2,600; IQR 780-1,100)
Low birth weight, n (%)	9 (19.1)
Median 5th minute APGAR score	6 (range 3-9; IQR 4-7)
Presence of umbilical catheter, n (%)	41 (87.2)
Presence of central venous catheter, n (%)	31 (66.0)
Need for respiratory support, n (%)	43 (91.5)
Comorbid conditions, n (%)	
Respiratory distress syndrome	43 (91.5)
Patent ductus arteriosus	17 (36.2)
Necrotizing enterocolitis	18 (38.3)
Previous antibiotic exposure, n (%)	
Ampicillin	47 (100.0)
Aminoglycoside	35 (74.5)
Cephalosporin	36 (76.6)
Carbapenem	45 (95.7)
Amphotericin	35 (78.7)
Vancomycin/teicoplanin	47 (100)
Concomitant antibiotics, n (%)	
Aminoglycoside	21 (44.6)
Cephalosporin	3 (6.4)
Carbapenem	38 (80.8)
Amphotericin	21 (44.6)
Vancomycin/teicoplanin	39 (83.0)
The median duration of hospitalization (days)	76 (range 22-131; IQR 45-105)
Mortality rate, n (%)	4 (8.5)

IQR: interquartile range

Median ALT and AST levels were significantly increased after colistin use (Table II). There was no change in the frequency of ALT elevation with the use of colistin. However, the rate of patients with AST elevation before colistin was 23.4%, which increased to 44.7% with the use of colistin (p = 0.031).

Discussion

In our study, the incidence of AKI after colistin use was 17.0% in premature neonates. In a study evaluating extremely low birth weight prematures, the incidence of colistin-associated AKI was 14.3%.[18] In a case-control study published by İpek et al.[19], the frequency of nephrotoxicity was similar between prematures who had used colistin and those who had not (12.8% vs. 13.6%, respectively). In the literature, the incidence of colistin-related nephrotoxicity observed in premature neonates was lower than that found in adult and pediatric studies.[6,7,18,20] In a multicenter study from the United States, colistin-related nephrotoxicity was found to be 7 times higher in children ≥13 years of age than in younger children.[20]

In our study, gestational age was found to be a risk factor for colistin associated AKI in premature neonates. In a study evaluating 66 neonates by

Table II. Changes in serum electrolytes and liver function tests after colistin use in premature neonates.

Variables	Before colistin treatment	During colistin treatment	p
Serum creatine (mg/dl)	0.53 ± 0.11	0.61 ± 0.26	0.003*
Serum sodium (mmol/L)	138.31 ± 4.39	137.00 ± 3.81	0.055
Frequency of hyponatremia (%)	23.4	34.0	0.359
Serum potassium (mmol/L)	4.56 ± 0.56	4.28 ± 0.81	0.068
Frequency of hypokalemia (%)	4.3	27.7	0.007*
Serum calcium (mg/dl)	9.59 ± 0.84	9.20 ± 1.04	0.048
Frequency of hypocalcemia (%)	8.5	12.8	0.754
Serum magnesium (mg/dl)	1.70 ± 0.84	1.57 ± 0.29	0.017*
Frequency of hypomagnesemia (%)	78.7	91.5	0.031
ALT (U/L), median (min.-max.)	17 (5-76)	24 (6-132)	0.020*
Frequency of high ALT level (%)	17.0	21.3	0.754
AST (U/L), median (min.-max.)	33 (21-45)	40 (6-127)	0.001*
Frequency of high AST level (%)	23.4	44.7	0.031*

*: $p < 0.05$
ALT: alanine transaminase, AST: aspartate transaminase.

Table III. Evaluation of risk factors for acute kidney injury (n = 47).

Risk factors	Odds ratio	95% CI	p
Gestational age	0.446	0.238-0.832	0.011*
Birth weight	0.995	0.991-1.000	0.061
5th minute APGAR score	0.742	0.467-1.180	0.208
PDA	2.074	0.430-9.306	0.377
NEC	0.479	0.086-2.683	0.403
Concomitant aminoglycoside use	0.258	0.053-1.264	0.095
Day colistin started	0.945	0.875-1.021	0.154

PDA: patent ductus arteriosus, NEC: necrotizing enterocolitis OR: odds ratio, CI: confidence interval.

Ilhan et al.[18], colistin associated AKI incidence was 14.3% in neonates with birth weight <1,500 g and 2.6% in those with birth weight >1500 g; but this difference was not statistically significant (p = 0.15). In this study, we also found that colistin and aminoglycoside combination was associated with AKI development in premature neonates. The association between colistin-related nephrotoxicity and concomitant aminoglycoside use has been particularly emphasized in adult studies.[21]

In the current study, electrolyte imbalance developed in most of the premature neonates who had used colistin. With the use of colistin, both mean serum magnesium concentration decreased and the frequency of neonates with hypomagnesemia also increased. In accordance with our study, most of the previously published studies evaluating neonates reported low serum magnesium levels.[13,19,20,22] In the study by Alan et al.[13], serum magnesium concentration decreased significantly after the use of colistin; to such a degree that in 52% of patients, magnesium replacement was required. Since the change in serum magnesium concentration was observed especially in premature neonates, this condition was thought to be due to colistin-related nephrotoxicity, and the immature structure and lower number of nephrons.[18]

In our study, not only hypomagnesemia but also calcium and potassium imbalance were observed in premature neonates. In a study

evaluating 21 preterm neonates after colistin use, potassium and calcium replacement were required in 52% and 33% of patients, respectively.[13] Among premature neonates, low birth weight patients were found to have higher risk for potassium and calcium imbalance.[18] In the literature, a case mimicking tubular disorders after colistin use has been reported. A newborn with a gestational age of 28 weeks was presented with a Bartter-like syndrome developed after colistin use, and also metabolic alkalosis.[23]

In our study, we found a significant increase in both transaminase levels, especially AST, with the use of colistin. To our knowledge, hepatotoxicity has not been reported among the side effects of colistin. However, despite this finding, it is highly likely that this increase in transaminase levels is due to other drugs, clinical characteristics, sepsis, and multiple organ dysfunction syndrome.

The most important limitation of this study is its retrospective nature. Secondly, in this study, serum creatinine was used as a marker of renal damage. It has been shown that serum creatinine level does not increase unless 25-50% of renal functions are impaired in neonates.[24] Cumulative doses and drug interactions of all antimicrobial drugs used prior to colistin could not be evaluated within the scope of the study. Because of the prolonged renal effects of some antimicrobial drugs (amphotericin B, aminoglycoside, etc.) used just before the colistin, we may have been evaluated as the side effects of colistin. Finally, as the urine electrolyte levels were not evaluated, we thought that electrolyte imbalance after colistin use may be due to renal tubular loss. However, despite these limitations, we think that it will contribute to the literature as it investigates the side effects related to colistin use in neonates with low gestational age.

The current data suggests that the frequency of colistin associated AKI in premature neonates is lower than children and adults. It also seems that electrolyte imbalance is observed more frequently in this age group following colistin use; however, it is also possible that this change is associated with various other parameters. Nevertheless, we suggest strict serum electrolyte level monitoring, especially magnesium, in premature neonates that are receiving colistin.

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Original Article

Turkish validation of the maternal responsiveness global rating scale in slow-to-talk toddlers

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ABSTRACT

Background. The relation between maternal responsiveness and language development has been shown in previous literature however it is difficult to evaluate responsiveness because of the difficulties involved when coding the specific patterns of parent-child interactions. The maternal responsiveness global rating scale is important because it requires less time and expertise from professionals and predicts the language outcome of children. The aim of the study was to adapt the Maternal Responsiveness Global Rating Scale into Turkish, thereby making it accessible to a variety of professionals, and creating a way to use this useful scale.

Methods. Twenty-seven 18- to 42-month old children who had been admitted to the Developmental Pediatrics outpatient clinic with concerns of speech delay and had received a diagnosis of language disorder with the standardized language test were included in the study. The general development and language development of each participant was evaluated using Denver II, Bayley-third edition and Pre-school Language Scale-5. After the translation study of the Maternal Responsiveness Global Rating Scale, video collecting and rating procedures and finally reliability and validity analyses were implemented.

Results. The results of this study demonstrated that the Turkish translation of the Maternal Responsiveness Global Rating Scale shows strong evidence of adequate reliability and validity and is a feasible tool to measure responsiveness in routine child health care practice for children with language delay.

Conclusions. This in expensive, easy-to-use and reliable tool may be recommended in order to identify which slow-to-talk toddlers and their mothers need early intervention and may be used by community-based practitioners and researchers in Turkey to support language development during early intervention stages.

Key words: maternal responsiveness, language development, Turkish validation.

Language acquisition is a fundamental domain of child development and one of the main concerns in early childhood due to the high frequency of its delay and adverse consequences. The prevalence of language delay is known to be as high as 20 % in pre-school children.[1] Persistent speech and language delay causes poor academic achievement, and behavioral, emotional and social maladjustment. Therefore early identification and intervention is a priority for all nations.[2]

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It is widely accepted that the acquisition of language occurs through the interaction of biological and environmental factors. Within this context many studies have investigated the most significant predictive factors of language development.[2] Maternal responsiveness has been shown as a strong predictor of later language scores.[3] Since language learning is shaped by the experiences of a child, maternal responsiveness is an essential component of language development. Maternal responsiveness is identified as a kind of parenting behavior with ''the prompt, contingent and appropriate responses to a child's initiations''.[2] This pattern of parent-child interaction has been demonstrated to promote language development based on the reason

that if a caregiver is more responsive, the child is more likely to be involved in learning new language skills.[3]

Maternal responsiveness overlaps with the social interaction context of child development, in which language development occurs especially through interactions between the child and caregiver. Responsiveness supports reciprocal conversation and stimulates language learning by allowing the child to initiate the interaction and then to receive suitable meaningful responses from the parent. This course also streamlines the working memory process and makes the child more receptive to new inputs.[3,4]

Another important role of maternal responsiveness for language development is that it provides joint attention.[5] Rather than redirecting the attention, focusing on a child's current interest is a good opportunity to maintain a language rich environment for a child. Previous literature has shown the positive relation between the duration of joint attention and language development in child-parent interactions.[5,6] The content of parental responses is also related to joint attention. Using developmentally appropriate language for a child's communicative acts meets their developmental needs and affects responsiveness as a result.[7]

Although the relation between maternal responsiveness and language development has been demonstrated in previous literature, it is difficult to evaluate responsiveness because of the difficulties involved when coding the specific patterns of parent-child interactions; it requires training, time and expertise. On the other hand, global rating systems are less complex; require little training and are less time-consuming with the rater's subjective estimates for a specific behavior pattern.[3,8]

The maternal responsiveness global rating scale is important because it requires less time and expertise from professionals and predicts the language outcomes of children. The scale was based on Marfo's description of maternal responsiveness in 1992; ''The mother responds appropriately to the child's cues and signals, interests, and overt behavior''. Maternal behaviors should also be conducted in a developmentally appropriate way so as to be identified as responsive. In addition to maternal responsiveness, ''maternal directiveness'' which had been identified by Marfo as ''the extent to which the mother uses hints, requests, commands and other controlling behaviors or actions to get the child to do what she wishes and follow her lead'' has been considered and involved in the scale.[3,9]

In terms of the importance of the first 3 years of life, which involve rapid brain growth and constitute a sensitive period for neuronal plasticity; parental attitudes have a unique and powerful effect on developmental trajectories. Maternal responsiveness is an essential component of language development and predicts the language outcome of children.[3] This inexpensive, easy-to-use and reliable tool can be recommended even in busy clinics in order to identify which slow-to-talk toddlers and their mothers need early intervention. Moreover, it may be used by community-based practitioners and researchers in our country to support language development during the early intervention stages. However there is no valid measurement to evaluate maternal responsiveness specifically in Turkey.[10] Therefore the aim of the study was to adapt the Maternal Responsiveness Global Rating Scale into Turkish, thereby making it accessible to a variety of professionals, and creating a way to use this useful scale in our culture.

Material and Methods

Participants and Procedure

After receiving ethical approval (GO 16/499), the study was conducted at Hacettepe University Ihsan Dogramacı's Child Hospital, Division of Developmental Pediatrics. The study began after obtaining the approval of Penny Levickis who created the Maternal Responsiveness

Global Rating Scale[2,3] and, was conducted between August 2016 and January 2017. Twenty-seven 18- to 42-month- old children, who had been admitted to the Developmental Pediatrics outpatient clinic with concerns of speech delay and had received a diagnosis of language disorder with the standardized language test, were included in the study. The inclusion criteria were having no cognitive delay and no sensorial deficit (hearing and viewing). The exclusion criteria were a diagnosis of autism spectrum disorder, genetic, neurological and other chronic conditions. Each patient was examined by a developmental pediatrician and screened for eligibility for the study and the general development and language development of participants were evaluated using Denver II GTT[11], Bayley 3[12] and Pre-school Language Scale 5 (PLS-5).[13] After receiving written consent from their families, participants were requested to record videos while they were playing with their children following the instructions of the Maternal Responsiveness Global Rating Scale. During the study course the treatment and follow-up of the participants were continued in the Developmental Pediatrics outpatient clinic.

The translation study of the Maternal Responsiveness Global Rating Scale

The scale was translated into Turkish without any changes by two native speakers of Turkish. Turkish translations were examined by the study team and the most appropriate expressions were determined. The translation was then back-translated into English by two native speakers of English. The obtained scale was compared to the original scale by the study team, and the suitability of the translation and the similarity of meaning were discussed. Subsequently a definitive final translation was obtained.

Video collecting and rating procedure

Participants were instructed to follow the same procedure as in the original scale at their first appointment. All mothers were requested to play with their children for fifteen minutes in their home environment, as they would normally, using one of the two sets of toys (farm or nurturing set). In the original scale a free-play with a standard set of farm and nurturing toys was videotaped by a research assistant in the home environment for fifteen minutes. In this study, a different technique was used in which the participants were requested to play with their own sets of farm and nurturing toys and to make the recording by themselves. The creator of the scale Penny Levickis approved this technical difference by noting that it could potentially deal with the issue of parents behaving differently when they knew they were being watched by the researcher, so this could be a benefit of the method which we have used. We also considered the idea of being able to reach more people by more professionals. After the collection of all videos, they were watched twice over with twenty-five day intervals by two blind researchers. Interrater and intrarater consistency was calculated in this way. It was scored using the 5-point Likert scale as in the original scale in which 1 is ''very low'' responsiveness and 5 is ''very high'' (Table I).

Evaluation tools

Maternal responsiveness was evaluated by the Maternal Responsiveness Global Rating Scale in pursuance of the aim of the study. It accounts for the frequency of developmentally appropriate and desirable maternal responses to a child's verbalization/gestures, as well as for less desirable maternal directive behaviors (attempts to redirect the child's attention from the current activity).[14] In one community-based sample with slow-to-talk toddlers, 246 parent-child interactions were randomly and blindly coded for specific maternal behaviors. A detailed overall score of maternal responsiveness was derived by summing the mean frequencies per minute of four individual behaviors.[2] The higher scores showed more maternal responsive behaviors; and subsequently The Maternal Responsiveness Global Rating Scale was developed using the 5-point Likert scale by 2 independent researchers blinded to the children's language scores. The global

Table I. Maternal Responsiveness Global Rating Scale.

Rating	Definition
1 = very low	Mother rarely responds in a developmentally appropriate way either verbally or non-verbally to any of Child's gestures or verbalizations AND Mother attempts to redirect Child's behaviour, rather than following Child's interests
2 = low	Mother responds occasionally in a developmentally appropriate way either verbally or non-verbally to Child's gestures or verbalizations AND/OR Mother spends more time attempting to redirect Child's behaviour than following Child's interest
3 = moderate	Mother spends some time responding in a developmentally appropriate way either verbally or non-verbally to Child's gestures or verbalizations, and some time ignoring them AND/OR Mother spends equal time following Child's interest and redirecting Child's behaviour
4 = high	Mother often responds in a developmentally appropriate way either verbally or non-verbally to Child's gestures or verbalizations AND/OR Mother spends more time following Child's interest than redirecting Child's behaviour
5 = very high	Mother frequently responds in a developmentally appropriate way either verbally or non-verbally to Child's gestures or verbalizations AND Mother does not attempt to redirect Child's focus from the current activity, but follows Child's interests

Note: Specification of extent of maternal directiveness: 'redirecting the child's behaviour' refers to redirecting the child's attention away from their current play and interests at that point in time. Source: Adapted from Marfo (1992: 224).

rating scale was compared with the detailed responsiveness scores in the same sample by Down et al.[14] and strong evidence of moderate correlation between the global and detailed ratings of maternal responsiveness with Pearson correlation coefficients ($r(242)=0.44$; $p< 0.001$) was found. Furthermore, a substantial inter-rater agreement (0.61-0.80) was indicated with Cohen' s kappa of 0.79 (84.6%).[2,3,14] The global rating scale is on a Likert scale from 1 to 5 with 1 being very low responsiveness and 5 being very high responsiveness. While this global rating scale provides a measure of responsiveness that is less demanding on the rater and saves substantial time.[3]

Mother-child interaction was evaluated with the Piccolo.[15] The Piccolo is used to score parent–child interactions with 10-minute video recordings. A Turkish adaptation was conducted by Bayoğlu et al.[10] involving Turkish mothers interacting with their children and good reliability and validity were demonstrated. In this study the subdomain of Responsiveness was used separately.

The general development of each child was evaluated with Denver II and Bayley-Third Edition Developmental Assessment Tests. Denver-II- Developmental Screening Test was initially developed for the developmental screening of 0 to 6-year-old children by Frankenburg and Dodds.[16] The adapted Turkish version was used in the study.[11] The Bayley-Third Edition Developmental Assessment Test is one of the most frequently used developmental evaluation tools worldwide. The test assesses cognitive, language and motor development in children aged between 1– and 42 months confidently with high internal consistency and test-retest reliability.[12] There are no Turkish norms for the Bayley-III so we used original norms for the scoring as in general.

Language development was evaluated with the Pre-school Language Scale 5 (PLS-5). PLS-5 is a language test which is widely used in children aged between 0- and 7 years 11 months to assess receptive and/or expressive language skills. It was adapted for Turkish children by Sahlı and Belgin and found to be a valid and reliable language test in our cultural context.[13]

The questionnaire for the sociodemographic data was prepared by the study team. Socio-economic status (SES) was determined using the Hollingshead Redlich Scale which was based on the profession and training of both parents.[17]

All tests were conducted by an experienced and certificated developmental pediatrician and a child development specialist in the study team.

Data analysis

Numerical variables were evaluated for normality and parametric tests were used for data with normal distributions whereas non-parametric tests were used otherwise. Concurrent validity of the Maternal Responsiveness Global Rating Scale was analyzed with the Spearman rho correlation coefficient using the responsiveness sub-domain of the Piccolo. Known group validity was analyzed using the cut-off point of the responsiveness sub-domain of the Piccolo and the groups were categorized as responsive (group without risk) and non-responsive (group with risk). The distributions of Maternal Responsiveness Global Rating Scale were compared by Mann Whitney U test in each group. Receiver operating characteristics (ROC) analyses were used to determine the diagnostic accuracy of the Maternal Responsiveness Global Rating Scale. The area under the ROC curve (AUC) was calculated as a measure to assess the accuracy of the Maternal Responsiveness Global Rating Scale. Intra-class Correlation Coefficient (ICC) and Weighted Kappa were used for the reliability analyses. The Intra-class Correlation Coefficient (ICC) was calculated to determine both the intra and inter-rater reliability. For test-retest comparison and inter-rater reliability, we used the weighted Kappa coefficient. The power analysis of the study was made with NCSS 2007 in PASS programme. P value <0.05 was defined as the limit of significance. All statistical analyses were performed using IBM SPSS Statistics 23.0 software.

Results

Twenty-seven children with language delay and mother-child interactions were included in the study. However two records were excluded from the study. In one of them the parent playing with the child was the father and, in the other the quality of the video was too low to rate. One video from each participant was collected. Finally, twenty-five mother-child dyads were analyzed. The mean ages of patients were 31 ± 6.6 months with a range of 12-41 months and, 64% of them were male (n = 16). The mean maternal age was 30.3 ± 4.3, and the mean age of the fathers was 34.5 ± 5.3. Mothers were mostly educated ≤ 8 years (84%) and housewives (88%). The number of children in the household was usually 2 (56%) and they were mostly the second child (60%) in the family. Most of the participants were term (mean 38.4 ± 1,95 weeks) and normal birth weight (mean 3377 ± 627 gr). Our median rating of maternal responsiveness was 2 (range 1-4). Detailed sociodemographic data of participants, child language outcomes and maternal responsiveness ratings are shown in Table II.

Reliability Analyses

The intraclass correlation coefficient (ICC) and Weighted Kappa were used for the reliability analyses. ICC for intra-rater reliability were 0.912 and 0.827 ($p < 0.001$) of Rater-1 and Rater-2 for Time-1(T1) and Time-2(T2), respectively. ICC for inter-rater reliability were 0.897 and 0.762 ($p < 0.001$) of Rater-1 and Rater-2 for T1 and T2, respectively. Weighted Kappa was also used for the reliability. The values of kappa for intra-rater reliability were 0.8196 and 0.6753 ($p < 0.001$) of Rater-1 and Rater-2 for T1 and T2 showing 93.3% and 88% compliance, respectively. The values of Kappa for inter-rater reliability were 0.7222 and 0.6324 ($p < 0.001$) of Rater-1 and Rater-2 for T1 and T2, respectively. The reliability analyses are shown in Table III.

Validity Analyses

Concurrent validity of the Maternal Responsiveness Global Rating Scale was analyzed with the Spearman rho correlation coefficient using the responsiveness sub-domain of the Piccolo. For Rater 1 and Rater 2, $r = 0.887$ and $r = 0.816$, respectively with a significance of $p < 0.001$.

Known group validity was analyzed using the cut-off point of the responsiveness sub-domain

Table II. Characteristics of the participants, child language outcomes and maternal responsiveness ratings.

Variables	Total sample (N=25)
Children	
Mean Age (month ± SD)	31 ± 6.6
Male % (n)	64 (16)
Term born % (n)	92(23)
Number of children at home (median)(range)	2(1-4)
Number of the child (to be the second child) % (n)	60 (15)
PLS5 standard scores of children (mean ± SD)	
Expressive	74.3 ± 3.4
Receptive	92.1 ± 7.4
Total	84 ± 8.9
Mothers	
Maternal age (mean ± SD)	30.3 ± 4.3
Maternal education(≤8 years) % (n)	84 (21)
Maternal employment(housewife) % (n)	88 (22)
Married % (n)	100 (25)
SES*(range:2-4) % (n)	
Class 2	36 (9)
Class 3	48 (12)
Class 4	16 (4)
Maternal Responsiveness Global Rating(median)(range)	2(1-4)
% (n)	
1= very low	40(10)
2= low	28(7)
3=moderate	24(6)
4=high	8(2)
5=very high	0(0)

PLS5: Preschool Language Score_5, SES: Socioeconomic status SD: standard deviation *: Hollingshead Redlich Scale provides categorical results according to the profession and training of both parents and lower levels of the classes demonstrate higher SES.

Table III. The reliability analyses of the Maternal Responsiveness Global Rating Scale.

	ICC	WKappa
intra-rater reliability*		
Rater 1	0.912	0.8196
Time 1-Time 2		
Rater 2		
Time 1-Time 2	0.827	0.6753
inter-rater reliability*		
Time 1		
Rater1 –Rater 2	0.897	0.7222
Time 2		
Rater1 –Rater 2	0.762	0.6324

ICC= Intraclass Correlation Coefficient and WKappa= Weighted Kappa
*: $p<0.001$ for all reliability analyses.

of the Piccolo and the groups were categorized as responsive (group without risk) and non-responsive (group with risk). The distribution of the results of the Maternal Responsiveness Global Rating Scale was analyzed with the Mann Whitney U test in each group. Maternal Responsiveness Global Rating scores of Rater-1 and Rater-2 were compared with the groups of the PİCCOLO which were categorized as group with risk and group without risk and found to be p <0.001 for both raters (Table IV).

Receiver Operating Characteristics (ROC) analyses were also used to observe the performance of the scale for classification. Area under the curve (AUC) can take values between 0.5- 1.0. As the value is closer to 1.0, the classification performance of the test increases. The closer the curve to the left upper corner, the better the test.[18] Area under the curve for Rater 1 and Rater 2 were 0.961 and 0.951, respectively (Fig. 1). The standard error for Rater-1 and Rater-2 were 0.033 and 0.039, respectively and p <0.001 for both of them.

There was no association between the sociodemographic characteristics and maternal responsiveness in the correlation analysis. Additionally the power analyses of the scale were implemented via the performance of classification of the scale and found to be 100% power.

Discussion

The results of this study demonstrate that the Turkish translation of the Maternal Responsiveness Global Rating Scale shows strong evidence of adequate reliability and validity and is a feasible tool to measure responsiveness in routine child health care practice for children with language delay in Turkey.

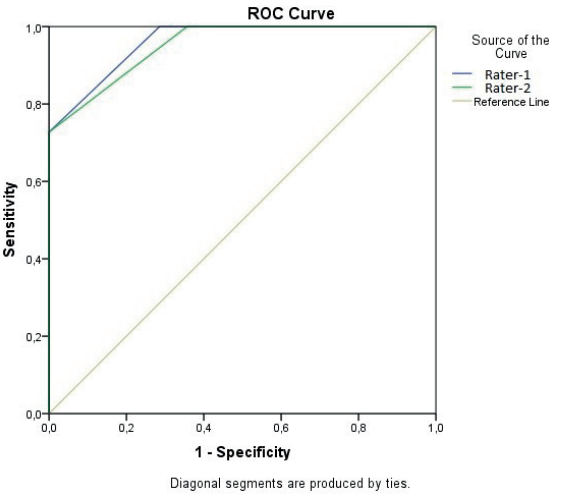

Fig. 1. Receiver Operating Characteristics (ROC) Curve of Maternal Responsiveness Global Ratings of Rater-1(R1) and Rater-2(R2). The area under the curve (AUC) for both raters are close to 1.0 and the curves are close to the left upper corner; R1_AUC:0.961, R2_AUC: 0.951.

The contribution of maternal behaviors to early language development and the predictive potential of the scale have been previously shown at the population level by Levickis et al.[2] and Hudson et al.[3] With the adaptation of this scale to Turkish we hope to open the way to use it in studies with new hypotheses and facilitate any research in this area. It is important as this method does not require any transcription or coding software, unlike detailed coding methods, and therefore reduces both cost and time and is of a user-friendly nature for health professionals since only brief training is necessary. Furthermore as the free-plays were videotaped in a home environment, the settings are thought to be more representative of the natural parent-child dyad rather than laboratory

Table IV. Known group validity analyses of the Maternal Responsiveness Global Rating Scale.

Maternal Responsiveness Global Rating scores	Group with risk n=14	Group without risk n=11	p
Rater1(median)(range)	1(1-1)	3(2-4)	0.000
Rater2(median)(range)	1(1-2)	3(3-4)	0.000

The groups were identified with risk and without risk according to the cut -off point of the Piccolo and, Maternal Responsiveness Global Rating scores were analyzed by the Mann-Whitney U test.

or clinic-based observations.[3,14,19] The technical difference in our study which includes playing with their own toys (sets of farm and nurturing toys) and recording by themselves may provide a closer approach to the natural environment for such interactions. Besides, enabling its' use by many professionals should be considered in low and middle- income countries where technical difficulties have been present.

In the population-based study with slow-to-talk toddlers the average rating of maternal responsiveness has been found to be 3.3 ± 0.9 and one-fifth of mothers have been rated 'low' or 'very low' on the global scale.[3] In our study we found that 68% of mothers were rated 'low' or 'very low' and the median rating of maternal responsiveness was 2 (range 1-4). Our average rating of maternal responsiveness was lower and the number of mothers with high responsiveness was less than in the original study. Additionally, our children's mean expressive language scores were lower than those of the original study (74.3 ± 3.4 compared to 90.5 ± 12.1). Uncontrolled variables such as socio-economic level between two study samples could be the reason for these differences. Another possible explanation of this finding is that, as has been noticed in the study, low maternal responsiveness is associated with lower language scores.[3] This result also contributes to existing literature by providing evidence regarding the importance of maternal responsive behaviors for early language development. Several descriptive studies have described the causal influence of parental responsiveness on child development. Children whose parents display a high level of responsiveness have been reported to have better communication, cognitive and socio-emotional functioning.[20-23] Therefore interventions including responsive behavior teaching strategies have been recommended to encourage parents to promote dimensions of engagement. It has been asserted that these behaviors maintain the processes of developmental learning that depends on the increasing frequency of using such behaviors.[24] Furthermore, in our study, the use of a video-recording procedure in their own environment at home and without an observer may have reflected more natural behavior of the participants. There are some methodological issues relating to direct observation of parent-child interaction in literature.[19] The type of task imposed by the observer, directing the parent and child to play rather than observing spontaneous interaction and the location of the observations such as in a clinic or laboratory rather than at home have been technical concerns discussed in observational studies. Although it has been suggested that the presence of an observer does not disturb the nature of interactions, a review of a number of studies in this area has reported that interactions in structured or artificial settings are not necessarily representative of those normally taking place at home.[19]

To the best of our knowledge this is the first time that low maternal responsive ratings have been demonstrated in children with language delay in Turkey. As the early identification and intervention of language delay are crucial, evaluating maternal responsiveness is noteworthy to enable the support of both parents and children during the critical developmental stages.

The small sample of the study was the main limitation. Cultural differences should be considered in this context. Our patients were not willing to record their natural home environment. One of the videos was excluded from the study because the person who was playing with the child was the father despite the precise instructions. However, the power analysis of the study has demonstrated that the sample was large enough to enable the adaptation of the scale with this video recording procedure.

We have investigated the reliability and validity of the Maternal Responsibility Global Rating Scale and indicated good reliability and validity of the scale in Turkey. This inexpensive, easy-to-use and reliable tool may be recommended in order to identify which slow-to-talk toddlers and their mothers need early intervention and

may be used by community-based practitioners and researchers in Turkey to support language development during the early intervention stages.

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Original Article

Neonatal outcomes of early- and late-onset preeclampsia

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ABSTRACT

Background. The aim of the current study was to demonstrate the neonatal outcomes of infants born to mothers with early-onset preeclampsia (EP) and late-onset preeclampsia (LP), and compare the neonatal outcomes before and after 34 weeks of gestation in EP group.

Methods. In this retrospective study, we evaluated preeclamptic mother and child pairs who were followed-up at Hacettepe University Hospital between the years 2010 and 2017. The pregnant women were classified as having EP if diagnosed before 34 weeks of gestation (n=91) and LP if diagnosed after 34 weeks of gestation (n=34). The women in the EP group were further divided into subgroups according to the gestational week at birth, including those who gave birth before 34 weeks of gestation (early birth; n=57) and after 34 weeks of gestation (late birth; n=34). Necessary clinical and demographic data were withdrawn from the electronic registry and patient files.

Results. Neonates in the EP/late birth subgroup had significantly lower gestational age and birthweight. Small for gestational age (SGA) frequency was higher in the early-onset subgroup born after 34 weeks' gestation compared to the late-onset preeclampsia group (p= 0,016). The incidence of neutropenia was significantly higher in the EP/late birth subgroup than in the LP group (p= 0.002). After correcting for gestational week and birth weight, neutrophil count was still significantly lower in the EP/late birth subgroup (p= 0.002). EP/late birth subgroup and LP group had comparable outcomes regardless of neutrophil count and SGA rate.

Conclusions. Close follow up and postponing delivery in stable and appropriate pregnant women with preeclampsia would be beneficial for neonates.

Key words: newborn, early-onset preeclampsia, late-onset preeclampsia, neutropenia, small for gestational age.

Preeclampsia, an important cause of maternal morbidity and mortality, is a progressive multisystem disease characterized by the new onset of hypertension, proteinuria, or hypertension and end-organ dysfunction with or without proteinuria after 20 weeks of gestation.[1,2] The incidence of preeclampsia in developing countries is about 3-5 per 100 live births.[3,4]

Preeclampsia is classified as early-onset or late-onset depending on whether it appears before or after 34 weeks of gestation.[5] In terms of its pathophysiology, early-onset preeclampsia (EP) is caused by intrinsic placental factors, whereas late-onset preeclampsia (LP) is attributed mainly to maternal factors. Thus, these two conditions are thought to be different conditions which have different etiological factors.[6,7]

The only treatment option for preeclampsia is delivery. Timing of birth depends on gestational week, maternal and fetal condition, and severity of the disease; so every patient should be evaluated individually.[8] If mother and fetus are stable with no end-organ damage, delivery should be postponed as long as possible to enable fetal growth and maturation with close follow up.[9]

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Preeclampsia results in preterm birth in 20% and intrauterine fetal growth restriction may be detected in 12% of cases.[10] There are several complications such as intraventricular hemorrhage (IVH), respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), and retinopathy of prematurity (ROP) among premature infants of preeclamptic women.[10,11] Moreover, studies showed that infants born to preeclamptic mothers have an increased risk of elevated blood pressure and high body mass index in childhood, also there is a nearly two-fold increase in the incidence of stroke in adulthood.[12,13]

We compared neonatal outcomes of infants born to mothers with EP and LP in this study. We also compared the outcomes of infants in early preeclamptic mothers regarding gestational week at delivery.

Material and Methods

In this retrospective study, we evaluated pregnant women who were followed up and delivered due to preeclampsia at Hacettepe Univerity Hospital between 2010 and 2017, and neonatal outcomes of these pregnancies were analyzed. The study protocol was approved by the Hacettepe Ethical Committee (GO 17/532). Initially, a total of 249 pairs of mothers and infants were identified by screening medical records. Two women with eclampsia, 37 women and 13 neonates with incomplete records or missing data, and 72 neonates with incomplete laboratory test results were excluded. The study was completed with the remaining 125 pairs of mother and infant.

The pregnant women were classified as having EP if diagnosed before 34 weeks of gestation (n= 91) and LP if diagnosed after 34 weeks of gestation (n= 34).[2] The women in the EP group were further divided into subgroups, including those who gave birth before 34 weeks of gestation (early birth; n= 57) and after 34 weeks of gestation (late birth, n=34).

Antenatal corticosteroid therapy was defined as 2 doses of betamethasone (12 mg, intramuscular) at least 24 h before delivery.[1] Abnormal ultrasonography was defined in the presence of absent or reversed end-diastolic flow in the umbilical artery on Doppler ultrasound examination.[14] The neonates were assessed in the delivery room by a neonatologist and follow up was performed in the neonatal intensive care unit if necessary.

The subjects' demographic characteristics, gestational week at preeclampsia diagnosis, time interval between diagnosis and birth, maternal comorbidities, medication during pregnancy, and manner of delivery, as well as the neonates' gestational age at birth, Apgar score at 5th minutes, neonatal findings, complete blood count values in the first 24 h after birth, length of hospital stay, and survival status were recorded. Neutropenia was defined as the absolute neutrophil count (ANC) < 1500/μL.[15]

Statistical analysis

Statistical analyses were performed using IBM SPSS software. The variables with a normal distribution were evaluated with an independent samples t-test, and the variables not conforming to a normal distribution were analyzed using the nonparametric Mann-Whitney U test. P values less than 0.05 were considered statistically significant. Categorical variables were analyzed using Fisher's exact and Pearson's chi-square tests. The results were expressed as the median and 25th-75th quartile values. Analysis of covariance was used to correct for gestational week and birth weight.

Results

Among the 125 neonates in the study, 91 were in the EP group and 34 in the LP group. The mean gestational age at birth was 33.5 ± 3.8 (22.6-40.0) weeks, and the mean birth weight was 1987 ± 984 (480-4750) g. Comparison of the demographic and neonatal characteristics of the pregnant women and infants are shown in Tables I and II.

Table I. Demographic characteristics of the patients.

Characteristic	Early-onset preeclampsia (n= 91)	Late-onset preeclampsia (n= 34)	Total n= 125	p
Sex (Male/Female)	46/45	21/13	67/58	0.316
n/n (%/%)	(50.5/49.5)	(61.8/38.2)	(53.6/46.4)	
Gestational week *	32.6 (30.0–34.6)	37.7 (36.7–38.4)	34.4 (31.1–36.7)	< 0.001
Birth weight (g)*	1540 (960–1920)	3135 (2850–3440)	1800 (1160–2850)	< 0.001
ART, n (%)	18 (19.8)	2 (5.9)	20 (16.0)	0.097
Corticosteroid administration, n (%)	62 (68.1)	3 (8.8)	65 (52.0)	< 0.001
Abnormal Doppler, n (%)	12 (13.2)	2 (5.9)	14 (11.2)	0.347
CS / VD n/n (%/%)	87/4 (95.6/4.4)	33/1 (97.1/2.9)	120/5 (96.0/4.0)	1.000
Apgar at 5th min	9 (7–10)	10 (10–10)	9 (8–10)	< 0.001
Need for resuscitation at birth, n (%)	40 (44.0)	1 (2.9)	41 (32.8)	< 0.001
SGA, n (%)	43 (47.3)	0 (0.0)	43 (34.4)	< 0.001

*median (25^{th}–75^{th} percentile); ART: assisted reproductive technique; CS: cesarean section; VD: vaginal delivery; SGA: small for gestational age.

Table II. Neonatal characteristics of the patients.

Characteristic	Early-onset preeclampsia (n= 91)	Late-onset preeclampsia (n= 34)	Total n= 125	p
MV duration (days)*	5 (2–10.5)	-	-	-
RDS, n (%)	31 (34.1)	1 (2.9)	32 (25.6)	< 0.001
TPN duration (days)*	10 (4–18)	-	-	-
Hospital stay (days)*	10.0 (5.0–26.0)	2.0 (2.0–2.0)	6.0 (2.0–17.0)	< 0.001
Gestational age at diagnosis of preeclampsia (weeks)*	30.0 (27.8–32.0)	37.0 (36.0–37.7)	32.0 (28.8–35.0)	< 0.001
Time interval between diagnosis and birth (days)*	14 (1–28)	0 (0–6)	7 (0–22)	< 0.001
Neonatal pneumonia, n (%)	16 (17.6)	1 (2.9)	17 (13.6)	0.039
IVH, n (%)	6 (6.6)	0	6 (4.8)	0.188
Pneumothorax, n (%)	4 (4.4)	0	4 (3.2)	0.574
PDA, n (%)	15 (16.5)	0	15 (12.0)	0.011
Sepsis, n (%)	24 (26.4)	0	24 (19.2)	< 0.001
NEC, n (%)	18 (19.8)	0	18 (14.4)	0.003
ROP, n (%)	4 (4.4)	0	4 (3.2)	0.574
BPD, n (%)	10 (11.0)	0	10 (8.0)	0.061
Apnea, n (%)	2 (2.2)	0	2 (1.6)	1.000
Death, n (%)	12 (13.2)	0	12 (9.6)	0.035

*median (25^{th}–75^{th} percentile); MV: mechanical ventilation (intubation); RDS: respiratory distress syndrome; TPN: total parenteral nutrition; IVH: intraventricular hemorrhage; PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; ROP: retinopathy of prematurity; BPD: bronchopulmonary dysplasia.

No statistically significant differences were found between the EP and LP groups with regard to neonatal gender, use of assisted reproductive techniques, abnormal Doppler findings, manner of delivery, incidence of pneumothorax, ROP (> Grade I), BPD (defined as oxygen requirement either at 28 postnatal days or 36 weeks postmenstrual age), IVH (>

Grade I), and apnea (p= 0.316; p= 0.097; p= 0.347; p= 1.000; p= 0.574; p= 0.574; p= 0.061; p= 0.188; p= 1.000, respectively).

The EP group had a significantly lower gestational age at the time of diagnosis, gestational age at birth, birth weight, and 5th min Apgar score compared to the LP group (p < 0.001 for all). Neonates in the EP group had a significantly longer hospital stay and time interval between diagnosis and birth; as well as a significantly higher rate of steroid therapy, need for resuscitation, SGA, pneumonia, PDA, sepsis (proven sepsis), NEC (> Grade I), RDS, and mortality (p < 0.001; p < 0.001; p < 0.001; p < 0.001; p < 0.001; p= 0.039; p= 0.011; p < 0.001; p= 0.003; p < 0.001; p= 0.035, respectively).

The comparison of neonatal complete blood count values in the first 24 hours after birth in infants in the EP and LP groups is shown in Table III.

Hemoglobin, hematocrit, white blood cell, thrombocyte, and neutrophil counts were significantly lower in the EP group compared to the LP group (p < 0.001; p < 0.001; p= 0.003; p=0.011; p < 0.001, respectively). The incidence of neutropenia was 24.2% in the EP group and 8.8% in the LP group, but the difference was not statistically significant (p= 0.078).

A comparison of infants in the LP group (n= 34) and those in the EP group that were delivered after 34 weeks of gestation (n= 34) is presented in Table IV. The neonates in the EP/late birth subgroup had significantly lower gestational age and birth weight (p < 0.001; p < 0.001, respectively). However, no statistically significant differences were found between these two groups in terms of gender, abnormal Doppler, manner of delivery, need for resuscitation, or rates of RDS, pneumonia, pneumothorax, and mortality (p= 0.806; p= 0.493; p= 1.000; p= 0.197; p= 1.000; p= 1.000; p= 1.000; p= 1.000, respectively) (Table IV).

In the EP/late birth group, use of assisted reproductive techniques, antenatal steroid therapy, and SGA status were significantly more common, and hospital stay and time between preeclampsia diagnosis and delivery were significantly longer than in the LP group (p= 0.045; p <0.001; p <0.001; p <0.001; p <0.001, respectively). The Apgar score was higher in the LP group (p= 0.016) (Table IV).

Table V shows the comparison of the complete blood count values in the first 24 h after birth between neonates in the EP group born after 34 weeks of gestation and infants in the LP group.

The comparison of complete blood count values in the first 24 h after birth between EP infants born after 34 weeks of gestation and LP infants revealed no statistical differences in hemoglobin or hematocrit (p= 0.076; p= 0.053, respectively), but white blood cell, thrombocyte, and neutrophil counts were significantly lower in the EP group (p= 0.039; p= 0.008; p= 0.001, respectively). The incidence of neutropenia was significantly higher in the EP/late birth

Table III. Hematologic parameters of infants born to mothers with early-onset and late-onset preeclampsia.

Complete blood count values	Early-onset preeclampsia (n= 91)	Late-onset preeclampsia (n= 34)	p
Hemoglobin (g/dl)*	16.3 (15.1–18.5)	18.6 (17.0–20.4)	< 0.001
Hematocrit (%)*	50.2 (45.8–54.6)	55.7 (52.2–61.3)	< 0.001
White blood cell count (/mm^3)*	10000 (5500–14400)	12700 (10200–16800)	0.003
Thrombocyte count (/mm^3)*	164000 (106000–219000)	212500 (156000–256000)	0.011
Neutrophil count (/mm^3)*	2200 (1150–6050)	8050 (5400–12100)	< 0.001
Neutropenia, n (%)	31 (34.1%)	3 (8.8%)	0.006

*Median (25th–75th percentile); Neutropenia=absolute neutrophil count < 1500 mm^3.

subgroup than in the LP group (p= 0.002). After correcting for gestational week and birth weight, neutrophil count was still significantly lower in the EP/late birth subgroup (p= 0.002), and no differences were observed in the other hematological parameters.

Table IV. Comparison of infants in the early-onset preeclampsia group born after 34 weeks of gestation and infants in the late-onset preeclampsia group.

Characteristic	Early-onset preeclampsia + late birth (n= 34)	Late-onset preeclampsia (n= 34)	p
Gender (Male/Female)	19/15	21/13	0.806
n/n (%/%)	(55.9/44.1)	(61.8/38.2)	
Gestational age (weeks)*	34.9 (34.4–36.2)	37.7 (36.7–38.4)	< 0.001
Birth weight (g)*	1980 (1740–2380)	3135 (2850–3440)	< 0.001
ART, n (%)	9 (26.5)	2 (5.9)	0.045
Intrauterine steroid therapy, n (%)	32 (94.1)	3 (8.8)	< 0.001
Abnormal Doppler, n (%)	0	2 (5.9)	0.493
CS/NSVD, n/n (%/%)	32/2 (94.1/5.9)	33/1 (97.1/2.9)	1.000
Apgar at 5th min*	10 (8–10)	10 (10–10)	0.016
Need for resuscitation at birth, n (%)	5 (14.7)	1 (2.9)	0.197
MV duration (days)*	2 (2–3)	-	-
RDS, n (%)	1 (2.9)	1 (2.9)	1.000
TPN duration (days)*	4 (3–8)	-	-
Hospital stay (days)*	6 (2–10)	2 (2–2)	< 0.001
Gestational age at preeclampsia diagnosis (weeks)*	32.0 (29.0–33.0)	37.0 (36.0–37.7)	< 0.001
Time between diagnosis and birth (weeks)*	4.0 (2.7–5.9)	0.0 (0.0–0.9)	< 0.001
SGA, n (%)	17 (50.0)	0 (0.0)	< 0.001
Pneumonia, n (%)	1 (2.9)	1 (2.9)	1.000
Pneumothorax, n (%)	1 (2.9)	0	1.000
Death, n (%)	1 (2.9)	0	1.000

*median (25^{th}–75^{th} percentile); ART: assisted reproductive technique; CS: cesarean section; NSVD: normal spontaneous vaginal delivery; MV: mechanical ventilation (intubation); RDS: respiratory distress syndrome; TPN: total parenteral nutrition; SGA: small for gestational age.

Table V. Hematological parameters of infants of mothers with early-onset preeclampsia born after 34 weeks of gestation and neonates of mothers with late-onset preeclampsia.

Complete blood count values	Early-onset preeclampsia + late birth (n= 34)	Late-onset preeclampsia (n= 34)	p	p_s
Hemoglobin (g/dl)*	17.3 (15.6–19.2)	18.6 (17.0–20.4)	0.076	0.307
Hematocrit (%)*	52.1 (50.1–58.7)	55.7 (52.2–61.3)	0.053	0.371
WBC (/mm3)*	10400 (7300–15700)	12700 (10200–16800)	0.039	0.918
Thrombocyte count (/mm3)*	149000 (103000–212000)	212500 (156000–256000)	0.008	0.083
Neutrophil count (/mm3)*	1600 (1000–4500)	8050 (5400–12100)	< 0.001	0.002
Neutropenia, n (%)	15 (44.1%)	3 (8.8%)	0.002	-

*Median (25^{th}-75^{th} percentile); Neutropenia=absolute neutrophil count <1500 mm^3; p_s: p value calculated after correcting for gestational age and birth weight

Discussion

The early onset of preeclampsia is associated with increased risk of life-threatening maternal complications and fetal death, whereas late onset preeclampsia is usually associated with minor placental involvement and a milder clinical presentation.[7] In addition, EP is associated with lower gestational age at birth, thus preterm birth complications are also more common in this group. Although previous studies compared hematological and neonatal features in EP and LP,[16-18] to the best of our knowledge, ours is the first study in the literature to compare these variables in neonates born to mothers with LP and neonates of mothers with EP born after 34 weeks of gestation.

As our hospital is a tertiary care center that receives referrals of high risk pregnancies, more patients were presented in the EP group. Consequently, median gestational age at birth and birth weight were lower in the EP group than in the LP group (median, 25th-75th percentile: 32.6, 30.0-34.6 weeks vs. 37.7, 36.7-38.4 weeks; 1540, 960-1920 g vs. 3135, 2850-3440 g, respectively). Nevertheless, we repeated the statistical comparisons after correcting for these differences in gestational age and birth weight.

Neonatal morbidity and mortality risk is higher with decreasing gestational age and birth weight. Besides prematurity and low birth weight, complications such as IVH and BPD are other factors that increase the risk of morbidity, particularly neurodevelopmental delays, in preterm infants.[19] In our study, the EP group had higher rates of neonatal morbidities (RDS, NEC, sepsis, PDA, and pneumonia), placental insufficiency findings (abnormal Doppler ultrasonography), SGA, lower Apgar scores, need for resuscitation, longer hospital stay, and mortality rate. These findings support the higher risk in preterm infants of mothers with EP than in those with LP.

These results are somewhat expected for preterm infants with lower gestational age and birth weight. However, neonatal risks can be further reduced, especially in women with EP, by carefully planning delivery time with close prenatal follow-up in a tertiary center with an experienced team consisting of perinatologists and neonatologists. Similar to the study of Ni et al.[17] we observed no significant difference in the incidence of IVH between the EP and LP groups. However, studies including larger sample numbers and analyses of long-term neurodevelopmental follow-up data are still needed.

A higher prevalence of abnormal Doppler findings in the uterine artery was reported in EP in some studies,[7,20] but no such difference was observed between the EP and LP groups in our study (p= 0.347). However, abnormal uterine artery Doppler findings are strong indicators of placental deficiency; they may not be a direct indicator of preeclampsia-related placental deficiency, chronic intrauterine hypoxia, or fetal growth restriction. In our study, the SGA rate was higher in the EP group (p <0.001). Madazli et al.[7] evaluated 154 pregnant women, 91 (59%) with EP and 63 (41%) with LP, and reported that although no statistical differences were found between the EP and LP groups, the EP group had higher SGA frequency and lower Apgar score and mortality rates. Iacobelli et al.[18] reported no statistical difference between infants born to mothers with EP and LP in terms of the first min. Apgar score and SGA rate after correcting for gestational age. Our findings are compatible with the literature; however comparison of EP/late birth subgroup and LP group was not available in those studies. Moreover, hematological parameters of the neonates born to preeclamptic mothers were also investigated in our study.

In our study, gestational age and birth weight were significantly lower in neonates of EP mothers born after 34 weeks of gestation (n= 34) than in those of LP mothers (n= 34) (p <0.001; p <0.001, respectively). SGA frequency and 5th min Apgar scores were significantly higher in the EP group (p= 0.016; p <0.001, respectively). However, neonatal morbidity and mortality rates were significantly lower in EP infants

born after 34 weeks of gestation. This finding shows that, pregnant women diagnosed with EP should be closely followed up, and delivery should be scheduled after 34 weeks whenever possible to reduce the neonatal morbidity and mortality.

Our results show that infants of EP mothers born at gestational ages over 34 weeks had comparable hemoglobin and hematocrit values with infants born to mothers with LP. Although we expected these values to be lower in the EP group because of their lower mean gestational age and birth weight, we believe that the similar values in the two groups could be attributable to the EP infants having increased erythropoiesis secondary to the higher rate of SGA.[20]

There are some studies which investigated the hematological parameters in infants of preeclamptic mothers in the literature,[22-23] only a small number of studies have compared the hematological parameters in babies born to mothers with EP and LP.[16] Herzog et al.[16] analyzed the complete blood count data from the cord blood of infants born to 11 EP and 12 LP mothers and found that leukocyte, neutrophil, and thrombocyte counts were significantly lower in the EP group. The median gestational age and birth weight were 31.0 weeks and 1155 g in the EP group and 37.4 weeks and 3238 g in the LP group, respectively. After correcting for gestational age and birth weight, only reduced neutrophil count was significantly associated with EP.[16] Similarly, we found that white blood cell, thrombocyte, and neutrophil counts were significantly lower in the EP group (p= 0.039; p= 0.008; p= 0.001, respectively). The incidence of neutropenia was higher in the EP subgroup born after 34 weeks of gestation than in the LP group (p= 0.002). After correcting for gestational week and birth weight, neutrophil count remained significantly lower in the EP/late birth subgroup (p= 0.002), and no differences were observed in the other hematological parameters.

To the best of our knowledge previous studies compared only EP and LP groups,[17-18] whereas we also compared neonatal and hematological outcomes between EP and LP infants and also EP/late birth and LP. The limitations of our study include the retrospective design and the lack of long-term neurodevelopmental results. Prospective clinical studies including larger sample numbers and evaluating long-term outcomes are needed to further improve prenatal follow-up and prevent neonatal morbidity and mortality.

In conclusion, EP/late birth subgroup and LP group had comparable outcomes regardless of neutrophil count and SGA rate. Close follow up and postponing delivery in appropriate preeclamptic pregnant women could be beneficial for neonates.

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Original Article

Duration of treatment with oral rehydration salts for vasovagal syncope in children and adolescents

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ABSTRACT

Background. Oral rehydration salt (ORS) is a first-line medication for vasovagal syncope (VVS) in children and adolescents. We retrospectively investigated the treatment with ORS-I (Na 90 mmol/L) for VVS in children and adolescents to define appropriate duration of treatment.

Methods. All patients with a diagnosis of VVS, based on the first head-up tilt test (HUTT) response, and who accepted ORS-I treatment were enrolled. ORS was stopped when the HUTT response turned negative. Patients were followed for six months after cessation of ORS treatment.

Results. The study group included 129 patients (57 male, 72 female; mean age, 11.8 ± 2.0 years, age range, 7.0-17.0 years). Median duration of VVS was 4 months (range, 1 week to >10 years). The number of syncope ranged from 2 times to >20 times. Mean follow-up time was 27.8 ± 6.9 weeks (range, 26-33 weeks). It took to 2~13 weeks for HUTT response to turn negative, with an average time of 8.4 weeks (95% confidence interval, 6.89~9.84 weeks). There was no statistical difference for the time to negative HUTT response according to age groups (<12-year-old vs. ≥12-year-old), syncope type (vasodepressor vs. mixed), and the syncope frequency. No patient experienced syncope after cessation of ORS treatment.

Conclusions. Our findings suggest that ORS-I is an effective measure to treat children and adolescents with VVS. We recommend a treatment course of 2 months.

Key words: oral rehydration salts, vasovagal syncope, medication course, children, adolescents.

Vasovagal syncope (VVS) is the most common type of unexplained syncope in children and adolescents. Oral rehydration salts (ORS) is one of the first-line non-pharmacological measures. Increasing dietary intake of salt is the main measure of rehydration,[1] however, clinically, it involves problems such as intake of water and salt is hard to control and their excessive intake can affect blood pressure. ORS have been used for more than 40 years in the prevention and treatment of mild dehydration caused by acute or chronic diarrhea. As an alternative measure to increase dietary salt intake, ORS has been increasingly used in clinics.[2-5] However, there is as yet no report on the medication course of ORS both at home and abroad. This paper aims to retrospectively analyze the clinical data of the children and adolescents with confirmed VVS in our hospital and discuss the medication course of ORS for VVS.

Material and Methods

Between March 2006 to October 2013, a total of 129 children and adolescents (57 males and 72 females; mean age, 11.8 ± 2.0 years; range, 7.0-17.0 years) with unexplained syncope for a course of 1 week to >10 years (median course of 4 months) and syncope episodes of 2 to >20 times

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(3.5 ± 3.3 times on average; ≥2 episodes in half a year) who visited Pediatric Syncope Clinic in the Second Xiangya Hospital of Central South University, China were enrolled. The inclusion criteria included the following: 1) other syncope diseases were ruled out on routine examination; 2) initial head-up tilt test (HUTT) response was positive and ORS therapy was given once VVS (including vasodepressor type and mixed type, excluding cardioinhibitory type) was confirmed. Patients whose diseases were failed to be confirmed on detailed inquiry of medical history, physical examination, biochemical examination, routine 12-lead ECG, 24-hour dynamic ECG, 24-hour ambulatory blood pressure, EEG, and skull CT or MRI, etc. were excluded. Patients were classified according to age (<12-year-old and ≥12-year-old), frequency of syncope (2-5 episodes and ≥6 episodes). The patients were followed-up at 2-4 weeks as directed by medical order for collection of detailed medical history including recurrence of syncope after treatment, patients' compliance with ORS treatment etc. All patients showed good compliance with ORS, and HUTT was performed in every follow-up until negative HUTT response was shown. Syncope recurred no more during 6-month follow-up after ORS withdrawal.[6-8]

The informed consent was obtained from all the subjects directly or their guardians prior to enrollment. The study protocol (no. 2014-012) was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University, China.

Head-up tilt test (HUTT) was used for assessing patients with suspected syncope but lack of confirmed diagnosis after an initial assessment, or a differential diagnosis of convulsive syncope from epilepsy or pseudo-syncope from VVS. The head-up tilt period was performed under two conditions: passive (without any provocative drugs), and pharmacological (with drug challenge). Subjects were tilted at 60° head upward, heart rate (HR), blood pressure (BP) and ECG were recorded continuously until either 45 min. duration, or development of syncope or intolerable near syncope symptoms. If syncope occurred, patients were rapidly placed in the supine position. If syncope or presyncope did not occur, tilted posture was maintained, subjects were medicated with adjunctive agents, such as isoproterenol, nitrates, and clomipramine, and HR, BP and ECG were recorded for 20 min. or syncope or presyncope occurred.

Syncope or pre-syncope symptoms accompanied with one of the following conditions during HUTT was defined as VVS: (1) BP <80/50 mmHg (10.66/6.67 kPa) or >25% reduction of mean BP relative to baseline BP; (2) HR <75 bpm for children aged 4-6 years, <65 bpm for those aged 6-8 years , <60 bpm for those aged above 8 years; (3) ECG showing sinus arrest or premature junctional contractions; (4) atrioventricular block, asystole up to 3 sec. VVS was classified as: vasodepressor type (significant reduction in BP but insignificant change in HR), cardioinhibitory type (significant reduction in HR but insignificant change in BP), or mixed type (significant reduction both in BP and HR).[9,10]

Therapeutic regimen

Patients who have been diagnosed as having VVS by HUTT were treated with ORS-I [Drug specification: 14.75 g/bag; composition: glucose of 11.00 g, sodium chloride of 1.75 g, potassium chloride of 0.75 g, sodium bicarbonate of 1.25 g, and osmotic pressure of 311 mOsm/L, sodium 90 mmol/L; Manufacturer: Fuzhou Haiwangfuyao Pharmaceutical Co., Ltd. (Approval Number: H35021107)]. Dosage & Administration: All the subjects were administered with ORS-I (14.75 g) that dissolved in 500 ml (per day) of warm water or cold water in divided oral doses. ORS discontinued when negative HUTT response was shown.

Evaluation on curative efficacy

The curative efficacy was evaluated based on clinical syncope episodes: Improvement was defined as decrease in syncope episodes or even no reoccurrence of syncope after treatment; no improvement was defined as no change or even increase in syncope episodes as compared with that before treatment.

Statistical analysis

The data were analyzed using SPSS17.0 software, with measurement data expressed as mean ± standard deviation and counting data expressed as rate. Variance analysis was carried out for group comparison. A p-value <0.05 was considered statistically significant.

Results

Curative efficacy

When negative HUTT was shown in 129 VVS cases during follow-up, syncope reoccurred no more in 110 and significantly less frequent in 19 than before medication, with clinical response rate of 100%. Negative HUTT response was noted at 2-13 weeks (8.4 weeks on average, 95% confidence interval: 6.89- 9.84).

Time to achieve negative HUTT response according to age groups and different hemodynamic types

No significant differences were noted in the time to achieve negative HUTT response between the <12-year-old group and ≥12-year-old group (p = 0.068), or between vasodepressor type and mixed type (p = 0.218), and the interactions between age and the time to negative HUTT response or between hemodynamic types and the time to negative HUTT response were non-significant (p = 0.944) (Table I).

Time to achieve negative HUTT response according to age groups and frequency of syncope episodes

No significant differences were noted in the time to achieve negative HUTT response between the 2-5 syncope episodes group and ≥6 syncope episodes group (p = 0.212). The interactions between age and syncope episodes were non-significant (p = 0.115) (Table II).

Discussion

VVS is a condition that is marked by sudden loss of consciousness due to transient insufficient cerebral blood flow resulting from

Table I. Vasovagal syncope reaction type according to age groups; time to negative head-up tilt test response according to vasovagal syncope reaction type and age groups.

Age groups	VVS reaction type, n (%)		Time to negative HUTT response according to VVS reaction type, weeks	
	Vasodepressor type	Mixed type	Vasodepressor type	Mixed type
<12 years (n = 48)	37 (77)	11 (23)	5.9 ± 3.7	8.1 ± 5.6
≥12 years (n = 81)	56 (69)	25 (31)	8.9 ± 7.0	10.1 ± 8.7

HUTT: head-up tilt test, VVS: vasovagal syncope

Table II. Frequency of syncope according to age groups; and time to negative head-up tilt test response according to frequency of syncope and age groups.

Age groups	Frequency of syncope, n (%)		Time to negative HUTT response according to frequency of syncope, weeks	
	2 to 5 episodes	≥6 episodes	2 to 5 episodes	≥6 episodes
<12 years (n = 48)	44 (92)	4 (8)	6.2 ± 4.2	5.0 ± 1.0
≥12 years (n = 81)	70 (86)	11 (14)	8.3 ± 6.5	9.0 ± 7.1

HUTT: head-up tilt test

diffident factors and fainting due to inability to maintain muscle tone. Studies have shown that Bezold-Jarisch reflex, hypovolemia and baroreflex dysfunction might be involved in the pathogenesis of VVS. Although VVS is self-limited with favorable prognosis, it causes recurrent syncope in 70% of pediatric and adolescent cases, and can even lead to syncope-related somatic accidental injury thus affecting their physical and mental health, study and life. Therefore, VVS in children and adolescents still need intervention in an effort to reduce syncope episodes so as to improve their quality of life.[11,12]

Non-pharmacological approaches are the first-line intervention measures in the management of VVS, aiming at increasing circulating blood volume, enhancing autonomic neuroregulatory function, and increasing orthostatic tolerance etc. Increasing intake of water and salt is the basic measure of non-pharmaceutical treatment. According to the guidelines for the diagnosis and management of syncope, expanding blood volume by increasing water and salt intake is the key to the management of VVS and is thus recommended to be one of the basic measures for VVS treatment.[1,3,4]

In the study of rehydration and salt supplementation for unexplained syncope, Cooper et al.[13] reported symptom relief and significant increases in orthostatic tolerance and baroreceptor sensitivity in 178 adult patients with syncope or threatened syncope after oral administration with sodium chloride 100 mmol/day (5.85 g) for 3 months. El-Sayed et al.[14] made a study on 20 syncope patients (40-60 years) by treating the patients with sodium chloride 120 mmol/day for 8 weeks and noted a significant increase in plasma and blood volumes (100-500 ml) in 80% patients and increased orthostatic tolerance in all, much as in those whose 24-hour urinary sodium excretion was below 170 mmol/day. Mtinangi et al.[15] found increase in orthostatic tolerance within 3 days after salt loading. Shichiri et al.[16] reported increase in blood volume and significant increase in orthostatic tolerance in a 14-year-old orthostatic hypotension child after 48-hour treatment with sodium chloride at a dosage of 3 g/day, b.i.d. In addition, studies have shown that supplementation of salt and fluid is also effective for syncope in children and the elderly.[17,18] Bellard et al.[19] reported that increasing hydration alone does not improve orthostatic tolerance in patients with neurocardiogenic syncope (NMS). Wieling et al.[20] suggested that adult patients with orthostatic syncope should increase their salt intake by ≥1 g/ time (three times a day) and fluid intake by 2-2.5 liter per day. Guzman et al.[21] pointed out that in the absence of contraindication, symptomatic patients should be encouraged to increase their salt and fluid intake to at least 2 g/day of sodium and 2 to 3 L/day of water, which is probably a cost-effective and safe strategy that should be used as first-line therapy. The study made by Claydon et al.[22] on the effect of salt supplementation on syncope patients by using HUTT and Doppler ultrasound showed significant improvement in symptoms, orthostatic tolerance and cerebral autoregulation, yet non-significant change in resting heart rate or blood pressure. The underlying mechanism might be the increase in orthostatic tolerance following increase in salt loading, which might be associated to the increase of plasma volume as well as the enhancement of sympathetic control on peripheral vascular system and the improvement of cerebral vascular autoregulation. The increase in responsiveness of vascular resistance during orthostatic period may be related to the increased baroreceptor sensitivity resulting from salt supplementation, which might pose a direct effect on vascular resistance in that it has been shown in some animal experiments that increasing salt intake could lead to enhanced response of isolated blood vessels to norepinephrine.[23] However, the mechanism underlying the improved cerebral autoregulation remains unclear, with the only finding that the cerebral blood flow velocity depends less on blood pressure after salt load increase.

The curative efficacy of ORS for VVS has gained general consensus, and it has been recommended as one of the basic measures

for the treatment of VVS by the guidelines for the diagnosis and management of syncope. In traditional therapy, increasing dietary salt intake is the main measure of ORS, yet it poses the risk of increasing blood pressure and has its limitations including changing the taste of food thus affecting food intake. Accurate control of water and salt intake can effectively reduce possible complications. ORS as an alternative to increase dietary salt intake has been gradually applied in clinics. However, there is yet no report on the medication course of ORS. At present, ORS has three generations (I, II, and III), and they were ranked in descending order in terms of osmotic pressures. ORS-I with a cheap price has been widely used in clinics since the 1970s when it was recommended by the World Health Organization (WHO) for clinical prevention and management of mild dehydration induced by acute or chronic diarrhea. In this study, ORS-I was selected for treating VVS in children and adolescents, with clinical syncope recurrence episodes as the criteria for evaluation of clinical symptom improvement and HUTT negative conversion as the end point to analyze the medication course of ORS for VVS. Our study on 129 children and adolescents with VVS showed that when negative HUTT was achieved after treatment with ORS therapy, syncope occurred no more in 110 cases, significantly less frequent in 19 than before treatment, with a response rate of 100%. HUTT converted to negative at 2-13 weeks (8.4 weeks on average, with 95% confidence interval of 6.89 - 9.84).

No significant differences were noted in the time to achieve negative HUTT response between the <12 years old group and ≥12 years old group, between the vasodepressor type and mixed type, or between the 1 syncope episode group and ≥6 syncope episodes group, and the effects of their interactions on the time to achieve negative HUTT response were all non-significant.

In short, ORS is an effective therapy for VVS in children and adolescents. It is advisable to adjust the medication course of ORS for VVS in children and adolescents in accordance with the improvement of clinical symptoms and HUTT results. The recommended course of treatment for ORS is 2 months.

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Case Report

A novel compound heterozygous variant in *CYP19A1* resulting in aromatase deficiency with normal ovarian tissue

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ABSTRACT

Background. Aromatase deficiency leading to virilization in mother and female fetuses during pregnancy is a rare disease. It is characterized by impaired estrogen production, increased gonadotropins, and ovarian cysts.

Case. Herein, we report a clinical phenotype of the virilized female due to a novel compound heterozygous variant in *CYP19A1* [IVS10 + 1 G> A; c.344 G> A (p.R115Q)], with normal gonadotropin levels at the time of admission and histologically normal ovarian tissues.

Conclusion. Aromatase deficiency should also be considered even if the initial follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels are normal, and ovarian cysts are lacking.

Key words: disorders of sex development, p450 oxidoreductase deficiency, clitoromegaly, hirsutism, ambiguous genitalia.

Aromatase is a type II cytochrome P450 enzyme located in the endoplasmic reticulum, which catalyzes the conversion of C19 steroids (testosterone, 16-alpha-hydroxytestosterone, and androstenedione) into C18 steroids (17 beta-estradiol, estriol, and estrone). It is encoded by the *CYP19A1* gene located on chromosome 15q21.1.[1] Aromatase deficiency is a rare autosomal recessive disorder that leads to increased androgen levels in both the fetus and the mother. The latter condition results in specific signs of maternal virilization including cystic acne, hirsutism, clitoromegaly, and deep voice.[2]

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It was first described by Shozu et al.[3] in 1991. Subsequent experimental studies showed that aromatase-knockout female mice developed a male body habitus, had small or polycystic ovaries with no corpora lutei, small uteri, and they were infertile.[4] Until now, nearly 40 cases with varying clinical presentations from various ethnic groups were reported.

Female cases with aromatase deficiency have ambiguous genitalia at birth, failure to enter puberty, a propensity to develop ovarian cysts, increased gonadotropins and pronounced virilization.[1] During childhood, both serum basal or gonadotropin-releasing hormone (GnRH)-induced follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels are expected to be elevated. Thereby, high concentrations of circulating gonadotropins and increased intra-ovarian androgen levels usually cause large and polycystic ovaries from the infancy period.[1]

Herein, we report a virilized female infant with normal ovarian morphology and initially normal gonadotropin levels associated with a novel compound heterozygous variant [IVS10 + 1 G> A; p.R115Q (c.344 G> A)] in *CYP19A1*.

Case Report

A 4-month-old girl was referred to our outpatient clinic due to clitoromegaly, which was decreasing in size since birth. She was born with a birth weight of 2710 grams at 35th week of gestation. Her parents were unrelated. Her mother had developed acne, hair loss, voice change, and hirsutism during pregnancy. There was no similar case in family history.

Physical examination revealed normal weight [6.8 kg; 0.4 standard deviation score (SDS), 50-75p] and length (64 cm; 0.5 SDS, 50-75p). Clitoris was 1 cm long and labia minora were fused posteriorly. There were no gonad-like structures in the inguinal region. The remaining systemic examination was normal.

A number of laboratory tests already performed before admission to our unit were interpreted as normal [16th day of life, adrenocorticotropic hormone 29.6 pg/ml (0-46), cortisol 1.57 μg/dl (0.55-19.8 μg/dl), 17-OH progesterone 9.89 ng/ml (<20 ng/ml), androstenedione 1.15 ng/ml (0.3-3.3 ng/ml), anti-Mullerian hormone 0.04 ng/ml (<4.7 ng/ml for females)]. Gonadotropin levels were normal at the time of admission (Table I). Ultrasonography revealed a normal uterus and but no ovarian tissue, confirming previous findings. Karyotype was identified as 46,XX. Fluorescence in situ hybridization (FISH) analysis demonstrated no Y chromosome among 200 nuclei. The testis-determining gene (*SRY*) was not detected via the polymerase chain reaction (PCR). At 6th month of age, vaginoscopy and laparoscopy were performed in order to plan surgery and vagen, uterus, and ovaries were considered normal. In addition, gonadal biopsies were performed during the laparoscopy due to investigate the gonadal karyotype and for differential diagnosis of ovotesticular syndrome. The biopsy specimens from both gonads were histologically consistent with normal ovarian tissue (Fig. 1). The karyotype analyses of those specimens revealed 46,XX in both gonads.

The diagnosis of aromatase deficiency was considered according to these findings. Cytochrome p450 oxidoreductase deficiency can also be thought in similar circumstances, however, normal 17-OH progesterone levels are uncommon in this situation. Genetic analysis of the case revealed a compound heterozygous variant in *CYP19A1* [novel IVS10 + 1 G> A; novel p.R115Q (c.344 G> A)] (Fig. 2). *In silico* analyzes categorized the variants to be pathogenic. The mother and the father were heterozygous

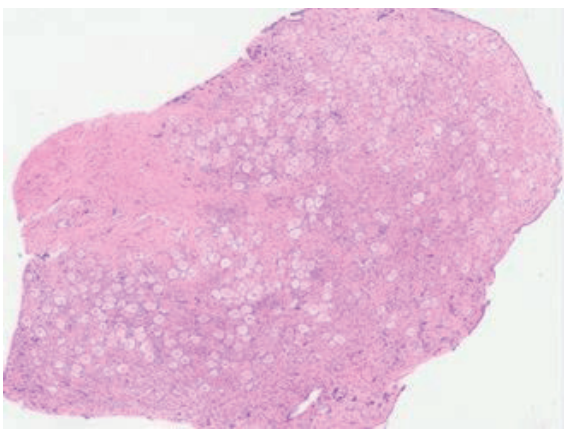

Fig. 1. Normal ovarian tissue histology from the biopsy specimens of both gonads.

Table I. Hormone levels of the case at different time points.

	16th day	4th month	6th month	8th month	11th month	17th month
FSH (mIU/ml)	6.4	7.02	36.3	18.7	27.9	75.1
LH (mIU/ml)	0.53	0.97	4.36	1.27	0.77	15.64
Estradiol (pg/ml)	5.0	<20	<20	<20	<20	<20
Total testosterone (ng/dl)	78	<10	<10	-	-	-

FSH: follicle-stimulating hormone, LH: luteinizing hormone.

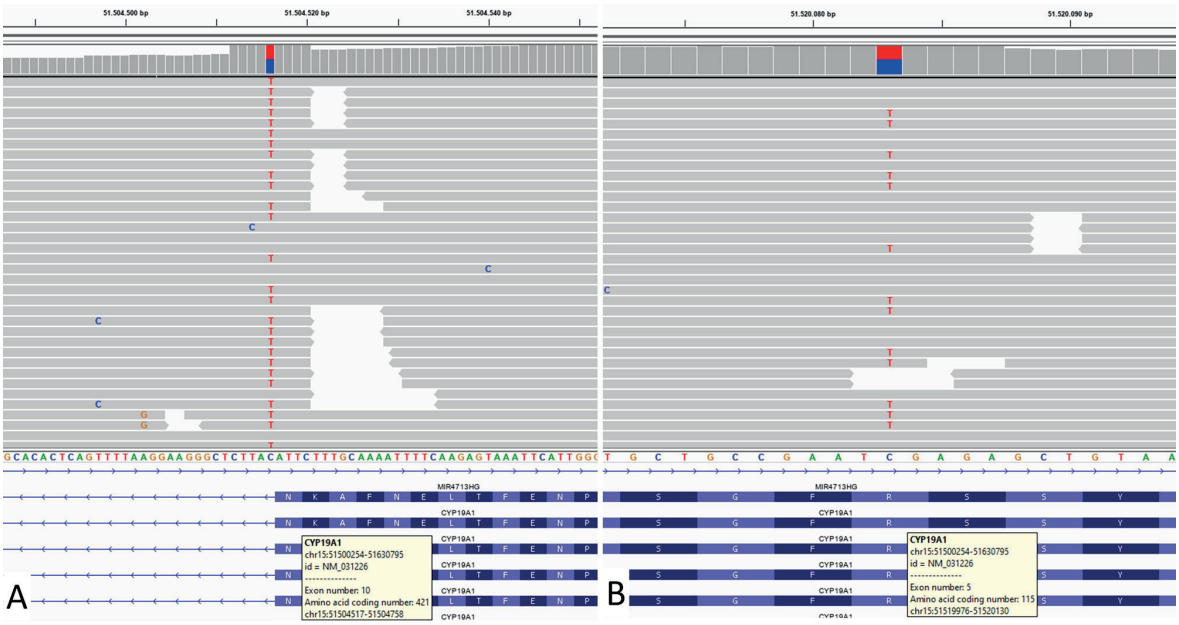

Fig. 2. Partial sequences of CYP19A1 of the patient demonstrating (a) heterozygous IVS10 + 1 G> A, (b) heterozygous c.344 G> A.

carriers of p.R115Q (c.344 G>A) and IVS10 + 1 G>A variant, respectively.

During the follow-up, the fusion at the posterior of the labium minus was surgically corrected. No ovarian cyst was observed with pelvic ultrasonography until the last visit at the age of 3 years. Her weight was 17.2 kg (1.6 SDS, 85-95p), and height was 100 cm (0.9 SDS, 75-85p) on the last visit (BMI 17.5 kg/m^2, 1.19 SDS, 85-95p). Written informed consent was obtained from the parents for publication of the case.

Discussion

Aromatase deficiency is a rare disease characterized by a decrement in estrogen synthesis, due to reduced aromatase activity. Until today, more than 32 variants in the *CYP19A1* gene, including missense, nonsense, small deletions and insertions, splice site variants, and one large intragenic deletion, have been described in patients with aromatase deficiency.[1,5-8] The majority of variants were missense and located in exons 9 and 10, which encode the substrate (androgen)-binding site and haem-binding domains, respectively.[5] In the current study, we identified a novel compound heterozygous variant in *CYP19A1* [IVS10 + 1 G> A; p.R115Q (c.344 G> A)]. In contrast to the previous cases reported from our country, the locations of variants detected in our case were different.[6-8] The missense variant (c.344 G> A) is localized in exon 4 and IVS10 + 1 G> A is a splice site defect localized in 10th intron of the *CYP19A1*.

Due to placental aromatase deficiency, the conversion of androgen into estrogen decreases, and therefore high levels of androgen circulates in maternal blood.[2] The mother with a fetus, who has aromatase deficiency, develops progressive virilization symptoms during pregnancy such as an increase in acne, voice thickening, clitoromegaly, frontal baldness, and facial hirsutism. These findings in the mother regress after birth.[2,9,10] Similarly, the mother of our patient developed virilization symptoms, including acne, hair loss, voice change, and hirsutism during pregnancy.

Aromatase deficiency results in varying degrees of virilization in the external genitalia in newborn girls due to high intrauterine androgen concentration. Gonads are non-palpable and

female internal genitalia differentiation was not affected. On the other hand, there is no change in the external genitalia in boys at birth. All infants born full-term with adequate weight for gestational age.[1,3,11,12] The birthweight of our patient was normal for gestational age (2710 grams at 35th gestational week). She had clitoromegaly, posterior labia minora fusion, and but normal uterus and ovaries.

In female cases with aromatase deficiency, changes in the hypothalamic-pituitary-gonadal axis cause some clinical issues. During childhood, both serum basal and GnRH-induced FSH-LH levels are expected to be elevated, starting from 2nd month of age.[1] However, the estradiol and estrone levels tend to be remarkably low during this same period.[9,11] In our case, gonadotropin levels were normal at the time of admission but started to increase by the age of 6 months. Excessive virilization and lack of the start of puberty are expected in affected females, with primary amenorrhea and also absence of breast development. The pubertal spurt is lacking and bone age delayed.[10-12] Increased intra-ovarian androgen levels and high concentrations of circulating gonadotropins usually cause large and polycystic ovaries from the infancy period.[1] However, in a total of six cases from four studies, hypoplastic or non-cystic ovaries have been reported. Gagliardi et al.[5] reported streak ovaries in a case and commented that it was due to estrogen treatment before the diagnosis. Lin et al.[10] reported a case with streak ovaries and suggested that the streak ovaries may be a constitutional presentation of *CYP19A1* deficiency. Other patients from two reports (age, 19 months-7 years of age) had not received estrogen treatment, and no cystic structure was noted.[6,7] When the laparoscopy was required for our patient, it was considered to take a sample from the ovary in terms of the possibility of ovotestis.[13] Although a normal gonadal appearance was seen, this would not always indicate a normal histology as a demarcation line was not remarkable in some patients with ovotestis described in literature.[14] Unlikely to most of the patients with aromatase deficiency, the biopsy specimens from both gonads of the present case were histologically consistent with normal ovarian tissue, and no cystic structure developed during the follow-up. Thinking retrospectively, we would not need to perform an ovarian biopsy if a genetic diagnosis could be made earlier.

Treatment in aromatase deficiency consists of estrogen replacement in girls; however, there is no concurrence on the dosage or age of beginning. Estrogen treatment normalizes bone maturation, gonadotropin secretion with a feedback mechanism.[9,15,16] It is also useful for glucose and insulin metabolism and decreases lipid levels.[9,16] We have not started estrogen replacement yet, as our case did not have any ovarian cysts or metabolic syndrome.

In conclusion, we presented a case with aromatase deficiency caused by a novel variant in the *CYP19A1* gene. Aromatase deficiency should be considered in children with ambiguous genitalia who have a history of maternal virilization during pregnancy and karyotype of 46,XX, even if the initial FSH and LH levels are normal and ovarian cysts are lacking.

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Case Report

A rare cause of hepatomegaly and dyslipidemia: lysosomal acid lipase deficiency

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ABSTRACT

Background. Lysosomal acid lipase deficiency (LAL-D), also known as cholesteryl ester storage disease or Wolman disease, is a multi-systemic autosomal recessive genetic disorder caused by mutations in the lysosomal acid lipase gene (*LIPA*).

Case. A 14-year-old female patient was diagnosed as LAL-D with the findings of hepatomegaly, splenomegaly, elevated liver enzyme levels, and abnormal lipid profile. Her sister had similar laboratory and ultrasonographic findings. Both siblings had a homozygous c.894 G>A mutation in the *LIPA* gene, and their parents were heterozygous for this mutation.

Conclusions. This case is one of the similar reports in the literature regarding clinical, biochemical, and genetic findings. It is well-known that LAL-D has overlapping clinical manifestations, and early diagnosis is quite challenging. Therefore, most patients die in the first year of life. After the determination of novel mutations in LAL-D patients, it is thought that LAL-D can present with heterogeneous signs and symptoms.

Key words: Lysosomal acid lipase (LAL), Lysosomal acid lipase deficiency (LALD), cholesterol ester storage disease (CESD).

Lysosomal acid lipase deficiency (LAL-D), also named as cholesteryl ester storage disease and Wolman disease, is a multi-systemic autosomal recessive genetic disorder caused by mutations in the lysosomal acid lipase gene (*LIPA*) that encodes the enzyme lysosomal acid lipase (LAL).[1,2] LAL-D is responsible for the accumulation of triglycerides (TG) and progressive cholesteryl esters (CE) in most cells, including liver and spleen.[3] LAL-D is historically divided into two chief clinical phenotypes: early-onset Wolman disease (WD) and late-onset cholesteryl ester storage disease (CESD).[4] WD is a rare and rapidly progressive form that unveils in the first week of life. It leads to severe early-onset disorders, including chronic malnutrition, hepatosplenomegaly, adrenal calcification, steatorrhea, emesis, and multiorgan failure.[5] Most of the patients with WD die within the first year of life. On the other hand, CESD is an unrecognized form that manifests later (between 3-15 years of age). It also leads to a broad spectrum of clinical manifestations, including accelerated atherosclerosis, hepatic steatosis, hepatosplenomegaly, and dyslipidemia. The incidence of LAL-D is estimated to be between 1/40.000 and 1/300.000.[6]

Additionally, a 36 kb *LIPA* gene is located on chromosome 10q22.2 and contains 10 exons. According to the literature, more than 60 *LIPA* defects have been identified in LAL-D patients, and new studies are still describing other mutations. The most common *LIPA* defect is c.894G >A.[3] On the other hand, progressive TG and CE accumulation is associated with characteristic liver damage, increased liver transaminases, serum low-density lipoprotein cholesterol (LDL-c) and triglycerides, and normal/low high-density lipoprotein cholesterol (HDL-c) levels.[2] Many patients with

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LAL-D suffer from accelerated atherosclerosis, hepatosplenomegaly, cirrhosis, liver fibrosis, and liver failure.[7] Enzyme activity assays from dried blood, and confirm by genetic sequencing in whole blood are the most preferred diagnostic techniques.

In this study, we report siblings with clinical and biochemical aspects of LAL-D with homozygous c.894G>A mutation in the *LIPA* gene.

Case Reports

A 14-year-old female patient was admitted to our hospital with abdominal distension due to hepatomegaly. The proband had a birth history of normal weight and height. Her family history revealed that the parents were first-degree cousins. On physical examination, the patient had a weight of 55.2 kg [0.27 standard deviation (SD)], a height of 148.4 cm (-2.02 SD), the liver was about 1 cm palpable, and other systemic examinations were normal.

The initial laboratory tests showed increased liver enzymes, including alanine aminotransferase (ALT) (63.1 IU/L; reference value < 50 IU/L), aspartate aminotransferase (AST) (53.8 IU/L; reference value < 50 IU/L), gamma-glutamyl transferase (GGT) (48 IU/L; reference value < 40 IU/L), and lactate dehydrogenase (LDH) 195 U/L (reference value < 247 IU/L). Furthermore, her creatine kinase (CK) level was 85 U/L (reference value < 145 U/L) total bilirubin value was 1.48 mg/dl (reference value < 1.2 mg/dl) and direct bilirubin value was 0.15 mg/dl (reference value < 0.2 mg/dl). Dyslipidemia was detected in addition to elevated liver enzymes. Her serum lipid profile findings showed a total cholesterol (TC) level of 280 mg/dl, a low-density lipoprotein (LDL) level of 210.5 mg/dl, and a high-density lipoprotein (HDL) level of 39.5 mg/dl, triglyceride level of 295 mg/dl which were compatible with dyslipidemia.

The abdominal ultrasound demonstrated grade 1 hepatosteatosis with hepatomegaly and splenomegaly (Fig. 1). There were no pathological findings on the echocardiography evaluation of the patient. At this stage of the investigation, LAL-D was suspected. The LAL activity was measured from a dried blood spot card, and LAL-D was diagnosed by verifying low acid lipase activity (<0.02 nmol/punch/hr; reference range: 0.37-2.30). Confirmatory diagnosis of LAL-D was performed with genetic sequencing, which showed a pathological homozygous mutation in c.894G>A. After the diagnosis of LAL-D was confirmed, her three year old sister who had the same complaints was evaluated. It was detected that the younger sister had similar laboratory and ultrasonographic findings. Her height was 91.8 cm (-0.9 SD), and her weight was 15.2 kg (0.56 SD). Physical examination was normal except for about 2 cm palpable liver. She also had increased liver enzymes (ALT: 56.1 IU/L, AST: 79.7 IU/L), and dyslipidemia (TC: 279 mg/dl, LDL: 218 mg/dl, HDL: 41 mg/dl, and triglyceride: 111 mg/dl). Furthermore, her abdominal ultrasound was compatible with hepatomegaly and splenomegaly (Fig. 1). She had a normal echocardiography evaluation.

Both siblings had a homozygous c.894G>A mutation in the *LIPA* gene, and their parents

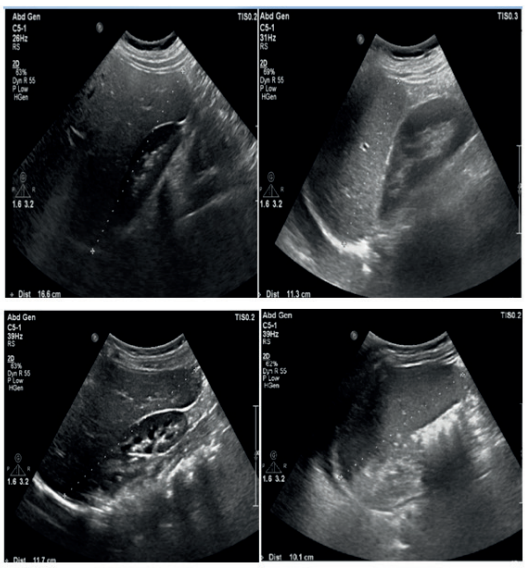

Fig. 1. Ultrasound images showing the maximum lengths of patients' (a:index case, b:sibling) liver and spleen.

were heterozygous for this mutation (Fig. 2A, 2B). However, there were no phenotypical changes in both siblings.

A low-fat diet and omega-3 supportive therapy were started both patients. We planned to start Sepelipase alpha enzyme treatment, which has proven efficacy in LAL-D patients. However, the drug could not be obtained due to the health practices policy in our country. In addition, statin therapy was added to the proband patient due to dyslipidemia. Despite the treatments applied in the follow-up of the patients, no positive response was observed, especially in terms of dyslipidemia, and liver enzyme elevations also persisted.

The parents of the patient were informed, and written and oral consent was obtained according to the principles of the Helsinki Declaration.

Discussion

LAL-D is known as a rare lipid storage disease with early mortality and significant morbidity. Its incidence is higher among individuals with Iranian–Jewish ancestry (1:4200).[8] The natural course of the late-onset form of the disease results in inflammation, and the findings from the hepatomegaly and splenomegaly biopsy specimens are indicative of CESD.[9] There is a wide range of signs and symptoms of CESD, including vomiting, diarrhea, low level of HDL cholesterol, liver steatosis, growth failure, hypercholesterolemia, as well as elevated AST, ALT, and GGT levels.[10] Additionally, according to the Human Gene Mutation Database, the most common *LIPA* mutation in CESD patients is c.894 G>A, which is a *LIPA* gene exon 8 splice junction mutation.[6]

In this study, we presented a patient with late-onset LAL-D, who had clinical, enzymatic, and lipid profile abnormalities. Increased AST, ALT, GGT, TC, LDL, together with decreased HDL and acid lipase activity, were detected in our patient. An exon 8 c.894G>A *LIPA* gene mutation was confirmed. Due to its low incidence in society and having similar signs and symptoms with other diseases, LAL-D is hard to diagnose.[7] Some studies suggested that LAL enzymatic activities should be measured in patients with abnormal lipid and enzyme profiles such as elevated LDL, TG, TC, AST, ALT, and reduced HDL.[6,7,11] Similar reports and suggestions are available in the literature. Kuranobu et al.[9] reported a case of an 11-year-old male CESD patient with abnormal lipid and

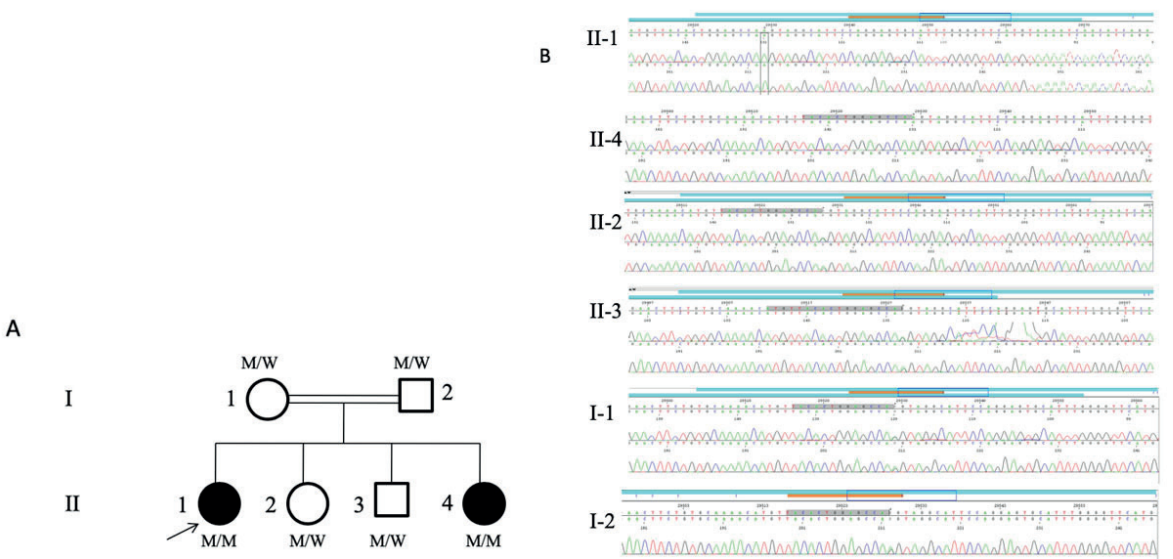

Fig. 2A, 2B. Pedigree and sequence analyses of family. Both siblings (II-1, II-4) homozygous, parents and other two siblings (I-1, I-1, II-2, II-3) were heterozygous for this mutation.

enzyme profiles and a novel mutation in the *LIPA* gene.

Similarly, Benevides et al.[12] reported a case series with seven patients with LAL-D (both WD and CESD). They detected abnormal lipid and enzyme profiles in all patients, as well as other expected symptoms. Additionally, diagnostic images such as liver/spleen ultrasound (US) and biopsy are necessary to show the changes in organ morphology. As seen in our case, hepatomegaly and splenomegaly were detected by ultrasonography. Hepatic morphological changes such as microvesicular steatosis with Kupffer cell involvement, fibrosis, and cirrhosis were reported by other researchers.[10,12-14]

On the other hand, homozygous *LIPA* gene mutation of c.894 G>A has been detected in more than 50% of patients with late-onset LAL-D. The same gene mutation was also detected in our patient. This mutation occurs in exon 8 of the *LIPA* gene that encodes a mutant enzyme with no residual LAL activity.[15] However, some researchers reported novel mutations in LAL-D patients such as c.607 G>C, c.791 T>C, c.266 T>C, and c.67 G>A.[9,12]

In conclusion, this rare disease should be suspected in presentations with hepatomegaly, fatty liver, and dyslipidemia. Our case is one of the similar reports in the literature regarding clinical, biochemical, and genetic findings. It is well-known that LAL-D has overlapping clinical manifestations, and early diagnosis is quite challenging. Therefore, most patients die in the first year of the disease. After the determination of novel mutations in LAL-D patients, it is thought that LAL-D can present with heterogeneous signs and symptoms, which might facilitate making an accurate diagnosis.

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Case Report

High-grade neuroepithelial tumor with medulloepithelioma-like areas out of the central nervous system in an infant with hemihypertrophy: a unique association

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ABSTRACT

Background. High-grade neuroepithelial tumor with areas resembling medulloepithelioma was diagnosed in an infant with coccygeal and inguinal masses. Hemihypertrophy is associated with Wilms tumor, hepatoblastoma and pancreatic tumors in children.

Case. The authors report on the first case of peripheral HNET associated with hemihypertrophy in an infant, with special discussion on histopathological differential diagnosis and management of this rare and highly malignant tumor.

Conclusions. HNET should be included into the list of hemihypertrophy associated tumors. Complete surgical excision with free margins is essential for the successful treatment of such cases and should be tried in suitable cases at the time of diagnosis. Continued treatment should be decided individually on a case to case basis.

Key words: high-grade neuroepithelial tumor, medulloepithelioma, inguinal, coccygeal, tumor, hemihypertrophy, infant.

The differential diagnosis of an inguinal mass in childhood include lymphadenitis, inguinal hernia with or without incarceration, spermatic cord cyst in boys and cyst of canal of Nuck that is its equivalent in girls, undescended testis, extravaginal testicular torsion and metastatic lymph node. Metastasis to inguinal lymph nodes may occur in lymphoma, rhabdomyosarcoma, testicular tumor and tumors of the sacrococcygeal area. Interestingly, we found a few cases with similar presenting locations which were diagnosed as peripheral medulloepithelioma but did not found a case with peripheral high-grade neuroepithelial tumor (HNET) associated with hemihypertrophy.

Hemihypertrophy is a predisposing abnormality for Wilms tumor and hepatoblastoma. The risk of Wilms tumor development is 3-4% in children with hemihypertrophy. To the best of our knowledge, HNET or medulloepithelioma associated with hemihypertrophy has not been reported previously.[1]

Authors report on an infant with this unique association of peripheral HNET and hemihypertrophy, and make special emphasis on histopathological differential diagnosis and management of this rare and highly malignant tumor.

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Case Report

A 16-month-old boy was admitted for swelling in the right groin of one-week duration. It was painless, not enlarging and not disappearing. Past medical history was unremarkable except buried penis anomaly.

Physical examination revealed a firm, immobile, non-tender mass of 3x4cm size in the right groin. An additional mass of 3x2.5cm size was found located close to the coccyx. It was firm, fixed and not extending to the rectal wall on digital rectal examination. Femoral part of the right lower extremity was larger when compared to the corresponding proximal part of the left lower extremity and accepted as partial hemihypertrophy. Both testicles were normal on palpation and in the scrotum. The remaining findings on physical examination were normal.

Complete blood count (hemoglobin; 10.9 g/dl, leukocytes; 9300/ml, platelets; 346.000/ml) revealed mild anemia, blood biochemistry was in normal ranges. Alpha-fetoprotein level was mildly elevated (11.74 ng/ml, N: 0-9) and beta-human chorionic gonadotropin level (<1.2 mIU/ml, N<5) was within normal limit. Urine catecholamine metabolites levels (VMA; 12.86 mg/g creatinine, N<18.8, HVA; 38.9 mg/g creatinine, N<32.6, 5-HIAA; 22.1 mg/g creatinine, N: 1.2-16.2) revealed mild elevation of HVA and 5-HIAA.

Ultrasound (US) examination of the inguinal region revealed a mass (3.6 x 2.7 x 3 cm) with solid and cystic component in the right groin. Neck and abdominal US showed normal findings. Magnetic resonance imaging (MRI) showed two similar masses with composed of cystic and contrast-enhanced solid areas in the right inguinal region and in the coccygeal region (Fig. 1). MRI also revealed that subcutaneous fat tissue and muscles of the right lower extremity was hypertrophic.

At surgery, coccygeal mass (3 x 2.5 cm) was totally excised together with the coccyx (Fig. 2). The tumor mass was extending into the

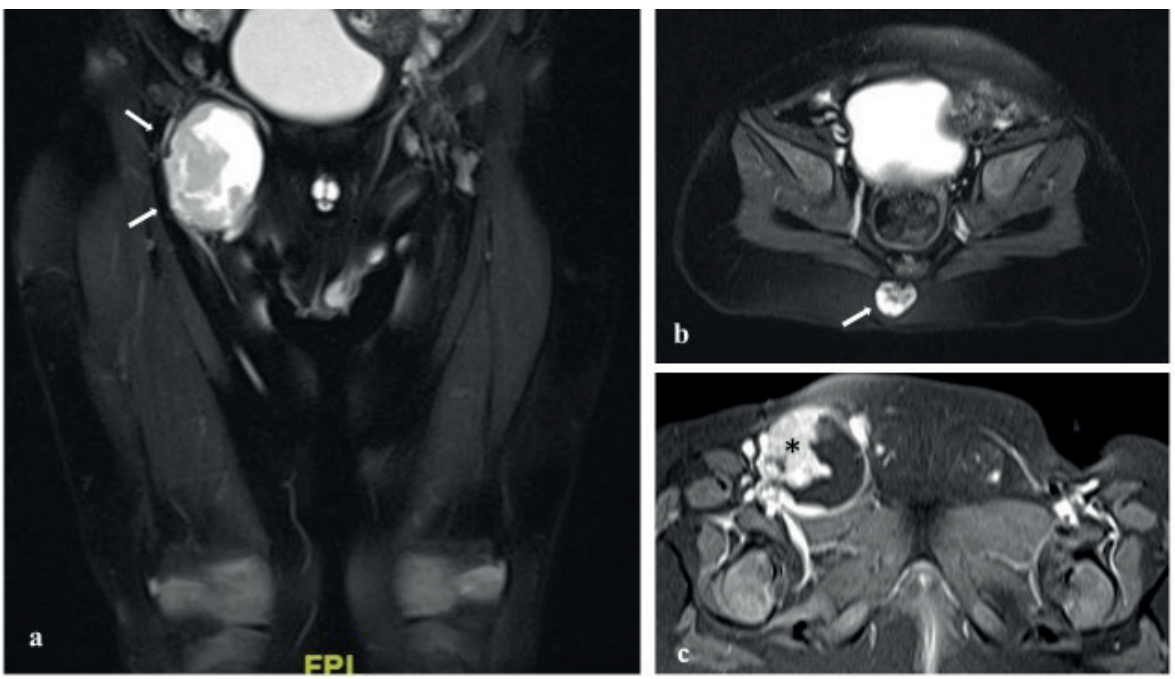

Fig. 1. Coronal (a) and axial (b) fat saturated T2-weighted MR images show radiologically similar masses (arrows) containing solid and cystic areas in the right inguinal region (a) and in the coccygeal region (b). Axial fat saturated post-contrast T1-weighted MR image (c) shows contrast-enhanced solid component (*) of the inguinal mass.

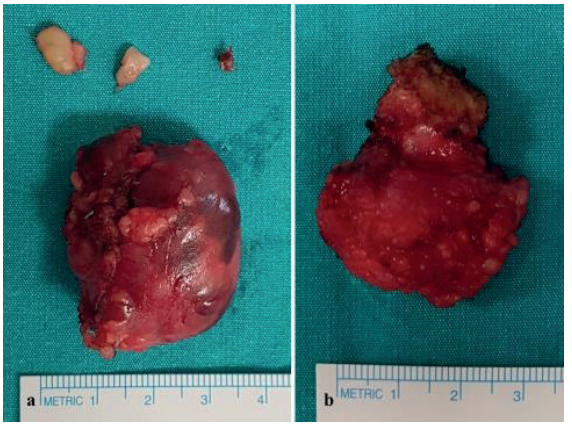

Fig. 2. Macroscopic view of inguinal tumoral mass with lymph nodes (a) and coccygeal tumoral mass (b).

gluteal muscles, well circumscribed and firm. The inguinal tumor mass was located in the femoral region, well circumscribed, firm and firmly attached to the great saphenous vein and femoral vein. The inguinal mass (3 x 4 cm) was apparently larger than the coccygeal mass and it was totally excised including adjacent two lymph nodes (Fig. 2). The margins of both operative areas were marked with hemoclips. Postoperative course was complicated by wound infection in the coccygeal area. It was managed by antibiotics and intensive wound care and healed in a week.

Histopathological examination of coccygeal mass showed stellated/spindle cells arranged into cords or scattered individually, and epithelioid cells composed multi-layer rosettes or papilla in a myxoid background (Fig. 3). Neoplastic cells had monotonous appearance and contained nuclei with multiple nucleoli, and contained clear chromatin especially in tubular areas. Remnant of embryonal notochord was encountered in a focus in the cartilage tissue. Inguinal mass showed similar histopathological findings with coccygeal mass and neoplastic tubules displayed neural tube-like organization. Tubules contained marked pseudo stratification and apical mitotic activity. Neoplastic cells stained diffusely with PGP9.5, EMA, and SALL4; focally positive with GFAP, Glipican-3, CD56 and S100; and negative with OCT-3/4, WT1, pan-keratin, PLAP, alpha-fetoprotein (AFP) and CD30 (Fig. 4). Ki67 proliferation index reached to 50% by focally. Staining with SMARCA4, NSE and synaptophysin were not informative. Marked membranous staining with LIN28A was encountered in the apical region of multi-layered rosettes. Loss of INI1 was not encountered. Surgical margins were clear in both samples.

Histopathological material was consulted with extra institutional pathologists. They reported that the tumor had two distinct components: a low-grade component with glial and ganglion cell differentiation, which predominated in sections of the sacrococcygeal excision and

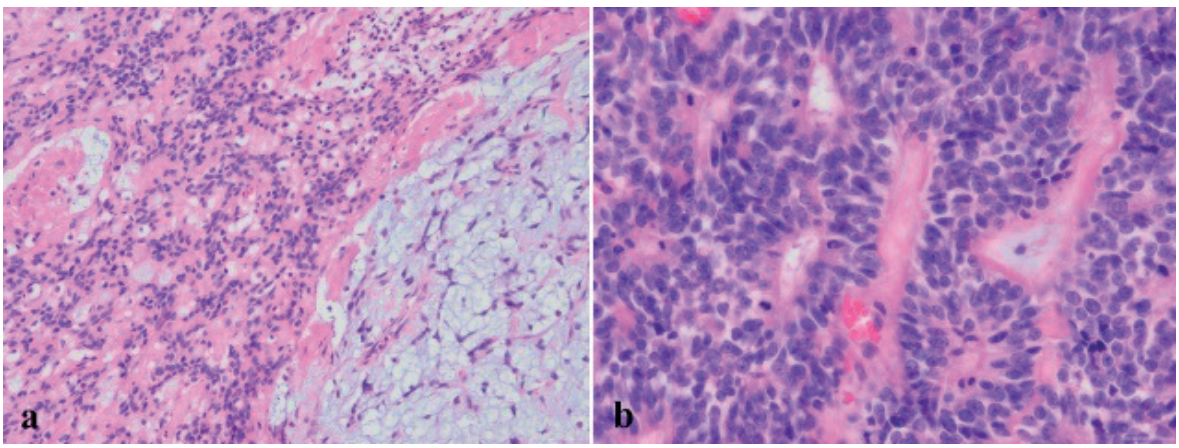

Fig. 3. Tumor cells showing diffuse infiltrative pattern (left side) and myxoid area (right side) (HE, X400) (a), tumor cells presenting rosette formation and mitotic figures (HE, x400) (b).

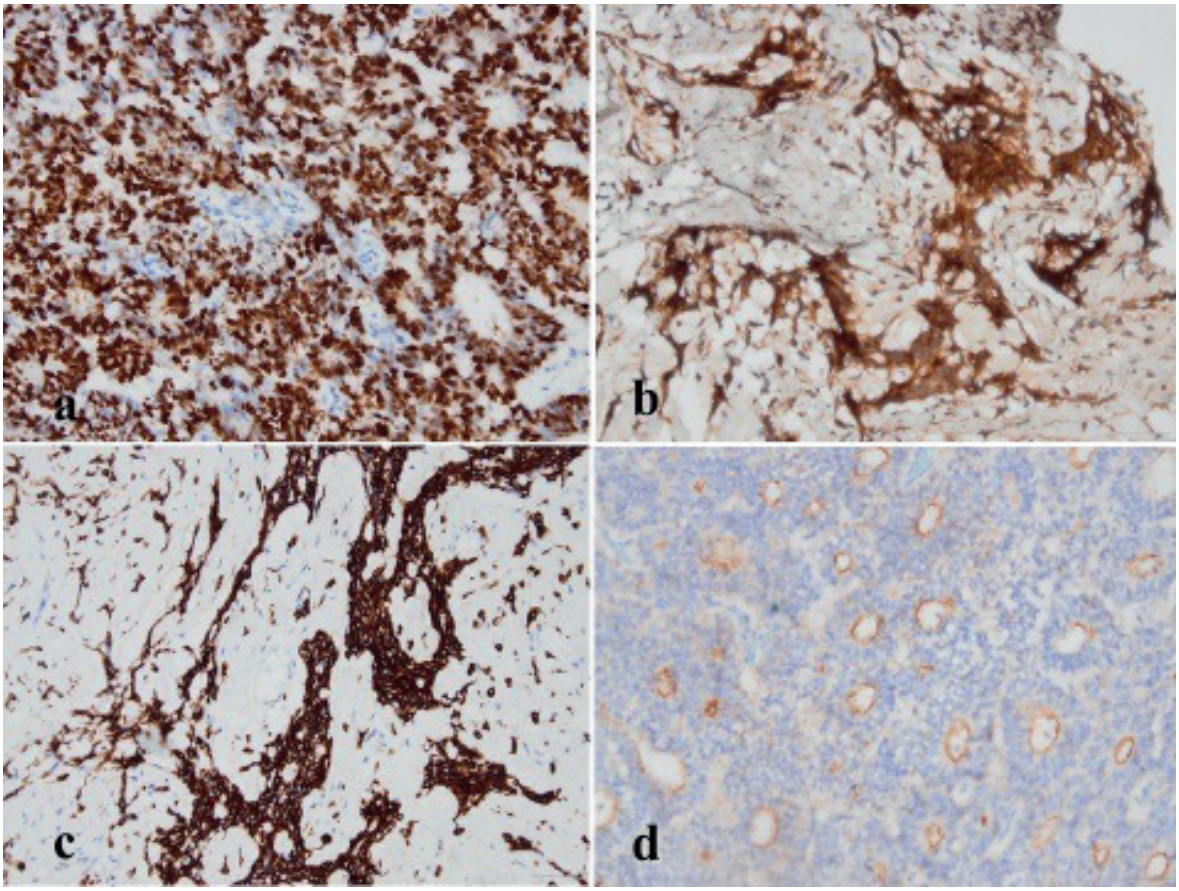

Fig. 4. Neoplastic cells showing nuclear staining positive with SALL4 (x200) (a), PGP9.5 (x100) (b), GFAP (x100) (c), and LIN28A (x100) (d).

characterized by monomorphic ovoid cells with low-grade nuclear atypia, low cellular density and low proliferation activity; and a high-grade neuroepithelial component, which was the predominant feature in the inguinal excision and characterized by hyperchromatic neuroepithelial cells with papillary and rosette-like structures. The presence of lymphoid tissue around the tumor in the inguinal resection suggested that this was the site of nodal involvement. Both resection margins were negative for tumor. FISH for chromosome 19 microRNA cluster (C19MC) (19q13.41) was negative for amplification. They made the diagnosis of HNET arising in a low-grade CNS-type glioneural neoplasm for sacrococcygeal mass and metastatic HNET for the inguinal mass.

The child received 6 cycles of chemotherapy composed of Cisplatin and etoposide. Cisplatin was replaced by carboplatin to avoid toxicity and additional 2 cycles of carboplatin and etoposide were given. Control abdominal MRI in the 5th and 11th postoperative months showed postoperative signal changes in the inguinal and coccygeal areas without recurrent lesion. Late (postoperative 7th month) PET revealed no activity in the paraaortic lymph nodes, inguinal area and inguinal lymph node but decreased activity in the coccygeal operative fields. The patient has been still under follow up without recurrent or metastatic disease for 18 months.

Informed consent was received from the family.

Discussion

This case is an interesting case due to simultaneous presence of inguinal and coccygeal masses, challenges in the histopathological differential diagnosis and presence of associating hemihypertrophy.

Naturally, the histopathological differential diagnoses for a tumor in the coccygeal region would include a PNET-like teratomatous tumor as well as a myxopapillary ependymoma. Morphologically, however, the features do not fit well for either diagnosis. In addition, despite the diffuse expression of SALL4 in the high-grade component of the tumor, there were no teratomatous elements and no immunohistochemical expression of more specific germ cell tumor markers, such as PLAP, AFP, OCT3/4 (per initial pathological report) or OCT2. Both consultants of pathology believed that the morphological features were more in line with a CNS-type neuroepithelium than a peripheral neuroectodermal tumor, despite the lack of spinal canal connection. The possibility of an embryonal tumor with multilayered rosettes and medulloepithelioma has been raised. According to the consultant, the morphological features are not entirely classical for either entity. In addition, FISH for chromosome 19 microRNA cluster (C19MC) (19q13.41) was negative for amplification, providing no support for the diagnosis.

The histopathological findings of the tumor revealed morphological similarities to medulloepithelioma and embryonal tumor with multilayer rosettes, but negative for C19MC. Final diagnosis was given as HNET with areas resembling to medulloepithelioma.

Detailed search for simultaneous presence of inguinal and coccygeal masses has also led us to focus on a few cases of peripheral medulloepithelioma (Table I a and b).[2-10]

Medulloepithelioma is a rare embryonal tumor characterized by tubular and papillary patterns like primitive epithelium of the neural tube and medullary plate. It occurs usually in the eye or in the central nervous system and peripheral location has been reported quite rarely. Intraocular medulloepithelioma (diktyoma) is a benign tumor and complete excision is

Table Ia. Clinical characteristics of children with neuroepithelial tumor around the coccygeal region.

Case no	Author, year	Age, sex	Primary and metastatic location(s) at presentation	Tumor size (cm)	Associated abnormality
1	Seemayer[2], 1975	5.5 y, F	Presacral	?	?
2	Nakamura[3], 1982	Birth, M	Course of sciatic nerve, dorsum of right foot, right leg mass	?	?
3	Figarella-Branger[4], 1992	17 y, F	Presacral	?	?
4	Bruggers[5], 1999	Term, F	Pelvic	NA	Complete absence of left hemipelvis and left kidney
5	Donner[6], 2003	12 y, F	Pelvic	19x9x1.5, 19x14x9	Not present
6	Somjee[7], 2004	3.5 y, M	Presacral, lung and liver metastases	5x5cm	Not present
7	Pillai[8], 2008	3 y, F	Presacral, inguinal LAP	6x4.5x3	Not present
8	De Pasquale[9], 2014	3 y, F	Presacral	7x6x6	Not present
9	Honnorat[10], 2019	2 y, F	Presacral	6.3x5x5.7	Not present
10	Karnak, 2019	16 m, M	Coccygeal, inguinal LAP	3x2.5, 3x4	Hemihypertrophy and buried penis

Y: year, m: month, F: female, M: male, LAP: lymphadenopathy, NA: not available.

Table Ib. Treatment and outcome in children with neuroepithelial tumor around the coccygeal region.

Case no	Histopathological diagnosis	Treatment	Outcome	
1	Medulloepithelioma	Surgery, RT, CHT (after metastases)	Local recurrence and liver metastases after 2 m	DOD 8 m after diagnosis
2	Medulloepithelioma	Surgery (right hemipelvectomy)	7 y	NED
3	Medulloepithelioma	Surgery, multiagent CHT and RT (after metastases)	Lung metastases 6 weeks after surgery	DOD 8 m after diagnosis
4	Medulloepithelioma	Surgery (subtotal resection), CHT	50 m	NED
5	Medulloepithelioma	Surgery (2 times debulkings), CHT	2 y	NED
6	Medulloepithelioma	CHT	8 m	On treatment
7	Medulloepithelioma	Surgery (total tumor and coccyx resection, lymph node biopsy, CHT, RT	5 m	NED
8	Medulloepithelioma	Biopsy, CHT, Surgery (total excision), RT	Local recurrence 6 m later, total reexcision, target therapy	DOD 5 m after recurrence
9	Medulloepithelioma	CHT, Surgery (total excision)	5 y	NED
10	HNET containing medulloepithelioma- like areas	Surgery (total excision), CHT	20 m	Under follow up

HNET: high-grade neuroepithelial tumor, CHT: chemotherapy, RT: radiotherapy, DOD: died of disease, NED: no evidence of disease.

frequently enough for cure. In contrast, CNS medulloepithelioma is an aggressive neoplasm and good prognosis is possible only in cases with complete excision of the tumor.[11]

Our case with the diagnosis of peripheral HNET has unique features such as the larger size of the inguinal metastasis than coccygeal primary neoplasm and the presence of hemihypertrophy. Hemihypertrophy is an alarming finding on physical examination and prompts urinary and hepatobiliary evaluation by ultrasound for Wilms tumor and hepatoblastoma respectively.[1] Hemihypertrophy may also associate with benign lipomatous tumors in children.[12] Although the exact mechanism could not be established, HNET should be included in the list of neoplasms associated with hemihypertrophy. The larger size of inguinal metastasis may be also explained by the increased blood flow to the right inguinal area along with hypertrophied proximal part of the right lower extremity.

The tumor location close to the coccyx may be due to tumor origin of multipotent cells located around the coccygeal region in the embryological life. There was no tumoral mass in presacral and abdominopelvic regions by detailed radiological examination, which is also an interesting finding in this patient.

HNET is a malignant tumor and may metastasize to the inguinal lymph nodes. The authors agree that complete excision of the tumor should be tried in suitable cases (no distant metastasis, no possibility of increased surgical morbidity). Multiagent chemotherapy is necessary for the treatment of peripheral HNET. Close follow up is essential as the clinical course is difficult to predict. One may argue the need of radiotherapy in such a case. The authors decided not to give radiotherapy in this case with safe surgical margins and potential side effects of radiotherapy in two different locations.

The authors presented a unique case of peripheral HNET in an infant with hemihypertrophy. HNET should be included into the list of hemihypertrophy associated tumors. Complete surgical excision with free margins is essential for the successful treatment of such cases and should be tried in suitable cases at the time of diagnosis. Decision making on the treatment of such cases are difficult due to rarity of the disease. We believe surgery, multiagent chemotherapy and radiotherapy must be decided individually in each case, then close follow up is necessary.

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Case Report

Temporal bone hemangioendothelioma as a rare vascular tumor in childhood: case report and review of the literature

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ABSTRACT

Background. Hemangiondothelioma is a rare vascular tumor that can occur in the bone. Temporal bone involvement has been reported extremely rare in the literature.

Case. Radiological examination of a one-year-old girl who was admitted due to facial paralysis revealed vascular tumor of the temporal bone and Galen vein aneurysm. Pathological examination showed retiform hemangioendothelioma. She was treated with propranolol, prednisolone, vincristine, and endovascular embolization followed by oral sirolimus. With sirolimus treatment, a partial response was obtained first, then the tumor remained stable and sirolimus treatment was discontinued. No progression was observed in the disease after discontinuation of treatment.

Conclusion. In this article, a case of hemangioendothelioma originating from the temporal bone is discussed in the light of other case reports in the literature.

Key words: hemangioendothelioma, temporal bone, galen vein aneurysm, sirolimus, childhood.

Primary vascular tumors of the bone are rare and consist of a wide range of different clinicopathological entities, from benign lesions to malignant tumors. Bone hemangioma is the most common benign vascular bone tumor. Vascular tumors vary from local aggressive tumors such as hemangioendothelioma to malignant tumors such as angiosarcoma.[1]

Hemangioendothelioma is the term used to name for vascular neoplasms that show a borderline biological behavior, intermediate between entirely benign hemangiomas and highly malignant angiosarcomas.[2] Hemangioendothelioma can occur at any age, but is more frequently seen in adults. These lesions mainly affect the long bones; more than half of them are located in the tibia or femur. Temporal bone involvement of hemagioendothelioma is extremely rare in the areas of the involved bone.

In this report, we present a child with hemangioendothelioma, a rare vascular tumor of the temporal bone, and a galen vein aneursym.

Case Report

An otherwise healthy one-year-old girl was admitted to our department with acute onset right peripheral facial nevre palsy for 10 days. According to the history obtained from her parents, 10 days prior her parents realized that she could not close her right eye while crying. She had no otalgia or otorrhea complaint at the time. On physical examination, the head was slightly asymmetric with a right temporal prominence, narrowing of the right external auditory canal, and right facial paresis. There was no pain in the lesion. There was no history of paresthesias and neurological deficit or similar lesions on other parts of the body. It was firm

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and non compressible. There was no regional lymphadenopathy, and the patient did not have any history of constitutional symptoms such as fever, weight loss or loss of appetite. Total blood count and biochemical analysis were within normal range. Cranial computed tomography (CT) scan showed expansile and lytic lesion on the right temporal bone (Fig. 1A). Cerebral magnetic resonance imaging (MRI), inner ear MRI and cerebral MRI angiography revealed fluid-fluid levels in the right mastoid cells and lytic lesion with pronounced hypervascular feature in an expanding character (Fig. 1 B). Anterior venous malformation between galen vein and posterior cerebral artery and also dilated feding and draining vessels suggesting galen vein aneursym was seen in cerebral MRI (Fig. 1C). Temporal bone biopsy was done. Histopathologic examination of the mass showed narrow arborizing vascular channels forming a retiform pattern and spindle cells which were focally obliterating the vessel wall. The vessels were lined by monomorphic hobnail-like endothelial cells, without significant pleomorphism. There were no mitoses and necrosis. Immunohistochemically, the tumor cells stained with CD31, CD34, and FLI-1 antibodies (Fig. 2. A,B,C,D,E). The diagnosis were compatible with retiform (intermediate grade) hemangioendothelioma.

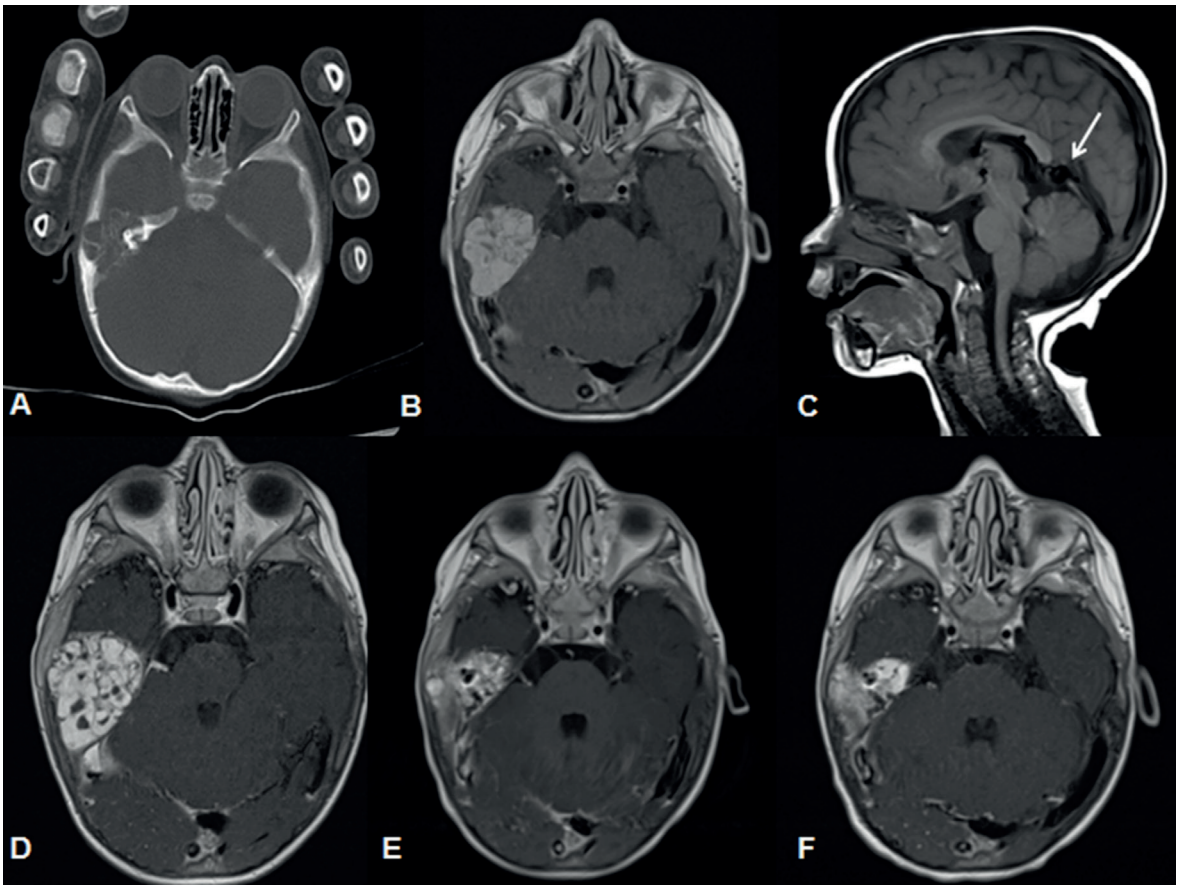

Fig. 1. (A): CT scan shows expansile and lytic lesion on the right temporal bone, (B): Contrast enhanced T1 weighted MR scan shows diffuse contrast enhancement of the lesion, (C): Sagittal view T1-weighted MR scan shows the enlarged median prosencephalic vein of Markowski, characteristic of vein of Galen aneurysmal malformation (*arrow*), (D): Contrast-enhanced T1-weighted MRI shows progression of the mass that completely fills mastoid air cells, (E): Contrast enhanced T1 weighted MR scan shows regression in mass size after embolization, (F): contrast enhanced T1-weighted MRI shows 9 months after sirolimus treatment minimal regression of mass size.

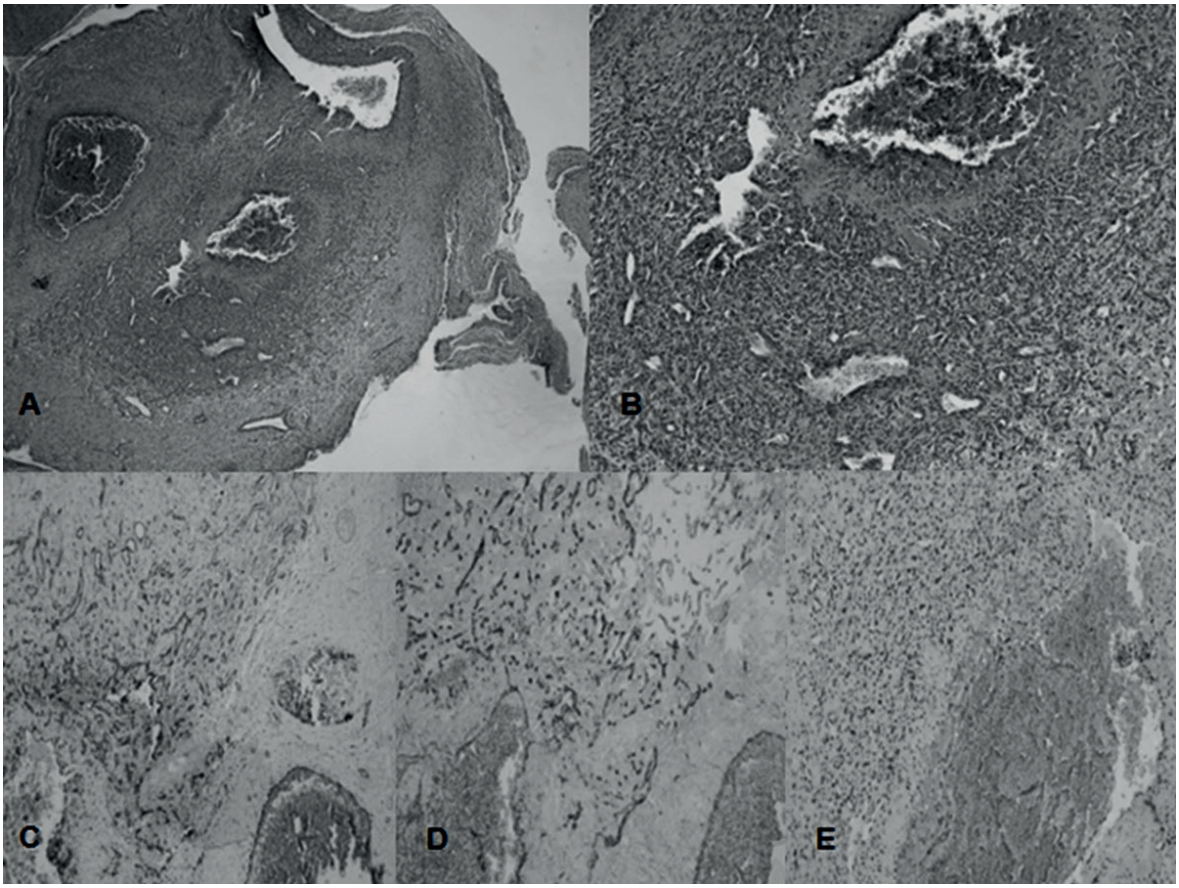

Fig. 2. (A): Tumor cells were obliterating the vessel wall (HE×100), (B): Tumor cells showed mild pleomorphism. Mitoses and necrosis were absent (HE×200), (C): Tumor cells showed staining with CD31, (CD31×100), (D): Tumor cells showed staining with CD34, (CD34x100), (E): Tumor cells showed diffusely staining with FLI-1 (FLI-1x200).

Oral propranolol was started at a dose of 2 mg/kg/day in two doses. One month after propranolol treatment, prednisolone was added to the treatment because of no significant change in the size of the mass on physical examination and minimal radiological progression. But her facial paralysis improved slightly. After one month follow-up period, cranial and inner ear MRI showed progression of the lesion, weekly vincristine (1.5 mg/m^2) treatment was added and embolization treatment was planned for the hemangioendothelioma (Fig. 1D). After 5F intraducer placement in the right femoral artery, the right anterior carotid artery and the outer carotid artery were reached, and the vascularity of the hypervascular lesion in the temporal bone and feeding vessels were detected, and embolization was performed from the two feeders (Fig. 3. A,B). After embolization, there was a slight decrease in the size of the lesion, there was a poor response to medical treatment (Fig. 1E). The treatment was switched to oral sirolimus. Sirolimus was started at 0.8 mg/m^2 per dose twice daily with plasma level monitoring target of 10–15 ng/ml. After nine months of sirolimus treatment, the lesion regressed and treatment response was defined as partial remission (Fig. 1F).

Sirolimus treatment was continued for a further three months after partial remission was achieved. Sirolimus treatment was discontinued after determining a stable disease in the evaluation of the tumor with control MR. No

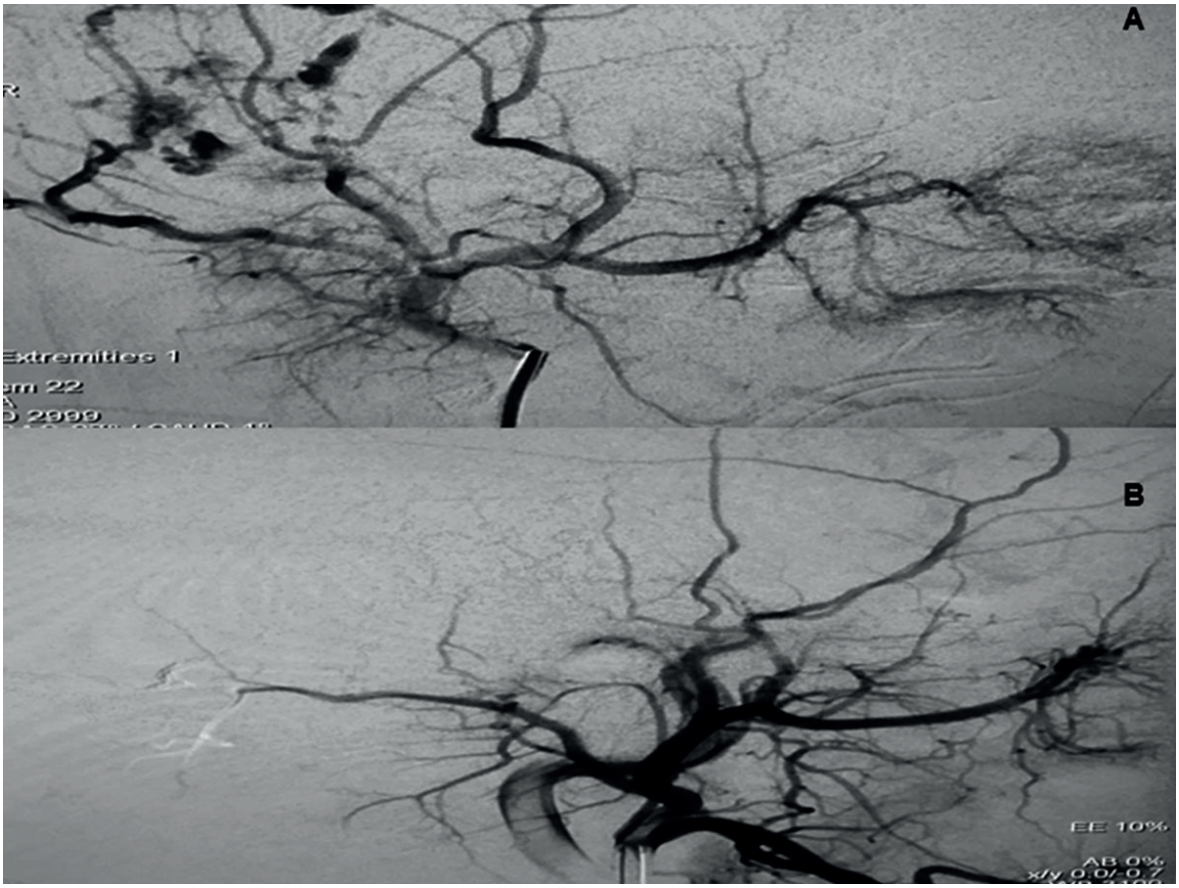

Fig. 3. (A): Lateral DSA image the external carotid artery shows vascular feeders of the lesion originated from frontal branch of the superfisial temporal artery and occipital artery, (B): Post-embolisation DSA images show nearly total oclussion of the feeder vessels and onyx cast.

progression was observed in the tumor three months after treatment was discontinued and the patient is still being followed up with stable disease Inform consent was received from the family

Discussion

Vascular tumors of the bone have a wide spectrum ranging from benign hemangiomas and epithelioid hemangiomas to intermediate grade hemangioendotheliomas to malignant angiosarcomas. For years, the classification of vascular tumors of the bone has been highly controversial, especially given the lack of consistent terminology, limited histological criteria, and limited correlations between clinical course and diagnosis.

Hemangioendotheliomas have a wide range of histological features and are classified as local aggressive or borderline tumors according to International Society for the Study of Vascular Anomalies (ISSVA).[3] In this group, caposiform, retiform, composite, papillary intralymphatic (also known as Dabska tumor) and pseudomyogenic types are included, whereas epitheloid hemangioendothelioma belongs to malignant vascular tumor group in ISSVA classification.

Retiform hemangioendothelioma (RH) is a very rare intermediate or borderline vascular tumor with unknown etiology. Lymphedema, previous radiotherapy and non-epidermal malignant tumors have been proposed in the etiology of RH, and in one case the relationship with human herpes virus 8 (HHV-8) has been

Table I. Temporal bone hemangioendothelioma cases in English literature.

Case	Author	Age (years)	Sex	Symptoms	Location	Angiography	Treatment	Follow-up
1	Jochaims et al.	19	M	Tinnitus Earache Discharge Hearing loss	Mastoid	NA	Surgery radiotherapy	3 year
2	Goldestien et al.	62	M	Tinnitus Hearing loss Vertigo Mass	Middle ear	Normal angiogram	Surgery radiotherapy	1 year
3	Eliashar et al.	3	M	Retroauricular swelling Tendeness Fever Lymphadenophathy	Mastoid	NA	A interferon	NA
4	Lalaji et al.	1	F	Mass	Mastoid	NA	Surgery predisolon	2 year
5	Ibarra et al.	5	F	Mass Facial plasy	EAM	Middle meningeal artery	Surgery	NA
6	Kim et al.	7	M	Mass	Mastoid	Branches of the middle meningeal artery	Partial Surgery Radiotherapy	Recurrent 2 year Radiotherapy Chemotherapy
7	Chang et al.	1	M	Facial palsy	Internal auditory canal	External carotid artery	Surgery Cortison Interferon	1 year
8	Panda et al.	38	M	Tinnitus Hearing loss Fullness of the ear Dizziness	Middle ear	NA	Surgery	6 months
9	Moskowitz et al.	6	F	Facial palsy Hearing loss Dizziness Tinnitus	Middle ear	NA	Surgery	NA
10	Tian WZ et al.	57	F	Mass	NA	NA	NA	Surgery
11	This case	1.5	F	Facial palsy Mass	Mastoid	External carotid artery	Propranolol Prednisolone Vincristine Embolization Sirolimus	3 years

reported.[4] It is characterized by a high rate of local recurrence and a low frequency of metastasis, and its biologic potential is between that of hemangiomas and angiosarcomas. Histologically, the tumor exhibits arborizing elongated blood vessels, hobnail monomorphic endothelial cells with scant cytoplasm and no significant atypia, prominent endovascular papillae with collagenous cores, and prominent lymphocytic infiltrate.[5,6] To our knowledge, no more than 50 cases have been described in the literature since the first description in all age groups according to diagnostic criteria. RH is usually seen in young and middle-aged people, but rarely in children. So far, the youngest patient described in the literature was 6 and the oldest was 78 years old. To our knowledge our patient is the youngest diagnosed case in the literature.[7]

Although RT originating from various cranial bones and sphenoid wing has been reported, to our knowledge RH originating from temporal bone like our case has not been reported to date. Temporal bone tumors especially vascular tumors are rare. Ramadan O.[8] analysed and reviewed 45 manuscripts of temporal bone origin malignant vascular tumors, which have been published so far and whose data are available, of 47 cases described in these manuscripts 9 (19%) were diagnosed as hemangioendothelioma. To our knowledge, to date, 13 cases of hemangioendothelioma originating from temporal bone have been reported, none of them were RH histology.[9-21] Eleven of 13 cases whose data can be accessed including our case are summarized in the table (Table I). Seven of these cases are under 18 years of age. The ratio of males to females is 6/5 in the reported cases. Mastoid bone was reported to be the most common site as in our case. Surgical treatment was performed in eight patients.

The most frequent radiographic finding of hemangioendothelium is an osteolytic sharply demarcated lesion but calcification and periost reaction are not usually seen.[16] MR findings of hemangioendothelioma are nonspecific. The signal intensity of these vascular structures may display as either high flow (low signal intensity on images of all pulse sequences) or low flow (high signal intensity on the T2-weighted images).[16] In the CT scan images of our case, the mastoid air cells were almost completely filled with contrast enhanced mass on the right side. Cranial contrast-enhanced MRI showed a lytic hypervascular mass that almost completely filled the right mastoid air cells but did not cause destruction. Hyperintense hemosiderin residues were found in the mass in T1-weighted images, and fluid-liquid levels were found in T2-weighted images and and the mass was markedly enhanced in contrast-enhanced images of our case. According to these radiological findings, our patient was thought to have a tumor of vascular origin. Although it was thought to be a vascular tumor radiologically, a biopsy was performed to determine the definitive diagnosis and treatment option and pathological examination of the lesion revealed retiform hemangioendothelioma.

The vein of Galen aneurysmal malformation (VGAM) is a rare congenital vascular malformation characterized by the shunting of the arterial flow into an enlarged cerebral vein of Galen. Its incidence is 1/25000 and constitutes 1% of all cerebral vascular malformations and 30% of vascular malformations in children.[22] Clinical presentations vary with the age of onset and vascular architecture. The main manifestations include congestive heart failure, hydrocephalus, and neurological symptoms.[22] Our patient was asymptomatic and galen vein aneursym was found incidentally on cranial MRI. According to our knowledge, galen vein aneursym and temporal bone hemagioendothelioma have not been reported in the same patient before. We think that this coexistence is a coincidental finding.

Treatment for hemangioendothelioma may depend on the histologic type and the risk of recurrence or metastases. In our patient, medical treatment was planned due to its unsuitable locationfor surgery.

In recent years, propranolol has been widely used in the treatment of infantile hemangioma and vascular tumors.[23,24] Although the exact mechanism of action of propranolol is not clear, it has been reported that it acts on infantile hemangiomas by increasing vasoconstruction and apoptosis, reducing angiogenic factors and modulating renin-angiotensin system. Moreover, propranolol induces apoptosis and disrupts the migration of malignant vascular tumor cells. Infantile hemangiomas express high levels of beta adrenergic receptors potentially explaining their sensitively to propranolol, and these receptors have been reported to be strongly expressed in hemagioendothelioma and vascular malformations.[25] In our case, we preferred propranolol treatment as the first-line treatment. However, no response was obtained despite one month of treatment of propranolol. Firstly prednisolone then weekly vincristine was added to the treatment. Four months after the initial treatment, cranial MRI showed minimal progression in the lesion, so embolization was performed and sirolimus treatment was initiated.

The mammalian target of rapamycin (mTOR) is a serine threonine kinase regulated by phosphoinositide 3 kinase (PI3K) and protein kinase B (Akt) that activates protein synthesis. mTOR plays a key role in the pathogenesis of various vascular anomalies, leading to angiogenesis and lymphangiogenesis by increasing VEGF expression, except that it plays an important role in cell growth and proliferation.[26] mTOR inhibitors directly inhibit mTOR, blocking downstream protein synthesis and presenting antitumoral and antiangiogenic effect. Sirolimus, also known as rapamycin is a mTOR inhibitor. Clinical data on the use of sirolimus in patients with vascular anomalies are rare and most of them are case reports.[27,28]

Since our patient did not respond to propranolol, prednisolone and vincristine treatments and the lesion showed slight progression, it was decided to try sirolimus treatment after embolization. An MRI was performed one month after embolization and regression of tumor volume was detected. MRI performed six months after sirolimus treatment showed significant regression in tumor size which defined the response to treatment as partial remission. Sirolimus treatment was continued. There were no complications during the treatment. This suggests that sirolimus is an option in the treatment of hemangioendothelioma.

In conclusion, hemangioendothelioma of the temporal bone origin is an extremely rare vascular tumor in childhood and its association with Galen vein aneurysm has not been previously described. In addition, sirolimus seems to be a safe and efficient treatment option for the treatment of hemangioendothelioma, but its effect should be supported by studies conducted in large series.

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Case Report

Atypical presentation in patients with 17 α-hydroxylase deficiency caused by a deletion in the *CYP17A1* gene: short stature

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ABSTRACT

Background. Patients with 17α-hydroxylase deficiency (17 OHD) usually present with tall stature and eunuchoid features, rather than growth retardation. However, unlike the classic form of the disease, short stature due to a lack of pubertal growth spurt and sex hormone deficiency was present in our four cases. We wanted to emphasize that short stature might be the cause of first presentation in patients with 17 OHD.

Cases. We report five patients of Kurdish origin with 17 OHD, four of whom had short stature; two presented because of short stature and two were detected as having short stature. The external genitalia had a female appearance and was prepubertal in all cases. Hypertension was also detected in four of the patients. Serum biochemical and hormonal analyses were performed for each patient. Laboratory data suggesting severe growth hormone (GH) deficiency were obtained from one patient, while the other had a familial history suggesting constitutional delay of growth and puberty (CDGP). Whole exome sequence analysis of the CYP17A1 gene was performed on all patients. STR fragment analysis and multiplex ligation dependent probe amplification (MLPA) analysis was also performed to detect mutations associated with congenital adrenal hyperplasia (CAH) in the CYP17A1 gene. No mutation was detected in the whole exome sequence analysis of the CYP17A1 gene in all five patients, although wide deletions were identified in the 1st–6th exons of this gene at MLPA analysis.

Conclusions. Patients with 17α-hydroxylase deficiency can present with short stature because they have no pubertal growth spurt during adolescence. Therefore, 17 OHD should be considered in the differential diagnosis of patients with delayed puberty and short stature.

Key words: 17α-Hydroxylase deficiency, plasma CYP17A1 gene, MLPA analysis, short stature.

17α-hydroxylase deficiency (17 OHD) is a rare autosomal recessive form of congenital adrenal hyperplasia (CAH) with an estimated incidence of 1:50,000-100,000, accounting for 1% of all CAH cases.[1,2] Blockage of the pathway catalyzed by 17α-hydroxylase stimulates adrenocorticotropic hormone (ACTH), leading to overproduction of corticosterone and 11-deoxycorticosterone (11-DOC).[3] These steroids, which exhibit mineralocorticoid activity, lead to water and sodium retention, and inhibition of renin and aldosterone production. Levels of 17-OH-pregnenolone, 17-hydroxyprogesterone (17OHP), 11-deoxycortisol, cortisol, dehydroepiandrosterone, androstenedione, and testosterone decrease.[4] Increased sodium reabsorption and high blood volume suppress the renin angiotensin system. Reduced aldosterone levels are therefore observed in most patients with 17 OHD.[5]

17 OHD is characterized by sexual infantilism due to an inability to produce gonadal steroids, primary amenorrhea in women (46XX), and

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disorders of sex development (DSD) in men (46XY). Decreased production of glucocorticoids also increases ACTH secretion, leading to overproduction of mineralocorticoids, and resulting in hypertension and hypokalemia.[6] Gonadotropin levels are high during the pubertal period, and androgen and estrogen levels are low. The growth rate in affected patients is slow, but due to delayed epiphyseal plate fusion, these patients keep growing into adulthood and subsequently exhibit tall stature.

In this study, we describe five patients of Kurdish origin with 17 OHD. Short stature was present in four of these patients with 17 OHD. Delayed puberty was also present in all patients and hypertension in four. MPLA analysis revealed wide deletions in the 1st-6th exons of the CYP17A1 gene.

Case Reports

Five patients who were diagnosed as having 17 OHD were included in this case series. These patients were followed from 2016 to 2019 in the Pediatric Endocrinology Unit of Adıyaman Training and Research Hospital. 17-OHD was suspected in the presence of delayed puberty, hypertension and/or hypokalemia. Diagnosis of the disease was established based on clinical observations, typical laboratory findings, and molecular features. Routine serum and urine biochemical analyses and hormonal evaluations including cortisol, ACTH, progesterone, 17OHP, androstenedione, DHEA-S, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), testosterone, aldosterone, and plasma renin activity (PRA) levels were conducted for each patient. All patients underwent karyotype analyses and mutational analysis of the CYP17A1 gene. The study protocol was approved by the Institutional Ethics Committee of Adıyaman University Faculty of Medicine (Decision number 2-3/20.03.2018). Written informed consent was obtained from all participants.

Genomic DNA Preparation and PCR Amplification of the CYP17A1 Gene

Genomic DNA was isolated, and whole exome sequence analysis of the CYP17A1 gene was performed. Additionally, STR fragment and MLPA analysis were performed for the detection of mutations (large deletions/duplications) associated with CAH in the CYP17A1 gene. A SALSA MLPA P0334 Gonadal kit (MRC, Holland) was used for the MLPA test.

Case 1 (Index patient)

A 12-year-old girl presented with hypopotassemia. The parents were first-degree cousins, but the family history was otherwise unremarkable. The family pedigree is shown in Figure 1. In a physical examination, her external genitalia was female appearance and prepubertal. Her blood pressure was high (148/96 mm Hg). The patient's weight was 34.2 kg (-2.46 SDS), height was 144.4 cm (-2.23 SDS), with a target height of 165 cm (0.32 SDS), and the bone age was delayed. Laboratory investigations revealed a low serum potassium concentration and renin level, and a high progesterone level (17.22; normal range: 0.1-1.3 ng/mL). Androgen levels are shown in Table I. The maximum cortisol response to the standard-dose ACTH stimulation test was insufficient (Table II). The patient's karyotype was 46 XY and SRY (+). A deletion in the 1st – 6th exons of the CYP17A1 was detected following STR fragment and multiplex ligation dependent probe amplification (MLPA) analysis (Fig. 2).

Case 2

A 13-year-old girl presented to the pediatric endocrinology outpatient clinic with short stature. The parents were second-degree cousins. The family pedigree is given in Figure 1. In a physical examination, her external genitalia was female appearance and prepubertal. Her blood pressure was high (130/76 mm Hg). The patient's weight was 39.4 kg (-1.92 SDS), height was 143.4 cm (-2.70 SDS), with a target height of 151 cm (-2.06 SDS), and the bone age was

Table I. Clinical, biochemical and genetical features of the cases.

Case	Case 1	Case 2	Case 3	Case 4	Case 5
Cause of admission	Hypopotassemia	Short Stature	Amenorrhea	Short Stature	Hypertensiyon
Age	12.9	13.6	15.5	12.9	14.9
Bone age	9	10.5	10.5	10.5	9.5
Hypertension	+	+	+	-	+
Breast development (Tanner stage)	1	1	1	1	1
Pubic hair (Tanner stage)	1	1	1	1	1
Karyotype	46,XY(SRY+)	46,XX	46,XY (SRY+)	46 XX	46 XY(SRY +)
Na, mmol/l (135-145)	133	143	143	138	139
K, mmol/l (3.5-5.5)	2.4	2.9	3.3	4.3	3.8
Testosterone, ng/ml (0.1 – 0.75)	0.36	0.23	0.08	0.12	0.23
LH, IU/l	22.16	33	29.92	34.65	21.64
FSH, IU/l	100.61	80.87	79.23	79.37	74.53
Estradiol, pg/ml	4	33	12	15	12
Aldosterone, ng/dl (3.5-30)	10.4	55.49	30	23.2	20.48
Renin, mcg/ml/saat (0.5-5)	0.15	0.31	0.11	0.10	0.13

ACTH: adrenocorticotropic hormone, LH: luteinizing hormone, FSH: follicle-stimulating hormone.

Table II. ACTH stimulation test results.

Case	Case 1	Case 2	Case 3	Case 4	Case 5
ACTH, pg/ml (0-46)	66.7	379	1250	1000	597
Cortisol (μg/dl) (5-25)					
Basaline	0.44	0.75	0.26	0.23	0.22
Peak	0.74	1.01	0.31	0.26	0.25
Progesterone (ng/ml) (0.3-1.5)					
Basaline	17.22	14.89	10.99	8.51	7.81
Peak	19.32	16.46	15.12	12.89	13.16
17-OH progesterone (ng/ml) (0.2-1.3)					
Basaline	0.88	1.18	0.61	0.88	0.76
Peak	0.94	1.23	0.76	1.09	1.01
Androstenedione (ng/ml) (0.75-3.1)					
Basaline	0.16	0.06	0.08	0.02	0.1
Peak	0.21	0.09	0.1	0.04	0.15
DOC (ng/dl) (0.12-0.6)					
Basaline	1.35	3.66	2.64	1.24	1.82
Peak	1.84	3.88	2.7	1.26	2.24
DHEA-S (μg/dl) (35-430)					
Basaline	3.1	4.7	3.4	4.8	4.4
Peak	3.4	5.2	3.8	5.4	4.9

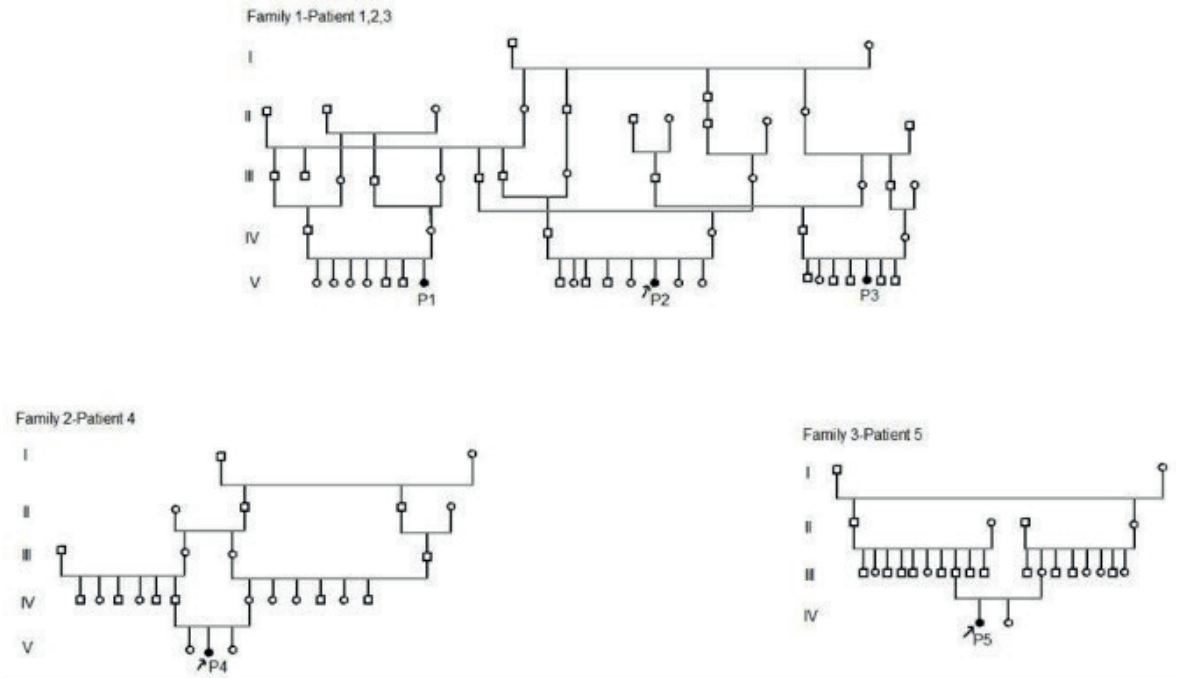

Fig. 1. Family pedigree of the cases.

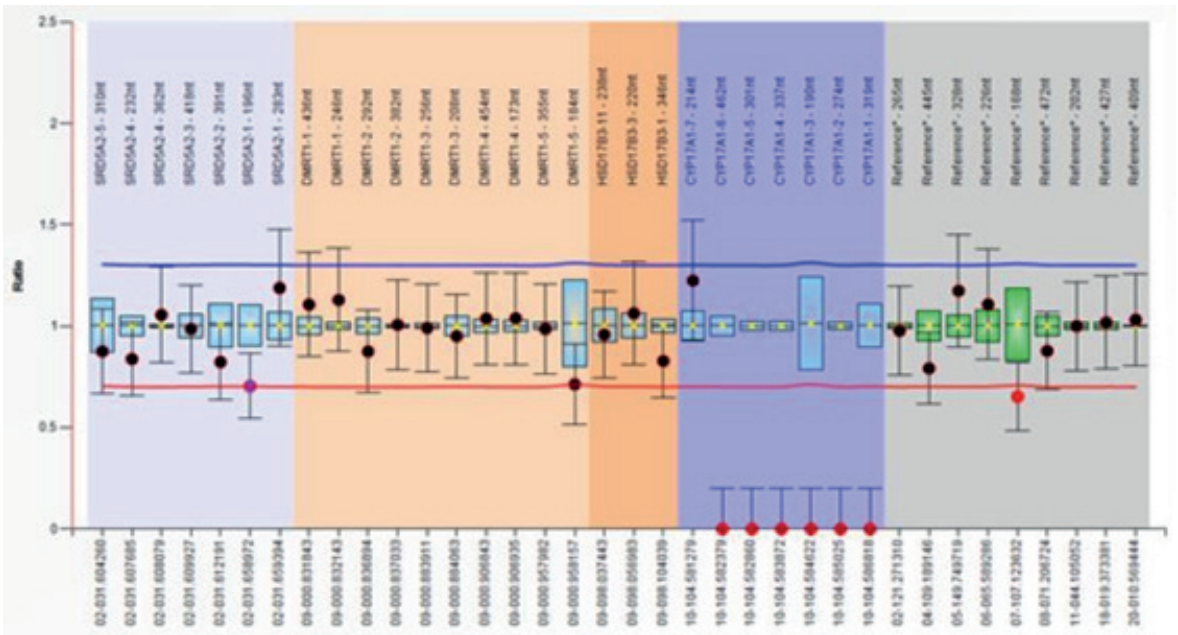

Fig. 2. Detected deletion in 1st – 6th exons of the *CYP17A1* gene.

delayed. Laboratory investigations revealed a low serum potassium concentration and suppressed renin level, and a high progesterone concentration (14.89 ng/ml). The maximum cortisol response to the standard-dose ACTH stimulation test was insufficient (Table II). The maximum response to the growth hormone (GH) stimulation test, which was performed in an external center, was insufficient (L-dopa: 0.6 ng/mL, and clonidine: 1.2 ng/ml). Priming

in this patient with delayed puberty was performed with the administration of 40 μg / m^2/day ethinyl estradiol for three days, after which a sufficient GH response was achieved in the GH stimulation test (L-dopa: 11.2 ng/mL). The patient's karyotype was 46 XX. A deletion in the 1st – 6th exons of the CYP17A1 gene was detected following STR fragment and MLPA analysis (Fig. 2).

Case 3

A 15-year-old girl was referred from the gynecology outpatient clinic due to hypergonadotropic hypogonadism and 46 XY sexual development disorder. The parents were first-degree cousins. The family pedigree is shown in Figure 1. In a physical examination, her external genitalia was female appearance and prepubertal. Her blood pressure was high (148/96 mm Hg). The patient's weight was 42.3 kg (-2.35 SDS), height was 158.5 cm (-0.61 SDS), with a target height was 166 cm (0.49 SDS), and the bone age was delayed. Laboratory investigations showed a low serum potassium concentration and suppressed renin level, whereas the progesterone was high (15.12 ng/mL). The maximum cortisol response to the standard-dose ACTH stimulation test was insufficient (Table II). The patient's karyotype was 46 XY and SRY (+). A deletion was detected in the 1st – 6th exons of the CYP17A1 gene following STR fragment and MLPA analysis (Fig. 2).

Case 1 (index patient), case 2, and case 3 presented to the pediatric endocrinology outpatient clinic independently of each other and at different times. As a result of clinical and laboratory evaluations, it was noticed that these patients, who were diagnosed as having 17-OHD, had the same surname, and in detailed questioning, these three patients were found to be distant relatives.

Case 4

A 12-year-old girl presented because of short stature. The parents were first-degree cousins, but the family history was unremarkable. The family pedigree is shown in Figure 1. In a physical examination, her external genitalia was female appearance and prepubertal. Her blood pressure was normal (110/70 mm Hg). The patient's weight was 37.9 kg (-1.26 SDS), height was 143.5 cm (-1.83 SDS), with a target height was 162 cm (-0.19 SDS), and the bone age was delayed. Laboratory investigations showed normal serum sodium and potassium concentrations, but high progesterone levels (8.51 ng/mL). The maximum cortisol response to the standard-dose ACTH stimulation test was insufficient (Table II). The patient's karyotype was 46 XX. A deletion in the 1st – 6th exons of the CYP17A1 gene was detected following STRE fragment and MLPA analysis (Fig. 2).

Case 5

During hospitalization for the correction of prominent ears, a 14-year-old girl was detected as having hypertension. We learned that the parents were first-degree cousins, and that the patient had undergone surgery at the age of 3 years for bilateral inguinal hernia. The family pedigree is shown in Figure 1. The mother reported that her first child, a girl, reached puberty late, had late growth, and that this child who had no chronic disease had regular menstruation and had similar height as her peers. At physical examination, her external genitalia was female appearance and prepubertal. Her blood pressure was high (135/100 mm Hg). The patient's weight was 38.6 kg (-2.65 SDS), height was 149.5 cm (-1.94 SDS), with a target height was 164 cm (0.15 SDS), and the bone age was delayed. Laboratory investigations showed normal serum sodium and potassium concentrations, but her progesterone level was high (7.81 ng/mL). The patient's karyotype was 46 XY and SRY (+). A deletion in the 1st – 6th exons of the CYP17A1 gene was detected following STR fragment and MLPA analysis (Fig. 2).

The clinical and laboratory features of all patients at presentation are shown in Table I. The ACTH stimulation test was started at 08:00

A.M. Initial blood samples were collected, and 30 and 60 minutes after intravenous 250 μg synthetic ACTH 1-24 administration.

Discussion

CYP17A1 gene mutations occur in different patterns including point mutations, small deletions and insertions, splice site alterations, and rarely, large deletions.[7] These mutations may exhibit ethnic variations.

Most patients with 17 OHD typically present with hypertension and primary gonadal insufficiency during adolescence and adulthood. Hypertension may be detected at the time of diagnosis, from infancy to the fifth decade of life, and usually in patients presenting due to delayed puberty.[8,9] When diagnosis is established in these patients, adequate treatment should be initiated in order to prevent complications such as hypertension and hypokalemia, and in order to ensure the appearance of secondary sexual characteristics in an appropriate time.[10]

The defect in sex steroid production causes ambiguous genitalia or female external genitalia (46 XY sexual development disorder) in affected males and delayed puberty and primary amenorrhea in affected females.[11]

Tall stature may be seen at initial presentation in patients with 17 OHD.[12] The apparently tall stature in individuals with 17 OHD and XY karyotype can be due to using growth charts developed for females. Height is dependent upon linear bone growth.[13] Regulation of linear bone growth in children and adolescents involves a complex interaction of hormones and growth factors. GH is considered as the key hormone regulator of linear growth in childhood.[14] Sex steroids play a crucial role in pubertal growth, both at the systemic level via the GH/insulin-like growth factor-1 (GH/IGF-1) axis, and at the local level of the epiphyseal growth plate. Estrogen is a critical hormone for controlling growth plate acceleration and fusion in both sexes.[15] Epiphyseal closure is delayed in patients with 17 OHD because of the lack of estrogen in the growth plate, and the final height is therefore above average in late adolescent or adult patients. In contrast, Ma et al.[13] reported a patient with 17 OHD and short stature at the time of admission, whose height reached 152 cm following glucocorticoid and sex steroid replacement. In addition, Kardelen et al.[16] determined short stature at presentation in three out of six patients (50%) with 17 OHD. One common feature of these patients is that growth can occur without the need for GH. Similarly, in the present study, four of our patients had short stature at presentation. Sex steroids could not be started in case 1 because the family could not be convinced of the benefits of drug therapy. The growth rate in case 1 was therefore lower compared with the other patients who did receive sex steroids. A number of variables can directly or indirectly affect the pubertal growth spurt, including sex, genetics, nutrition, endocrine regulation, physical activity, and ethnicity.[17] The absence of a pubertal growth spurt caused by an absence of sex steroids in patients with 17 OHD may explain the short stature in these cases.

Sex steroids regulate GH release either directly, or else indirectly through IGF-1, and GH secretion increases with pubertal development. Some studies have reported rare transient partial growth hormone deficiency as a result of GH stimulation testing in patients with delayed puberty.[18,19] GH test outcomes may be low such as to occasionally suggest growth hormone deficiency in patients with 17 OHD presenting with prepubertal short stature. This may derive from sex steroid deficiency. Priming with sex steroids should therefore be performed in these patients before GH stimulation tests. Diagnostic confusion may be experienced at first presentation in patients with 17 OHD with short stature, as in our case 2, in which the GH stimulation test suggested severe GH deficiency, and case 5, with a history of constitutional delay of growth and puberty (CDGP), and with delayed puberty and delayed bone age.

This study reports five cases from three different Kurdish families with partial deletions between exons 1 and 6 in the CYP17A1 gene in order to add their clinical and molecular characteristics to the existing literature. All our patients were living in the Adıyaman province of Southeastern Turkey, and this identical gene deletion seen in different families may be explained by the founder effect.

In conclusion, wide deletions in exons 1-6 of the CYP17A1 gene disrupt 17 alpha / 17,20 lyase enzyme activity, leading to severe phenotypic alterations. Causes of presentation in patients with same genotypic changes may range from delayed puberty and hypertension to hypopotassemia and short stature. 17 OHD should be ruled out with clinical and laboratory investigations including blood pressure measurement in patients presenting with delayed puberty and short stature before diagnosing CDPG or GH deficiency. Further multicenter studies are now warranted in order to better evaluate the relationship between CYP17A1 gene mutations and short stature.

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Case Report

Successful treatment of pediatric post-liver transplant Kaposi's sarcoma with paclitaxel

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ABSTRACT

Background. Kaposi's sarcoma (KS) is a complication of immunosuppressive therapy for transplant recipients. Unlike adult recipients, KS in pediatric organ transplantation is quite rare. Treatment is usually withdrawal of immunosuppression; non-responders often receive chemotherapy.

Case. We have reported a child with post-liver transplant visceral KS which has progressed despite withdrawal of immunosuppressive therapy, who has been treated with Paclitaxel for three weeks. KS has regressed completely after four cycles of Paclitaxel.

Conclusion. Paclitaxel should be considered as an effective first line treatment option for patients with post-transplant KS.

Key words: Kaposi's sarcoma, children, paclitaxel.

Kaposi's sarcoma (KS) is a rare type of visceral and soft tissue sarcoma related to human herpes virus (HHV)-8 characterized by the proliferation of spindle shaped cells, mainly of vascular origin.[1] Clinically, KS is a slowly progressing malign lesion commonly manifesting with cutaneous lesions with or without involvement of internal visceral organs and/or lymph nodes.[1] It is classified clinicopathologically into four subgroups based on epidemiological data which are classical, endemic (African type), epidemic (AIDS-related), and iatrogenic (transplantation-related) KS. The type observed in the organ transplant recipients is known as a type that is considered to be related to immunosuppression and may regress after the discontinuation of the therapy or may show an aggressive and multifocal course compared to classical type.[2] Both sporadic and opportunistic forms of KS are associated with the presence of the HHV-8 genome in the neoplastic cells. Although its incidence varies across different types, the most common presentation is purple, blue, red or dark brown, black macula, papule, plaque and nodular skin lesions and accompanying lymphedema, especially in the lower extremities.[1] Systemic therapy may be considered in the patients with symptomatic visceral and mucosal involvement. For systemic therapy, in addition to the drugs such as anthracyclines, vinca alkaloids, bleomycin, oral etoposide and gemcitabine, taxanes have also being used during recent years for their antiangiogenic effects.[3,4] Moreover, several studies have shown that paclitaxel monotherapy is a successful second-line treatment for both AIDS-associated KS and KS in a therapeutically immunosuppressed patient.[4] Additionally, for patients with epidemic or iatrogenic KS, the most effective treatment requires correcting the underlying immunodeficiency.[2]

In this report, we present a patient with disseminated visceral KS after liver

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transplantation who has showed a dramatic response to Paclitaxel therapy.

Case Report

A seven year-old girl with chronic liver failure secondary to progressive familial intrahepatic cholestasis type III (PFIC III) was transplanted with a left lateral segment from her mother. The patient was third of four children from a first degree-cousin marriage. Her older brother and cousin were also diagnosed with PFIC III, and both were liver transplanted. According to current immunosuppression protocol, she received IV methylprednisolone at the dose of 10 mg/kg during surgery and the dose was decreased to 2 mg/kg/day at the end of the first week. Then, steroid was tapered to the dose of 0.5 mg/kg/day in the post-transplant first month and withdrawn at the end of the post-transplant third month. She was given tacrolimus during the post-transplant period. One year after transplantation, she was presented with ascites. On physical examination of the oral cavity, there were 2x3 cm brown-blue-red nodule posterior of lower incisors (Fig. 1). Complete blood count revealed pancytopenia. Coagulation profile together with blood biochemistry was unremarkable. Serological tests for CMV and EBV IgM, CMV antigen testing, PCR for CMV and EBV were all negative. In addition, HIV antigens were negative. Abdominal CT revealed multiple enlarged mesenteric lymph nodes. Endoscopic examination revealed multiple polypoid brown-blue-red lesions on esophagus, stomach and duodenum. Multiple biopsies from said lesions revealed an infiltration of neoplastic cells with round-oval nuclei and moderate-sized eosinophilic cytoplasm, and red blood cells among them. In immunohistochemical assessment, the neoplastic cells displayed CD34, CD31 (Fig. 2a) and HHV-8 immunoreactivity (Fig. 2b). The histopathological examination of biopsy was consistent with HHV-8 related KS. Tacrolimus was switched to sirolimus at a dose of 2 mg/m^2 to keep the serum levels around 5 ng/ml. In due course, no regression of the lesions was observed. Paclitaxel therapy at a dose of 100 mg/m^2 per three weeks was started.[5] Informed consent was received from the family. Oral lesions regressed rapidly following first cycle; after four cycles of paclitaxel, all lesions were resolved completely (Fig. 3). The gastrointestinal endoscopic examination was macroscopically absent of any kaposiform lesion and histopathology of multiple biopsies was completely normal. Paclitaxel was tolerated well by the patient. Bone marrow suppression which developed during therapy was easily controlled by supportive therapy without any clinical manifestation. Now the patient is free of disease for at least 18 months and completely healthy with good hepatic graft function.

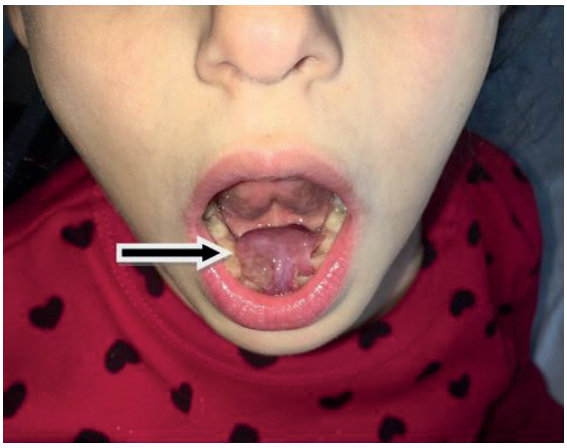

Fig. 1. Brown-blue-red nodule in the oral cavity.

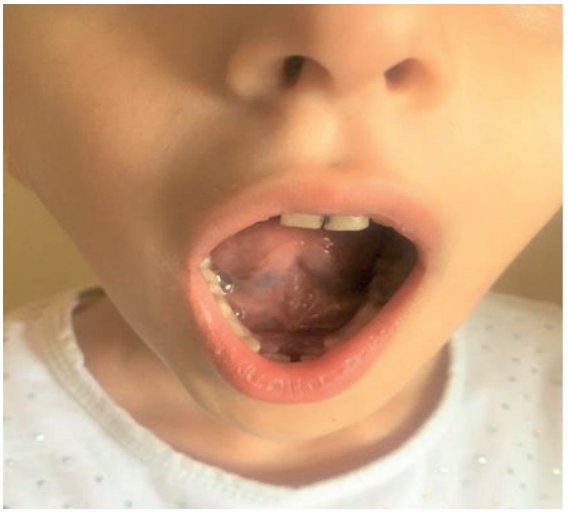

Fig. 3. After four cycles of paclitaxel therapy, the lesion in the oral cavity regressed totally.

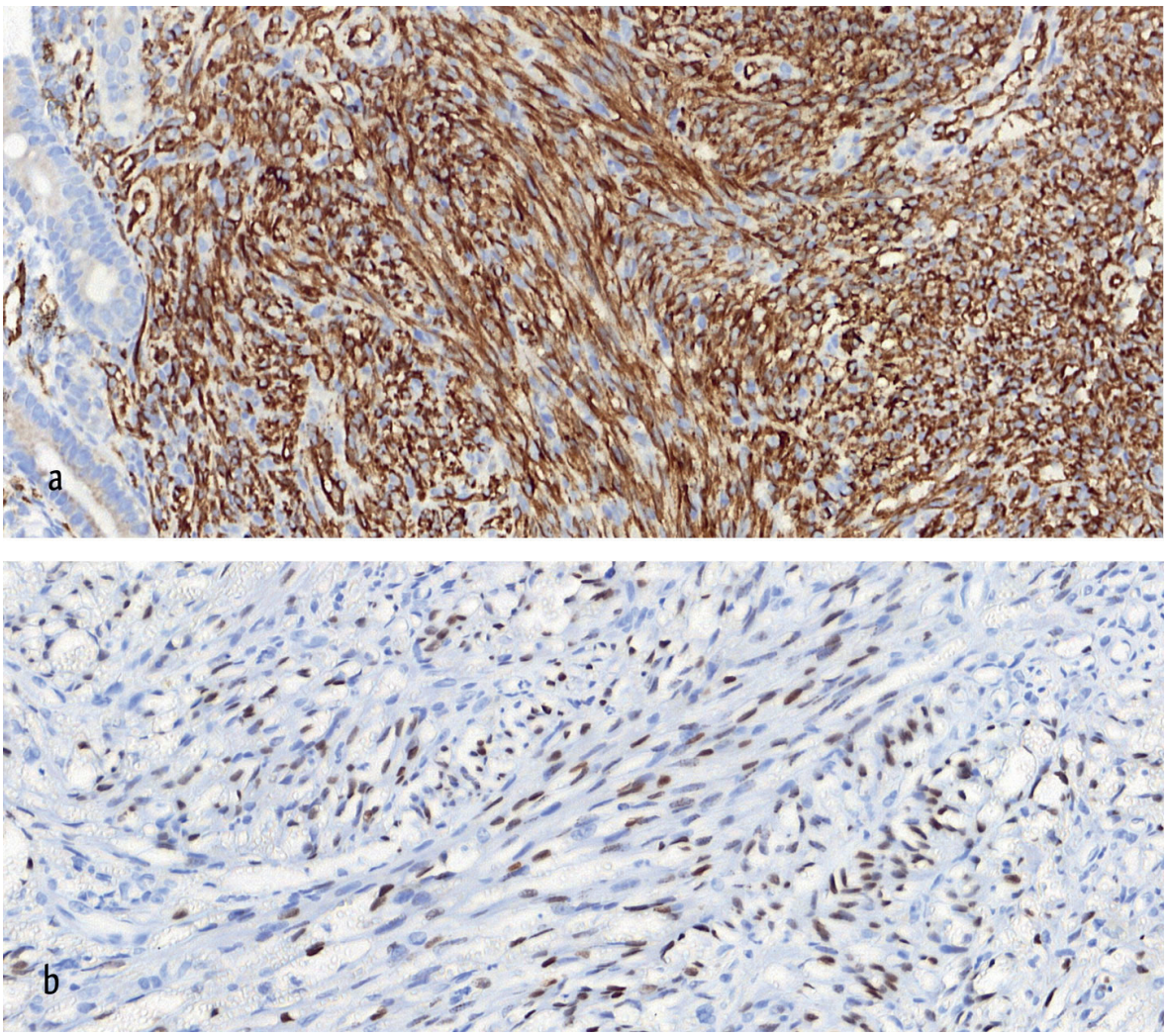

Fig. 2. Immunohistochemical staining of biopsy specimens. Figure 2a and 2b show CD31 positivity and HHV-8 immunoreactivity, respectively.

Discussion

Because of the rarity of pediatric KS, there is a paucity of literature on treatment and outcome stratification with most treatment modality options extracted from literature of adults treated for KS. To date, there is no established consensus group therapeutic guidelines for the treatment of all four forms of pediatric KS.

Strong correlation between the immunosuppressive status of the patient and the development of KS is very well demonstrated, and it is now a major and increasing problem in organ transplant recipients.[6] Unlike adult recipients, KS in pediatric organ transplantation is quite rare and mainly observed in renal allograft recipients.[6] It is reported rarely after liver transplantation in children.[7,8] Compared with renal transplant recipients, visceral involvement is more frequent among liver transplant recipients.[9] So, the tendency to dissemination and systemic nature of KS in liver transplant recipients is considered to be responsible for shorter survival than those who have undergone renal transplantation.[10]

Reduction or discontinuation of immunosuppressive treatment is the first step in the treatment of iatrogenic KS and it has been associated with the disappearance of lesions.[11,12]

In addition, there is evidence that patients with iatrogenic KS have had tumor regression when immunosuppression has been switched to sirolimus, a mammalian target of rapamycin (mTOR) inhibitor sharing both immunosuppressive and anti-neoplastic effects.[13] Unlike other immunosuppressants, sirolimus has potent antitumor activity in vitro and in vivo. Sirolimus having antiproliferative and antitumor effect has been increasingly reported in the management of the disease.[7,9] In the presented case, tacrolimus has been withdrawn and sirolimus has been started. However, lesions have remained unaltered and chemotherapy has commenced.

Therapeutic options for KS are based upon disease stage, progression pattern and distribution, clinical type, and immune status.[14] For KS patients with more widely disseminated, progressive or symptomatic disease, systemic therapy with cytotoxic chemotherapy is generally warranted. The present case was not eligible for local therapy because she had multiple visceral and mucosal lesions. Several single agent therapies have been reported to be active in all subtypes of KS (vincristine, vinorelbine, etoposide, adriamycin, liposomal doxorubicin, epirubicin, bleomycin, docetaxel and paclitaxel) with an overall response rate ranging from 30 to 70%, although most of them have been partial responses.[3]

Taxanes (paclitaxel and docetaxel) are now promising agents in the treatment of KS with tolerable side effects. They have potent antiangiogenic activities, which may explain their efficacy on Kaposi lesions.[15] Paclitaxel is a drug affecting the microtubules and cellular vital processes in nonmitotic phases of the cell cycle and it inhibits the growth of proliferating tumors either rapidly or slowly.[14] The efficacy of paclitaxel given as second-line or third-line therapy has been evaluated well in association with highly active antiretroviral therapy in patients with AIDS-related KS.[16,17] Recently, some articles about its efficacy in the treatment of classical, endemic and post-transplantation types of KS have also been published.[18] Patel et al.[5] have demonstrated prompt and durable response in two renal transplant recipients.

Limited cases of pediatric iatrogenic KS have shown promising response to mTOR inhibitor sirolimus, and paclitaxel chemotherapy.[7,8] Based on these data, because of promising results and tolerable side effects, we decided to start paclitaxel monotherapy as first line treatment. We continued to give sirolimus after the completion of chemotherapy regimen as maintenance, because it is an effective agent in both treatments of KS and prevention of organ rejection.

Pediatric patients with post-liver transplant who develop KS may face a dismal prognosis.[8,19] Celtik et al.[8] describe a 15-month-old child who underwent liver transplant with subsequent pancytopenia, lymphadenopathy, edema, and subconjunctival bleeding. A cervical lymph node biopsy confirmed the diagnosis of disseminated KS. The first chemotherapy choice was weekly vinblastine, but the child was unresponsive. Second-line chemotherapy with, actinomycin D and cyclophosphamide was not effective. Then durable response was ensured by paclitaxel. Similar findings of multi-visceral KS after pediatric liver transplant have also been described in Mexico, France, and Turkey; many with fatal outcomes.[7,19] Anthracyclines are another treatment option but limited effects with cardiotoxic side effects restrict its preference.[3] When evaluating child and adult data on post-transplant KS, cytotoxic chemotherapy does not appear to be very effective. In addition, serious side effects associated with these treatments have been observed. Recently, efficacy of paclitaxel in post-transplant type KS has been published.[5] In a review of Dow et al.[20] paclitaxel was the first chemotherapy option in pediatric post-transplant KS. Paclitaxel-related side effects were also much fewer than other chemotherapeutic agents.[4]

In the present case, KS regressed completely after four cycles of Paclitaxel and remained in remission for 18 months.

In conclusion, KS continues to cause significant morbidity and mortality worldwide in both pediatric and adult populations. Our experience suggests that paclitaxel is a promising and effective first line option for treatment of iatrogenic KS with tolerable side effects and sustained response. Further studies are required to standardize the paclitaxel treatment schedule and dosage in the disseminated iatrogenic type of KS.

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Case Report

Ascites: a loadstar for the diagnosis and management of an intracranial tumor

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ABSTRACT

Background. Ascites is defined as abnormal fluid retention in the peritoneal cavity and it can be encountered at any age including fetal life. Ascites mostly results from cirrhosis, chronic renal disease and heart failure in childhood. However, there are various reasons for cirrhotic and non-cirrhotic ascites in the pediatric age. Cerebrospinal fluid ascites is one of the rarest.

Case. A 3.5- year- old Sudanese boy who underwent right-sided ventriculoperitoneal shunt surgery for hydrocephalus 10 months ago was admitted to the Neurosurgery Intensive Care Unit for intracranial tumor surgery. He had neurologic deterioration and ascites accumulation for the last 4 months. He was consulted with the pediatric gastroenterologist to exclude the reasons causing ascites after admission. No chronic liver, renal or heart disease was shown. The gross appearance of ascitic fluid was so clear that it resembled the cerebrospinal fluid and laboratory analysis results were compatible with transudate. The magnetic resonance imaging identified a mass in the left lateral ventricle. From the pediatrician's perspective, overproduction of cerebrospinal fluid from a tumor was assumed and shunt exclusion was proposed to alleviate ascites. After the externalization of the stunt and external ventricular device implementation, no further ascites occurred. The patient had a successful tumor excision and discharged after gaining oral feeding ability and sufficient weight gain.

Conclusion. In case of intractable ascites occurrence after a ventriculoperitoneal shunt placement, a pediatrician should consider etiologies resulting in imbalance of absorption and secretion function of cerebrospinal fluid.

Key words: ascites, ventriculoperitoneal shunt, choroid plexus papilloma.

Peritoneal fluid is the product of ultra-filtration activity of the peritoneum.[1] In healthy individuals, visceral and parietal layers of the peritoneum are flushed with a small volume of peritoneal fluid, typically 5-20 mL or up to 100 ml at most.[1,2] Ascites is the abnormal accumulation of peritoneal fluid secondary to the imbalance between peritoneal secretion and absorption functions.[2] Cirrhosis is the most common cause of pediatric ascites. However, non-cirrhotic factors should be reviewed in patients with ascites and good functioning livers. Here, we present a patient referred to our hospital with a symptom that accelerated the diagnosis and treatment of an intracranial tumor; resistant ascites.

Case Report

A 3.5- year- old Sudanese boy was admitted to our Neurosurgery Intensive Care Unit for the management of his intracranial tumor. He was consulted with the Department of Pediatric Gastroenterology due to his history of intractable ascites for the last 4 months. According to his brief medical report from Sudan, he was in a good state of health until he was 2 years old. He presented to a local hospital in Sudan 18 months ago with complaints of

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fever and irritability. Antimalarial medications were empirically given due to the high incidence of malaria in Sudan. In the following 6 months, increase of the head circumference and neurological deterioration such as loss of abilities to sit up, walk, and swallow occurred gradually. Ten months ago, the patient underwent right-sided ventriculoperitoneal (V-P) shunt surgery to relieve his hydrocephalus but the intracranial mass excision could not be performed concurrently. His neurologic deterioration progressed and ascites developed. Serial paracentesis during the last 4 months in Sudan did not improve his symptoms and he was referred to our hospital's Department of Neurosurgery and admitted to Intensive Care Unit. At admission, he was lethargic. Vital parameters showed no fever but tachypnea, tachycardia and hypotension. On physical examination, macrocephaly, setting-sun eye phenomenon, severe malnutrition with muscle wasting, dyspnea, severe abdominal distention, umbilical hernia as well as the findings of dullness on percussion and transmitted fluid wave were determined (Fig. 1). Liver and spleen could not be balloted. No other physical stigma revealing chronic liver disease were observed. Despite his oral automatisms and deterioration of consciousness, cranial nerve functions and deep tendon reflexes showed no abnormalities. Pathologic reflexes were negative.

In laboratory analysis, transaminases (ALT: 15 U/L, AST: 23 U/L), tests for liver synthesis functions (albumin: 4.15 g/dL, prothrombin time: 13.7 seconds, INR: 1.12, ammonia: 52 ug/dL) and renal functions were normal. Viral serology results for Hepatitis B and C, Toxoplasma, Rubella, Cytomegalovirus, Herpes viruses excluded active or chronic infection. Further work up for autoimmune liver disease, Wilson's disease and other metabolic diseases were negative. Neither findings of chronic liver disease and renal disease nor a shunt related pseudocysts were determined on the abdominal ultrasonography. The only positive finding was massive ascites. Portal and hepatic vein flow parameters excluded portal hypertension and Budd-Chiari syndrome. Echocardiography was normal.

Biochemical as well as microbiological analysis of cerebrospinal fluid (CSF) and ascites were evaluated. Despite the high level of CSF protein (1723 mg/L), glucose level of CSF was normal (CSF glucose: 116 mg/dL and serum glucose: 105 mg/dL). The gross appearance of ascitic fluid was so clear that it resembled the CSF and according to laboratory analysis it was classified as transudate. Microbiological and cytological studies of CSF were negative. Ziehl-Neelsen staining of the ascites was negative. No malignant cells, bacteria or parasites were determined in cytological evaluation. Cultures of ascites were negative for bacterial agents including tuberculosis. The cranial magnetic resonance imaging identified significant

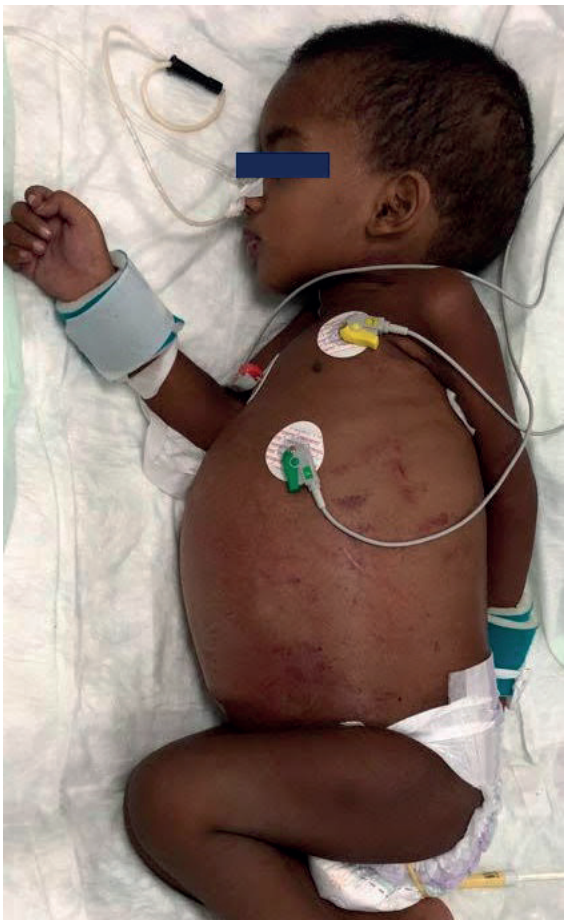

Fig. 1. The patient before externalization of the ventriculoperitoneal shunt.

communicant hydrocephaly, compression of cerebral and cerebellar parenchyma and a multi-lobulated hypervascular mass (49x49x53 mm) located in the left lateral ventricle. Excessive amount of CSF produced by a tumor and drained into the abdomen via V-P shunt was hypothesized to be the underlying reason. Hence, shunt externalization was suggested to the neurosurgeon in order to improve clinical situation before tumor surgery. In the first operation, the surgeon externalized the abdominal end of V-P shunt from the neck and performed large volume paracentesis. Ten days later, the V-P shunt was totally removed and external ventricular drainage (EVD) was placed. Approximately 1000-1200-mL/day of CSF was drained from EVD and no ascites developed afterwards (Fig. 2). Three weeks later the intraventricular tumor was totally excised. The pathologic assessment revealed choroid plexus (CP) papilloma. In the following days, the patient started to swallow liquids effectively and was discharged on the 40th day of the curative surgery after sufficient weight gain.

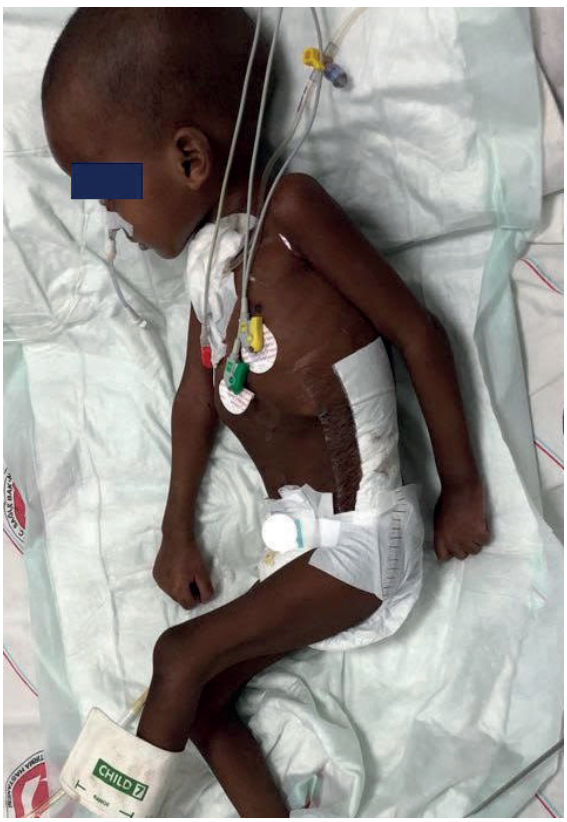

Fig. 2. The patient after choroid plexus excision surgery.

Written informed consent regarding clinical data and any accompanying photographs was obtained from the patient's parents.

Discussion

Ventriculoperitoneal shunt surgery was developed to manage hydrocephalus.[4,5,6] Due to its safety, convenience, and lower systemic infection risk, the procedure that is targeting to benefit from the absorption function of the peritoneum, diverting the CSF into the abdomen by a V-P shunt, is preferred.[5] However, several complications of V-P shunts from the first day to 12th year of the surgery have been reported.[3] Infection, malfunction and occlusion of the shunt are the most common complications. Shunt migration into the thorax (hydrothorax) and scrotum (hydrocele), shunt pseudocyst, intracranial haemotoma, intestinal perforation and shunt/CSF ascites are rarely encountered after V-P shunts.[3,5]

This patient was referred to our hospital for the management of his two problems; an intracranial tumor and ascites, which is a more significant problem that enabled us to reach the main diagnosis. After exclusion of other common non-cirrhotic ascites' reasons (nephrotic syndrome, Budd-Chiari syndrome, tuberculosis, and cardiac failure), the possibility of CSF overproduction was considered in the case of intracranial tumor and V-P shunt implantation.

Reasonable amount of CSF in the abdomen is expected after V-P shunts. However, CSF ascites is an infrequent complication of V-P shunt. While the pathophysiology is still not clear, two possible mechanisms accused for CSF ascites formation are exaggerated secretion of CSF from a tumor and reduced absorption of CSF due to peritoneal infection and/or inflammation.[3]

Other contributing factors and their possible mechanisms for CSF ascites are listed as;

1) Peritoneal seeding of a malignant tumor via increase in:
   - CSF protein level and oncotic pressure within the peritoneal cavity
   - Peritoneal permeability by over secretion of vascular permeability factor
2) Chronic infection such as tuberculosis meningitis via increase in CSF protein level
3) Abdominal surgery or repetitive shunt revisions via formation of adhesions and inflammation
4) Chemotherapy via inflammation related malabsorption
5) Immunizations via immune response related inflammation
6) Catheter rejection via eosinophilic inflammation.[3,4,6]

Nonetheless, some cases still remain idiopathic.[4]

Intracranial pathologies such as CP tumor, craniopharyngioma, and optic-hypothalamic glioma have been reported to result in cerebrospinal ascites after V-P shunt implantation.[5,6]

Choroid plexus tumor of the central nervous system, a spectrum disorder based on tumor grading, covers CP hyperplasia, CP papilloma, and CP carcinoma.[7] Choroid plexus papillomas are benign tumors located mostly in lateral ventricles and that can be cured by surgical resection.[8] They mainly present in the first 10 years of life, especially in the first two years, with signs of increased intracranial pressure and hydrocephalus.[7] Choroid plexus is made of tufts of villi producing 100-150 mL of CSF daily in healthy adults and nearly half of that volume in children.[4] Besides, CP papilloma produces huge amount of CSF such as 400-5000 mL daily.[9] After we implanted the EVD, 1000-1200 mL of CSF was discharged daily, and no longer intra-abdominal fluid accumulated as a supporter of the CSF overproduction hypothesis.

On the top of the differential diagnosis list, there is CP hyperplasia, a low-grade form of the CP tumor. Choroid plexus hyperplasia (diffuse villous hyperplasia of the choroid plexus) is defined as the bilateral symmetrical enlargement of the CP with a normal choroidal morphology and distinguished from CP papilloma based on the pathologic assessment.[7] Infection-related pseudocyst of the shunt should also be excluded in case of V-P shunt and excessive abdominal fluid association.[5]

The literature about the treatment for CSF ascites reveals that many of the cases related to various etiologies other than infections have resolved by diverting the shunt into the atrium (ventriculoatrial shunt)[4] or rarely into the gallbladder.[10] Ventriculopleural shunt is not advised due to the structural similarity of the pleura with the peritoneum in order to prevent hydrothorax. In case of infection, EVD is advised besides the aggressive antibiotherapy.[5] The optimal treatment for CP papilloma is surgical resection but the vascular structure of the tumor makes it challenging.[7]

In conclusion, massive sterile ascites is a rare complication of V-P shunt. The clinician should be suspicious about an etiology that affects the balance between absorption and secretion of CSF in case of intractable ascites accompanying a V-P shunt. The macroscopic feature of the ascitic fluid such as clarity may be the first clue. Differential diagnosis of ascites should be done carefully because the management of the various etiologies is different. Ruling out infections, pseudocyst and malignancy by imaging techniques and ascitic fluid analyses should be the first steps of the CSF ascites algorithm. The shunt externalization gives the advantage of relieving symptoms besides identifying the source of ascites.

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Case Report

Nosocomial pneumonia caused by water-born *Legionella pneumophila* in a pediatric hematopoietic stem cell transplantation recipient for thalassemia major

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ABSTRACT

Background. Nosocomial pneumonia caused by *Legionella pneumophila* serogroup 2-14 occurred in a 7-year-old patient following allogeneic hematopoietic stem cell transplantation for thalassemia major.

Case. The patient was diagnosed with nosocomial *Legionella pneumophila* by polymerase chain reaction (PCR) examination of the bronchoalveolar lavage and culturing *Legionella pneumophila* serogroup 2-14 from the patient's room faucet water. *Legionella pneumophila* was eradicated from our hospital's water distribution system by superheating and chemical eradication methods (hyper-chlorination and hydrogen peroxide). We did not detect any other case after this event.

Conclusion. Early recognition of contamination of the hospital water system with *Legionella* proves the importance of prevention in new cases.

Key words: Hematopoietic stem cell transplantation, *Legionella pneumophila*, pneumonia.

Legionella pneumophila is an intracellular gram-negative bacillus that requires special microbiological culture media. It seems that the risk of *L. pneumophila* pneumonia may be increasing in immunocompromised patients.[1,2] Patients who receive bone marrow transplantation are sensitive to *Legionella* infection due to prolonged intervals of neutropenia and abnormalities in cell-mediated immunity. Therefore, *Legionella* infections in an immunocompromised patient can easily be severe and cause high mortality.[3] Nosocomial *Legionella* infections are often transmitted via contaminated aerosol or aspiration of contaminated water. Relevant aerosol sources for hospital-acquired legionellosis include therapeutic devices and water distribution system outlets.[4-6] Some practices for eradicating *Legionella* from the hospital water distribution system are superheating, hyper-chlorination, ultraviolet light, and the addition of copper and silver electrodes to the water.[3,5] Herein, we report the management of an immunocompromised child with nosocomial *Legionella pneumophila* pneumonia acquired from the contaminated hospital water system.

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Case Report

A 7-year-old girl followed-up with the diagnosis of thalassemia major (thalassemia mutation analysis IVS 1.110 G>A homozygote mutant) was hospitalized due to a second allogeneic hematopoietic stem cell transplantation (HSCT). The patient had undergone HSCT at the age of 4.5 years from her sibling with full compatible tissue type, but after three months, secondary graft rejection occurred, and recurrence of thalassemia major was considered. After three

years, the patient underwent a 2nd HSCT again from her sibling with full compatible tissue type. Because of the patient's fever (38.5 C°) during anti-thymocyte globulin (ATG) therapy administered in the period of HSCT preparation, intravenous (IV) cefoperazone-sulbactam therapy was initiated. Blood, catheter, and urine cultures collected from the patient. Fever's focus was negative, and her fever did not persist. Since expected engraftment was not achieved, the patient underwent bone marrow aspiration biopsy and chimerism examination. According to the results, the case was evaluated as primary rejection. Upon having a fever that exceeded 39 C° on the 26th day after transplantation, blood, catheter, throat and urine cultures were collected, IV cefoperazone-sulbactam therapy was terminated and, IV meropenem and IV teicoplanin were initiated. Because the duration of neutropenia was prolonged, prophylactic fluconazole therapy was replaced with 1mg/kg IV liposomal amphotericin B therapy on alternate days. At follow-up, the patient had complaints of non-productive coughing, abdominal pain, and headache. On the 3rd day of fever, the respiratory sounds were decreased in the right middle and lower zones. A chest X-ray examination was carried out, and pneumonic infiltration on the lower lobe of the right lung was detected (Fig. 1). In this period, white blood cell (WBC) count, hemoglobin (Hb) level, and platelets (Plt) count were found as 100/mm^3, 9.4 g/dL, and 17000/mm^3, respectively and C-reactive protein (CRP) was 200mg/L. Serum galactomannan was found as 0.3 s/co and cytomegalovirus (CMV) DNA was negative. Blood, catheter, urine, and throat cultures were also negative. Thoracic computerized tomography (CT) of the patient revealed a consolidated area in the right lung lower lobe superior segment and minimal pleural effusion (Fig. 2). Although the patient was under beta-lactam antibiotics treatment, fever persisted. Because of persistent fever, bronchoscopy was performed. Bronchoalveolar lavage (BAL) fluid was negative for bacterial and fungal cultures. BAL galactomannan level was found as 0.38 s/co, and rare leukocytes and lymphocytes were observed among alveolar macrophages. Infectious mononucleosis PCR, CMV PCR, *Pneumocystis jirovecii* PCR, *Mycobacterium tuberculosis* PCR, respiratory virus PCR panel were negative, while *L. pneumophila* PCR was positive. *L. pneumophila* antigen was negative in the urine. IV levofloxacin and PO azithromycin were added to her treatment for *Legionella* pneumonia. Autologous cells of the patient were infused because of long-term neutropenia and severe pneumonic infiltration. The patient's fever resolved on the 15th day of fever, and clinical signs of pneumonia disappeared gradually. She received azithromycin for 14 days and levofloxacin for 21 days.

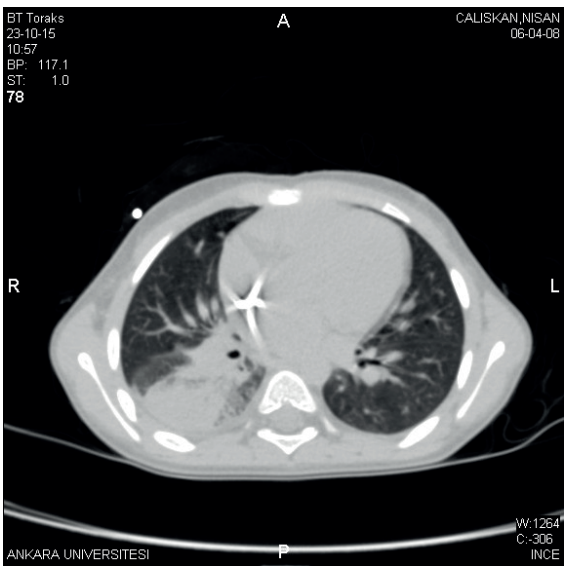

Fig. 1. Pneumonic infiltration of the lower lobe of right and left lung, chest X-ray 2.

Upon *L. pneumophila* PCR being detected in *the* BAL fluid in this period, the case was reported to the local public health officials. Tap water of all floors, air conditioning systems, hot and cold water tank outlet temperatures, and chlorine levels were examined in our pediatric hospital. Cultures were collected for *Legionella spp.* Water temperature was between 39-68.4 C°, and the chlorine level varied between 0.26-0.47ppm. From the cultures collected, 12000cfu/L colonies of *L. pneumophila* serogroup 2-14 was isolated from the culture of the tap water of the patient's room. Additionally, 550000cfu/L-9000000 cfu/L

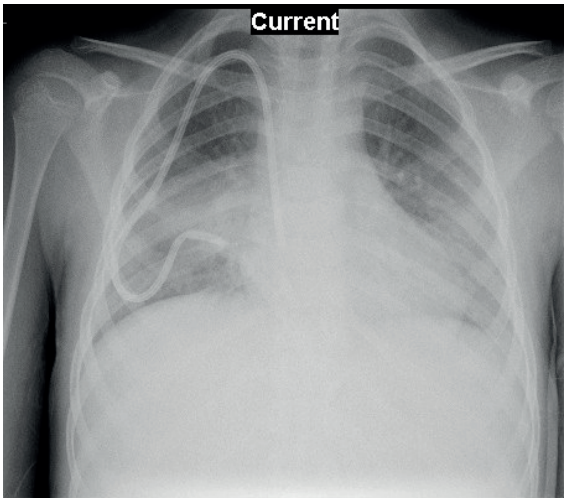

Fig. 2. Thoracic computerized tomography (CT) of the patient revealed consolidated area 3 and minimal pleural effusion in the right lung lower lobe superior segment.

colonies were isolated from the culture of the tap water of treatment room of neonatal intensive care unit (NICU) and the intermediate NICU.

Following the examination of *L. pneumophila*, superheating, hyper-chlorination, and hydrogen peroxide (H_2O_2) were implemented for decontamination purposes. All faucets and showerhead filters were flushed with descaling agents. These processes were repeated two times with one-week intervals. Temperature, chlorine level, and culture samples were collected again from the same places after two weeks.

Discussion

We report nosocomial pneumonia of *L. pneumophila* in a 7-year-old female patient in the HSCT unit. Informed consent was obtained from the family. Because *L. pneumophila* was only detected with PCR in the patient's BAL fluid, we could not reveal its genotype. The increasing risk of *L. pneumophila* pneumonia in immunocompromised patients has previously been reported.[1-4,8,9] In our patient, azithromycin, and levofloxacin were added to treatment without waiting for the results of water system culture samples since the patient was immunocompromised and did not give a clinical response to beta-lactam antibiotics. After detecting the PCR positivity, potential *Legionella* case was reported, and 22 samples were collected from the water systems. The water temperature of the tap water in the patient's room was found as 39 C° and chlorine level as 0.26 ppm. 12000cfu/L colonies of *L. pneumophila* serogroup 2-14 was isolated from the culture of the tap water of the patient's room. In a study by Orsi et al.[10] concerning *Legionella* control in the water system of antiquated hospital buildings, similar to our water characteristics, low level of residual free chlorine and water temperatures being between 20 C° and 45 C° were reported as risk factors. Nosocomial *Legionella* infections are often transmitted via contaminated aerosol or aspiration of contaminated water. Relevant aerosol sources for hospital-acquired legionellosis include humidifiers, nebulizer masks, showers, taps, cooling towers.[4-6,11-13] In our case, we suggested that the infection was acquired by microaspiration of contaminated faucet water. *L. pneumophila* can create a biofilm layer, persisting for years in the water distribution system and requires biocides to be cleaned.[12,14] Infection control for *L. pneumophila* is challenging and includes periodic inspections, cleaning, and maintenance of the water distribution systems, and decalcification of showers/taps. Routine microbiological surveillance and chemical monitoring of the water supply is necessary.[10,12,15] For example, some practices for eradicating *Legionella* from the hospital water distribution system are superheating, hyper-chlorination, ultraviolet light, and the addition of copper and silver electrodes to the water.[3,7] In our study, after the reproduction of *L. pneumophila*, the temperature of hot water was increased to 70 C° for decontamination. Hyper-chlorination and H_2O_2 were applied in the water system and tanks. All faucets and showerhead filters were cleaned with decalcification. These processes were repeated two times with 1-week intervals. Temperature, chlorine level, and culture samples were collected again from the same places after two weeks and examined. Water temperature differed between 61.9-70 C° and

chlorine level between 0.36-0.67 ppm. Oren et al.[3] also reported the control of the *Legionella* outbreak in their hospital, which was derived from the water system in the HSCT unit by superheating and hyper-chlorination. Likewise, the follow-up cultures collected in our hospital were negative, and the collection of water cultures was planned to take place once every two months for two years.

In brief, a nosocomial *Legionella* pneumonia outbreak originating from a water distribution system was prevented by early diagnosis, rapid treatment, and management of outbreak control with the collaboration of local public health officials. In order to prevent such outbreaks, inspections, cleaning, and maintenance of the water distribution systems, and decalcification of showers/taps should be performed periodically. There must be routine microbiological surveillance and chemical monitoring of the water supply in hospitals.

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Case Report

Abusive head trauma: two cases and mini-review of the current literature

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ABSTRACT

Background. Abusive head trauma (AHT) is the leading cause of fatal head injuries and are responsible for more than half of serious or fatal traumatic brain injury cases in children younger than 2 years of age. Long-term outcomes of AHT are death, spastic hemiplegia or quadriplegia, intractable epilepsy, microcephaly with cortico-subcortical atrophy, visual impairment, language disorder and cognitive, behavioral and sleep disorders.

Cases. Herein we present two cases of AHT (7-month-old boy, 7-month-old girl) according to forensic analysis, and discuss them in light of the current literature and share our experience. Inconsistency between the presenting history and the clinical findings were typical in both cases; follow-up histories and detailed workup revealed the diagnosis of AHT. The first case was deceased; the second case was discharged with neurological deficits.

Conclusion. A multidisciplinary approach is critical for the prevention, diagnosis and treatment of AHT.

Key words: shaken baby syndrome, retinal hemorrhage, subdural hemorrhage, forensic.

Abusive head trauma (AHT) is the leading cause of fatal head injuries and responsible for more than half of serious or fatal traumatic brain injury cases in children younger than 2 years of age.[1] According to Chevignard and Lind[2], long-term outcomes of AHT are death (20-25%), spastic hemiplegia or quadriplegia (15-64%), intractable epilepsy (11-32%), microcephaly with cortico-subcortical atrophy (61-100%), visual impairment (18-48%), language disorder (37-64%) and cognitive, behavioral and sleep disorders (23-59%). Prevention of AHT is of utmost importance for saving children from mortality and morbidity. The estimated lifetime cost of 4,824 cases at ages 0-4 years in 2010 was $13,5 billion in the USA.[3]

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In 1946, Caffey[4] first documented six children with chronic subdural hematoma and fractures of long bones in his study. After that other authors mentioned this phenomenon.[5-7] In 1962, Kempe et al.[8] named it as "battered-child syndrome". In 1971, Guthkelch[9] suggested that subdural hematoma (SDH) in infants could be created by whiplash forces which tear the bridging veins. He made this suggestion based on Ommaya's study in 1968 on primates using rotational movement of the head on the neck to produce concussion and SDH.[10] In 1972, Caffey[11] coined the term "whiplash shaken infant syndrome" defined by infants with SDH and/or subarachnoid hemorrhage (SAH), retinal hemorrhages (RH), with little or no external marks of trauma to the head. In 1987, Duhaime et al.[12] hypothesized that based on clinical, pathological data and biomechanical models, the rotational acceleration/deceleration whiplash injuries do not provide enough force to account for the severe injuries of these

children and that in severe cases blunt trauma must be involved. After this article, the term shaken infant/shaken impact emerged. There are still debates over whether shaking alone or shaking with blunt trauma is required.[13-15] The etiology of injury is multifactorial (shaking, shaking and impact, impact alone, etc.) so that the current best and most inclusive term is AHT, as recommended by the American Academy of Pediatrics.

The aim of this study was to share our experience about how to diagnose and handle AHT which is rarely diagnosed, and review the literature. An informed consent was received from the legal guardians. This study was carried out in concordance with international ethical standards and the World Health Organisation Helsinki Declaration. It was approved by The Presidency of The Council of Forensic Medicine Education and Scientific Research Board (Approval No:21589509/2018/709) and the local ethics committee of the Medical Faculty of Marmara University (Protocol No:1016).

Case Reports

Case 1

A 7-month-old male infant was brought to the emergency department with complaints of inability to breathe and cyanosis of the lips. At the first interview, the mother stated that "After I had fed the baby, I left the room. Ten minutes later I found him in a color of purple, lying face down". On the presentation to the hospital, the infant had tachycardia and tachypnea. Glasgow Coma Score (GCS) was 7, his body temperature was 36.7 °C. He was intubated and was referred to the intensive care unit. His blood and cerebrospinal fluid cultures were negative for microbiological agents. Complete blood count, electrolytes, coagulation tests, liver and kidney function tests were also normal. Cranial computerized tomography (CT) depicted subdural hemorrhage on falx cerebri and surface of bilateral cerebellar hemispheres, predominantly on the left side. Brain magnetic resonance (MR) imaging scan verified the CT findings. The neurosurgeon opted conservative treatment approach with anti-edema and anti-seizure medication. When the retina was examined, bilateral intra and pre-retinal hemorrhages were found. Both transfontanel and abdominal ultrasound (US) findings were normal for any other traumatic injuries. Also, the full body skeletal survey was negative.

The pregnancy period of the mother was uncomplicated and the infant was born by vaginal delivery at term. The birth weight of the infant was 2,875 g; there was no ante-, peri-, or post-natal medical problems.

Then the infant was directed to the Child Protection Unit in the hospital for the evaluation of suspected child abuse. In several interviews at the Child Protection Unit; the mother reported that she was 31-year-old and had two marriages. Both of these marriages ended in divorce. She had four children from these marriages. Two of the children were from the first husband. The parental rights of those two children were with the biological father. The infant was born as the fourth child of the mother from her second marriage. The parents had divorced and the children were living with their mother and the mother's new partner. The partner was an employee of a cargo company and was rarely at home, he didn't care about the children. The mother was emotionally and economically dependent on the partner. The mother didn't have alcohol or drug abuse. According to the history obtained from the mother, on that day, she fed the child and put him on her shoulders. While she was trying to hold the child, he fell down to the ground. Two to 3 days later, she realized the deviation of the eyes and loss of consciousness of the child. She decided to bring the child to the emergency unit. These statements of the mother were inconsistent with her first interview. The follow-up histories of the mother, presence of bilateral retinal and subdural hemorrhages and no other explanations for presented findings were diagnosed as AHT by the council. Then the case was reported to Directorate of Family and Social Policies Ministry and the public

prosecutor. After 20 days in the hospital, the infant was taken to state care by social workers. When he was being discharged from the hospital, as a consequence of global paralysis, he got stage 2 spasticity in the upper extremities, stage 3 spasticity in the lower right extremity and global blindness.

Two months after discharge, he died and an autopsy was performed posthumous, his brain was atrophic and the cause of death was pneumonia. Figure 1 shows the severity of brain atrophy.

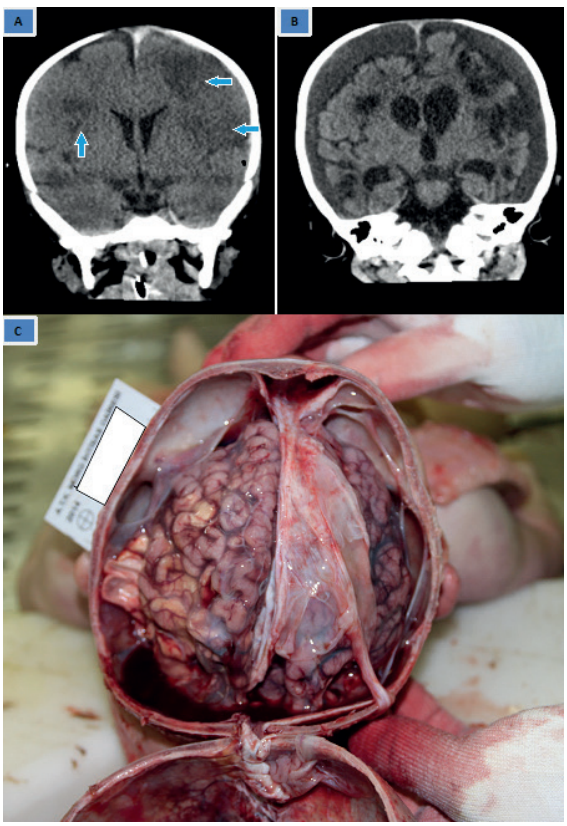

Fig. 1. Generalized cerebral atrophy progressions due to abusive head trauma of case 1: A) a coronal non-contrast computerized tomography (CT image), 4 days after trauma, revealed subacute hypodense lesions (arrows indicate lesions), B) a coronal non-contrast CT image, 68 days after trauma (obtained from Marmara University Hospital with permission), revealed generalized cerebral atrophy and chronic subdural effusion, C) autopsy image, 112 days after abusive head trauma (obtained from The Council of Forensic Medicine with permission) showed generalized cerebral atrophy.

Case 2

A 7-month-old female infant was brought to the emergency room with complaints of vomiting, seizures lasting 10-15 minutes and altered mental status. The infant was referred to our hospital for further evaluation and treatment. At the first interview, the mother stated that "the baby had weird movements. Then I shook her by holding her feet in order to bring her consciousness back and to stop the movements. But that attempt was not successful and my sister-in-law hit the infant's back."

The infant was presented as being conscious, hypotonic, lethargic and pale. Her GSC was 11. She was unable to hold her head in an upright position. The neurological exam showed that she had a right-sided hemiparalysis. Her cardiovascular and respiratory systems were normal. Cranial CT depicted subdural hemorrhage. The infant was referred to the intensive care unit to treat the recurrent seizures and cranial hemorrhage. Her blood and cerebrospinal fluid cultures were negative for microbiological agents. Complete blood count, electrolytes, coagulation tests, liver and kidney function tests were also normal. The neurosurgeon opted for conservative treatment approach with anti-edema and anti-seizure medication. When the retina was examined, bilateral pre-retinal hemorrhages were found. Brain MR angiography and venography were normal. Abdominal US was also normal for any other traumatic injuries. Full body skeletal survey was negative. The mother's pregnancy was desired and uncomplicated. The infant was born at the gestational age of 38 weeks with a birth weight of 2,200 g.

Then the infant was directed to the Child Protection Unit in the hospital for the evaluation of suspected child abuse. In the interviews in the Child Protection Unit; the mother stated that she was 39-year-old, had three children, and was not working at that time. The father was 35-year-old and he was working in a bar. The mother explained that the day had been very strenuous because the infant had cried

for long periods of time. While she was trying to put the infant to sleep, she started doing some spasmodic movements. Then the mother grabbed her by her feet and shook her several times. She described her shaking as mild. Psychiatric examination of the mother revealed that she had not shown any psychopathological mistreatment of her child.

The follow-up histories of the mother, presence of pre-retinal and subdural hemorrhages and no other explanations for presented findings were diagnosed as AHT by the Council. Then the case was reported to Directorate of Family and Social Policies Ministry. After spending 25 days at the hospital, her condition became more stable, seizures were under control and she was discharged from the hospital. During the medical treatment procedures, social investigation report about the family revealed that the family provided basic needs for children. The neighbors of the family stated that they had not witnessed any violence inside the family or against the children. Social services decided to support and educate the parents about child development and how to overcome difficult situations. And also social services provided them with advisory assistance about parental skills. The infant was left in her parents' care and the parents obligated to bring her to pediatric neurology and physical medicine and rehabilitation clinics for follow-up examinations. No legal action was taken against the family.

Table I shows the clinical short summary of the cases.

Discussion

The diagnosis of AHT is made like any other medical diagnosis, by getting all the information acquired via clinical history, physical examination, and laboratory and imaging. Inconsistency between the presenting history and the clinical findings is an important diagnostic tool for child maltreatment including AHT so detailed history including follow-up histories are vital for the accuracy of diagnosis.[16,17] In both of our cases, there was an inconsistency between presenting histories and follow-up histories. The most common history is a minor fall (less than 1.5 m).[18] As in case 1, the mother dropped the infant from her shoulder. But hitherto literature shows us that death from minor falls is extremely rare, and the majority of these have minor traumatic lesions or no lesion.[19,20] There is no pathognomonic finding for AHT. Compilation of injuries most often include SDH, complex retinal hemorrhage

Table I. Clinical short summary of cases.

Clinical features	Case 1	Case 2
Gender	Male	Female
Age	7 months	7 months
Presenting history	Infant lying face down	Infant had weird movements
Follow-up history	Mother dropped infant from shoulder to ground	Infant cried all day. Mother partly admitted to shaking her baby
Cranial computerized tomography images	Subdural haemorrhage on falx cerebri and surface of bilateral cerebellar hemispheres, predominantly on the left side	Subdural haemorrhage
Retinal examination	Bilateral intra and pre-retinal haemorrhage	Bilateral pre-retinal haemorrhage
Social condition of the family	Mother had complex psychosocial issues (divorced, male-dependent low economic income)	Exhausted mother spent all her time with infant and house work.
Result	Infant was taken from mother's care, but died after two months.	Social services supported family regarding child development and parental skills. Infant was followed by schedule at hospital

and/or retinoschisis, rib, metaphyseal or other fractures, and soft-tissue injury leads to the diagnosis. Findings inconsistent with the provided history of trauma and the severity and age of the findings provide clues to the diagnosis. Subdural hemorrhage is the most common finding in AHT, is most commonly parafalcine in location and is seen in 90-95 % of fatal cases and in 40-55 % of living patients where it is imaged by either CT or MRI[12,19,21,22], but brain parenchymal injury is the most important cause of morbidity and mortality.[23] In both of our cases, the babies had subdural hemorrhages and in case 1, SDH was on falx cerebri and surface of bilateral cerebellar hemispheres. A review by Maguire[24] mentioned that any combination of 3 or more of the apnea, retinal hemorrhage, rib, skull, and long-bone fractures; seizures; and head and/or neck bruising yielded an Odds ratio of >100 (positive predictive value for AHT >85%).

Palusci and Covington[25] identified that a trigger to AHT's death was crying (20%), disobedience (6%), domestic arguments (5%), toilet training (4%) and feeding problems (3%). In case 2, the mother was exposed to crying for a long period of time.

Clinicians should perform a detailed examination of external lesions. Lesions to the head and face have been associated with AHT, and patterns of injury consistent with grabbing, choking and blunt trauma should be sought. Also, clinicians should keep in mind that the absence of external lesion is common.[26]

The skeletal survey according to guidelines should be performed for all children that are suspicious for AHT, especially those younger than 2 years.[27] Suspicion of AHT that warrants full-body imaging, does not have a similar diagnosis procedure with low-risk blunt head trauma.[28] In both of our cases, the skeletal surveys were found to be normal. If the child is brought to a hospital in acutely ill condition with severe neurologic symptoms, it is recommended to start imaging with unenhanced CT with 3-D reformatted images of the calvarium[29] followed by a full multi-sequence MRI of the brain and the cervical, thoracic and lumbar spine as soon as possible. MRI is more sensitive than CT in showing parenchymal lesions and early diagnosis of brain parenchymal injury is very important for preventing morbidity and mortality so children who are in a good condition neurologically, can be imaged with MR first.[30] Also, MRI is better in characterizing extra-axial bleeds and defining cerebral contusion, laceration and other parenchymal brain injuries than CT.

Younger children get more severe cognitive and motor function deficits than will older children.[31] Also, young infants have increased the risk for upper cervical injury (generally soft tissue or ligamentous).[32]

Ocular findings in AHT can be orbital and lid ecchymosis, subconjunctival hemorrhage, anisocoria, and disconjugate eye movements and retinal hemorrhages. In the absence of head trauma findings, retinal hemorrhage is more specific for AHT.[33] Retinal hemorrhages (RH) are very common findings in AHT and are found in 84% of cases.[34] The RHs which are mostly associated with AHT tend to be very multiple in the layers of the retina and which can extend to the ora serrata.[35,36] The RHs are generally bilateral but in some cases appear in only one eye. Early detection of retinal hemorrhages is very important because they can fade rapidly. Generally, intraretinal hemorrhages fade rapidly but preretinal hemorrhages could persist for many weeks. It is recommended that eye examination should be done as early as possible, preferably within 24-48 hrs.[37] In case 1, the infant had bilateral retinal hemorrhages, in case 2, the infant had bilateral pre-retinal hemorrhages.

Differential diagnosis should be done by clinicians. Accidental head trauma, medical or surgical manipulations, pre- and peri-natal conditions, birth trauma, metabolic disorders, clotting disorders, tumors, autoimmune disorders, infectious diseases, long-term shunting of hydrocephalus, and miscellaneous

other conditions need to be distinguished from AHT. It is compulsory to consider other causes and to examine all aspects of the history, physical examination, radiological imaging, and laboratory studies to exclude other causes and the question to be answered is "Is there any cause other than AHT to explain the child's medical findings and condition?".

When accidental head trauma and AHT are compared: 1- skull fractures are equally common, but the complex skull fractures are more common in AHT; 2- epidural hematomas are more common in accidental trauma; 3- subdural hematomas are far more common in AHT; and 4- subarachnoid, intraparenchymal and intraventricular hemorrhage are equally common in both AHT and accidental trauma.[14,38-41]

Prevention should be the main aim of dealing with AHT. Healthcare providers who work in pediatric and emergency departments must be educated and trained about identifying parents at high risk of child abuse and the parents should be taught how to deal with a crying baby and the danger of shaking or blunt impact on an infant with an undeveloped brain. Diagnosis of AHT is also important in countries like Turkey where it is rarely diagnosed. Due to the fact that diagnosis of AHT is not just a constellation of medical symptoms or finding, a multidisciplinary approach is needed. This multidisciplinary team should include pediatricians, neurosurgeons, radiologists, ophthalmologists, forensic medicine experts, social workers, and other health care professionals.

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Case Report

Takayasu arteritis presenting with spontaneous pneumothorax

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ABSTRACT

Background. Takayasu arteritis (TA) is an idiopathic chronic inflammatory arteritis that affects the large blood vessels. Pulmonary involvement was considered an uncommon manifestation of the disease and spontaneous pneumothorax has not been previously described in association with TA.

Case. We report a 13-year-old female who had TA complicated by spontaneous pneumothorax during treatment. She was admitted to the hospital reporting difficulty standing from a squatting position and inability to walk without support. She had been diagnosed with dilate cardiomyopathy four years ago and cardiac functions had deteriorated over time. Catheter angiography revealed diffuse narrowing of the abdominal aorta. In magnetic resonance angiography, total-subtotal occlusion of the infrarenal abdominal aorta in a 2 cm area and subtotal occlusion of the left renal artery were detected without pulmonary artery involvement. Methotrexate, azathioprine, and prednisolone were administered. Tension pneumothorax developed on the left side while she was on prednisolone treatment.

Conclusion. To our knowledge, this is the first case of spontaneous pneumothorax associated with TA to be reported in the literature.

Key words: spontaneous pneumothorax, Takayasu arteritis.

Takayasu arteritis (TA) is an idiopathic chronic inflammatory arteritis that affects the large blood vessels, predominantly the aorta, its major branches, and the pulmonary artery.[1,2] The clinical signs and symptoms of TA are quite variable and depend on the localization, and degree and severity of vascular involvement.[3] The pulmonary manifestations of TA include cough, dyspnea, chest pain, and pulmonary hypertension.[4] In the past, because of the rare presentation of pulmonary symptoms, pulmonary involvement was considered an uncommon manifestation of the disease; however, the prevalence of pulmonary arterial involvement is now estimated to be between 50% and 80%.[5]

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Pneumothorax is defined as the presence of air in the pleural space.[6] Spontaneous pneumothorax is a type of pneumothorax that occurs without trauma.[7] Pneumothorax is classified as both primary and secondary; primary pneumothorax occurs without underlying lung disease and secondary pneumothorax occurs in patients with underlying pulmonary disease.[8]

Spontaneous pneumothorax can be observed in various types of lung disease; however, rheumatologic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, anti-neutrophil antibody (ANCA) with associated vasculitis, scleroderma, and dermatomyositis are rarely associated with pneumothorax.[9] The majority of the information reviewed for this research was obtained from a series of case studies and reports. To our knowledge, spontaneous pneumothorax has not been

described in association with TA in previous literature.

Case Report

This case was of a 13-year-old female who was admitted to the hospital reporting difficulty standing from a squatting position as well as the inability to walk without support, which she had had for at least two months. Symptoms of vomiting, nausea, and loss of appetite were also reported. At the age of nine years, the patient had been diagnosed with dilated cardiomyopathy and was treated with diuretics, angiotensin-converting enzyme inhibitors, and digoxin. For the following 4 years, as the patient's cardiac function worsened, catheter angiography was performed, which revealed diffuse narrowing of the abdominal aorta. Acute- phase reactants were high and oral steroid (1 mg/kg/day) and azathioprine (100 mg/day) were started because of a probable diagnosis of TA. One month later, acute-phase reactants were still high and azathioprine treatment was changed to methotrexate (15 mg/ week). After three weeks, she developed pancytopenia and received three pulses of intravenous methylprednisolone (500 mg). Subsequently, the patient was referred to our hospital for further evaluation. On admission, her height and weight were 140 cm and 28 kg, respectively. On physical examination, she was irritable, her body temperature was 39°C, heart rate was 100 beats per minute, blood pressure was within normal range in the four extremities, lower-limb pulses were weakly palpable, upper-limb pulses were present and equal. A 3/6 systolic murmur was audible over the cardiac apex. Her muscle strength was grade 4/5 in the upper limbs and grade 3/5 in the lower limbs. The remaining physical examination was unremarkable.

Her laboratory findings showed leukopenia [white blood cells (WBC): 1900/μl], anemia [hemoglobin (Hb): 10.6 g/dl], and thrombocytopenia (platelets: 51,000/μl). Inflammatory biomarkers such as C-reactive protein and the erythrocyte sedimentation rate (ESR) were both elevated [44.9 (normal: 0-0.8) mg/dl and 60 (normal: 0-20) mm/h, respectively]. Blood glucose, liver and renal function parameters, and muscle enzymes were all within the normal range. The tests for anti-neutrophil cytoplasmic antibodies (ANCA), anti-nuclear antibody, anti-double stranded DNA (anti-dsDNA), and rheumatoid factor were negative. Complement levels were normal. Echocardiography showed severe generalized left ventricles dysfunction with an ejection fraction of 25%.

Magnetic resonance (MR) angiography revealed total-subtotal occlusion of the infrarenal abdominal aorta in a 2 cm area, and subtotal occlusion of the left renal artery. Pulmonary arterial involvement was not detected and the diagnosis of Takayasu arteritis was confirmed. Methotrexate treatment was stopped because of pancytopenia and prednisolone was continued at a dosage 1 mg/kg/day. Nontyphoidal salmonella group D was identified through a blood culture and amikacin and ciprofloxacin were started. The muscle weakness was thought to be due to steroid myopathy. She was not a candidate for anti-tumor necrosis factor (TNF) or anti-interleukin (IL)-6 agents as immunosuppressive therapy owing to her impaired cardiac function and thrombocytopenia. During her follow-up on antibiotic and steroid treatment, the patient was evaluated for lung involvement because she required oxygen supplementation.

Respiratory osculation and the chest radiography findings were normal. A pulmonary function test revealed forced expiratory flow in one second (FEV_1) 1.62 liters (70% predicted), forced vital capacity (FVC) 1.36 liters (68% predicted) and FEV_1/FVC 83%. As a result, extensive diagnostic tests were planned and the examination of the patient was remarkable for the absence of breath sounds in the left lung. Chest X-ray revealed left-sided pneumothorax (Fig. 1). Chest computed tomography (CT) was performed to evaluate the pulmonary parenchyma, which showed tension pneumothorax on the left side of the lung (Fig.

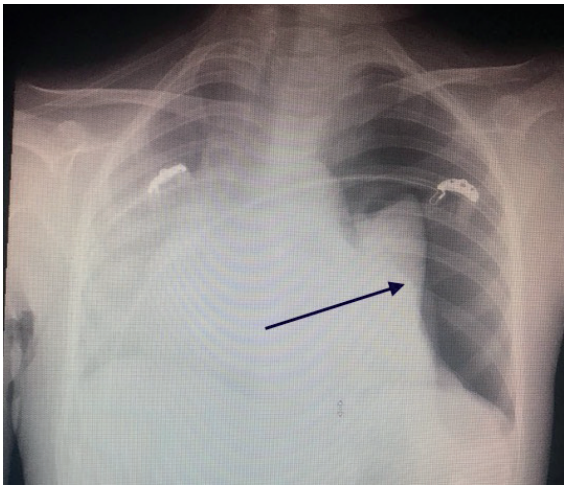

Fig. 1. Posteroanterior chest X-ray showing pneumothorax on the left site.

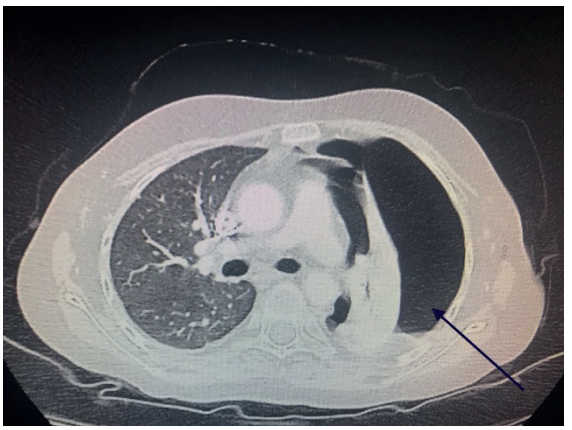

Fig. 2. Pulmonary CT scan reveals tension pneumothorax on the left side.

2). A chest tube was placed and the lung was re-expanded while the pleural cultures were negative. The chest tube was then removed after five days. Extensive viral tests were also negative.

After 14 days of antibiotic therapy, the patient's cytopenia improved and tocilizumab (10mg/kg) treatment was started and as a result the steroid treatment dose was reduced. After 3 months on tocilizumab treatment, the patient died of heart failure.

Informed consent was received from the families for publication of the case.

Discussion

Even though pulmonary involvement is seen in more than half of all patients with TA, predominant pulmonary involvement and its symptoms are not common. Most of the pulmonary symptoms mentioned in the literature were due to the pulmonary arterial involvement of the disease. Pulmonary parenchymal involvement is also rarely seen in TA; however, in the literature, there are reports of unusual manifestations of pulmonary involvement.[10] A case of TA with severe respiratory failure associated with bilateral parenchymal infiltrations that responded well to steroid treatment was described by Cilli et al.[11]

Takahashi et al.[2] evaluated the CT findings of the pulmonary parenchyma in 25 patients with TA and found low attenuation areas in the lungs of 11 patients, subpleural reticulolinear changes in 12 patients, and pleural thickening in nine patients. Their results suggested that regional hypoperfusion was responsible for the appearance of low attenuation pulmonary areas. They also made speculations regarding pulmonary thromboembolism in other CT findings for the pleura and adjacent lung.

The interesting feature of this case is the development of spontaneous pneumothorax, which is rarely associated with systemic inflammatory disease.[9] According to different studies, the incidence of pneumothorax in Wegener's granulomatosis (WG) is between 3 to 5%.[12,13] In certain reported cases of WG, the occurrence of pneumothorax was attributed to the rupture of cavitary lesions and immunosuppressive treatment.[9]

In a literature review by Tanaka et al.,[13] 11 patients with recurrent pneumothorax with SLE were evaluated. All patients had underlying pulmonary involvement such as interstitial lung disease, pulmonary suppuration, pulmonary hemorrhage, and pulmonary embolism or cyst formation. Additionally, all but one of the 11 patients in this study used glucocorticoid treatment.

Glucocorticoids have been shown to cause tissue fragility by creating adverse effects on both growth factors, as well as collagen deposition during wound healing.[13] Tissue fragility may be among the factors contributing to pneumothorax. On the other hand, the occurrence of pneumothorax has only been reported in a few cases, and the use of methotrexate and azathioprine, and their potential contribution to pneumothorax is unclear.[14] There is no data in the literature that suggest that methotrexate-induced parenchymal lung disease can predispose to the development of pneumothorax and bronchopleural fistula.[15] Gotsman et al.[16] described a case of pneumothorax secondary to the cavitation of a rheumatoid nodule during methotrexate treatment. It was speculated that methotrexate might have aggravated the previous pulmonary involvement. Also, there are no case reports indicating an association of azathioprine with pneumothorax. This association should be investigated further because it remains unclear.

Acute bacterial pneumonias caused by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, have all been associated with pneumothorax. Also, infectious agents such as *Pneumocystis jiroveci pneumonia*, fungal pneumonia and tuberculosis have also been associated with pneumothorax.[17] In these situations, pulmonary cavity formation due to infections are risk factors for the development of pneumothorax.[18] In our patient, there was no evidence of viral or bacterial pneumonia, but she had salmonella bacteremia, which has not previously been shown associated with pneumothorax development.

The underlying pathologic mechanism of pneumothorax in our patient has not yet been fully determined. We think that many factors such as glucocorticoids use, other immunosuppressive treatments, and the pulmonary involvement of TA may have contributed to the occurrence of pneumothorax. As a result, close monitoring of patients is important because pneumothorax, which occurs in rheumatic diseases, tends to be recurrent.[13]

To our knowledge, this is the first description of a patient with spontaneous pneumothorax associated with TA. Although there are other factors that may contribute to the development of pneumothorax, this finding can be considered as part of the wide spectrum of TA.

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Case Report

Rituximab-induced serum sickness and anaphylaxis in a child with nephrotic syndrome

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ABSTRACT

Background. Rituximab is effective for treatment of children with refractory nephrotic syndrome (NS). However, the drug may cause serum sickness characterized by fever, rash, and arthralgia 10-14 days after primary antigen exposure or within a few days after secondary antigen exposure. Rituximab may also lead to anaphylaxis. It is important to recognize rituximab-induced serum sickness (RISS) clinically, as it may mimic various infectious or vasculitic diseases.

Case. A six-year-old male with NS treated with rituximab presented with diffuse arthralgia and myalgia eight days after the first dose. He developed an urticarial rash and arthralgia one week after the second dose, while he had swelling of lips and periorbital regions, choking sensation and erythematous rash in whole body within minutes after the third dose of rituximab. The first two reactions resemble typical serum sickness whereas the third reaction seem to be an anaphylaxis/anaphylactoid reaction.

Conclusions. Although rituximab-induced serum sickness is typically self-limited, further infusions of rituximab should be avoided as it may provoke more severe symptoms. Most of the previous reported cases of RISS are patients with autoimmune or hematologic disorders. We present the first pediatric case with membranous nephropathy and RISS. The patient also developed anaphylactoid reaction during the third rituximab infusion.

Key words: rituximab, nephrotic syndrome, serum sickness, anaphylaxis.

Nephrotic syndrome (NS) is a renal disease characterized by massive proteinuria, hypoproteinemia and generalized edema. In NS, first line treatment is prednisolone. However, other treatments such as cyclophosphamide and cyclosporine A are used in frequently relapsing or steroid-resistant NS, respectively. Recently, rituximab has been used in both forms of NS when the response to other treatment options is inadequate.[1]

Rituximab is a chimeric murine/human anti-CD20 monoclonal antibody binding to the B-cell surface antigen CD20. Although its mechanism of action remains unclear, rituximab is effective for preventing relapse in refractory NS while avoiding steroid side effects.[2,3] On the other hand, rituximab has adverse events such as fever, cough, dyspnea, and erythema in patients with NS.[4-6] Rituximab may also rarely induce serum sickness.[7]

Rituximab-induced serum sickness (RISS) is a rare type III hypersensitivity reaction which occurs after injecting foreign antigens and is characterized by fever, rash, and arthralgia. RISS is relatively common among patients who have autoimmune and hematologic diseases. So far, only two cases of RISS in patients with NS (17 and 50-years-old) have been reported.[8,9] IgE-mediated hypersensitivity reactions, confirmed by positive skin tests, have also been reported.[10]

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Case Report

A 6-year-old boy was diagnosed with NS at 2 years of age. Renal biopsy performed for steroid resistant NS revealed membranous nephropathy. Evaluation for secondary causes of membranous nephropathy (HBV, HCV, HIV, syphilis serologies; ANA, anti-dsDNA, C3, C4) was inconclusive. As the parents did not accept cyclophosphamide treatment, calcineurin inhibitor was instituted. He responded to steroid plus cyclosporine A, but experienced frequent relapses. Thus, rituximab treatment was instituted as 375 mg/m^2 intravenously over 4 hours. He was premedicated with chlorphenoxamine 5 mg intravenously (IV) and acetaminophen 10 mg/kg IV. Trimethoprim-sulfamethoxazole (150 mg/m^2/day in 3 divided doses) was started for Pneumocystis jirovecii pneumonia prophylaxis. He had no infusion reactions. Eight days after the first dose, the patient presented with diffuse incapacitating arthralgia and myalgia, and he was hospitalized. Physical examination and laboratory tests were normal at that time. His symptoms improved within 24 hours without any intervention.

Two months later, the second rituximab infusion (in the same manner as the previous dose with premedication) was given as the number of CD19 and CD20-positive cells increased. The patient presented with diffuse rash and arthralgia the following week (Fig. 1). This time, his symptoms resolved spontaneously within two days.

Three weeks later, during the third cycle of rituximab (with the same previous dosing schedule), he developed swelling of lips and periorbital regions, choking sensation, generalized erythematous rash during rituximab infusion leading to discontinuation of the infusion (Figs. 2-4). On physical examination, the patient had a mild fever (37.8 °C) and was hemodynamically stable with a pulse rate 96/min, respiratory rate of 22/min, and blood pressure 105/65 mmHg. There was no sign of acute infection. He was treated with intramuscular methylprednisolone and IV adrenaline with rapid resolution of symptoms. His laboratory tests were as follows: leukocyte 5000/mm^3 with 43% neutrophils and 3.5% eosinophils, erythrocyte 4.4×10^6/mm^3, platelet 347×10^3/mm^3, C-reactive protein 0.7 mg/L, Anti-nuclear antibody negative, IgG 1102 mg/dl (N:700-1600), IgM 108 mg/dl (N:20-200), IgA 147 mg/dl (N:70-400), IgE 147 IU/ml (N:0-52), C3 116 mg/dl (N: 81-157), C4 13.9 mg/dl (N:13-39). Urinalysis and viral serology were normal.

The symptoms and signs of the patient were attributed to RISS (the first and second infusions) and to anaphylaxis (third infusion) based on the temporal relation of the clinical findings with rituximab infusion and rapid clinical improvement. Thus, rituximab treatment was discontinued.

Informed written consent was obtained from the parents for reporting the case.

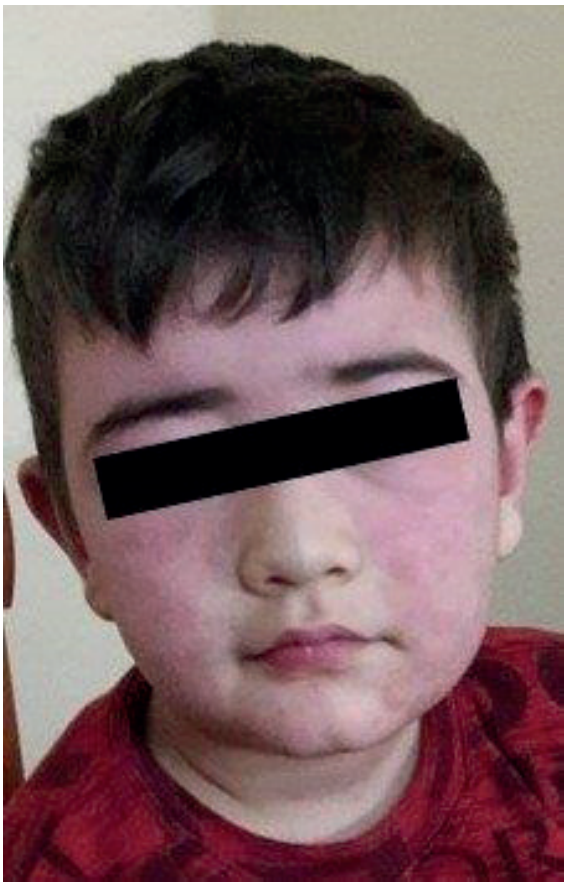
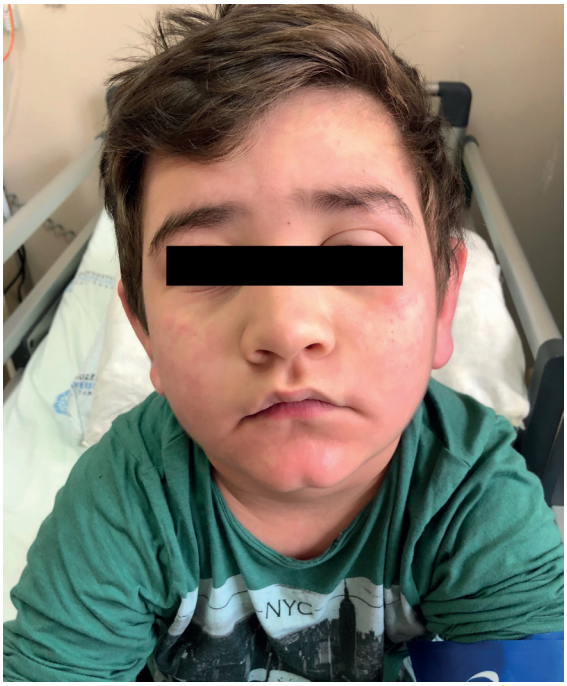
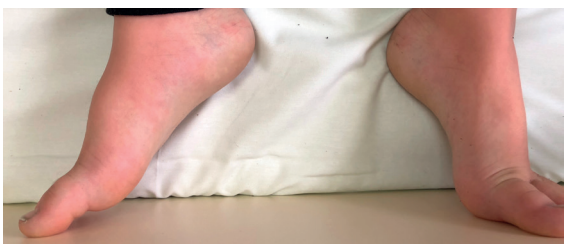
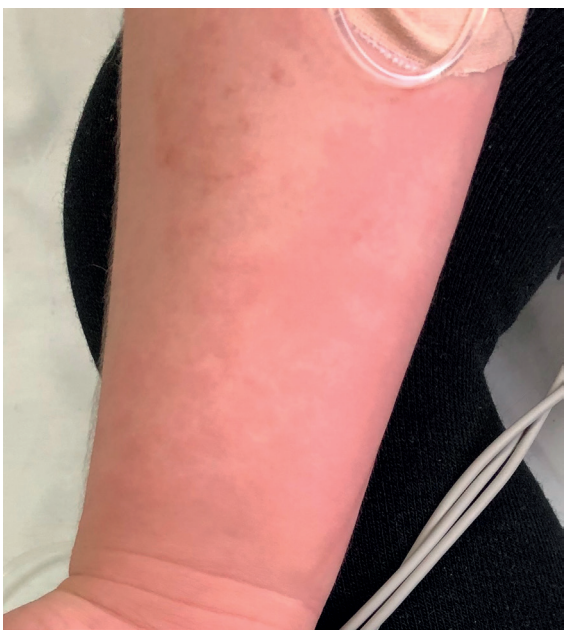


Fig. 1. The rash associated with the second dose of rituximab.







Figs. 2-4. The rash associated with the third dose of rituximab on the face, feet and forearm.

Discussion

Rituximab is a chimeric anti-CD20 mouse-human monoclonal antibody. Once bound to CD20, rituximab eliminates B cells by direct, complement and antibody-dependent cellular cytotoxicity.[11] Rituximab has been generally used to treat various autoimmune diseases and hematological malignancies in which B cells or CD20-bearing cells are participants.[12,13] While it is frequently licensed for these diseases, it is not yet licensed for nephrotic syndrome in many countries.

Rituximab reactions mostly occur with the first infusion. These are associated with general infusion reactions, including fever, chills, and rigors. However, it may also be allergic/anaphylactoid spectrum reactions such as urticaria, angioedema and hypotension.[14] Simple reactions are thought to be due to a cytokine release syndrome potentially triggered by the murine element of the antibody.[14]

RISS was first reported in 2001 in a patient treated with rituximab for refractory autoimmune polyneuropathy. Since then, about 50 cases of RISS have been published in patients with rheumatological and hematological diseases.[14,15] However, there are only two case reports of RISS associated with NS, one being a 17-year-old girl with refractory steroid-dependent NS and the other was a 50-year-old female with membranous nephropathy.[8,9] Nevertheless, to the best of our knowledge, no pediatric case with RISS has yet been reported in membranous nephropathy.

RISS usually occurs 10-14 days after primary antigen exposure or within a few days of secondary antigen exposure. Clinical findings include fever, arthralgia, urticarial rash, lymphadenopathy, proteinuria and gastrointestinal symptoms.[14] It may also be accompanied by elevated inflammatory markers, high immunoglobulin and decreased complement levels.

The mechanism of RISS is not exactly known, but it is considered that B-cells sensitized to

rituximab are lysed, resulting in the release of antibodies to rituximab and formation of immune complexes.[16] After that, formation of these immune complexes result in immune complex-mediated type III hypersensitivity, resulting in complement activation and mast cell degranulation. It is considered that human anti-chimeric antibodies (HACAs) may be associated with RISS and HACAs development may provide important clues to diagnosing serum sickness. However, not all of the patients with RISS developed HACAs in previous studies.[14] Furthermore, absence or presence of HACAs has not been consistently associated with RISS development.[17] Therefore, the diagnosis of RISS should be made based on history and symptoms.[14] Our patient presented with diffuse arthralgia and myalgia eight days after the first rituximab dose. He developed an urticarial rash and arthralgia one week after the second rituximab dose, while he had swelling of lips and periorbital regions, choking sensation and erythematous rash in whole body within minutes after the third dose of rituximab. It is important to recognize the temporal relationship of symptoms with the dose of rituximab infusion (usually 7 days), as RISS is a clinical diagnosis.[14] The first two reactions resembled typical serum sickness with regard to both the clinical findings and the temporal relation to infusion. However, the third reaction that developed during infusion of rituximab seemed to be an anaphylaxis/anaphylactoid reaction clinically. IgE-mediated hypersensitivity reactions to rituximab have been reported even during 4th or 5th infusions previously. Positive skin test was reported in half of the cases.[10] We could not measure serum HACAs level and the RISS diagnoses was made according to the history and symptoms. We also did not attempt to perform a skin test for confirmation of hypersensitivity to rituximab.

RISS has been reported at a higher rate in patients with autoimmune diseases compared to those with lymphoma and NS.[4] Thus, it has been proposed that rituximab may be more immunogenic in autoimmune diseases due to the highly activated B lymphocytes.[18]

Steroid treatment is the mainstay of management in RISS.[8,14] Antihistaminics may also provide extra benefit. A systemic review showed that symptoms resolve in 2.15 ± 1.34 days.[14] Rituximab is usually discontinued in patients who developed RISS. On the other hand, rituximab was continued in four RISS cases along with steroid therapy in a previous study.[14] We also used intramuscular methylprednisolone with adrenaline and the next day the reaction subsided almost completely. However, we did not continue rituximab therapy in our patient.

In conclusion, RISS is often a benign condition and usually resolves with steroid therapy. However, it is important to recognize and distinguish RISS clinically from the other side effects of rituximab. If RISS is not recognized and the patient is re-exposed to rituximab, more serious clinically consequences may develop.

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Case Report

A rare cause of inguinal abscess: perforated appendicitis due to foreign body in Amyand's hernia

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ABSTRACT

Background. Amyand's hernia is rarely noted in children, and appendicitis caused by a foreign body in Amyand's hernia is even rarer.

Case. A 2-year-old girl presented with recurrent conglomerate lymph node enlargements and an abscess in the right groin existing for one year despite medical treatment. Direct radiography revealed a foreign body in the right inguinal region. Computed tomography showed a foreign body and soft tissue inflammation in the inguinal canal. Laparotomy was performed, and Amyand's hernia was diagnosed. A foreign body was found in the lumen of the appendix vermiformis causing perforated appendicitis. This case is presented because of its rarity and unusual clinical presentation.

Conclusion. Amyand's hernia should be considered in paediatric cases with the history of recurrent inguinal abscesses.

Key words: Amyand's hernia, inguinal abscess, perforated appendicitis, foreign body.

Amyand's hernia (AH) was first described in 1735 by Claudius Amyand in an 11-year-old male patient.[1] The incidence of acute appendicitis (AA) within an inguinal hernia is approximately 0.13%.[2,3] Preoperative diagnosis is rather difficult despite advances in imaging modalities.[3,4] This case is presented to emphasise this rare comorbidity and to highlight that AH should be considered in cases with a foreign body in the inguinal canal and inguinal abscess.

Case Report

A 2-year-old girl was admitted to a paediatric haematology clinic one year ago with complaints of swelling and redness in her right groin, and antibiotic treatment was administered. Clinical findings improved, and the patient was discharged. Two months previously, antibiotic treatment was started again due to the same complaints. Since needle biopsy results were compatible with abscess, the patient was referred to our clinic. The general condition of the patient was well, and her body temperature was 38.5°C. Physical examination revealed swelling, redness and local temperature increase in the right inguinal region (Fig. 1). Blood biochemistry values were normal. White blood cell count was 16530/mm^3, erythrocyte sedimentation rate was 60 mm/h, and C-reactive protein level was 71.5 mg/L. Direct abdominal radiography revealed an opacity in the right inguinal region, which was attributed to a foreign body (Fig. 2). Inguinal ultrasonography (US) performed with linear probe revealed a fluid collection with high-density content in the edematous subcutaneus fat tissue and accompanying multiple lymph nodes. Pelvic computed tomography (CT) revealed a 31×35×42 mm (anteriorposterior x

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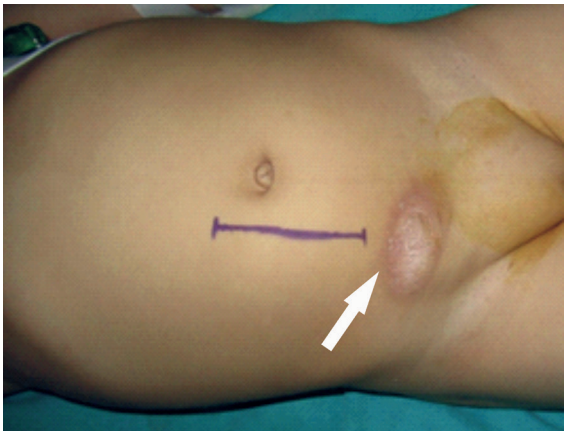

Fig. 1. Perioperative view (abscess and incision site in the right inguinal area).

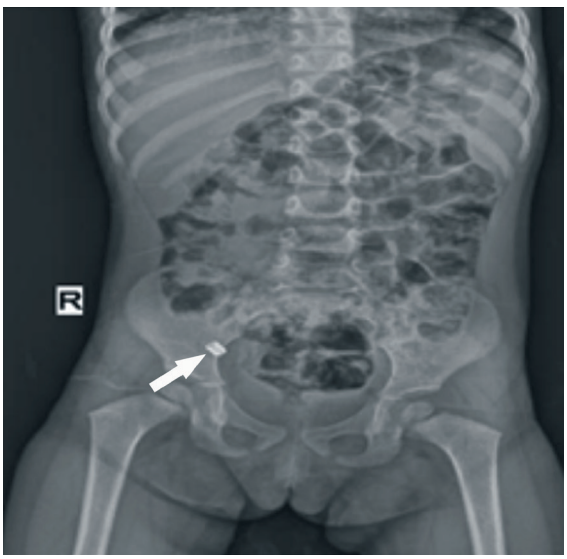

Fig. 2. Abdominal X-ray image demonstrates a radiopaque foreign body (arrow) in the right lower quadrant.

transverse x longitudinal) mass with soft tissue density in the subcutaneous compartment of right lower quadrant. A 10-mm foreign body with hyperdense appearance was located posterior to this lesion and close to the anterior abdominal wall in the abdomen. On laparotomy, the intra-abdominal area was clean. The appendix was found to be herniating in the hernial sac (AH) through the opening in the right internal inguinal ring. The appendix was reduced and noted to be perforated, and an abscess in the inguinal canal was detected. Appendectomy, abscess drainage and inguinal hernia repair were performed. The foreign body (metal lid??) found in the lower right quadrant on perioperative radiography was not detected on radiography following appendectomy. Examination of the removed appendix showed that the foreign body was located distal to the appendix (Fig. 3). The patient was discharged on 6th postoperative day.

Informed consent was obtained from the patient's parents for publication.

Discussion

Faecal deposits, infections, tumours, and rarely foreign bodies (0.005%) constitute underlying causes in the pathophysiology of appendicitis.[5-7] AH is a rare pathology.[2,3] It is defined as the inflamed or non-inflamed appendix entering the inguinal canal through the inguinal hernial sac.[1,2] AH occurring with a foreign body is a much rarer condition.[8] AH is frequently detected in boys and on the right side due to the anatomical location of the ovarian tissue in girls.[9] The vast majority of AH cases are diagnosed within the first 6 months of life (85.7%).[3]

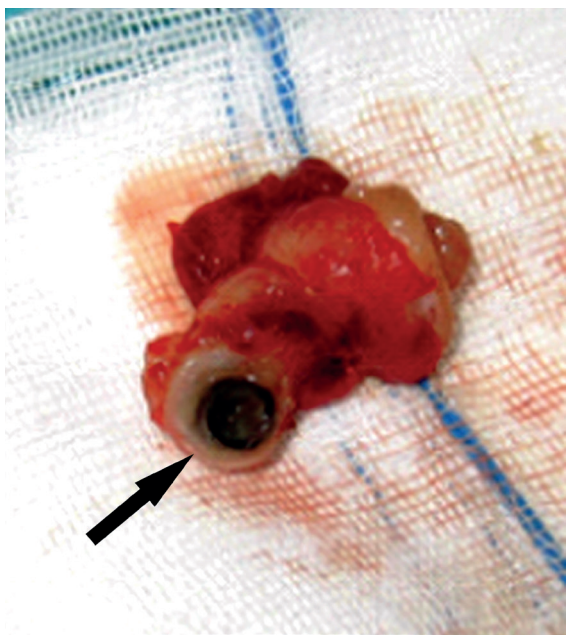

Fig. 3. Image of the foreign body (arrow) in the appendicular lumen.

AH is usually identified during surgery.10 Ultrasonography and CT are used for the identification of AH. However, imaging modalities are not routinely used for the diagnosis of inguinal hernias.[4,11] Unlike those in the literature, the present study reported the case of a 2-year-old female. The appendix had entered the right inguinal canal, and perforated appendicitis and abscess had developed due to the foreign body in it. The clinical picture, which was treated at the haematology clinic one year ago, was probably due to the same cause. However, radiography was not performed at that time. Foreign body in the right inguinal region and associated abscess development was predicted before the surgery; however, AH was defined perioperatively.

Differential diagnosis of inguinal masses in children include hernia (inguinal or femoral), lymphadenopathy, hydrocele, cyst of the canal of Nuck, abscess, hematoma, and malignant soft tissue tumors such as sarcoma.[12] The reasons for inguinal lymphadenopathy were local infections, bugbite, diaper dermatitis, syphilis, lymphogranuloma venereum, autoimmune diseases, storage disorders and malignancies.[13] Abdominal X-ray examinations may be performed if hernia is suspected and visualization of herniated bowel aimed. Most frequently preferred imaging technique in evaluation of inguinal mass is US. With its real-time imaging ability US is a mainstay imaging technique in inguinal hernia since herniating bowel or intraabdominal fat content may be visualized during valsalva maneuvre. US may be also used as a first-line imaging technique in the diagnosis of other inguinal mass-forming lesions such as enlarged lymph nodes, hydrocele, abscess and hematoma. CT or MRI may be required to interrogate the origin of the inguinal abscesses. Soft tissue sarcomas involving inguinal region should be evaluated with CT to determine the extent of these masses in the abdomen.[14]

The prevailing opinion in the treatment of AH is that appendectomy should not be performed in cases in which AA findings are absent, and the appendix can be reduced into the abdomen.[2] Amyand hernia is frequently detected incidentally during inguinal hernia operations. For this reason, inguinal approach is performed in AH cases. Appendectomy through the sac and high ligation procedures can be performed through inguinal transverse incision performed during inguinal hernia treatment. In the present case, laparotomy was planned due to the presence of a foreign body in the inguinal region extending into the abdomen. Appendectomy, abscess drainage and debridement and intra-abdominal inguinal hernia repair were performed.

In conclusion, AH should be considered in cases with recurrent inguinal abscess regardless of the gender of the child.

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Are homozygous *SLC19A3* deletions non-responsive to thiamine/biotin?

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With interest, we read the article by Değerliyurt et al.[1] about a 5yo male with biotin-thiamine-responsive Leigh syndrome due to a homozygous deletion in *SLC19A3*. Despite treatment with thiamine and biotin and a triple anti-seizure drug (ASD) therapy the patient presented clinically with severe developmental delay, intractable seizures, intermittent dystonia, axial hypotonia, and quadruspasticity and became progressive.[1] The cerebral MRI showed bilaterally symmetric T2/FLAIR hyperintense lesions in the basal ganglia (initially sparing the caudate nucleus), the pons, and bilaterally symmetric DWI hyperintensities in the peri-rolandic areas.[1] We have the following comments and concerns.

The main shortcoming is that genetic studies had been carried out only in the index patient and not in other affected or non-affected first-degree relatives. Since the deletion occurred in the homozygous form and one sibling had died from Leigh syndrome at age 2y, it is quite likely that either parent each carried the variant in the heterozygous form. In this respect, we should know if any of the parent's relatives were clinically affected by a neurodegenerative or multisystem disease.

A second shortcoming is that it remains unexplained why the patient was progressive despite receiving an appropriate treatment. We should know if the patient truly adhered to the therapy or if thiamine and biotin were not regularly taken after some time as indicated in the report.[1] It is also conceivable that the patient adhered to the treatment but that the condition was non-responsive to treatment. It is also conceivable that the dosage was too low or that there was an interference between the vitamins and other drugs (e.g. ASDs)[2] which prevented adequate resorption of the vitamins or induced accelerated elimination after resorption.

A third shortcoming is that the patient's triple antiepileptic medication was not mentioned. Initially, he received phenobarbital (PB) and phenytoin (PHT). From both these ASDs it is well-known that they are mitochondrion-toxic.[3] We should know if these ASDs were discontinued and replaced during the course or if the patient was still taking them at the last follow-up. It is conceivable that mitochondrion-toxicity of ASDs contributed to the progression of the phenotype and the involvement of the caudate nucleus on follow-up MRIs despite initial improvement of the imaging findings.

A fourth shortcoming is that no discussion was hold about the possibility that all the lesions seen on MRI were multi-locular stroke-like lesions (SLLs).[4] Since at least the supra-tentorial lesion were hyperintense on DWI and also the infra-tentorial lesions were described as DWI hyperintense, we should know if corresponding ADC maps were hyperintense, isointense, or hypointense. Knowing if a cytotoxic or vasogenic edema was present is crucial as therapy may be different from that applied in this case. If the lesions were interpreted as SLLs, NO-precursors could have been beneficial.[5] If it is was a cytotoxic edema, cardiac embolism, thus involvement of the heart in the metabolic disorder, needs to be excluded.

Overall, this interesting case has a number of shortcomings which need to be solved

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before final conclusions can be drawn. Drug-adherence, drug toxicity, nature of imaging abnormalities, and causes of progression need to be extensively discussed. It is also crucial for genetic counselling of the family that the genetic status of other first degree relatives, in particular the parents, is known.

Key words: SLC19A3, mitochondrial, thiamin, mtDNA, mitochondrial, biotin.

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Response to "Neonatal form of biotin-thiamine-responsive basal ganglia disease. Clues to diagnosis"

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Thank you for your interest in our report[1] and the commentary you provided.

If we start with genetics, this case has a homozygote whole gene deletion. In such a gross deletion case, family screening for probable heterozygote cases is frequently done using the MLPA method. MLPA commercial kits are produced by a single company all over the world and there is no kit for the SLC19A3 gene. PCR gel electrophoresis method can be used for family screening but it cannot make differential diagnosis between heterozygote deletions and those that are normal. Gap-PCR testing is not useful for whole gene deletions as detection of breakpoints is very difficult. Whole genome sequencing or Q-PCR may be used for this study but these applications are very expensive. In such homozygote cases when parents are relatives, the possibility of UPD or de novo deletions are "extremely low" and are mostly not present in daily routine. On the other hand, gross deletion is a very well-known mechanism for this disorder and there is no confusion concerning if this is a disease causing variant or not. As there was no confusion, healthy sibling screening was not a deficiency in showing if this deletion was disease causing for this patient or not. In summary, family screening is conducted in all cases with single gene disorders in our daily routine but in this case, due to the type of mutation and limitations of techniques worldwide, we did not screen this family.

Biotin-thiamine-responsive basal ganglia disease (BTBGD) is a progressive disorder that can result in severe sequela and death if not treated. Such poor prognosis is especially common in patients with early neonatal Leigh-like syndrome and the early infantile-onset form of BTBGD, as in our patient, even when treated with biotin and thiamine.[2,3] The fact that these patients show neurodevelopmental problems in various degrees despite early treatment[4,5] indicates that it may be too late to prevent permanent brain damage even when treatment is started at the time of the first symptoms.[6]

As we also mentioned in the article, our patient had not regularly used the vitamin treatment started during the neonatal period until he was diagnosed when 3 years old. The disease had therefore progressed until the time of diagnosis and the patient had developed dystonia in addition to motor retardation. He used the treatment regularly once the definite diagnosis was made and the symptoms did not worsen afterwards. However, he did not fully respond to the treatment and the sequela remained as the basal ganglia damage was already irreversible. The lack of response to treatment following the development of permanent damage in the cerebral areas as a result of a long untreated period has also been demonstrated previously for this disorder.[7]

Despite the lack of a general consensus, the recommended doses in BTBGD treatment are 10-40 mg/kg/d for thiamine (with increased doses suggested during the acute crisis period) and 5-10 mg/kg/d for biotin.[2,8] The doses of 30 mg/kg/d thiamine and 10 mg/kg/d biotin started after our patient was 3 years old are within the recommended ranges in the literature. Our patient is now 7 years old and the clinical picture is stable. Head control is present but he still needs support to sit and can only say a few words. The intermittent dystonia that appears with stimulus and the infrequent seizures continue. He is being treated for the seizures with topiramate, clobazam and levetiracetam, which have been cleared for use in BTBGD.[2]

Our patient has been undergoing cardiac investigations including echocardiography since the neonatal period for the metabolic disorder and there is no cardiac pathology.

Classic BTBGD presents with typical MRI findings demonstrating dominant involvement of the bilateral caudate heads and the putamen, as originally reported by Ozand et al.[9] There may be various degrees of cortex, subcortical white matter, thalamus, cerebellum and brainstem involvement in addition to significant symmetrical edema of the bilateral caudate heads and the putamen in the acute stage.[10,11] Once the acute stage is over, the abnormal signal changes at the caudate head and the putamen are usually permanent. It is not possible for a cerebrovascular accident or a stroke to cause bilateral and symmetrical involvement of so many different and extensive areas. Our patient had no cardiac involvement that could cause an embolus. Signal changes together with significant edema in the thalamus and putamen, bilateral cortical involvement, and brainstem involvement were present at the time of first presentation in the newborn period, as seen in almost all BTBGD patients with the acute neonatal form of the disorder. There was no caudate involvement in the MRI at the time of the first presentation. What we wanted to emphasize in this paper was the absence of caudate involvement, although involvement of the putamen, cortex, thalamus, and brainstem was present bilaterally, in the acute stage in patients with neonatal type BTBGD in contrast to the classic form. Although this has been mentioned in some previous articles, it has not been emphasized as a characteristic of the neonatal form.[12,13] We would like to emphasize once again that basal ganglia disease responsive to biotin and thiamine treatment, one of the treatable types of Leigh syndrome, should be considered and treatment started immediately if the MRI shows no caudate involvement despite cortex, putamen, thalamus, and brainstem involvement bilaterally on MRI in a newborn patient with encephalopathy and lactic acidosis.

In closing, we would also like to pay our respects to the eminent Turkish pediatrician Dr. Pinar Ozand who was the first to introduce this metabolic disorder, for which recognition is of great significance as it is treatable, into the medical agenda with the original article he wrote following his careful observations.

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NOTICE TO AUTHORS

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All papers are subject to editorial revision for the purpose of conformity to the style adopted by The Turkish Journal of Pediatrics, and authors may be requested to shorten or revise their papers. Statements and opinions expressed in the articles are those of the authors and not necessarily those of the Editor or publisher; the Editor and publisher disclaim any responsibility or liability for such material. Neither the Editor nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication; neither do they guarantee any claim made by the manufacturer of such product or service.

Preparation of Manuscript: Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts. Manuscript should be uploaded in a blinded manner; any affiliation must be covered.

Format: Manuscripts should be typed in 12pt (Times New Roman), double-spaced, with wide margins, and pages must be numbered on the right upper corner.

Covering Letter: The covering letter is important to help the Editor in his/her preliminary evaluation, please indicate why you think the paper is suitable for publication. A statement that each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript; if more than 6 authors, an explanation of the contributions of each author must be provided. The covering letter will be uploaded as a separate file.

Title Page: A separate title page should include authors' names and academic degrees; departmental and institutional affiliations of each author, and sources of financial assistance, if any. Designate one author as the correspondent, and provide an address as well as business and home telephone numbers. Galley proofs and order forms for reprints will be sent to the corresponding author, at a later date. On the title page please add a short running title, word count (excluding abstract and references), number of tables and figures.

Types of Articles:

Original Article: Original articles should report scientific findings within the pediatric field of interest. They must include a unstructured abstract of less than 300 words, to appear after the title page. The manuscript should be structured with the following headings: introduction, material and methods and Results. The introduction should put the study in context with the current literature and reflect the purpose of the study. The material and method should include the study methodology, the setting for the study (although affiliation must be covered), the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The Results section should include the outcome of the study and statistical significance, if appropriate. The Conclusion(s) states the significance of the results and limitations of the study.

Case Reports: Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Case Reports should consist of an unstructured abstract that summarizes the case(s), a brief introduction (recommended length, 1-2 paragraphs), a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process.

Review Articles: Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission.

Structured manuscript for systematic reviews are recommended. Headings should include: Unstructured abstract, Introduction, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions. The general instructions regarding submission (including cover letter, title page requirements, journal style guidance, and conflict of interest statements) also apply to Review Articles.

Short communication: Short communications are opinion pieces consisting of a main point and supporting discussion. They may address general issues or controversies in the field of pediatrics. No abstract is needed but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to short communications.

Clinicopathological exercises: Authors can submit for consideration an clinicopathological exercises that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to clinicopathological exercises.

Letters to the Editor: Letters to the Editor should pertain to papers published in The Turkish Journal of Pediatrics within the past year. Provide a unique title for the Letter on the title page with complete contact information for the author(s). Double-space the text of the Letter. References, including reference to the pertinent article(s) in The Journal, should conform to style for manuscripts. No abstract is needed for a letter to editor. Letters to the Editor may be sent to the authors of the original article to which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same issue as the Letter to the Editor.

Summary / Abstract and key words: An informative summary not exceeding 300 words must accompany each manuscript, Unexplained abbreviations and references should not be given. The abstract should state the objective (the purpose of the study and hypothesis) and summarize the rationale for the study. Following the material and methods it is critically important that all significant findings should be presented in the abstract. The abstracts should be unstructured.

The authors should list three to five key words or phrases taken from Index Medicus

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Abbreviations: Abbreviations must conform to accepted standards. Laboratory slang, clinical jargon and colloquialisms must be avoided. The full term for an abbreviation should precede its first use in the text. Generally, abbreviations should be limited to those defined in the AMA Manual of Style, current edition

Informed Consent: The Materials and Methods section should include a statement that the research was reviewed and approved by an institutional review board, and that participation involved informed consent. Please give information concerning where and when the study was approved, with the report number. The ethics committee report should also be uploaded to the online system.

Written consent from the family is required before a case report can be published. On submission, authors must attest that they have written consent from the family. Instances where there are extenuating circumstances in which family consent may be problematic will be handled on a case-by-case basis. A sentence stating that informed consent was received from the family should be added to the case section of the main document.

Conflicts of Interest: A conflict of interest is defined as any financial, personal, academically competitive or intellectual relationship between the author, referee or editor that involves article submission, referee evaluation, editorial decision and author-referee-editor correspondence and that could lead to a prejudiced and unfair action during the evaluation stage.

The authors must declare any financial relationship or conflict of interest areas that need to be known regarding the study. All financial contributions and sponsorships for the study and all financial relationships and conflict of interest areas should be included in the copyright transfer form that accompanies the article. The authors must explain the study sponsor's role in collecting, evaluating and analyzing the data, writing the article and submitting it for publication. Declaration of conflict of interest should be given at the end of the manuscript. If there is no conflict of interest, this section must include a "The authors declare no conflict of interest" statement at the end of the manuscript. All sources of funding should be mentioned in the manuscript.

The evaluation process for the article is not started if a conflict of interest statement is not present at the time the article is sent. If a conflict of interest becomes apparent after the article is published, the journal may make an official correction or withdraw the entire article.

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Tables: Tables should be numbered with Roman numerals according to the order of mention in the text, each table must be typed double-spaced. A title for each table must be given. The first letter of each word should be capitalized. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, â footnote must give full credit to the original source. Tables should be added as a separate file.

Figures: Figures and illustrations should be of direct interest to the subject of the paper and numbered with Arabic numerals according to order of reference in the text. Art should be created / scanned and saved as either TIFF or JPEG format, submitted as a separate file, and not embedded in the text file. All lettering must be professional and in proportion to the drawing, graph, or photograph. A list of legends for the figures and illustrations must also be added as a separate file.

References: References to the literature should be limited to those cited by the author, arranged and numbered according to order they appear in the text; do not alphabetize. In text, tables, and legends, identify references with superscript Arabic numerals at the end of the sentence (after the full stop). List all authors up to 6; if more than 6, list the first 3 followed by "et al."

If reference to an author is made in the text the reference must be given after the name of the author rather than at the end of a sentence.

Example: In a study by Bier DM et al.23 the prevelance of…….

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References to journal articles:

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References to books:

Example: 2. Praat RTC, The Genetics of Neurological Disorders. London: Oxford University Press, 1967: 173-174.

References to chapters in books:

Example: 3, Kissane M. Development of the kidney and congenital malformations. In: heptinstall RH (ed). Pathology of the Kidney (2nd ed) Vol. 1. Boston: Little, Brown and Co, 1974: 69-109.

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Submission of article: All manuscripts must be submitted on-line through the web site http://www.turkishjournalpediatrics.org. The corresponding author should upload the "Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form" with the main manuscript. The copyright transfer form can be downloaded during the online submission process.

Announcements: Announcements of scheduled meetings, symposia, or postgraduate courses may be sent to the publisher for consideration at least 5 months in advance of the meeting date.

Footnote: If the study has been presented at a scientific meeting or is part of a thesis this can be added to the end of the manuscript as a footnote.

No manuscript will be considered for review until all authors have completed an uploaded the copyright form.

Checklist

- Please check your article as described below before sending it to the Editor.
- Covering letter
- Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form.
- Title page
  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
  - Word count and number of tables and figures
- Article
  - Original articles: Summary, Introduction, Material and Methods, Results, Discussion,
  - Case Reports: Summary, Introduction, Case Report, Discussion,
  - Key words
- Manuscript should be uploaded in a blinded manner; any affiliation must be covered.
- Declaration of informed consent in the material methods section
- Declaration of conflict of interest at the end of the manuscript.
- References on a separate page
- Tables should be uploaded separately
- Figures should be uploaded separately

CASE REPORT

LETTER TO EDITOR

Printed by METEKSAN in Ankara, Turkey